

Fitness Physiology:
Selection of
Physiological Characteristics

SYMPOSIUM PROCEEDINGS

Jay Nelson

Don MacKinlay

International Congress on the Biology of Fish
Tropical Hotel Resort, Manaus Brazil, August 1-5, 2004

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For copies of these Symposium Proceedings, or the other 20 Proceedings in the Congress series, contact:

Don MacKinlay, SEP DFO, 401 Burrard St
Vancouver BC V6C 3S4 Canada
Phone: 604-666-3520 Fax 604-666-0417
E-mail: mackinlayd@pac.dfo-mpo.gc.ca

Website: www.fishbiologycongress.org

PREFACE

Little is known about how or when physiological characteristics of fish confer any selective advantage in the wild. Research over the past few decades has demonstrated that most whole-animal physiological measurements have extensive variability within individuals and populations of a given species. Whether this variability is, in turn, related to fitness of individuals in the field is poorly known for most animals and environments. However, knowledge of biological factors that control recruitment of individual fish and their ancestors to the reproducing population could vastly improve management models for economically important fishes and should be an important element of any ecosystem approach to future fisheries management. Moreover, although links between environmental constraints, performance, physiological and biochemical support of performance and behaviour can be elucidated in laboratory studies, we can only start to understand their interactions, ecological significance and implications for management of fish in the wild through nascent field studies. Recent developments in technology have allowed many studies evaluating the success of animals of known physiological phenotype to be gauged under natural or near-natural conditions. This symposium focuses on studies of this nature.

Symposium Organizers:

Jay Nelson, Towson University

Don MacKinlay, Fisheries & Oceans Canada

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.

Congress Chairs:

Adalberto Luis Val
National Institute for Research
in the Amazon, INPA,
Manaus, Brazil

Don MacKinlay
Fisheries & Oceans Canada
Vancouver, Canada

TABLE OF CONTENTS

Swimming physiology plasticity in Fraser River sockeye salmon: effects of kidney parasite infection. <i>Glenn Wagner</i>	1
Some loading and limiting factors that can affect swimming performance in fish <i>Ted Taylor and David J. McKenzie</i>	7
Energetic adaptation and fitness in freshwater resident and anadromous Arctic charr. <i>Tallman, R.F. and T. Loewen</i>	11
Energetic and fitness consequences associated with truncated selection for angling vulnerability in largemouth bass <i>S.J. Cooke</i>	17
Selection on morphology and swimming performance of European sea bass (<i>Dicentrarchus labrax</i>) in an experimental estuary <i>Jay Nelson, Handelsman, C. A. and Claireaux G.</i>	19
Dispersal across oxygen gradients: Fitness trade-offs for an African cyprinid <i>Lauren J. Chapman, Sarah Schaack, and Colin A. Chapman</i>	25
Enzyme activities in the gulf killifish, <i>Fundulus grandis</i> , during long- term hypoxic exposure <i>Mery Martinez</i>	31
Impacts of hypoxia on juvenile fish growth: evidence from laboratory and field studies. <i>Kevin L. Stierhoff and Timothy E. Targett</i>	37
Gut length and mass in prickleback fishes (Stichaeidae): ontogenetic, dietary, and phylogenetic effects <i>Michael H. Horn and Donovan P. German</i>	43

Gene expression of amylase and trypsin in prickleback fishes: ontogenetic, dietary, and phylogenetic effects <i>Anna Gawlicka,</i> <i>Kelly H. Kim, and Michael H. Horn</i>	47
Effects of acclimation period to different salinities on the bioenergetic budget of juveniles of <i>Centropomus parallelus</i> (Poey) <i>Arthur José da Silva Rocha</i>	51
The influence of environmental variables on <i>Squatina guggenheim</i> (Chondrichthyes: Squatinidae) distribution in the Argentine- Uruguayan Common Fishing Zone (AUCFZ) <i>Rodolfo Vögler Santos</i>	61
Effects of pressure on eel physiology : how are eels pre-adapted to migrate at depth? <i>Sebert Philippe, Vettier Aurélie And Pequeux* André</i>	67
Trimethylamine oxide as an organic osmolyte in deep-sea fishes: correlations with depth and stabilization of proteins under pressure <i>Paul H. Yancey</i>	75
Effects of hydrostatic pressure on G protein signaling in deep-sea fishes <i>Joseph F. Siebenaller</i>	79
<i>Integrated in situ and in vitro studies of deep-sea fish physiology</i> <i>David M. Bailey, Bertrand Benard, Jean-Francois Rees, Martin</i> <i>Collins, Philip Bagley, Alan Jamieson and Imants Preide</i>	81

