

Fish
Locomotion

SYMPOSIUM PROCEEDINGS

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PREFACE

Locomotion in fish consists of a set of motions and behaviours that are determined by the morphology, anatomy, physiology, and motivation of the particular species in question, as well as extrinsic factors such as water temperature, velocity, concentration of dissolved gasses, and contaminant load. Early studies on the subject typically employed laboratory-based experiments and forced swimming tests to study questions related to fish locomotion, and our understanding of swimming capacity and exercise physiology has benefited (and continues to benefit) immensely from this work. However, there has been an increasing interest in studying fish movements under more natural, and less confining, conditions using new and innovative techniques and technologies. These studies have (1) introduced novel protocols for measuring swimming capacity and physiology, (2) questioned long-held theories related to fish locomotion, (3) integrated behaviour, performance, muscle mechanics, cardiovascular dynamics, energetics, and exercise physiology, and (4) shed new light on complex behaviours such as foraging, migration, and predator avoidance. This symposium seeks to highlight some of these new and exciting areas of research, such that we can continue and build on the rich body of knowledge that has been generated by fish locomotion researchers over the past century.

Symposium Organizers:

Steve Peake, University of New Brunswick
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CONGRESS ACKNOWLEDGEMENTS

This volume is part of the Proceedings of the 6th International Congress on the Biology of Fish, held in Manaus, Brazil in August, 2004. Ten years have passed since the first meeting in this series was held in Vancouver, BC, Canada. Subsequent meetings were in San Francisco, California; Baltimore, Maryland; Aberdeen, Scotland; and again in Vancouver, Canada. From those meetings, colleagues from over 30 countries have contributed more than 2,500 papers to the Proceedings of over 80 Congress Symposia, all available for free viewing on the internet.

We would like to extend our sincere thanks to the many people who helped us organize the facilities and program for this 6th Congress.

The local arrangements team worked very hard to make this Congress a success. The leaders of those efforts were Vera Almeida Val, Adriana Chippari-Gomes, Nivia Pires Lopes and Maria de Nazare Paula Silva (Local Arrangements); Marcelo Perlingeiro (Executive Secretary) and Maria Angelica Laredo (Fund Raising). The enormous contribution of time and effort that was required has led to an unforgettable experience for the participants, thanks to the imagination, determination and dedication of this team.

Many sponsors helped ensure the success of the meeting through both monetary and in-kind contributions, including: Fundação Djalma Batista, Honda, Merse, Cometais, Turkys Aquarium, Banco da Amazônia, Banco do Brasil, FUCAPI, SEBRAE/AM, IDAM/SEPROR, FAPEAM, SECT-AM, SUFRAMA, PETROBRÁS, CAPES, FINEP, CNPq, the Physiology Section of the American Fisheries Society, UFAM - Federal University of Amazonas, Fisheries and Oceans Canada and INPA - National Institute for Research in the Amazon.

Travel arrangements were ably handled by Atlantic Corporate Travel (special thanks to Maria Espinosa) and Orcal Planet, and the venue for the meeting was the spectacular Tropical Hotel Conference Center in Manaus.

The Student Travel Award Committee of the Physiology Section of the American Fisheries Society, led by Michael Redding, evaluated 65 applications from 15 countries and awarded 40 Travel Grants, after an ambitious and trying fund-raising effort. Special thanks must go the US Department of Agriculture, the US Geological Survey, US National Science Foundation and the World

Fisheries Congress for providing funds. In addition, the American Fisheries Society contributed books to be used as prizes for the best student papers.

The editorial team compiled the short abstracts into an abstract book and formatted and compiled the papers for the Symposium Proceedings. Thanks to Karin Howard, Christie MacKinlay, Anne Martin, Callan MacKinlay and Marcelo Perlingeiro.

In particular, we would like to extend a sincere 'thank you' to the organizers of the individual scientific Symposia and their many contributors who took the time to prepare a written submission for these proceedings. Their efforts are very much appreciated. We hope that their participation will result in new insights, new collaborations and new lines of research, leading to new papers to be presented at the 2006 Congress in St. John's, Newfoundland.

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**ABNORMAL MIGRATION TIMING AND ENROUTE MORTALITY OF
LATE RUN FRASER RIVER SOCKEYE SALMON – FROM
OBSERVATIONS TO MECHANISMS**

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Since 1995, several stocks of Fraser River sockeye salmon (*Oncorhynchus nerka*) have begun upriver spawning migrations significantly earlier than previously observed. In some years, the timing of peak migration has shifted more than 6 weeks (Figure 1). Coincident with this early migration are high levels of en route and pre-spawning mortality, occasionally exceeding 90% (Cooke et al. 2004; Figure 2). These phenomena pose risks to the perpetuation of these fisheries resources.

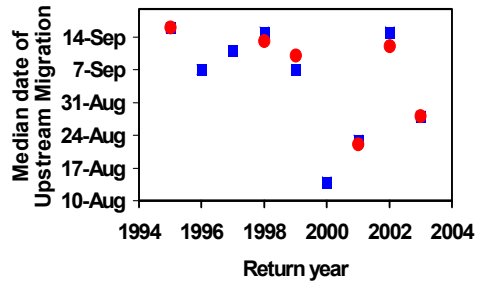


Figure 1. Median dates of upstream migration for late run Fraser sockeye salmon from the Adams (circles) and Weaver (squares) stocks between 1995 and 2003. Upstream migration is based upon passage at Mission, BC. In 2000 and 2001, migration timing was nearly six weeks earlier than historical values collected prior to 1995. Normal median date of migration would be ~September 22nd for Adams River fish and ~ October 1st for Weaver Creek fish.

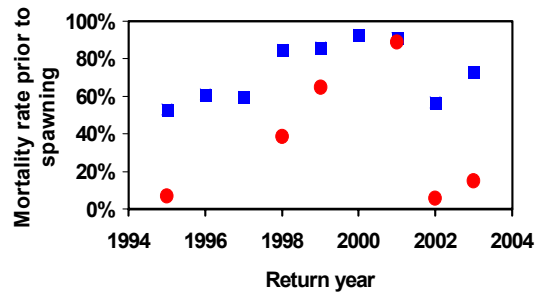


Figure 2. Annual mortality prior to spawning for late run Fraser sockeye salmon from the Adams (circles) and Weaver (squares) stocks between 1995 and 2003. Before 1995, mortality prior to spawning rarely exceeded 5%.

At present, although there are many competing hypotheses (e.g., energetics, osmoregulatory dysfunction, oceanic conditions, parasites) that may account for early migration and high mortality, there are no definitive answers, nor any causal evidence that link these issues. With poor predictive ability in the face of uncertainty, fisheries managers have been unable to effectively allocate harvest quotas, while ensuring that sufficient fish are able to not only reach the spawning sites, but also successfully reproduce. If trends in mortality rates continue, several important sockeye salmon fisheries and stocks could collapse. Indeed, one sockeye stock has already been emergency listed as endangered under Canadian legislation (Committee on the Status of Endangered Wildlife in Canada). Although the stock has yet to be listed under the new Species at Risk Act, a recovery team has already been formed.

Our research group has embarked on an interdisciplinary, multi-agency research program, funded largely through a Natural Sciences and Engineering Research Council of Canada Strategic Grant, to explore differences between abnormal and normal migrants and examine the intergenerational consequences of abnormal migration timing. The team brings together individuals with backgrounds in ecology, parasitology, behaviour, physiology, energetics, oceanography, conservation, and management. This comprehensive approach is intended to enable the exploration of hypotheses and mechanisms that cross traditional boundaries. Funding from the Pacific Salmon Commission and Canadian Department of Fisheries and Oceans is enabling complementary questions to be addressed, such as the large-scale telemetry and disc tagging programs. There is an urgent need to research this problem through strategic experimentation and continually monitor the physiological condition and performance of a set of representative stocks in different regions. This effort could help to identify management steps that could ultimately save millions of dollars in lost harvest opportunities while ensuring the perpetuation of the many diverse sockeye salmon populations.

Here, we provide an overview of this problem and describe our efforts to understand what is responsible for these phenomena after two seasons of field research. Even with two years of data, our findings are somewhat preliminary owing to the magnitude and complexity of the problem. However, through observation and experimentation we have been able to eliminate some hypotheses and focus efforts on promising research topics. We will provide an integrative perspective of how factors including water temperature, estuarine salinity, parasite load, osmoregulatory dysfunction, and maturation may interact to produce the patterns of timing and mortality observed since 1995. It is our

hope to move beyond the documentation of current patterns to understanding the mechanisms responsible for them.

References

Cooke, S.J., S.G. Hinch, A.P. Farrell, M. Lapointe, M. Healey, D. Patterson, S. MacDonald, S. Jones, and G. Van Der Kraak. 2004. Early-migration and abnormal mortality of late-run sockeye salmon in the Fraser River, British Columbia. *Fisheries*. 29(2):22-3

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**COUPLING NON-INVASIVE PHYSIOLOGICAL AND ENERGETIC
ASSESSMENTS WITH TELEMETRY TO UNDERSTAND INTER-
INDIVIDUAL VARIATION IN BEHAVIOUR AND SURVIVORSHIP OF
SOCKEYE SALMON**

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We developed a strategy for non-invasively sampling adult sockeye salmon (*Oncorhynchus nerka*) for physiological and energetic variables and then

releasing these fish with gastrically implanted radio and acoustic transmitters. If successful, the coupling of telemetry with physiological and energetic assessments would provide a robust approach for understanding inter-individual variation in behaviour and survival.

Fish were captured using a purse seine boat in the summer of 2003 in Juan de Fuca Strait and the Strait of Georgia along the coast of British Columbia, Canada. Our efforts focused on sockeye salmon that were attempting to locate their natal streams to spawn. For a number of reasons, we were unable to anesthetize fish prior to gastric tagging and bio-sampling so we had to manually restrain live fish in a trough supplied with flow-through water (Figure 1).



Figure 1. A member of the research team uses a vacutainer to draw a blood sample from a sockeye salmon while the fish is immobilized by two other

team members. Fresh water enters the trough near the mouth of the fish and spills out over the back of the trough near the tail.

All sockeye salmon were implanted with radio transmitters by using a plunger to push the transmitter through the esophagus and into the stomach. A subset of the telemetered fish were also bio-sampled which included drawing blood from the caudal peduncle (3 ml), biopsy of gill tissue (0.3g) and quantification of energetic status using a micro-wave fat probe. Fish were held for less than an hour after this procedure and then were released to continue on their upstream journey.

We used several independent experiments to test the hypothesis that the physiological and energetic sampling of telemetered fish had a negligible effect. In the first experiment, we found no difference in the survival (both 100%) or tag retention (both 100%) of the two treatment groups when fish were held in pens for 24 hours in the marine environment. In other experiments, we noted no difference in the proportion of fish that passed in-river telemetry checkpoints indicating similar levels of mortality and/or tag retention. Also, there were no statistical differences in the travel times of fish in the two treatment groups. Thus, the evidence derived from these independent assessments collectively indicates that it is possible to bio-sample telemetered sockeye salmon without any deleterious effects on survival or behaviour.

The approach detailed here enabled us to couple telemetry with physiological and energetic assessments to provide a robust approach for understanding inter-individual variation in behaviour and survival. We will present data from 2003 on ~550 fish to examine the energetic and physiological patterns underlying abnormal migration timing and high en-route mortality of sockeye salmon migrating to the Fraser River BC (Cooke et al. 2004). Data collected from the fat probe included percent lipid and gross somatic energy. The gill biopsy was used for gill ATPase assays. Plasma was assayed for reproductive hormones (including gender assignments), cortisol, glucose, ions, and osmolality. Using this technique we were able to discount a number of existing hypotheses related to the early migration and high prespawning mortality phenomena of late run sockeye salmon. For example, fish migrating early did not have lower energy reserves and there were no differences in the energetics of fish that lived or died. In this presentation we detail the procedures used to bio-sample live fish and then examine the physiological and energetic factors that were associated with different behaviours and fate. To our knowledge, this is the first such experiment to occur on fish on such a large scale. We feel that the approach that

we detail will be applicable to other fish species and we encourage other animal ecologists using telemetry to consider using this strategy to link the physiological and energetic status of individuals with their behaviour and fate.

References

Cooke, S.J., S.G. Hinch, A.P. Farrell, M. Lapointe, M. Healey, D. Patterson, S. MacDonald, S. Jones, and G. Van Der Kraak. 2004. Early-migration and abnormal mortality of late-run sockeye salmon in the Fraser River, British Columbia. *Fisheries*. 29(2):22-33.

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BIOCHEMICAL RESPONSES OF MATRINXA (*Brycon cephalus*),

SUBMITTED TO LONG-TERM SUSTAINED SWIMMING

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Abstract

The long-term exercise and fish performance correlation is still controversial and the responses are different for each species. No data are disposable among the Neotropical fishes concerning this subject. We have studied the metabolic profile of matrinxa submitted to continuous exercise for 37 and 72 days, swimming at $42\text{cm}\cdot\text{s}^{-1}$. The growth of fish exercised for 72 days was 38% greater than the other exercised group, and the catabolism of protein and sugars decreased in the white muscle. Body mass increased and was followed by lipid catabolism. We concluded that exercised fishes at moderate speed growth faster than that living in lentic environments. No large influence in their metabolic responses was observed.

Introduction

Water flux is a natural component of the environments of reofilic fish, like matrinxa. These species are forced swimming against the water current for long periods. However, exercise at moderate speeds has been reported to provide several advantages for fish farming and growth enhancement is related with it (Azuma et al, 2002; Davison, 1997; Jobling, 1993; Young and Cech Jr, 1994, Jobling, 1994).

The severity of the exercise regimen is extremely important, and even low speeds of swimming may produce greater growth rates and better-feed conversion efficiency (Davison, 1997; Totland et al, 1987; Jobling, 1994) what including fish that are less active for swimming, since the speed be adequate for its species (Ogata and Oku, 2000). The ideal swimming rates for rainbow trout and salmon are well defined in literature, altering between $9,68 \text{ cms}^{-1}$ and $35,5 \text{ cms}^{-1}$ (Ristori and Laurent, 1985; Butler et al, 1986; Weber, 1991; Azuma et al, 2002). However, the knowledge about the swimming capacity of Neotropical fish remains few explored.