**SWIMMING PHYSIOLOGY PLASTICITY
IN FRASER RIVER SOCKEYE SALMON:
EFFECTS OF KIDNEY PARASITE INFECTION**

G.N. Wagner^{1,2*}, L. Kuchel¹, A. Lotto², S.R.M.Jones³, D.A.Patterson⁴, S.J. Cooke², J.S. Macdonald⁴, G. Van der Kraak⁵, M.C. Healey⁶, K. English⁷, S.G. Hinch², and A.P. Farrell¹

¹Department of Biology, Simon Fraser University, Burnaby, BC, V5A 1S6

²Department of Forest Sciences, The University of British Columbia,
Vancouver, BC, V6T 1Z4

³Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, BC

⁴Department of Fisheries and Oceans, Co-operative Resource

Management Institute, Simon Fraser University, Burnaby, BC V5A 1S6

⁵Department of Zoology, University of Guelph, Guelph, ON, N1G 2W1

⁶Institute for Resources and Environment, University of British Columbia,
Vancouver, BC V6T 1Z2

⁷LGL Limited, 9768 Second St. Sidney, BC, V8L 3Y8

EXTENDED ABSTRACT ONLY: DO NOT CITE

The myxosporean kidney parasite *Parvicapsula minibicornis* is contracted by sockeye salmon (*Oncorhynchus nerka*) in the Fraser River estuary and may compromise the osmotic balance of sockeye at an escalating rate the longer their residence time in freshwater prior to spawning. This residence time has increased from historical values by 3-6 weeks for Late-run sockeye since 1996 (Pacific Salmon Commission, 2001). Subsequently, en route and pre-spawning mortalities for this stock have increased up to 90 %, a level that would preclude sustainable populations from those stocks. The impact of *P. minibicornis* on the migratory ability of sockeye was estimated by measuring repeated prolonged maximum swimming performance (U_{crit}) and metabolic rates of strong, weak, and non-infected sockeye (measured by PCR analysis) over an eight week period. Analysis of data from a separate telemetry study was used to compare the estimated parasite impact on migration ability with actual migrations of early-entry and normally behaving sockeye.

The U_{crit} recovery ratio of strongly infected fish was significantly lower (=8 %) than weak and uninfected fish, while metabolic rate recovery ratios were unchanged (Fig. 1). The reduction in U_{crit} recovery ratio of strongly infected fish may have been caused by increased energy use to maintain ion balance during exercise. Sockeye with strong parasite infections were exposed to 250-490 degree days (daily cumulative temperature above 0 °C) after infection with *P. minibicornis*, but exposure for >400 degree days may be necessary to reach the pathogenic stage of the parasite.