Fish organism is almost 60% muscle (Jürs and Bastrop, 1995; Jobling, 1994) and it represents the major fraction of energy consume. Usually, during prolonged exercise proteins and lipids are the main fuel, and carbohydrate oxidation is considered minimal, but recent studies have shown an increasing role of sugars for swimming at 55-85% U_{crit} (Weber and Haman, 1996; Van den Thillart and Van Raaji, 1995; Jobling, 1994; Shangavi and Weber, 1999).

Muscle glycogen stores are not the primary fuel for steady state or moderate exercises, but they are very useful supporting burst and sustained maximal exercises (Moyes and West, 1995; Weber and Haman, 1996), however, salmon exercised for eight month increases their glycogen stores (Totland et al, 1987). In the same way, sustained swimming does not stimulate hepatic glucose release from liver, and in rainbow trout glucose exhibits decrease (Shangavi and Weber, 1999). During aerobic swimming, if lactate was generated, production and oxidation keep their balance inasmuch as it is not important as a fuel for energy supply in low and moderate exercises (Jobling, 1994; Moyes and West, 1995; Weber and Haman, 1996; Richards et al (2002).

Lipid oxidation is an important energy fuel for fish, meanly during prolonged exercise, and some species can mobilize its more than others (Van den Thillart and Kesbeke, 1978; Ogata and Oku, 2000; Forster and Ogata, 1996; Van den Thillart and Van Raaji, 1995; Weber and Haman, 1996). Free fatty acid is important oxidative fuel during endurance swimming (Moyes and West, 1995), and lipid is stored in well-perfused tissues, which contains high density of mitochondria, being used for metabolism especially during low speed sustained swimming. However, the studies about exercise and lipid mobilization are very conflictive, because BERNARD et al (1999) showed that triacylglycerol hydrolysis and fatty acid release are not affected by endurance for prolonged exercise beyond the control values, and RICAHRDS et al (2002) demonstrated

that rainbow trout swimming at 30 and 60% U_{crit} oxidize more lipids than controls.

Protein was estimated to provide 80% of total substrates utilized by the fish during the rest and until 90% during sustained swimming (Jürs and Bastrop, 1995), and ammonia excretion reflects amino acid trans-deamination, which can increase during activity (Moyes and West, 1995).

There are some biochemical indices, which are used for both, indicating the metabolic state of fish and estimating their growth rates. In accordance with some authors, glycolytic enzymes, as lactate dehydrogenase (LDH) and piruvate kinase (PK), have strong positive correlation between their activities and growth, which could be correlated with swimming capacity improvement (Pelletier et al, 1995; Burness et al, 1999). The liver-somatic index (LSI), indirect measurement of growth rate, is used to correlate the nutritional status of fish and the growth rate (Busacker et al, 1990). The ratio RNA:protein is an index of ribosomal capacity for protein synthesis and links directly RNA concentrations and protein syntheses. Following the growth, is expected RNA content allows major protein syntheses, since food keep on being available (Burness et al, 1999; Pierce et al, 1999; Busacker et al, 1990; Houlihan et al, 1993).

Because the lack of evidences about Neotropical fish in concern with exercise and growth it is necessary more checking about their biochemical responses and growth rates when they are exercised at different spans and speeds. Matrinxas are fish largely used in aquaculture, explored by their fast growth potential and easy commercial ration adaptation. Brycon genus is spread for the main Brazilian hydrographic basins, and they have high capacity of swimming against current during migration (Margarido and Galetti, 2001; Mendonça, 1996; Castagnolli, 1992). Because of these evidences they have become ideal subjects for exercise and growth studies.

The purpose of this work was to analyze the metabolic responses of white muscle of matrinxa submitted to long-term sustained swimming for 37 and 72 days at $42 \text{ cm}\cdot\text{s}^{-1}$, and the difference in growth rates between all groups, non-exercised and exercised fish.

Materials and methods

Matrinxas (*Brycon cephalus*) were obtained by a commercial fish farm in Mococa, SP, Brazil, and was maintained in 5000L aquarium for a month. Eighty fish were previously anesthetized (Inoue, 2003), weighed and measured, and the averages were $45,5 \pm 17,1$ g and $14,2 \pm 1,7$ cm. They were randomly shared in four groups and transferred for exercising tanks, which had the following characteristics: four 250L fiber aquaria were interconnected to a biofilter, constituting a water recirculation closed system.

Two of this tanks had a submersed pump, responsible for producing current with angular speed of 42 cms⁻¹, which was weakly monitored, as well as the physical and chemical water parameters. The average of this values were: pH 7,4, temperature 24°C and pO₂ 5,7mg/L. Fish were fed twice a day with a commercial ration in a rate of 2% of the biomass for non-exercised fish and 3% to exercised one.

Two of these groups, referred as controls, C30 and C70, were kept in tanks with lentic water for 37 and 72 days, respectively, with any type of exercise and their values were used to be compared with exercised groups. The other two groups were classified as exercised, E30 and E70 and remained in exercising tanks for 37 and 72 days, respectively; swimming against the current with no interruptions, except for cleaning tanks and changing water. At the end of the first part of the protocol, C30 and E30 groups were anesthetized with eugenol, followed by weigh and measure of the fish. Afterwards, the animals were killed, the liver was excised and weighed, and a sample of white muscle and the liver were immediately frozen in liquid nitrogen for further analysis. After 72 days, other two groups were equally sampled.

Frozen muscle was weighed and used for the following determinations: ammonia (Gentzkow and Masen, 1942), glucose (Dubois, 1956), lactate (Harrover and Brown, 1972), protein (Lowry et al, 1951), glycogen (Bidinoto et al, 1997), free amino acids (Copley, 1941), total lipids (Folch et al, 1957), LDH and GDH (HOCHACHKA, 1978), PK (Staal et al, 1975) and the ratio RNA:protein (Pierce et al, 1999).

Results

Growth parameters: Weight values increased in all groups, non-exercised and exercised: C30 grew 15% and E30 20,4%. C70 grew 21,6%, while the growth of the E70 was 37,9% (fig.1). Length differences and liver somatic index (LSI) were not significant for any group (tab.1.) Rate RNA:protein decreased in E30 and feed conversion rate (FCR) enhanced in exercised fishes.

White muscle: Glucose, protein and ammonia remained with no differences when exercised groups were compared with their respective controls (tab.2.). However, lactate levels decreased significantly in E70 not only in comparison with its control but in relation with E30 too (tab.2). Glycogen was not different, but the relation glycogen/total weight decreased in E70 (tab.2.) There were decrease in PK and GDH activities in E70 and an increase in PK in E30. LDH remained with no different values. Total lipids content decreased more than 40% in E70, what could not be observed in E30 (fig.2).

Discussion

Growth parameters: Exercised matrinxas grew more than controls, being the E70 values higher than 37%, while C70 was only 21,6%, the same value reached by E30 in just a month (tab.1). Final length, in spite of significantly different in comparison with initial values, did not reached the same difference between treatments (tab.1). AZUMA et al (2002) related that salmon were positively affected by exercise enhancing their growth.

The liver-somatic index was not different among the treatments, what could indicate that there was no accumulate energy in the liver in glycogen form (tab.1).

It is known that matrinxa is aggressive and maintains dominance hierarchies, however, this fish, when exercised, changed their behaviour, including a reduction in the frequency of aggressions. This information indicates that domination is less strong in exercised fish than in non-exercised one. This has been mentioned for others species (Jobling, 1994; Davison, 1997). Those authors propose that the lesser the aggression the better use of food energy, what is corroborated by the better-feed conversion rate in exercised fish (tab.1.). This index is an indicative of weight gain per unit of food consumed, and fish forced to swim at moderate speeds, for prolonged periods, presents greater conversion than non-exercised fish (Jobling, 1993; Jobling, 1994).

Table. 1. Growth performance of matrinxãs non-exercised and exercised for 37 days and 72 days (mean value \pm sd). Different letters mean statistical differences.

	TREATMENTS			
	C 30	E 30	C 70	E 70
Body weight (g)				
Initial	41,5 \pm 14,5 ^{1a}	45,7 \pm 12,8 ^{1a}	44,7 \pm 17 ^{1a}	50,9 \pm 18,3 ^{1a}
Final	48,8 \pm 16,4 ^{1b}	57,4 \pm 10,3 ^{1c}	57,1 \pm 17,2 ^{1c}	82,1 \pm 24,7 ^{1d}
Body length (cm)				
Initial	13,7 \pm 1,5 ^{2a}	14,3 \pm 1,9 ^{2a}	14,1 \pm 1,6 ^{2a}	14,7 \pm 1,9 ^{2a}
Final	15,3 \pm 1,7^{2b}	16,4 \pm 1,9 ^{2b}	18,8 \pm 9,5 ^{2b}	18,4 \pm 1,8 ^{2b}
LSI	0,01 \pm 0,003	0,011 \pm 0,002	0,013 \pm 0,004	0,013 \pm 0,004
FCR (g)	7,1	4,84	4,28	1,94
		24,06 \pm 3,52 ^{3b}	18,59 \pm 3,73 ^{3c}	22,73 \pm 6,21 ^{3c}
RNA: protein	32,25 \pm 4,49^{3a}			
	(μg/g protein)			

LDH and PK activities (fig.1), when correlated with growth rates, did not exhibited the same responses reported (Pelletier et al, 1995; Burness et al, 1999). It should be expected an increase of their activities when body mass enlarge. LDH was not affected by exercise, while PK was less in E70. This fact may be, in part, because theses exercised fish did not triggered the glycolytic path to produce more ATP.

The rate RNA:protein did no show any evidence of positive relationship between growth and exercise. Actually, its value was less in E30, but this is not an indicative of growth decrease because body size was higher. Jobling (1994) reported the same, indicating a somatic growth with diminished growth rate, pointing some evidences on the correlation of protein synthesis and oxygen consume. This may be altered by the size. Some authors, however, were able to show positive correlation (Mathers, 1999; Pierce, 1999), making believe that each species have different ribosomal capacity for protein synthesis. (Jobling, 1994).

White muscle: Glycogen levels were not altered by exercise (tab.2), what indicates that it is not a preferential energetic fuel for sustained swimming. This

agrees with others authors (Moyes and West, 1995; Weber and Haman, 1996) The relationship between glycogen and final weight (tab.2) displayed slight decrease in E30 but larger in E70, probably indicating that carbohydrate still remains used for the whole organism. This fact was previously mentioned by Richards et al (2002). Glucose was not mobilized from white muscle, and lactate values are diminished in E70, which indicates that oxidized glucose was used by aerobic metabolism because it was not converted into lactate (tab.2.).

Protein value did not show differences for any group, however, the amino acids retention in E70 was very high indicating that protein oxidation in exercised fish was minimal (tab.2.). Similar results were found for rainbow trout that swam at 55-85% U_{crit} , where lipid oxidation was the prime energy fuel followed by carbohydrates and proteins (Richards et al, 2002). Ammonia values were the same, meaning exercise did not promote amino acid trans-deamination for using them as fuel (tab.2.), effect related by others (Van Den Thillart and Kesbeke, 1978; Moyes and West, 1995). These are the facts that contribute for major growth of fish exercised, since muscular protein deposition is higher in fish under constant movement.

Table 2. Metabolic responses of white muscle in non-exercised and exercised matrinxas (mean \pm sd). Letters mean statistical differences.

	TREATMENTS			
	C 30	E 30	C 70	E 70
Glycogen ($\mu\text{mol glycosilg}^{-1}$)	21,18 \pm 4,78	21,4 \pm 3,63	44,43 \pm 6,82	42,5 \pm 4,77
Glycogen (g)/ weight (g)	0,49 \pm 0,21 ^{1a}	0,39 \pm 0,11 ^{1a}	0,88 \pm 0,38 ^{1b}	0,58 \pm 0,23 ^{1c}
Glucose (μmolg^{-1})	49,9 \pm 9,74	52,92 \pm 12,64	34,58 \pm 4,44	30,11 \pm 5,7
Lactate (μmolg^{-1})	63,7 \pm 15,6 ^{2a}	68,5 \pm 12,9 ^{2a}	57,3 \pm 5,4 ^{2a}	45,8 \pm 10 ^{2b}
Protein (μmolg^{-1})	283,1 \pm 26,1	244,7 \pm 41	201,5 \pm 53,1	201,3 \pm 24,1
Free amino acids (μmolg^{-1})	36,47 \pm 11,2 ^{3a}	38,4 \pm 9,68 ^{3a}	42,6 \pm 11,36 ^{3a}	51 \pm 6,95 ^{3b}
Ammonia (μmolg^{-1})	29,3 \pm 1,82	31,6 \pm 2,6	30,13 \pm 2,66	32,72 \pm 3,7

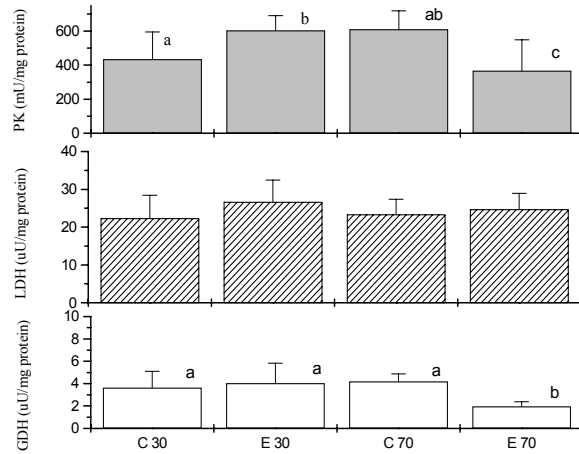


Fig.1. PK, LDH and GDH activities in white muscle of non-exercised and exercised fish.

It is evident that lipid was used as a fuel for E70 group (fig.2), which exhibited 43.4% of decrease in total lipid content. This demonstrates that matrinxas exercised for 72 days are more able to oxidize lipids than fish maintained in lentic environments. Previous studies of Forster and Ogata (1996); Ogata and Oku (2000) showed content lipid decrease during migration and exercise, however, Bernard et al (1999) and Young and Cech Jr (1994) did not observe the same results. According with Van den Thillart and Van Raaji (1995), high plasma FFA and lipid oxidation plus no alterations in white muscle glucose and lactate are indirect measures for indication of moderate speed. Glycolytic and amino acids catabolic enzymes can give information about fish energetic metabolism (graf.1). LDH did not show difference for any exercised groups, but lactate was very low, facts that demonstrate the less importance of this path for generating energy.

Activity PK decreased in E70, which shows the fish less dependence of glycolytic path for generating energy in preference to lipid fonts. Acetil-CoA and chain long fatty acids inhibit activity PK, diminishing more carbohydrates

as energy fuel (Lehninger, 1995). Its activity was high in E30, indicating that for 37 days of exercise the glycolytic metabolism still remains important to supply energy (Richards et al, 2002).

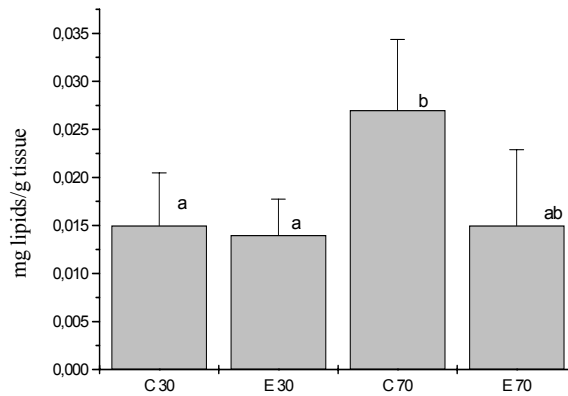


Fig. 2. Total lipid content in white muscle of non-exercised and exercised fish.

GDH activity was more reduced in E70, at the same time that amino acids were elevated. If GDH is responsible for amino acid trans-deamination and ammonia is the end answer of amino acids catabolism, we can deduce that fish exercised for 72 days decreased their use of protein and amino acids for energy supply, favoring their use to biosynthesis and consequently growth.

We have concluded that exercised fish did not suffer great metabolic differences in comparison with non-exercised fish, what improve their growth; exercised fish grow faster than non-exercised fish; E70 group showed best results in relation to lipid oxidation and greater amino acid retention than E30, followed by lactate, GDH and PK decrease; final weight were higher in exercised fish mainly in exercised for 72 days; LDH and PK did not show growth improvement, and rate RNA:protein was diminished in E30. However, fish grew as shown by the final weight differences; feed conversion rate was strongly decreased what could have favored fish growth, inducing thinking less food is necessary to enhance growth when fish are exercised at moderate speeds for long periods.

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DOES PHYSIOLOGY OR ENVIRONMENT

LIMIT SWIMMING PERFORMANCE?

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

The sustained swimming ability of fishes depends on many extrinsic and intrinsic variables (Hammer, 1995), especially those that affect oxygen uptake and delivery to aerobic musculature (Jones, 1971; Gallagher et al., 1995). Temperature is a particularly important factor that presents compounding problems for sustained swimming because it increases metabolic oxygen demand while simultaneously decreasing oxygen availability. Most fishes show a temperature dependent rise in sustained swimming velocity (U_{crit}) to a maximum value that usually corresponds to the preferred temperature for the species. U_{crit} subsequently declines at higher temperatures, presumably due to limitations associated with oxygen delivery. Jones (1971) showed that hypoxia or anaemia could decrease U_{crit} in juvenile trout at both high and low temperatures. However, we found that mild hypoxia (75% sat.) only affected U_{crit} at temperatures above the optimum value of 15°C (Bannon and Ling, 2004). Moreover, fish acclimated at 20°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. To test this theory we separately investigated the effects of mild hypoxia and severe anaemia on juvenile trout in sustained swimming trials at temperatures from 10°C to 20°C.

Materials and methods

Hatchery-reared rainbow trout, *Oncorhynchus mykiss* (Walbaum), 14 ± 0.5 cm, were randomly allocated to treatment groups and acclimated to 10°C, 15°C and 20°C (± 0.5 °C) for at least 21 days. Fish were fed daily to satiation. Treatments included fish at rest or fish swum to exhaustion at each temperature under normoxic ($>96\%$ sat.) or hypoxic conditions (6.8 mg O₂/L, being 75% of the saturated value at 20°C).

Anaemia was induced in some fish acclimated at 15°C and 20°C by caudal venesection. Fish were anaesthetised, weighed, and total blood volume calculated (4% of total weight). 75% anaemia was induced by a single withdrawal of 25% of total blood volume. 57% anaemia was induced by a second withdrawal of a further 25% of total blood volume 24 hours later. Anaemic fish were allowed to recover for 7 days prior to Ucrit determinations.

Critical swimming speeds (Ucrit) of individual fish were measured in a 230 L, variable velocity, recirculating flume. Fish were transferred to the flume and swum for 2 hours at 0.5 body lengths (BL)/s to aid recovery from handling and transfer stress (Milligan et al., 2000), and swum to exhaustion by increasing water velocity in 0.5 BL/s increments every 15 minutes. Ucrit was 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 (0.5 BL/s), T_i is the interval time elapsed at fatigue velocity and T_{ii} is the interval time (15 min).

All fish were bled immediately following exhaustion (~ 200 μ L) and analysed for haematocrit, red blood cell count, whole blood haemoglobin and cortisol.

Results

The critical swimming speeds of normocythaemic and anaemic trout under normoxic and hypoxic conditions are shown in Figure 1. Combined hypoxia and anaemia resulted in a significant reduction in U_{crit} at 20°C only. Although not statistically significant, anaemia or hypoxia independently resulted in slight reductions in U_{crit} at both 15°C and 20°C.

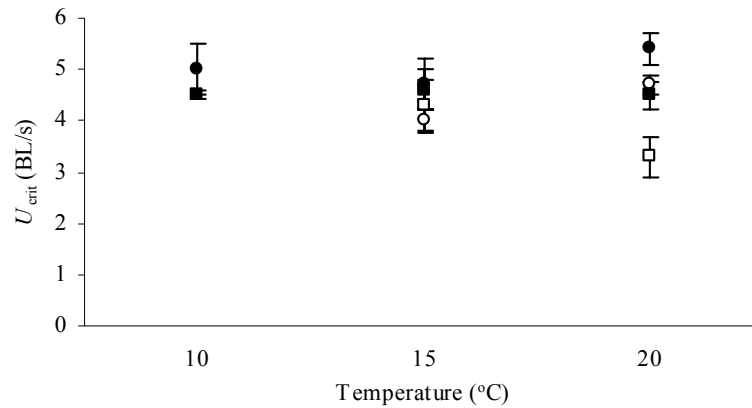


Figure 1. Sustained swimming speeds of normocythaemic (closed symbols) and anaemic (57% of normocythemia; open symbols) juvenile trout at different temperatures in normoxia (>96% sat.; circles) or mild hypoxia (6.8 mg/L; squares). Values are means \pm S.E.M. * = significantly different from other treatments at 20°C ($P < 0.05$).

Haematological responses of all treatment groups are given in Table 1. Compensatory haematological responses to increase oxygen carrying capacity are apparent in exercised fish including splenic release of stored erythrocytes and erythrocytic swelling.

Discussion

The sustained swimming velocity of juvenile trout was maintained from 10°C to 20°C, and hypoxia or anaemia, either singly or in combination, did not result in

significant reductions in Ucrit except at 20oC. Surprisingly, a small reduction in ambient oxygen had as great an effect on Ucrit 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. Although exercised normocythaemic fish do respond by compensatory changes in haematology, including splenic release of erythrocytes and erythrocytic swelling, it would appear that these may play relatively little role in improving aerobic exercise performance.

Variable	Temp (°C)	Control	Control	Normoxic	Normoxic	Hypoxia	Hypoxia
		Resting Normocyth.	Resting Anaemic	Exercised Normocyth.	Exercised Anaemic	Exercised Normocyth.	Exercised Anaemic
PCV (%)	10	28.0 (3.0)	ND	36.4 (2.2)	ND	38.0 (2.1)	ND
	15	28.8 (1.0)	15.6 (2.0)	44.0 (3.0)	16.8 (1.2)	41.6 (0.6)	18.2 (1.7)
	20	30.4 (1.3)	14.8 (1.7)	40.8 (2.5)	16.6 (1.1)	45 (3.0)	19.6 (3.4)
[Hb] (g/L)	10	76.0 (8.5)	ND	83.3 (5.8)	ND	81.5 (4.9)	ND
	15	71.3 (4.0)	34.3 (3.3)	91.3 (5.8)	34.8 (2.6)	91.5 (1.3)	36.8 (3.4)
	20	77.1 (4.0)	32.6 (3.2)	93.8 (4.8)	36.8 (3.0)	102 (6.3)	35.3 (6.5)
MCHC (g/L)	10	272 (8)	ND	229 (7)	ND	215 (4)	ND
	15	245 (8)	223 (8)	211(14)	205 (3)	220 (5)	203 (6)
	20	255 (11)	220 (10)	232 (9)	219 (6)	226 (4)	179 (8)
MCH (pg)	10	80.3 (6.9)	ND	70.2 (2.2)	ND	59.9 (2.3)	ND
	15	71.0 (5.1)	66.2 (4.3)	70.9 (6.1)	73.0 (5.0)	72.3 (6.3)	71.6 (3.0)
	20	68.3 (4.6)	59 (4.5)	74.7 (8.9)	73.5 (7.0)	87.4 (6.4)	56.9 (5.0)
MCV (fL)	10	294 (22)	ND	307(13)	ND	279 (8)	ND
	15	291 (22)	297 (18)	336 (14)	358 (24)	328 (26)	353 (19)
	20	269 (16)	267 (12)	324 (33)	334 (27)	393 (32)	318 (26)
RBCC (x 10 ¹² cells/L)	10	0.99 (0.15)	ND	1.18 (0.05)	ND	1.36 (0.05)	ND
	15	1.03 (0.13)	0.49 (0.06)	1.31 (0.10)	0.48 (0.05)	1.30 (0.10)	0.57 (0.08)
	20	1.15 (0.09)	0.68 (0.10)	1.30 (0.12)	0.51 (0.04)	1.19 (0.14)	0.60 (0.06)
Cortisol (nmol/L)	10	41 (26)	ND	717 (84)	ND	808 (85)	ND
	15	15 (7)	13 (6)	931 (193)	547 (75)	861 (122)	742 (171)
	20	42 (12)	5 (3)	1073 (242)	749 (78)	1030 (150)	1024 (97)

Table 1. Haematological values of normocythaemic and anaemic juvenile trout acclimated to 10oC, 15oC and 20oC and sampled at rest or following exhaustive exercise (Ucrit) under normoxia (>96% sat.) or hypoxia (6.8 mg/L). Values are means with S.E.M. in parentheses, N=5 for all treatments, ND = not done. 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. Indications of statistically significant differences between treatments have been omitted for clarity.

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 simply a compensatory increase in oxygen carrying capacity as implied by Bannon and Ling (2004).

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**METABOLIC RATE IN RELATION TO SWIMMING SPEED,
BODY SIZE AND TEMPERATURE
IN THE GAG GROUPER, *MYCTEROPERCA MICROLEPIS***

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Factors such as temperature, swimming activity and body size are known to influence energetic or metabolic demands of fishes (Beamish, 1970). The measurement of oxygen consumption, via use of respirometry as an indirect measure of metabolic rate, has been an efficient means of accounting for these factors in many studies (Beamish, 1970; Tolley and Torres, 2002). Since it is predicted that all animals must function within the bounds of metabolic scope (Fry, 1947), estimates of standard metabolic rate (SMR) and scope for activity are also important for estimating energetic expenditure for activity, growth, and reproduction. Growth differences in gag grouper have been observed on two artificial patch reef sizes in the eastern Gulf of Mexico. Recent comparative, prey consumption estimates do not explain the growth differences adequately. The present study provides the first examination of oxygen consumption in relation to swimming speed, body size and temperature for gag grouper in the Gulf of Mexico. Estimates for SMR and scope for activity are also examined.

Methods

Gag (420-620mm TL) were captured and housed in several 570 and 680 l tanks. Fish were acclimated to three sequential temperature treatments of 15, 22 and 30 °C, over several months. Temperature was raised slowly over one week, and two additional weeks were allowed before any trials. Fish were deprived of food for at least 48 hrs prior to swim trials to ensure they were in a similar post absorptive state. Swimming trials were conducted in a 257 l modified-Blazka respirometer (Blazka et al. 1960) and generally followed the procedures outlined by Beamish, 1970. Water velocity was increased by increments of 15 cm·s⁻¹ for durations of 30 min and swimming responses ranged from hovering (10 cm·s⁻¹) to burst swimming at (80 cm·s⁻¹). A rest period of 20 min between increases in velocity was allowed, to ensure replenishment of oxygen in the respirometer and control temperature. Oxygen consumption was measured using a polarographic micro-electrode with an A/D computer interface (Strathkelvin 928). Water velocity was measured using a solid state flow meter (Marsh-McBirney, Flowmate). Temperature fluctuated a maximum of ±0.5 °C over the course of an individual trial and ±1 °C over the entire testing sequence. Oxygen consumption rate (VO₂) in mgO₂·kg⁻¹·hr⁻¹ was calculated at each test velocity by linear regression from a portion of the experiment trial where the fish was swimming in a stable manner for at least 15 minutes. Linear, exponential and power functions were fitted to the data for comparison.

Comment: Be more specific about minimum adaptation time and photoperiod.

Results

The exponential model, $y = ae^{bx}$, was found to best fit the oxygen consumption rate versus swimming speed data for gag grouper at 15, 22, and 30 °C (Fig. 1). The model, fitted to experimental data at 15 and 22 °C yielded R² values of .771 and .886 for n= 14 and 13, respectively. The preliminary 30 °C data (n=5) yielded an R² of .840. SMR was estimated at 41.785 mgO₂·kg⁻¹·hr⁻¹ at 15 °C with an exponential relationship of $VO_2 = 41.785e^{1.307v}$; models at 22 and 30 °C corresponded to $VO_2 = 51.706e^{1.220v}$ and $VO_2 = 89.484e^{0.975v}$. The maximum sustainable swimming speed (U_{crit}) averaged 1.04 bl·s⁻¹ ± 0.168 SE at 15 °C, 1.31 bl·s⁻¹ ± 0.214 at 22°C and 1.43 bl·s⁻¹ ± 0.256 at 30 °C. The maximum predicted oxygen consumption at sustained swimming speed (VO_{2max}) was 160.57, 255.64 and 359.79 mgO₂·kg⁻¹·hr⁻¹ for 15, 22 and 30 °C respectively. Scope for activity, calculated by subtracting SMR from VO_{2max} was 118.78, 203.93 and 270.31 mgO₂·kg⁻¹·hr⁻¹, respectively. Average observed minimum (routine) and maximum swimming (active) VO₂ were calculated to yield values

of 49.77 ± 9.19 , 67.42 ± 10.02 and 100.7 ± 24.72 , over the three test temperatures 15, 22.5, and 30 °C, respectively (Fig. 2). Mean active metabolic rates were 166.10 ± 26.42 , 269.72 ± 29.87 and 329.53 ± 37.87 , respectively. Oxygen consumption generally increased with increased swimming speed and temperature throughout the experiment. It was difficult to differentiate an effect of body size on oxygen consumption per kilogram.