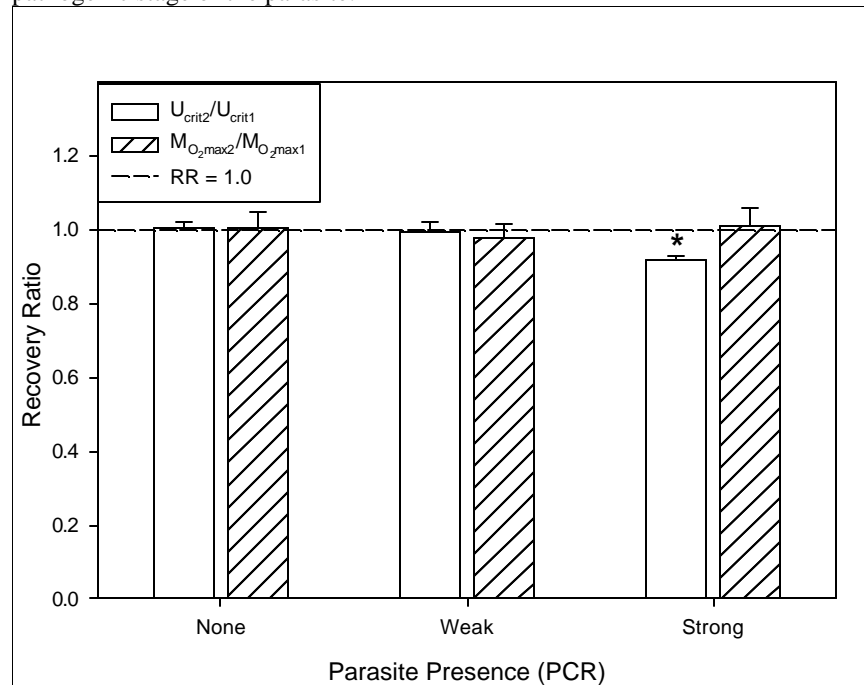


Figure 1: Maximum prolonged swimming performance (U_{crit}) and metabolic rate (M_{O_2max}) recovery ratios for sockeye salmon differentially infected with *P. minibicornis* (measured by PCR analysis) that performed repeated U_{crit} tests (45 min recovery period). The line of unity (dashed line) indicates $x = y$ (i.e. the predicted line if $U_{crit1} = U_{crit2}$, independent of temperature). An asterisk indicates a recovery ratio is significantly ($P < 0.05$) below the line of unity.

Radio transmitter data showed that early-entry Late-run sockeye salmon required a similar number of days as normally behaving fish to migrate from the mouth of the Fraser River to the lake holding area (Fig. 2). However, the former

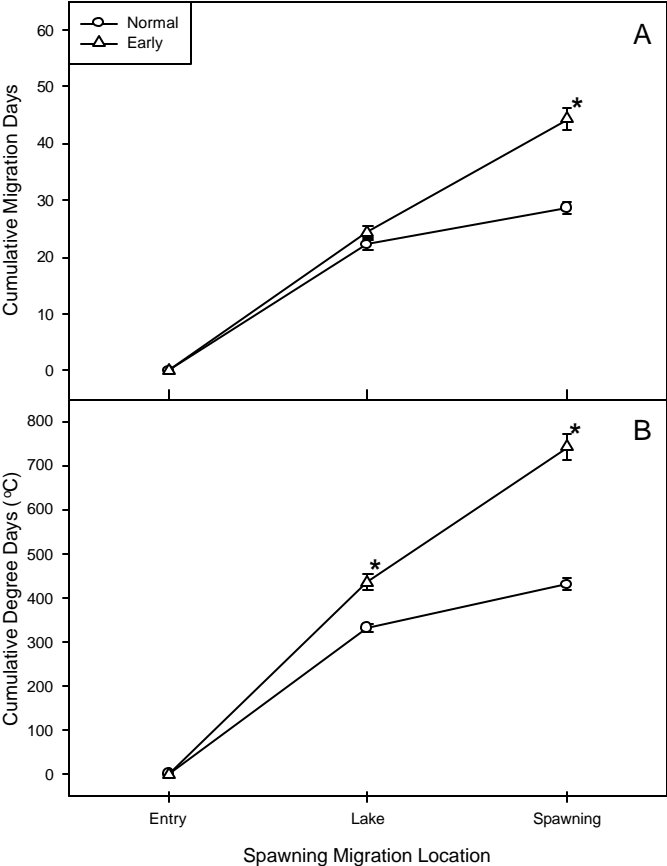


Figure 2: Radio transmitter tracking of the number of early and normal behaving Late-run sockeye salmon migration (A) from Fraser River to their lake holding area and to their final spawning grounds, as well as the number of degree day (B) exposure during this migration (calculated as the cumulative daily water temperature above 0°C in four locations along the migration

route). An asterisk indicates a value is significantly ($P < 0.05$) higher for early migrating sockeye.

group held in the lake system for a significantly longer period (20.0 ± 1.9 days) than the latter (6.5 ± 0.7 days) before migrating to their spawning grounds. Early migrants were exposed to significantly more degree days (436.0 ± 20.0 °C) than normal-behaving fish (333.0 ± 10.1 °C) during upriver migration to the holding lake and while holding within the lake prior to entering the spawning grounds (early: 309.1 ± 26.8 °C; normal: 96.6 ± 10.7 °C).