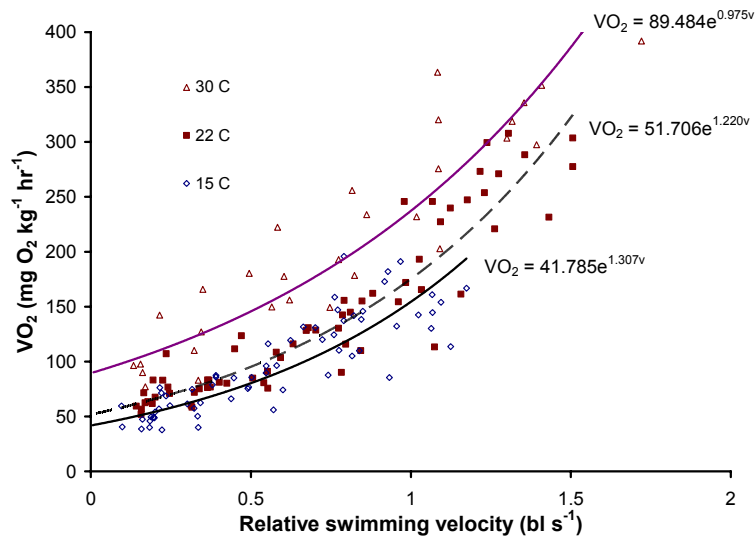


Figure 1. Exponential equations $VO_2 = SMR e^{bv}$ fitted to oxygen consumption data for three temperature treatments: $n=14$ for 15°C, $n=13$ for 22 °C and $n = 5$ for 30 °C.

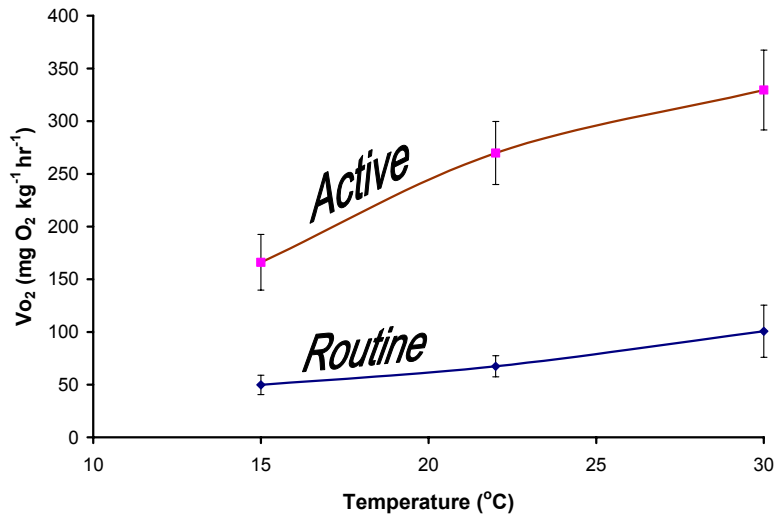


Figure 2. Average VO₂ +/-S.E. for routine and active metabolism at three temperatures: n=14 for 15°C; n=13 for 22 °C and n=5 for 30 °C.

Conclusion

The present data indicate that temperature has profound effects on metabolic rates and swimming performance of gag grouper. Oxygen consumption and maximum swimming speed increased with increasing temperature. However, the gag in the present study appear to have lower metabolic rates than other sit and wait predators such as common snook, *Centropomus undecimalis* (Tolley and Torres, 2002) or largemouth bass, *Micropterus salmoides* (Beamish, 1970) at ~22 °C. Temperature was also observed to increase SMR and scope for activity. Since gag performed best at the highest temperature tested, further testing at higher temperatures is indicated to determine if this positive relationship continues.

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**LOCOMOTORY PHYSIOLOGY AND GAIT TRANSITION IN
SMALLMOUTH BASS (*MICROPTERUS DOLOMIEU*) FOLLOWING
EXERCISE IN AN EXPERIMENTAL RACEWAY.**

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

The majority of research on exercise physiology of fish has been conducted on individuals forced to swim while confined in water tunnel respirometers (Beamish, 1978). We chose to study behaviour, gait transition and post-exercise physiology in smallmouth bass (*Micropterus dolomieu*) following a voluntary 25 m ascent through an experimental raceway against water velocities of varying intensity. Our objective was to establish relationships between exercise intensity and (1) behaviour, (2) locomotory gait, and (3) anaerobic fuel use.

Methods

Smallmouth bass were collected from the Winnipeg River and transported to a 25 m experimental raceway located in Pinawa, Manitoba, Canada (Peake and Farrell, 2004). Water velocity was adjusted to a value between 8 and 120 cm/s, and fish were placed in a holding tank that was continuous with the downstream end of the raceway. Ground speed of fish that voluntarily ascended was monitored by light-gate sensors placed at regular intervals along the longitudinal length of the raceway. Ground speed was calculated by dividing distance between the sensors by the amount of time that passed between consecutive

sensor hits.

The relationship between locomotory gait and swimming speed was assessed by filming fish as they swam through a 2.5 m Plexiglas-lined area of the raceway. Swimming gaits were classified as either steady or unsteady using criteria similar to those described by Rome et al. (1990). Post-exercise physiological measurements were made using plasma and muscle samples removed from smallmouth bass white muscle following a voluntary ascent through the raceway against various water velocities. Post-exercise oxygen consumption was also measured, for a sub-group of smallmouth bass that were anaesthetised following a complete ascent.

Results

The highest steady swimming speed observed defined the upper boundary of the steady zone in this study, while the lowest unsteady speed marked the lower boundary of the unsteady zone. The steady zone for 32 cm smallmouth bass extended to 77.5 cm/s, and the unsteady zone began at a swimming speed of 103.8 cm/s (Figure 1). Swimming speeds between 77.5 and 103.8 cm/s (termed the transitional zone) were supported through recruitment of steady and unsteady swimming. Mean ground speed increased significantly as fish moved from steady (18 cm/s) to transitional (22 cm/s) to unsteady (39 cm/s) swimming.

Muscle glycogen levels in fish that maintained speeds below about 90 cm/s were relatively high and variable (Figure 1A). In contrast, fish that maintained higher swimming speeds showed lower and somewhat more consistent readings. Glycogen levels remained relatively steady as swimming speed increased within the unsteady zone. Plasma lactate increased linearly with mean speed throughout the performance envelope (Figure 1B). Muscle lactate values were relatively steady in fish that maintained speeds lower than 140 cm/s (Figure 1C). However, muscle lactate and exercise intensity were positively correlated at higher speeds. Post-exercise oxygen consumption rates increased in a linear fashion with exercise intensity throughout the range observed (Figure 1D).

Discussion

This study is among the first to demonstrate that a link exists between exercise physiology and behaviour in fish. During ascents that predominately involved

steady swimming, smallmouth bass maintained a ground speed that resulted in a passage time of approximately 2 min. However, ground speeds demonstrated by fish swimming in the unsteady zone were much higher, and resulted in a 54% drop in passage time. This suggests that fish were actively trading exercise intensity for duration, likely in an attempt to conserve anaerobic fuel stores and/or mitigate physiological disturbances associated with anaerobic metabolism.

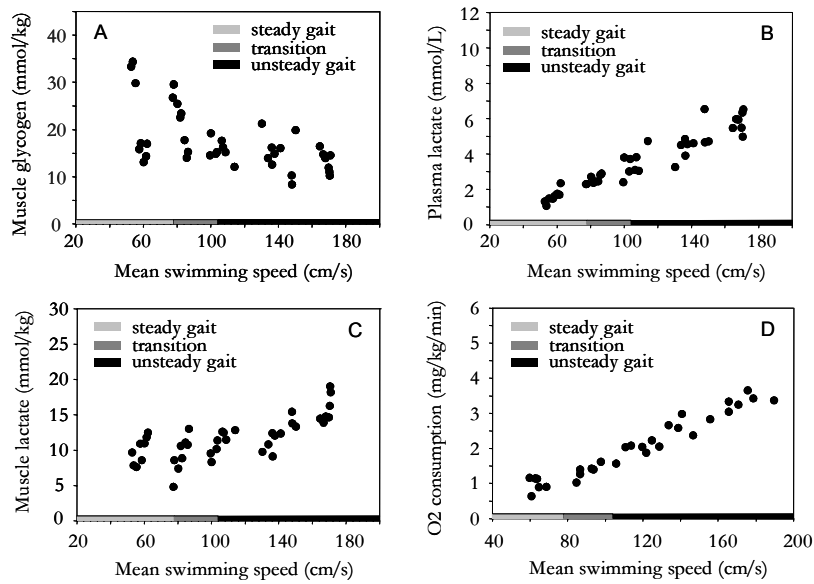


Figure 1. The relationship between post-exercise muscle glycogen (A), plasma lactate (B), muscle lactate (C), and oxygen consumption (D) and mean swimming speed in the raceway.

This hypothesis is supported by most of the post-exercise physiological data collected. Although plasma lactate and oxygen consumption data indicate that unsteady swimming was supported by anaerobic metabolism, and incurred an oxygen debt, muscle glycogen and lactate levels in the late and early portions of the transitional and unsteady zones, respectively, were relatively constant (Figures 1A and C) as swimming speed increased. Thus, fish were able to make

the 25 m ascent against progressively higher water velocities using similar amounts of anaerobic fuel, by adopting a more energetically efficient locomotory gait, increasing ground speed, and reducing passage time.

Conclusions

Smallmouth bass in the experimental raceway demonstrated distinct locomotory zones supported by steady, mixed, and unsteady swimming as exercise intensity increased. Ground speed increased and passage time decreased as water speeds “forced” fish to swim faster. This behaviour allowed individuals to conserve anaerobic energy stores and delay accumulation of glycolytic metabolites. Thus, free-swimming fish actively reduce energy expenditure when faced with various locomotory challenges, activity that is unlikely to occur in confined individuals.

Acknowledgments

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**THE COMBINED EFFECTS OF SWIMMING PERFORMANCE
AND ATTEMPT RATE ON PASSAGE SUCCESS
THROUGH VELOCITY BARRIERS**

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Models used to predict passage success of fish traversing velocity barriers are typically based on swimming performance data generated in controlled laboratory studies. Often, such data fail to accurately predict swimming performance in prolonged or sprint modes. This arises for several reasons, for example: constrained conditions in flow tanks or respirometers may not allow fish to exhibit the full range of behaviors available to them in the wild; fish are often coerced into swimming; and uncooperative fish are generally excluded from analyses (Brett 1964; Hammer 1995).

Elsewhere (Castro-Santos 2002; 2004), we have applied theory and methods of survival analysis to produce equations that control for some of these variables. This approach works by simultaneously incorporating attempt rate and swimming capacity into a single formula:

$$(1) \quad P(T, D) = 1 - \sum_{a=1}^k \left[N_a(T) \prod_{b=1}^a B_{b-1}(T, D) \right] + \prod_{a=1}^k B_a(T, D).$$

The proportion of a population passing an obstacle of length D in time T is a function of those that fail to stage attempt a before time T (allowing for k attempts; $N_a(T)$), and those that do stage attempts, but fail to negotiate the full distance of the barrier ($B_a(T, D)$). These factors combine to provide an estimate of the fish that remain below the barrier; the complement of this value is the proportion that pass. Note that, although some individuals may not stage any attempts by time T , others may stage more than one attempt, and in so doing increase their likelihood of successfully negotiating the barrier. Thus, given information about attempt rate and swimming capacity, it is possible to produce estimates of the proportion of available fish that will pass a barrier of known length in a set amount of time.

We studied sprinting performance in a suite of six migratory species: American shad (*Alosa sapidissima*), blueback herring (*A. pseudoharengus*), alewife (*A. aestivalis*), striped bass (*Morone saxatilis*), walleye (*Stizostedion vitreum*), and white sucker (*Catostomus commersoni*). Fish were allowed to sprint volitionally up a 23-m long open-channel flume against velocities of 1.5-4.5 m/s. We used these data to empirically quantify the relationship between flow velocity and distance of ascent, and between hydraulic conditions and timing and frequency of attempts, and demonstrate how to quantify the combined effects of these two factors on overall passage success. Most species were allowed only one hour to stage attempts. White sucker and walleye, however, were run for six hours, and so provide the most complete data for these analyses. Here, we focus on these two species plus American shad.

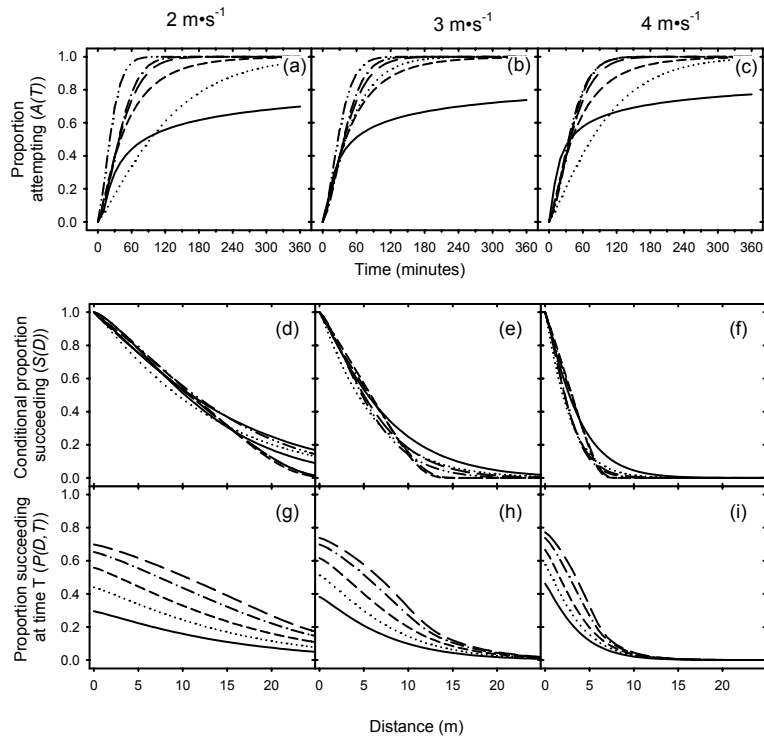


Figure 1. Model predictions of attempt time ($A(T)$; panels a - c) and conditional success probability ($S(D)$; panels d - f) for the first six attempts, and of combined probability of passage success ($P(T,D)$; panels g - i) over six hours. Models are for white sucker (*Catostomus commersoni*) ascending velocities of 2, 3, and 4 $\text{m}\cdot\text{s}^{-1}$ at 0.5 m depth. Predictions of $A(T)$ and $S(D)$ (panels a-f) are for attempts 1 (—), 2 (---), 3 (- · - ·), 4 (— · —), 5 (— · — ·), and 6 (- · - · - ·). Predictions of $P(D,T)$ are for 30 (.....), 60 (.....), 120 (- - -), 240 (- · - ·), and 360 (— —) minutes.