When compared with data analysed from the telemetry study, the results of the present study indicate that the migratory ability of early migrating sockeye can be impaired by *P. minibicornis* infection due to progressive exhaustion. Early migrating fish had a 31 % increase in temperature exposure and were within the level of degree days shown to affect the recovery of sockeye from exhaustive exercise. Although this reduction likely occurred near the end of their migration, increased migration stress also may impact the fecundity (Schreck et al. 2001) and progeny survival (Campbell et al. 1994) of infected fish because of diversion of blood flow to the muscles. This impact may be exacerbated by the >300 % higher temperature exposure of early-migrating fish while holding within the lake that would cause the proliferation of the parasite prior to fish entering the spawning grounds. This proliferation may cause further energy loss due to increased difficulty in maintaining osmoregulatory balance. Energy loss during migration or while holding in the lake system also could negatively affect the ability of early migrants to compete for space and mates on the spawning grounds.

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**SOME LIMITING AND LOADING FACTORS
AFFECTING SWIMMING PERFORMANCE IN FISH**

E.W. Taylor and D.J. McKenzie

Biosciences, University of Birmingham, UK and IFREMER, La Rochelle,
France.

EXTENDED ABSTRACT ONLY: DO NOT CITE

Factors that may limit swimming performance in fish were discussed by Fry (1971), who defined standard metabolic rate (SMR) as rate of oxygen uptake measured from a notionally stationary fish then described the increase in oxygen uptake in swimming fish as “scope for activity” (SFA). Factors that limit scope are by definition “limiting factors” while factors that increase SMR, thus reducing SFA, are “loading factors”.

Hypoxia, by limiting oxygen supply, clearly acts as a limiting factor. The range over which oxygen is limiting varies with oxygen demand, as it in turn varies between species and with temperature within species. Temperature limits swimming performance either above or below an optimum temperature that again varies between species and is reflected both in the functional properties of swimming muscle (Johnston and Ball, 1997) and the cardiovascular system (Taylor et al, 1997).

Changes in salinity may impose the costs of osmoregulation as a loading factor and in sturgeon SMR was increased in brackish seawater. However, maximum sustainable swimming speed (Ucrit) varied indirectly with plasma sodium concentration (McKenzie et al, 2001), so that lack of control of ion levels may be introducing a limiting factor on swimming performance.

Examination of a series of potential loading factors reveals a range of responses. Growth is likely to increase metabolic rate, acting as a loading factor. Growth hormone transgenic tilapia grew 1.5 times faster than a wild type control and had a higher SMR but SFA and Ucrit were unchanged, implying that the respiratory system had compensated for the increased demand. This was

confirmed by their responses to hypoxia (McKenzie et al, 2003). The cost of handling a meal (measured as SDA) certainly introduces a loading factor. Trout showed an increase in SMR and a reduced Ucrit when fed to satiation, compared to an unfed group (Alsop and Wood, 1997).

Diet can affect metabolic rate and may limit swimming performance. Salmon showed Ucrit values that varied directly with the content of canola oil in manufactured diets. This is rich in oleic acid, which is a favoured substrate for aerobic metabolism (McKenzie et al, 2000).

Recently, a direct relationship has been established between Ucrit and plasma ammonia levels in trout, swimming either in elevated levels of ammonia (Shingles et al, 2001) or in acid water containing copper (Beaumont et al, 1995), either of which cause an increase in plasma ammonia. Although there were indications that aerobic metabolism may have been affected by elevated ammonia, the primary cause of the reduced swimming performance was found to be failure to recruit white muscle because it was partially depolarised by the elevated levels of ammonia in the tissues (Beaumont et al, 2001). Thus ammonia, a product of protein catabolism, can be considered a limiting factor.

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**ENERGETIC ADAPTATION AND FITNESS
IN FRESHWATER RESIDENT
AND ANADROMOUS ARCTIC CHARR**

Ross. F. Tallman,
Department of Fisheries and Oceans, Central and Arctic Region,
501 University Crescent, Winnipeg, Manitoba, CANADA R3T 2N6,
telephone: 204-983-3362, FAX 204-984-2403,
E-mail: tallmanr@dfo-mpo.gc.ca

Tracey N. Loewen,
Department of Fisheries and Oceans, Central and Arctic Region,
501 University Crescent, Winnipeg, Manitoba, CANADA R3T 2N6, t
elephone: 204-984-2535, FAX 204-984-2403,
E-mail: loewent@dfo-mpo.gc.ca

EXTENDED ABSTRACT ONLY - DO NOT CITE

Migration in fish species is thought to have evolved due to a mis-match in the habitat suitability for winter refugia, feeding or spawning (Northcote, 1992). Benefits of adopting a migratory life history must outweigh the trade-offs associated with the behaviour. Advantages to migration include increased food resources which lead to increased growth potential, increased survivorship, decreased predation, and decreased physiological stress upon reaching a large size. Increased growth will accommodate an increase in body size, and thus an increase in fecundity. A delay in maturation may evolve to facilitate energy allocation to growth instead of reproduction and further enhance an increase in body size. Tradeoffs to migration include an increased risk of mortality before reproducing and increased risk of disease and parasitism. The selection of life history traits will evolve to optimise energetic efficiency and reproduction while sustaining life in an organism's environment. Organisms will migrate when resources gained in the new environment will provide a significant increase to the organism's fitness (an increase in lifetime reproductive output).