Flow velocity was by far the most important factor in determining ascent distance (D_{max}), with distance decreasing with increasing flow among all species. The effects of hydraulics on attempt timing were more variable,

however: the data suggest that optimal hydraulic conditions for stimulating fish to stage attempts arise from both velocity and discharge, with optimal attraction occurring at flow velocity (U_f) $3.5 \text{ m}\cdot\text{s}^{-1}$ and discharge (Q) of $1.75 \text{ m}\cdot\text{s}^{-3}$. Species also differed in timing of first and subsequent attempts: American shad staged first attempts quickly, but took much longer to stage subsequent attempts, while just the opposite was true of white sucker and walleye.

The results of these combined analyses indicate that passage performance is not maximized solely by reducing flow velocity, but is strongly influenced by the rate of the first and subsequent attempts (Figure 1).

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**EVIDENCE FOR BEHAVIORAL OPTIMIZATION DURING HIGH-
SPEED VOLITIONAL SWIMMING IN FISHES**

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Migrating fish traversing velocity barriers are often forced to swim at speeds greater than their maximum sustained speed (U_{ms}). Existing models that predict optimal swim speeds based on cost of transport at sustained speeds (Weihs 1974; Trump and Leggett 1980) may be inappropriate in this situation. I propose a new model of a distance maximizing strategy for fishes traversing velocity barriers, derived from the relationship between swim speed and fatigue time ($\ln T = a + bU_s$; T = fatigue time, U_s =swim speed). This relationship undergoes a discrete shift as fish switch from prolonged to sprint mode, with distinct values of a and b .

By multiplying T by U_s less flow velocity (U_f), maximum distance of ascent (relative to the ground, D_{max}) can be calculated for a range of swim- and flow speeds. Plots of this relationship show a clear optimum swim speed at each flow velocity and swimming mode at which D_{max} is maximized (Figure 1). Within a given mode, this optimum value occurs at a constant groundspeed equal to $-b^{-1}$. This relationship holds for all flows exceeding U_{ms} . As fish switch from prolonged to sprint mode, however, the optimal groundspeed changes in accordance with the differing parameter values b_P or b_S (subscripted here to identify prolonged (P) and sprint (S) modes, respectively). The speed of flow at which fish should switch from prolonged to sprint mode is defined as $U_{fcrit} = (\ln(b_S \square b_P^{-1}) + a_P - a_S) \square (b_S - b_P)^{-1}$.

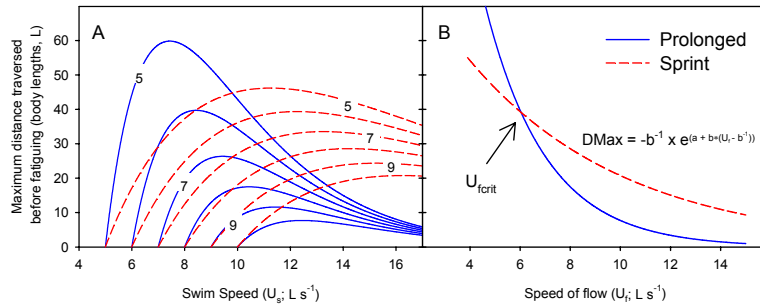


Figure 1. Effect of swim speed and fatigue time on D_{max} over a range of flow velocities (isopleths) and swim speeds (A); and at optimum swim speed within prolonged and sprint modes (B). Note that, depending on flow velocity, it may be more advantageous to swim one mode or the other.

I tested this hypothesis with data from six migratory fish species (anadromous clupeids: American shad, *Alosa sapidissima*, alewife, *A. pseudoharengus*, blueback herring, *A. aestivalis*; amphidromous: striped bass, *Morone saxatilis*; and potomodromous species: walleye, *Stizostedion vitreum*, white sucker, *Catostomus commersoni*) allowed to sprint volitionally against fixed flow velocities of $1.5 - 4.5 \text{ m} \cdot \text{s}^{-1}$ in an open-channel flume. Test conditions allowed for determination of mode shifts (prolonged to sprint) among American shad and the three nonclupeid species. Some blueback herring may have swum in prolonged mode, but this did not meet significance criteria; and alewife swam exclusively in sprint mode.

Of these six taxa, only anadromous clupeids exhibited the appropriate optimizing behavior (Figure 2). American shad made the switch from the optimal groundspeed for prolonged mode to that for sprinting at U_{crit} ; likewise, blueback herring and alewife selected the appropriate groundspeed for sprinting. The other species swam at the optimal groundspeed for prolonged mode. However, when they switched to sprint mode, they failed to switch to the optimal groundspeed for that mode, continuing instead to swim at the prolonged mode optimum. As a result, these fish failed to realize their maximum potential distance of ascent (Castro-Santos 2002).

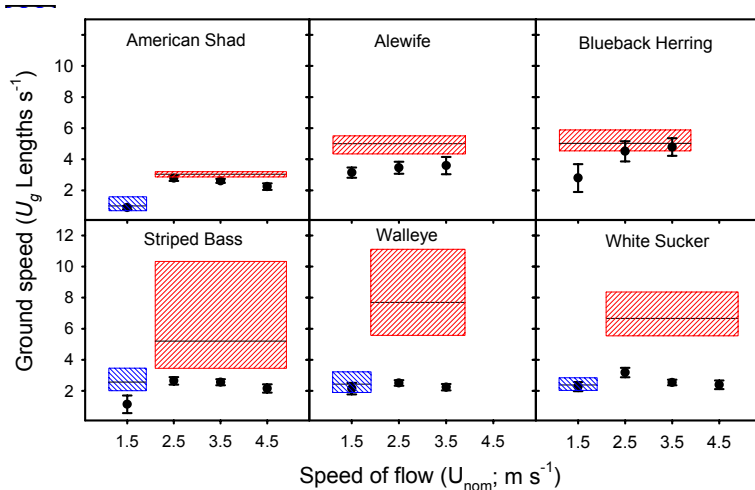




Figure 2. Groundspeeds of six species swimming against flow velocities of 1.5 – 4.5 $\text{m}\cdot\text{s}^{-1}$. Predicted optima are indicated by shaded areas ( = prolonged, and  = sprint mode); points and error bars (± 2 SE) indicate observed groundspeeds.

These results suggest that among the nonanadromous migrants, sprinting may not constitute a migratory behavior. It may instead be optimized for other behaviors, such as prey capture or escape, which follow different rules from distance maximization. If these species typically use only sustained and prolonged mode as part of their routine migratory behaviors, then selection may not have acted to produce a distance-maximizing strategy. Moreover, because these species are able to spawn in a greater variety of habitats (and among striped bass the migration is for feeding, rather than spawning), the selective pressure for distance-maximizing behaviors is reduced.

Regardless of the cause, the variability in groundspeeds exhibited by all species has important implications for models predicting maximum distance of ascent through velocity barriers. The extent to which fish deviate from their optimal groundspeed, as well as the direction of this deviation can cause dramatic reductions in achieved distance of ascent. Predictions of ascent capacity that are derived from the swim speed-fatigue time relationship must therefore

incorporate the behavioral variability of the species in question, and must not assume that fish adopt a distance-maximizing strategy.

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**LOCOMOTORY PERFORMANCE AND ENERGETICS AS A
FUNCTION OF TEMPERATURE IN TWO SUBSPECIES OF
*FUNDULUS HETEROCLITUS***

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Killifish, *Fundulus heteroclitus*, are distributed along the East coast of North America typically inhabiting inshore bays and estuaries. These fish demonstrate remarkable tolerance to challenging environmental conditions, most notably to temperature extremes. Within the species, substantial variation exists in morphological, molecular, genetic, and physiological traits between populations (Powers and Schulte, 1998). This variation shows significant directional change with temperature/coastal latitude such that two distinct regional subspecies have been suggested - the northern form, *Fundulus heteroclitus macrolepidotus*, occurring from the Gulf of St. Lawrence, Canada to New Jersey, USA, and the southern form, *Fundulus heteroclitus heteroclitus*, distributed from Virginia, USA to the Northeastern coast of Florida, USA. Multidisciplinary approaches have shown that the physiological specializations and genetic variation between *F. heteroclitus* subspecies are likely to be adaptive responses to temperature or some other factor correlated with latitude (reviewed by Powers and Schulte, 1998; Schulte, 2001). To further examine these differences, we quantified and compared swimming performance of *F. heteroclitus* populations acclimated to temperatures between 5 and 32°C and determined the patterns of metabolic fuel use supporting exercise metabolism across this temperature range.

Critical swimming speed (U_{crit}) was determined for fish collected from New Hampshire and Georgia and acclimated to 5, 10, 15, 22, 29 or 32°C in the laboratory for 21 days. To investigate fuel availability and mobilization patterns during exercise, a second group of fish was acclimated to temperatures of 5, 15 and 29°C and exercised at speeds corresponding to 80% of their U_{crit} for 1.5 hours. Muscle and liver tissues were freeze clamped and assayed for several metabolites including: ATP, creatine phosphate (CrP), glycogen, glucose, lactate, glycerol, total lipids, and triacylglycerol.

Results of the swimming performance trials suggest that at all acclimation temperatures, northern fish perform significantly better than southern fish (Figure 1). Secondly, at temperatures at or above 10°C, within a population, critical swimming speed is constant and independent of acclimation temperature (Figure 1).

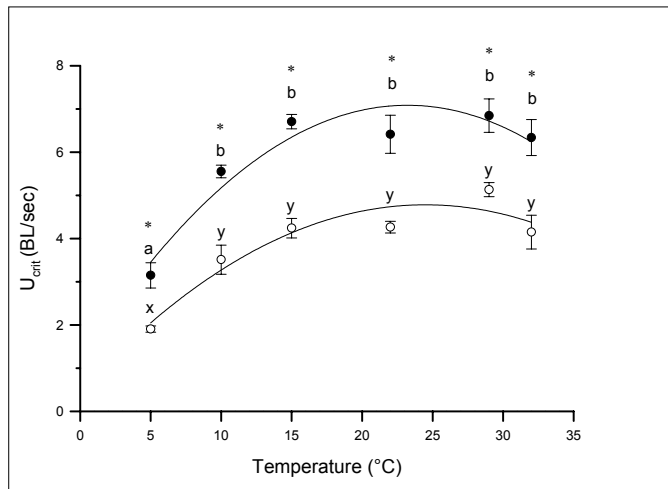


Figure 1: Critical swimming speed (U_{crit}) for northern (dark circle) and southern (open circle) killifish acclimated to temperatures ranging from 5°C to 32°C. Within a population, points with different letters are significantly different ($p < 0.05$) and asterisks represent significant differences ($p < 0.05$) between populations at a given temperature. Values are means \pm SE ($n=6$).

High-energy phosphates showed no significant differences between populations, acclimation temperatures, or in response to exercise for muscle ATP. Similarly, there were few differences in muscle [CrP] except that southern fish had slightly higher levels for both rest and exercise. In contrast, there was a strong effect of population on muscle glycogen such that glycogen concentrations were higher in northern than southern fish. Northern fish also accumulated significantly higher levels of lactate with exercise in both the 15 and 29°C groups, whereas southern fish did not accumulate lactate with exercise to a significant degree in any of the acclimation groups (Figure 2). These data suggest that northern fish may recruit white muscle during sustained swimming at 15 and 29°C to a greater degree than southern fish.

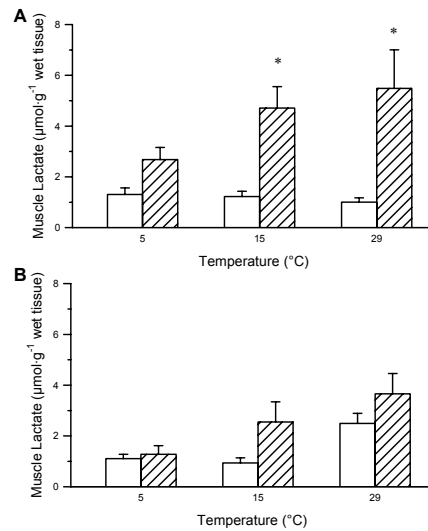


Figure 2: Northern (A) and southern (B) killifish muscle lactate concentrations for resting (open columns) and exercising fish (hatched columns) at 5, 15 and 29°C. Within a population, asterisks represent a significant difference ($p < 0.001$) between rest and exercise at a given temperature. Values are means \pm SE ($n=6$).

In summary, these data suggest that northern fish have both greater endogenous carbohydrate stores and an enhanced ability to utilize glycolysis during exercise

compared to southern fish that demonstrate a more limited glycolytic capacity. Experiments to explicitly examine burst performance (C-starts) in northern and southern fish are currently underway.

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**RECENT PROGRESS OF MECHANISMS
OF SALMON HOMING MIGRATION**

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A number of studies have investigated the amazing ability of salmon to migrate long distances from the ocean to their natal river for spawning (Ueda and Shoji, 2002). For a better understanding on the mechanisms of salmon homing migration, three different analyses have recently been applied using Japanese chum salmon (*Oncorhynchus keta*) migrating from the Bering Sea to Japan, and then to their natal river. The first is behavioral analysis on swimming speeds of homing chum salmon by means of a micro-data logger with a propeller, the second is endocrinological analysis on hormone profiles in the brain-pituitary-gonadal axis, and the third is olfactory analysis on discriminating ability of the natal river.

Swimming speeds in the oceanic phase can be one of the keys to understand the mechanism of chum salmon homing migration. We tagged a maturing chum salmon (fork length = 685 mm) which was considered to be of Japanese origin with a data logger (sampling intervals: speed and depth = 5 sec; temperature = 60 sec) in the central Bering Sea on July 9, 2000. This salmon was retrieved by a set net along the eastern Hokkaido coast 67 days after the release, and 51-days of subsequent data were recorded. The fish usually swam in the surface water column and rarely stayed deeper than 50 m. The average swimming speed was 60–70 cm per sec, and the horizontal rate calculated by an empirical relationship between the attack angle and vertical rate was 42.3–47.7 km per day. The estimated horizontal rate indicates that the chum salmon traveled 2,763 km in 67 days, which is almost equivalent to the distance between points of release and retrieval. This implies that the chum salmon moved to the coastal area near the spawning ground almost straightly from the Bering Sea, partly helped by currents. Vertical profiles of ambient temperature sampled by the salmon

suggest that the fish passed through the Okhotsk Sea around mid-August. All through the recording period, the chum salmon showed a clear foraging period in the daytime, which consisted of repeated short diving from the surface water column to the depth beyond the thermocline. This indicates that the chum salmon traveled searching prey patches during its oceanic migration. These results suggest that homing chum salmon migrated along the continental shelf of the Kuril Islands.

Gonadotropin-releasing hormone (GnRH) molecules produced in the various brain regions are considered to be involved in many physiological functions of the teleost life cycle. In order to clarify GnRH roles on salmon homing migration, measurements of two molecular types of GnRH, salmon GnRH (sGnRH) and chicken GnRH-II (cGnRH-II) in different brain regions, as well as gonadotropin (GTH) and steroid hormones were conducted using specific time-resolved fluoroimmunoassay (TR-FIA) systems (Yamada et al., 2002; Leonard et al., 2002). Maturing chum salmon were caught at nine points from the Bering Sea to the Chitose River. After decapitation, the brain was divided into six regions; olfactory bulb (OB), telencephalon (TC), diencephalon (DC), optic tectum (OT), cerebellum (CB), and medulla oblongata (MO). During spawning migration of chum salmon, sGnRH levels in OB, TC, and pituitary of both sexes were increased at the coastal sea to the branch point of the Chitose River from the Ishikari River. Moreover, sGnRH levels in the pituitary tended to increase at the same time of elevation in female pituitary GTH II and ovarian GTH I levels. The cGnRH-II level in MO was increased at the pre-spawning ground in both sexes, and levels in OT were also increased in males. Both GnRH levels in DC showed no significant changes during spawning migration. GTH II levels in gonads were not detected though the sampling period. Serum steroid hormone levels showed similar profiles as previous observations (Ueda 1999); estradiol-17 β in females and 11-ketotestosterone in males increased during vitellogenesis and spermatogenesis, respectively, and 17 β ,20 β -dihydroxy-4-pregnen-3-one increased dramatically at the time of final gonadal maturation in both sexes. It is quite interesting to note that both sGnRH content in TC and serum testosterone level showed coincident peaks at the branch point of the Chitose River from the Ishikari River. These results confirm the previous findings that sGnRH plays a role on GTH secretion in the pituitary of chum salmon, and sGnRH and cGnRH-II might be involved in brain region-dependent roles on sexual maturation and behavior in salmonid fishes.

For upstream homing migration from the coastal area to the natal stream, the olfactory hypothesis which was proposed by Hasler and Wisby (1951) has been

discussed in many behavioral and electrophysiological studies, but the odor substances of the home stream are still unknown. We found that the electrophysiological response to artificial stream water based on the compositions of amino acids and salts closely resembled the response to the corresponding natural water (Shoji et al., 2000), and we carried out behavior experiments to test whether amino acids mixtures of the home stream have attractive effects on chum salmon upstream movement. Mature male chum salmon (mainly 4 year olds) were captured at the weir in the Osaru River, Hokkaido, Japan, in the late spawning season of 2002, transferred to the Toya Lake Station, Hokkaido University, and reared for several days before experiments. Behavior experiments were conducted in a two-choice test tank. The artificial home stream water was prepared by the amino acid and related substance composition of the Osaru River and dissolved in artificial freshwater. A total of 44 chum salmon was tested, and 28 fish (63.6%) showed upstream movement to one of the choice arm. Among those that moved, 24 fish (85.7%) were found in the arm running the artificial home stream water, and 4 fish (14.3%) were observed in the arm running the natural lake water. These results demonstrate clearly that the artificial home stream water reconstituted by the amino acid composition of home stream has attractive effects on the chum salmon upstream selective movement. We concluded that amino acids dissolved in the home stream water are home stream odorants, and the hypothesis that amino acids dissolved in stream waters are home stream substances for salmon homing is strongly supported by these results.

These different new approaches will help to understand mechanisms of salmon homing migration and eventually to evaluate the stock dynamics of salmon in the North Pacific Ocean and Bering Sea.

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**REPEAT SWIMMING PERFORMANCE
AS A MEASURE OF DIETARY EFFECTS IN
ATLANTIC SALMON**

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EXTENDED ABSTRACT ONLY: DO NOT CITE

Repeated critical swimming performance trials (U_{crit}) were performed on Atlantic salmon (*Salmo salar*) to test the null hypothesis that the source of dietary lipids (fish-based, poultry-based, and plant-based) does not influence exercise and recovery performance. Swimming performance is an integrated assessment of several physiological processes (Plaut, 2001) and repeated swim tests assess the ability of fish to recover from maximum prolonged exercise (Jain et al., 1998). Diet is an environmental factor that, through its influence on the chemical composition of tissues, can alter physiology. Dietary lipid composition, for example, affects tissue fatty acid composition. Although the mechanisms of action are unknown, the relative proportions of saturated fatty acids (SFA) and highly unsaturated fatty acids of the n-3 series (n-3 HUFA) can alter fish cardiorespiratory physiology (see review by McKenzie, 2001).

Four diets were prepared by extensively replacing supplemental lipid from anchovy oil (AO; 100% AO at 150 g/kg) with cold pressed flaxseed oil (FO; 25% AO, 75% FO), sunflower oil (SO; 25% AO, 75% SO), or poultry fat (PF; 25% AO, 75% PF). These diets had equivalent protein and energy concentrations, but due to the different supplemental lipid sources, varied widely

in their fatty acid composition. Fish fed AO had a significantly higher ($P < 0.05$) first U_{crit} ($2.62 \pm 0.07 \text{ bl s}^{-1}$) than those fed PF ($2.22 \pm 0.12 \text{ bl s}^{-1}$) that had low muscle ratios of n-3 highly unsaturated fatty acids (n-3 HUFA) to saturated fatty acids (SFA) and arachidonic acid (AA, 20:4n-6), and high levels of oleic acid (18:1n-9). The AO diet group contained the highest muscle total n-3 HUFA levels and highest n-3 HUFA/SFA, eicosapentaenoic (EPA, 20:5n-3) /AA, and docosahexaenoic (DHA, 22:6n-3)/AA ratios that all correlated positively with increased U_{crit1} (Fig. 1, Fig. 2). In contrast, the PF diet group contained the lowest n-3 HUFA/SFA, EPA/AA, and DHA/AA ratios and the highest muscle oleic acid that correlated negatively with U_{crit1} . Fish in the FO and SO diet groups, the latter having high levels of linoleic acid (18:2n-6), swam as well as AO-fed fish in both swimming trials. The performance of fish fed AO decreased significantly ($P < 0.05$) during the second swimming trial (i.e. $U_{crit2}/U_{crit1} = 0.92 \pm 0.02$). No significant differences occurred between diet groups for the second swim trial. Individually, the highly unsaturated fatty acids (EPA and DHA) showed a significant positive linear relationship between dietary and muscle content, and U_{crit1} . Conversely, negative linear relationship occurred between the dietary and muscle content of oleic acid and U_{crit1} , and between the dietary content of AA and U_{crit1} . There was no relationship observed between levels linoleic acid in dietary and muscle content, and U_{crit1} .

The present results lead us to reject the null hypothesis because the poultry-based lipid supplement resulted in a poorer initial swimming performance (a 16 % decrease in U_{crit1}) relative to the AO group. However, dietary oil supplements primarily based on plant oils (flax and sunflower) did not significantly alter swimming performance. These results suggest that major replacement (75%) of the dietary fish oil supplement by plant-based oils has no significant effect on an integrated measure of cardiorespiratory physiology in Atlantic salmon. In addition, combinations of certain fatty acid types may interact to negate harmful side effects of high levels of individual fatty acids on tissue integrity and swimming performance.

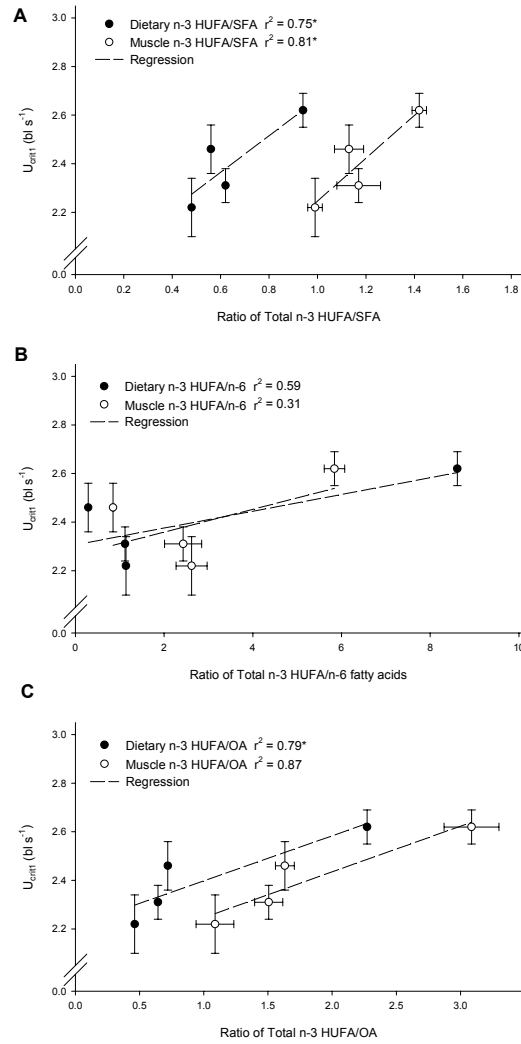


Figure 1: Least squares linear regression analysis of the relationship between n-3 HUFA/SFA ratio (A), n-3 HUFA/n-6 fatty acid ratio (B), and n-3 HUFA/OA ratio (C) in dietary and muscle lipids, and the first critical swimming trial (U_{crit1}) in the four dietary groups. HUFA, highly unsaturated fatty acids; SFA, saturated fatty acids; OA, oleic acid (18:1 n-9). An asterisk indicates a significant correlation at $P < 0.05$.

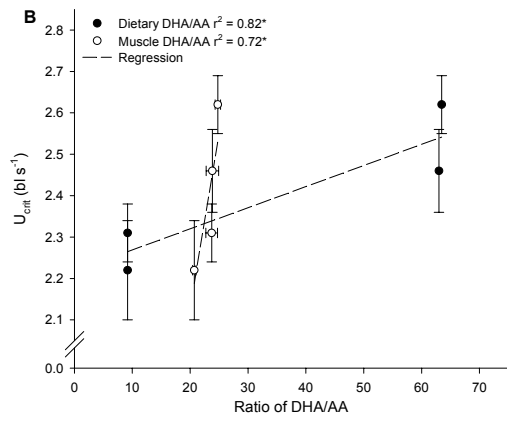
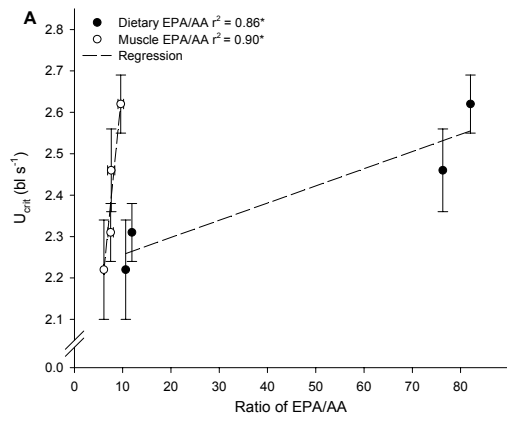


Figure 2: Least squares linear regression analysis of the relationship between EPA/AA ratio (A) and DHA/AA ratio(B) in dietary and muscle lipids, and the first critical swimming trial (U_{crit1}) in the four dietary groups. HUFA, highly unsaturated fatty acids; SFA, saturated fatty acids; OA, oleic acid (18:1 n-9). An asterisk indicates a significant correlation at $P < 0.05$.

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Acknowledgements

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**SWIMMING COST OF JUVENILE ATLANTIC SALMON
IN TURBULENT FLOW**

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Juvenile Atlantic salmon (*Salmo salar*; JAS) live in rivers characterized by highly turbulent flow. In these environments, flow turbulence is associated with a wide range of instantaneous flow velocities which may affect the energetic costs of habitat utilization. Although there is a vast literature documenting swimming performance of fish, nearly all experiments have considered fish swimming in steady homogeneous water flows despite the fact that fish live in environments where intense fluctuations of flow velocity occur. Only recently, work has started to consider the effect of turbulence on the swimming performance of fish (Pavlov et al. 2000; Enders et al. 2003; Liao et al. 2003). The purpose of our work was to develop a swimming cost model which especially accounts for the effects of velocity fluctuations. The objectives of this study were (1) to evaluate the effect of body mass, mean flow velocity, and descriptors of flow turbulence on the swimming costs of JAS, (2) to develop swimming cost models for fish swimming under turbulent flow conditions, and (3) to compare the prediction of turbulent swimming models to existing forced swimming models.

We attained our objectives by performing 40 respirometry experiments at 15°C during which we subjected individual fish to turbulent flow conditions. We estimated swimming costs under five turbulent flow conditions defined by the

selected combination of means (18, 23, 40 cm·s⁻¹) and standard deviations (5, 8, 10 cm·s⁻¹) of flow velocity.