Within the salmonid family anadromous (migratory) and residual (lake) forms of the same species co-exist within the same open freshwater system (Tallman *et. al*, 1996). Thorpe (1999) suggested that high-energy lifestyles and low energy lifestyles cause individuals to choose alternative life histories to optimise fitness. A threshold lipid reserve is needed to initiate maturation within fish and a threshold size must be attained to initiate smoltification. Juveniles with high metabolic rates will attain smolt size before they are able to attain the lipid threshold required for reproduction and thus adopt an andromous (high-energy) lifestyle. Juveniles with low metabolic rates are able to satisfy their energetic requirements within a freshwater habitat and will attain the lipid threshold necessary for reproduction. These individuals will mature before they reach smolt size and therefore adopt a resident lifestyle (low-energy lifestyle).

Due to energy constraints within the Arctic ecosystem and environmental uncertainty, Arctic charr have evolved great phenotypic plasticity, enabling them to adopt multiple life. Charr can exist as multiple resident forms, migrants or both within one lake system. In Norway, for example, four different forms (three varying resident and one anadromous) have been found to co-exist in one lake system (Tallman *et al.* 1996).

Reproductive events and over-wintering costs are extremely high for individual fish. For example, Atlantic salmon decrease lipid stores by approximately 50 %, Pacific salmon by approximately 80% in a single reproductive event, and anadromous Arctic charr use approximately 30 % of their energy reserves to overwinter (Thorpe, 1999). Physiological changes to accommodate maturation are thought to occur one year prior to the actual spawning event (Metcalf, 1998). At critical periods such as post-spawning (fall) and post over-wintering (spring), gonad development can be arrested through hormonal controls if the lipid threshold is not met (Metcalf, 1998).

Various stocks of Arctic charr in the northern Canada have resident populations (eg. Figure 1).

Anadromous Arctic charr must return to freshwater to overwinter and reproduce seasonally. Due to the Arctic climate, migratory charr only feed approximately 50 days at sea. Reproduction and overwintering are large energetically depleting events that each charr must endure for survival. Since fish that go to sea have increased metabolic rates, large lipid reserves may become depleted over the winter when food resources are not available. Anadromous fish are also known to have a three fold increase in fecundity in comparison to resident

counterparts (Roff, 1988). Thus, searun charr will incur higher lipid depletion during spawning events. Resident fish on the other hand are thought to have low metabolic rates and thus may not expend large amounts of energy for maintenance over the winter. Since resident individuals invest less into reproductive events, lipid depletion may only be minimal.

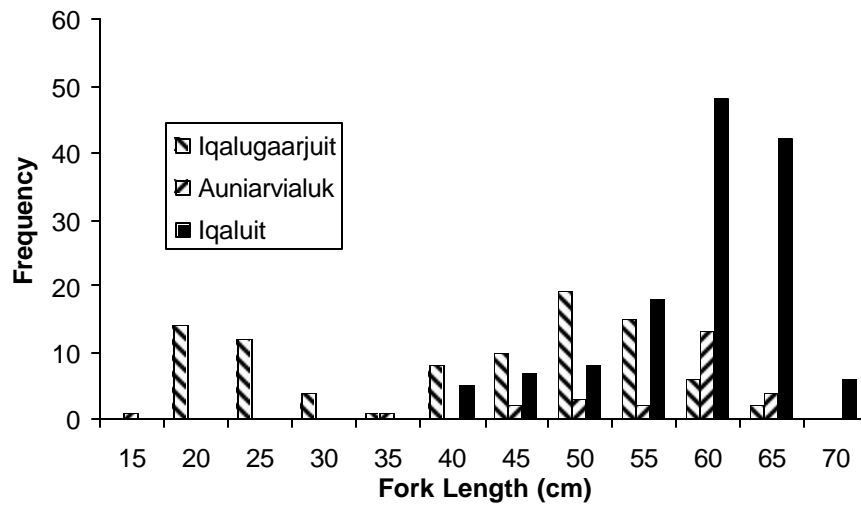


Figure 1. Length frequencies of mature charr in some Baffin Island. Note the presence of resident charr in the Iqalugaarjuit system.