A turbulent flow was created within the swimming chamber. The flow structure was quantified using an Acoustic Doppler Velocimeter (ADV, Sontek). The ADV allowed us to record the three orthogonal velocity components of the flow (streamwise, u ; vertical, v ; lateral, w) at a frequency of 25 Hz. The flow structure was recorded during 5 min. We obtained mean \bar{u} and standard deviation u_{SD} for the streamwise velocity for each flow condition. We also calculated the turbulent kinetic energy (TKE ; g·cm⁻¹·s⁻²)

$$(1) \quad TKE = 1/n \cdot \sum_{i=1}^n 0.5 \rho (u_i'^2 + v_i'^2 + w_i'^2)$$

where n is the number of instantaneous velocity fluctuations, ρ (g·cm⁻³) is the water density, u_i' , v_i' , and w_i' (cm·s⁻¹) represent the instantaneous velocity fluctuation of the three velocity components.

The five different flow conditions created by our apparatus during the experiments corresponded to the targeted \bar{u} and u_{SD} (Table 1). TKE ranged from 66.8 g·cm⁻¹·s⁻² to 416.2 g·cm⁻¹·s⁻². For any given flow condition, \bar{u} and u_{SD} did not vary significantly among the recordings performed before and after a suite of experimental observations (Stability test for \bar{u} : 0.42 < p < 0.98, variation = 0.0-0.3%; Stability test for u_{SD} : 0.12 < p < 0.94, variation = 0.2-2.1%).

Table 1. Number of respirometry experiments (n) per flow condition, range of mass (M) of fish and estimated swimming costs (C_R).

n	\bar{u} (cm·s ⁻¹)	u_{SD} (cm·s ⁻¹)	M (g wet)	C_R (mg O ₂ ·h ⁻¹)
8	18	5	5.6 – 15.5	1.6 – 3.5
8	18	8	5.2 – 15.0	2.0 – 4.3
8	23	5	4.9 – 16.0	2.7 – 6.0
8	23	8	5.1 – 15.0	3.7 – 8.0
8	40	10	4.3 – 14.0	5.0 – 14.9

The swimming costs varied 9.3-fold among our experiments (Figure 1). Statistical analysis indicated that mass ($p < 0.001$), \bar{u} ($p < 0.001$), and u_{SD} ($p < 0.001$) had a significant effect on swimming costs. The interaction term between \bar{u} and u_{SD} was not significant ($p = 0.71$). Multiple regression analyses allowed us to develop a model where mass, \bar{u} , and u_{SD} contributed, respectively, to 16%, 61%, and 15% of the variation in swimming costs.

$$(2) \quad \log C_R = 0.79 \log M + 1.47 \log \bar{u} + 0.47 \log u_{SD} - 2.45$$

(n = 40, $R^2 = 0.92$, $p < 0.001$)

Swimming costs also increased with mass ($p < 0.001$) and TKE ($p < 0.001$), resulting in a two-variable model with mass contributing 16% and TKE 58% of the variation in swimming costs.

$$(3) \quad \log C_R = 0.67 \log M + 0.68 \log TKE - 1.44$$

(n = 40, $R^2 = 0.74$, $p < 0.001$)

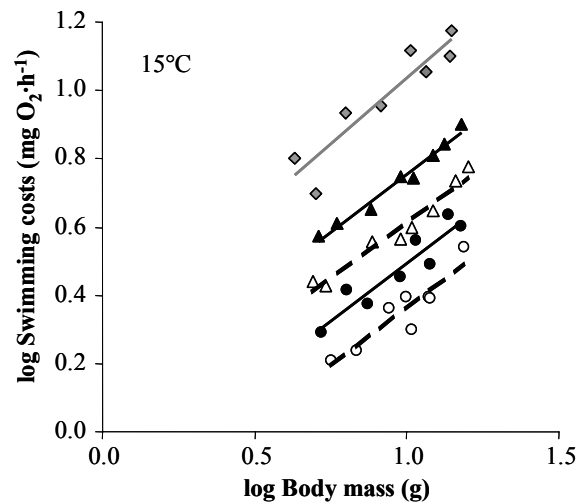


Figure 1. Swimming costs of JAS at 15°C under low (circle), medium (triangle), and high flow velocities (rhombus). The open symbols represent low, solid symbols medium, and shaded symbols high turbulence condition.

Regression lines of the log linear relation between body mass and swimming costs are presented.

Our study indicated that descriptors of turbulence explain a significant fraction of swimming costs. Standard deviation and *TKE* significantly contributed to the predictive power of the models developed. Most studies that estimate swimming costs of stream-dwelling fish use models based on experimental designs that minimize flow heterogeneity. Our results suggest that flow turbulence significantly increases the swimming costs, and consequently, that the use of such forced swimming models (e.g. Boisclair and Tang 1993) underestimate actual swimming costs. The swimming costs we estimated were 2.0- to 3.6-times higher than predicted by the forced swimming model.

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WHAT IS CRITICAL SWIMMING SPEED?

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

The critical speed (Ucrit) protocol is an increasing velocity test in which fish are confined in a swim tunnel respirometer and subjected to step-wise increases in swimming speed until fatigue occurs (Beamish, 1978). Although Ucrit is a measure of prolonged swimming capacity, it is also thought to be a reasonable estimator of maximum sustained speed. Despite the widespread use of the protocol, the nature and ecological relevance/significance of Ucrit have rarely been established in a quantitative manner. The objective of this study was to determine the degree to which critical speed estimates maximum sustained capacity of free swimming fish.

Methods

Smallmouth bass (*Micropterus dolomieu*; fork length: 24 to 45 cm) were collected from the Winnipeg River and transported to a research facility in Pinawa, Manitoba, Canada. Following a 24 hr recovery period, Ucrit was measured by placing fish in a respirometer, setting water velocity at 30 cm/s, and allowing fish to acclimate for 8 h. Individuals were then exposed to step-wise increases in velocity (15 cm/s) every 30 min until fatigue (impingement on the downstream screen) occurred.

The relationship between swimming speed and locomotory gait for confined fish (fork length: 32 cm) was also determined by placing individuals in the respirometer and acclimating them to water moving at 10 cm/s for 30 min. Following this, fish were observed visually for 10 s and the relative proportion of time spent swimming steadily or unsteadily was assessed. After this initial test, water velocity was increased by 10 cm/s, and fish were allowed to adjust to

the new speed for 5 min before gait recruitment was again recorded. Velocity increases were continued until swimming failure occurred.

Results

Critical swimming speed increased with fork length over the range tested (Figure 1). During gait experiments, all smallmouth bass that swam at 40 cm/s or less maintained position using steady swimming motions (Figure 2). At 50 cm/s, steady swimming still predominated; however, fish began to demonstrate some unsteady swimming movements. Fish moving at 60 cm/s spent most of the observation period swimming unsteadily, while this gait was recruited exclusively at speeds greater than 70 cm/s.

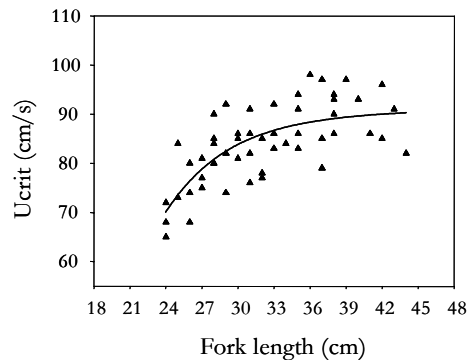


Figure 1. Critical speed of smallmouth bass.

Discussion

Mean critical speed of 32 cm smallmouth bass was about 84 cm/s (Figure 1). This suggests that the highest speed that fish could be expected to maintain using aerobic muscle would be about 75 to 80 cm/s (it is generally accepted that Ucrit overestimates maximum sustained speed by a small degree). This finding correlates well with that of Peake and Farrell (2004) for similarly sized, unconfined individuals of the same species. These authors established that the upper limit for steady swimming (supported by aerobic muscle) was 77.5 cm/s. From this, it appears that critical speed of confined fish is a good estimator of

maximum sustained speed for free swimming individuals. However, this finding was likely more fortuitous than not, because the upper limit of the steady swimming zone in confined smallmouth bass actually fell between 40 and 50 cm/s (Figure 2). This means that confined fish switched away from a steady gait

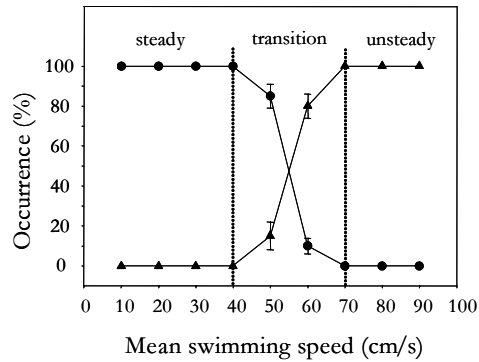


Figure 2. Gait transition in smallmouth bass.

much earlier than unconfined individuals. Fortunately, this difference was almost exactly made up for by the fact that the former were able to swim at speeds in excess of the gait transition value for the appropriate amount of time. Had confined fish stopped swimming sooner or later, critical speed would have underestimated or overestimated maximum aerobic speed in free swimmers.

Thus, the accuracy with which U_{crit} estimates maximum sustained speed probably depends on (1) how early confined fish transition away from a steady gait relative to that of free swimmers, and (2) the degree to which confined fish are willing to swim beyond gait transition. Only if these factors directly cancel out, will U_{crit} give a reasonable estimate of maximum aerobic capacity. While it is unclear why confined fish in the present study underwent an early gait transition, it is worth noting that swimming failure among individuals fell within the transitional zone in free swimmers (Peake and Farrell, 2004). These authors noted that fish rarely swam in the transitional zone, suggesting that swimming failure in the present study may have had a behavioural component.

Conclusions

Gait transition in confined smallmouth bass during an increasing velocity test occurred earlier than that demonstrated by free swimming individuals. In spite of this, the former continued to swim as velocity in the respirometer was increased. This cancelled out the premature gait transition and allowed U_{crit} to accurately reflect maximum aerobic capacity. However, there is no reason to suspect that these factors will always be similar, meaning that the degree to which these data reflect performance of fish in the field should be interpreted with caution.

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**BEHAVIORAL RESPONSES OF FISHES
TO DECLINING DISSOLVED OXYGEN:
AVOIDANCE, RECOVERY, AND ACCLIMATION**

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Estuaries provide nursery habitat for many ecologically and economically important fishes (Beck *et al.* 2001). However, abundant nutrients in estuarine waters can lead to eutrophication and hypoxia or low dissolved oxygen (DO) in estuarine habitats. Hypoxia can reduce fish growth rates, limit productive habitat, and increase mortality of young fishes. Despite a considerable number of laboratory and field studies on the detrimental effect of hypoxia on fish, the behavioral responses of fish to declining DO remains elusive.

The link between hypoxia and fish responses combines behavioral and physiological strategies that can mitigate the effects of exposure. For example, the ability of fish to sense and avoid a low DO event may limit exposure but may increase predation risk and decrease foraging (Kramer 1987). In order to understand the sublethal consequences of hypoxia, it is important to determine what DO levels fish avoid and their physiological responses, such as acclimation and metabolic depression.

Results of studies investigating fish behavior and declining DO generally conclude that fish employ an active and/or a passive strategy. Fish face a tradeoff between increasing their activity to escape low DO and decreasing their activity to save energy and avoid inefficient anaerobic metabolism. For example, Domenici (2000) observed that Atlantic herring initially increased swimming speed in response to declining DO, whereas Dalla Via (1998) observed that sole initially decreased activity. Domenici (2000) suggested that response to declining DO may be life history specific, but this has yet to be empirically determined.

This study investigated the swimming activity of two juvenile estuarine-dependent species (summer flounder, *Paralichthys dentatus* and weakfish, *Cynoscion regalis*) as a function of changing dissolved oxygen. Specifically, we investigated the species-specific changes in swimming speed in response to declining DO, recovery during increasing DO, and the effect of acclimation on weakfish behavior and survival.

Methods

A video camera attached to a frame grabber and associated image analysis software recorded voluntary swimming speed of both species in a mesocosm tank (2.4m diameter). Fish were introduced to the tank and acclimated for 1h prior to a trial. Each trial began at 7 mg O₂l⁻¹ (100% sat.) and DO was decreased by 1.4 mg O₂l⁻¹ (20% sat.) increments to 1.4 mg O₂l⁻¹ (20% sat.), at which point DO was reduced to 0.8 mg O₂l⁻¹ (15% sat.) and finally 0.4 mg O₂l⁻¹ (10% sat.); fish were exposed to each DO level for 30 minutes (Figure 1). Subsequently, DO was raised in the tank at the same rate to determine when fish resumed normal swimming activity. We tested 17 summer flounder and 18 weakfish. Ten control trials were run with each species for the same time period without DO adjustment. We also acclimated ten weakfish to diel-cycling hypoxia (1.0-11.0 mg/L) for ten days. We then observed their behavior with the same methods described above.

We used repeated measures ANOVA to test for changes in activity and trend analysis to characterize those changes.

Results and Conclusions

The DO conditions we simulated are commonly observed in the tidal headwaters of estuarine creeks which both species use as nursery grounds. It is interesting to

note that 24% and 39% of summer flounder and weakfish, respectively, did not survive treatment. However, when weakfish were acclimated to less severe diel cycling hypoxia and then exposed to treatment, there were no mortalities.

Neither summer flounder ($p = 0.093$) nor weakfish ($p = 0.54$) showed significant changes in activity during the control trials. However, when exposed to declining DO, summer flounder exhibited a significant decrease in activity beginning at ~ 4.2 mg/L ($p < 0.001$). In contrast, weakfish initially increased swimming speed and then decreased activity beginning at ~ 2.8 mg/L, ($p < 0.001$) (Fig. 1).

This interspecific difference in response can probably be attributed to their respective life-history strategies. Weakfish employ an active strategy followed by a passive strategy. The active strategy would allow weakfish to increase their search radius for more oxygenated habitat. In contrast, summer flounder are far less mobile and as such may not have the same behavioral latitude to escape a low DO event, therefore the onset of metabolic depression occurs earlier and lasts longer.

During subsequent exposure to increasing DO, summer flounder never resumed a level of activity observed in control trials. Weakfish, however, continued to decrease their activity even as DO was increasing, until DO reached ~ 4.2 mg/L, at which point they resumed a normal level of activity. Recovery time represents the latent effect hypoxia may have on a fish. Long recovery times, especially those observed from summer flounder, may have serious consequences in terms of increased predation risk and decreased foraging.

Preliminary results from weakfish acclimated to diel cycling hypoxia reveal that previous hypoxic exposure results in behavioral changes. Acclimated weakfish exhibit behavior more similar to summer flounder, with one notable exception: the decrease in swimming speed began at 1.4 mg/L (Fig. 2). Previous exposure may help to prime the respiratory system and lead to changes in the circulatory system that enable weakfish to survive low DO events. By forgoing the active response observed in non-acclimated weakfish, acclimated weakfish may also save energy and decrease the risk of mortality.

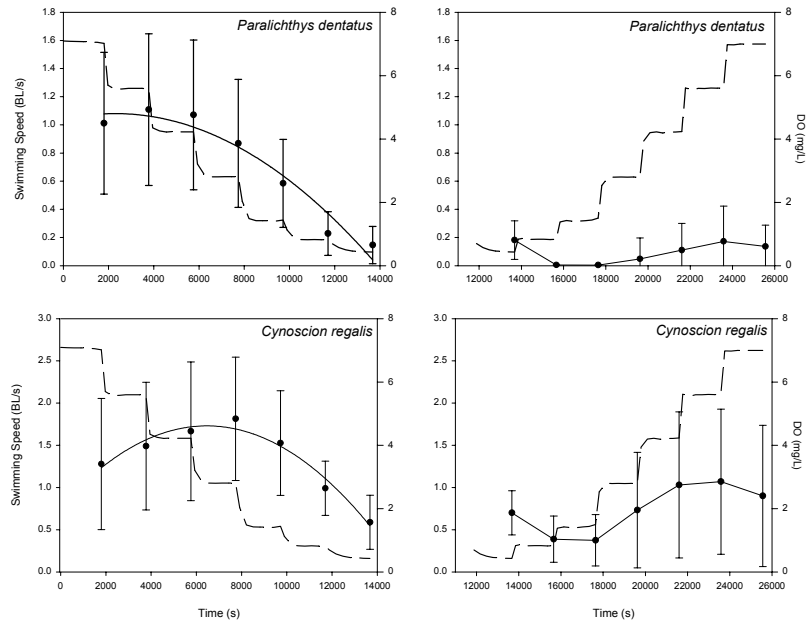


Figure 3. Mean (\pm SE) swimming speed of *Paralichthys dentatus* (top panels) and *Cynoscion regalis* (bottom panels) with associated DO exposure (dashed line).

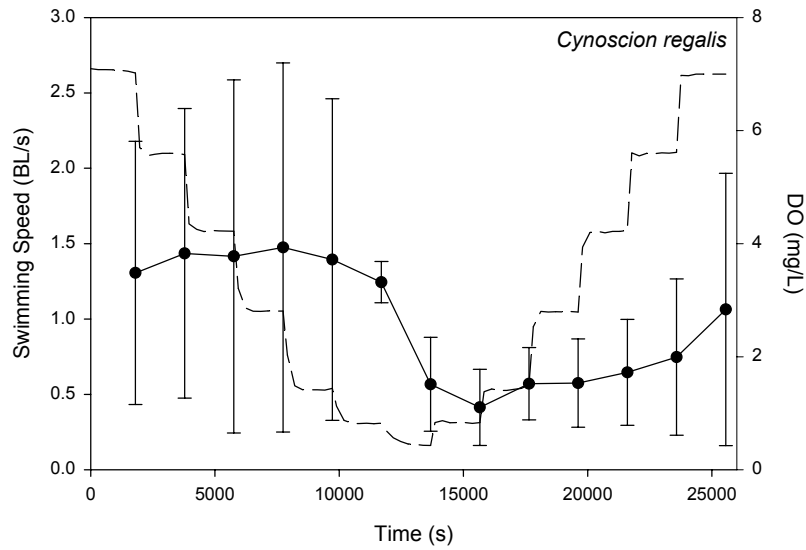


Figure 4 Mean (\pm SE) swimming speed of *Cynoscion regalis* (bottom panels) acclimated to diel cycling hypoxia (11 \rightarrow 1 \rightarrow 11 mg/L) with associated DO exposure (dashed line).

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**THE METABOLIC PHYSIOLOGY
AND STRESS RESPONSE OF
NORTH ATLANTIC TELEOST SPECIES**

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Active pelagic fish tend to have higher metabolic rates than sluggish benthic species (Webb, 1993). However, few studies have directly compared the metabolic capacity and the metabolic response to exercise in species with different lifestyles.

The post-exercise stress response of fish has been studied in a number of species, and most species show a well characterized response, with the release of high levels of cortisol and catecholamines into the circulation (primary response) and increased haematocrit (secondary response) (Butler *et al.*, 1986; Tang and Boutilier, 1988). However, some species with a less active lifestyle and a lower metabolic activity, like the starry flounder (Milligan and Wood, 1987) and sea raven (Vijayan and Moon, 1994) do not exhibit the typical stress response. These limited studies suggest that the magnitude of the post-exercise stress response in fish may be related to a species metabolic capacity (aerobicity).

The objectives of this study were: i) to measure the range of metabolic capacity in five North Atlantic teleost species (*Tautoglabrus adspersus*, cunner; *Macrozoarces americanus*, ocean pout; *Gadus morhua*, Atlantic cod); *Osmerus mordax mordax*, Atlantic rainbow smelt; and *Mallotus villosus*, capelin), with different life styles by measuring maximum oxygen consumption (MO_2 max.) for each species during intense exercise; ii) to study the stress response of the

five species when subject to exhaustive exercise; and iii) to examine the relationship between a species' aerobicity (MO_2 max), and its post-exercise stress response, cardiac morphometrics (relative ventricular mass, RVM), and haematocrit.

Methods

Metabolic rates (at 8°) were measured in a Blazka-type respirometer at rest (RMR), while swimming at low velocities, (10 and 15 cm s⁻¹; swimming metabolic rate, SMR), during intense exercise (active metabolic rate, AMR) and during 45 min. of recovery (ReMR) from intense exercise. MO_2 values were converted to mass-independent values using a mass exponent of 0.8. Metabolic scope (MS) was calculated as AMR – RMR.

In order to get maximal stress hormone levels (catecholamines and cortisol) and post-stress haematocrit levels, the fish were vigorously chased for 90 sec. The plasma catecholamines epinephrine and norepinephrine (measured immediately post-stress) were determined using high performance liquid chromatography with electrochemical detection after extraction with alumina. Plasma cortisol (measured 30 min. post-stress) concentrations were determined using a Coat-A-Count[®] Cortisol radioimmunoassay kit.

Results and Discussion

Similar values of RMR were observed for pout, cod and smelt (55-65 mg O₂ kg^{-0.8} h⁻¹). However, the cunner's RMR was lower as compared to these three species (~40 mg O₂ kg^{-0.8} h⁻¹) (P < 0.08), and the capelin's RMR (92.9±13.0 mg O₂ kg^{-0.8} h⁻¹) was significantly greater than all other species (P < 0.05). Significant inter-specific differences also existed in AMR (range 122 to 364 mg O₂ kg^{-0.8} h⁻¹), with the order: cunner < ocean pout = cod << rainbow smelt << capelin. Most species recovered from exhaustive exercise within 15 minutes, except for the cod, which required approx. 30 min. In conclusion, a 2.5 fold difference in metabolic capacity was found between the 5 species, and the metabolic values generally fit well with a species lifestyle and ecology. However, we were surprised that the cunner had the lowest value for AMR, and that the ocean pout's AMR and MS were comparable to the cod. Testable hypotheses arising from these latter results are: 1) the low Max. aerobic capacity of cunner is related to metabolic efficiency, or a consequence of being able to go

into torpor (severely depress metabolism); 2) the relatively high AMR of the benthic ocean pout is related to digestive costs/demands (i.e. consuming difficult to digest prey).

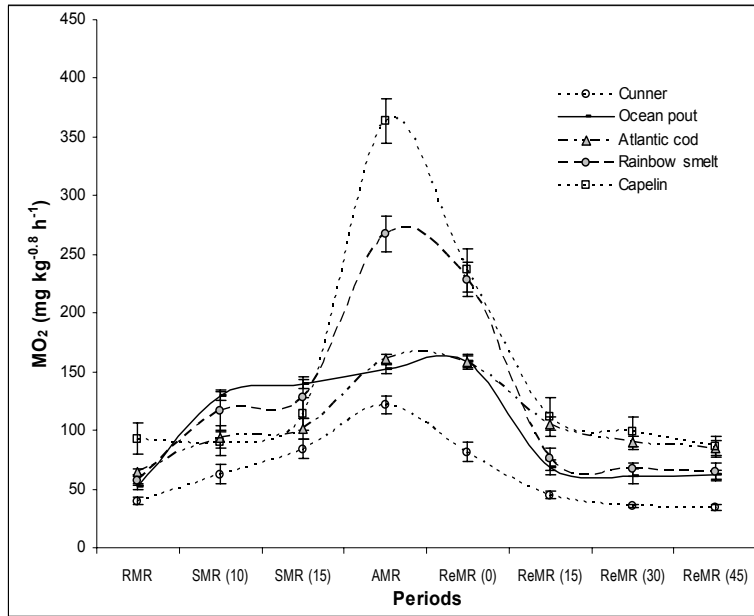


Figure 1. Variation in the metabolism of the five North Atlantic teleost species at rest (RMR); swimming at 10 (SMR (10)) and 15 cm s⁻¹ (SMR (15)); during a period of intense burst swimming (AMR); and during recovery from exhaustive exercise (ReMRs), 0, 15, 30 and 45 minutes after the stress period. Values are means ± standard error.

Table I. Stress hormone concentrations, post-stress haematocrit (Hct), relative ventricular mass (RVM.), mass (M) and length (L) for the five North Atlantic teleost species studied. Fish were chased vigorously for 90 sec., and blood samples for catecholamines and cortisol were taken immediately and 30 min., respectively, following the chase. Values are presented as means \pm S.E (N generally = 7-10). Dissimilar letters indicate differences between groups ($P < 0.05$, One-way ANOVAs followed by Tukey-Kramer post-hoc tests).

Species	Cunner	Ocean Pout	Cod	Smelt	Capelin
M (g)	111 \pm 11	51 \pm 3	73 \pm 6	34.0 \pm 4	23 \pm 1
L (cm)	19 \pm 1	22 \pm 1	21 \pm 1	18 \pm 1	16 \pm 0.3
R.V.M. (%)	0.07 \pm 0.002a	0.073 \pm 0.004a	0.096 \pm 0.003b	0.122 \pm 0.007c	0.137 \pm 0.004c
Cortisol (ng ml ⁻¹)	16 \pm 2a	7 \pm 2a	28 \pm 4b	34 \pm 5b	-----
Epi (nM)	80 \pm 16ab	58 \pm 15ab	188 \pm 35c	25 \pm 5a	148 \pm 33bc
NE (nM)	23 \pm 2a	41 \pm 5.a	47 \pm 9a	26 \pm 6a	149 \pm 20b
Total CA (nM)	103 \pm 18a	99 \pm 20a	235 \pm 42b	48 \pm 9a	297 \pm 52b
Epi:NE	3.2 \pm 0.5a	1.4 \pm 0.2b	4.2 \pm 0.6a	1.2 \pm 0.4b	0.9 \pm 0.1b
Hct (%)	35 \pm 1a	28 \pm 1b	28 \pm 1.4b	30 \pm 3ab	28 \pm 2b

Cod and capelin showed the highest total CA concentrations (>200 nM), similar to those reported for post-chased trout (eg. Butler *et al.*, 1986; Tang and Boutilier, 1988), whereas values for the cunner, pout and smelt were significantly lower (50 -100 nM). None of the species displayed a dramatic post-stress increase in cortisol, although values for the cod and smelt (< 15 ng ml⁻¹) were significantly higher than for cunner and ocean pout (~ 30 ng ml⁻¹). Interestingly, the species with the lowest AMR (the cunner), had a significantly higher post-stress haematocrit concentration (35%) as compared with all other

species ($\leq 30\%$). In summary, although the active cod and capelin showed a higher post-exercise hormonal response when compared to the less active species (cunner and pout), as predicted, the smelt (an active pelagic species) showed an unexpected low post-chase stress response.

No correlations were found between a species' aerobicity and the magnitude of the stress response (as measured in E, NE, Total CA, cortisol or haematocrit), and thus we must reject our hypothesis that the post-exercise stress response in fish is related to a species' metabolic capacity. In contrast, a strong relationship was observed between a species' MO_2 max and RVM ($r^2 = 0.90$, $P < 0.05$); confirming the importance of cardiac performance to fish metabolism and exercise capacity.

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**LINKING MIGRATION BIOLOGY OF SOCKEYE SALMON
WITH CONSERVATION AND MANAGEMENT
THROUGH MULTIVARIATE ANALYSES**

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EXTENDED ABSTRACT ONLY – DO NOT CITE

The recent phenomena of early migration and excessive enroute mortality in late run Fraser River, BC, sockeye salmon (*Oncorhynchus nerka*) has created management uncertainty and concern for the viability of some stocks (Cooke et al., 2004). The paucity of information regarding fundamental salmon migration biology has impeded the ability of scientists and managers to respond to these crises. Our interdisciplinary research group has been involved in a comprehensive assessment of Fraser River sockeye migration biology. In 2002, we collected biological samples on over 1,400 sockeye including data on

energetic status, gonadal development, endocrine parameters, osmoregulatory status, and parasite infection.

Using multivariate analysis of variance coupled with canonical variate analysis to assess differences among multivariate centroids we conducted exploratory analyses and tested specific hypotheses associated with the migration biology of sockeye salmon. Our analyses focused on examining which factors influenced or varied with run timing groups, stocks, gender, and sampling location. We used the same multivariate approaches to elucidate the mechanisms underlying abnormal migration timing. Collectively, these analyses provided a novel and comprehensive understanding of sockeye salmon migration biology and provided information that will aid in the management and conservation of these important fisheries resources.

Reference

Cooke, S.J., S.G. Hinch, A.P. Farrell, M. Lapointe, M. Healey, D. Patterson, S. MacDonald, S. Jones, and G. Van Der Kraak. 2004. Early-migration and abnormal mortality of late-run sockeye salmon in the Fraser River, British Columbia. *Fisheries*. 29(2):22-33.