Table 1. Trade-offs in fitness-related traits for anadromous and resident Arctic charr.

Fitness Related Trait	Anadromous	Resident
Metabolic Rate	High	Low
Fecundity	3 * greater	Low
Growth/Size	Increase/large size	Decrease/small size
Age at maturity	Delayed (8-10 years)	3-4 years
Resting Period	3-10 years ??	0 years ??
Maintenance Costs (Overwintering)	High	Low

Model For Balancing Fitness

The trade-offs required for anadromous life-style compared to a resident one are shown in table 1. While anadromous char must gain in growth and fecundity they lose in the recovery from winter conditions and subsequently in spawning frequency. As well, resident charr are less likely to have sudden mass mortality due to mis-timing of migration and other activities. Charr consistently produce small and large offspring even when under artificial selection for increased size. The production of smaller offspring must have fitness benefits to the parents. Thus, both forms persist.

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**ENERGETIC AND FITNESS CONSEQUENCES
ASSOCIATED WITH TRUNCATED SELECTION
FOR ANGLING VULNERABILITY
IN LARGEMOUTH BASS**

S.J. Cooke
Centre for Applied Conservation Research, University of British
Columbia, 2424 Main Mall, Vancouver, BC V6T 1Z4
604 228 1992; scooke@interchange.ubc.ca

C.D. Suski
Queen's University

K.G. Ostrand, D.H. Wahl, D.P. Philipp
Illinois Natural History Survey and the University of Illinois

EXTENDED ABSTRACT ONLY – DO NOT CITE

The evolutionary consequences of fishing-induced selection pressure are an emerging concern in fisheries science, and may represent a risk to the sustainability of fisheries resources. Reductions in long-term yield, age at maturity, size at age, along with other genotypic and phenotypic changes that are of interest to fisheries managers may arise from selective harvest. To date, research on fisheries-induced selection has focused on commercially exploited marine stocks and assessing the potential ability of those resources to persist under current levels of harvest. Recreational angling, however, may provide even stronger selection forces, particularly in inland freshwater systems where fishing pressure can be more intense and more targeted to certain individuals. Despite high rates of catch-and-release angling in many recreational fisheries, substantial mortality or sublethal physiological and fitness impairment may occur following angling, leading to opportunities for angling selection to operate even without harvest. Here, we report on a long-term (25+ year) study where largemouth bass were exposed to angling-induced selection.

Our results show that selection for angling vulnerability resulted in substantial differences in physiological and energetic attributes between vulnerable and non-vulnerable fish. Specifically, vulnerability to angling was a heritable trait; that covaried positively with factors including elevated metabolic rate, reduced metabolic scope, increased food consumption, and increased parental care activity. Ultimately, angling vulnerability appears to be a complex interaction of numerous factors leading to selection for different phenotypes. The strong angling pressure in many freshwater systems, and therefore the potential for this to occur in the wild, necessitates management approaches that recognize the evolutionary consequences of angling.

SELECTION ON MORPHOLOGY AND SWIMMING PERFORMANCE
OF EUROPEAN SEA BASS (*Dicentrarchus labrax*)
IN AN EXPERIMENTAL ESTUARY

Jay A. Nelson
Department of Biological Sciences, Towson University
Towson Md. 21252-0001 USA
Phone: 410-704-3945 Fax: 410-704-2405
Jnelson@towson.edu

Corey A. Handelsman
Department of Biological Sciences; Towson University

Guy Claireaux
Centre de Recherche sur les Ecosystèmes Marin et Aquacoles, Place du
Séminaire, L' Houmeau, 17137, France.

EXTENDED ABSTRACT ONLY - DO NOT CITE

Very little is known about how performance characteristics of economically important fishes relate to Darwinian fitness. In addition, fish physiologists have rarely studied variation of performance and its component processes, the raw material of natural selection. If variation in performance can be related to differential success in either wild or aquaculture situations, the mechanism(s) behind that variation will be of great interest.

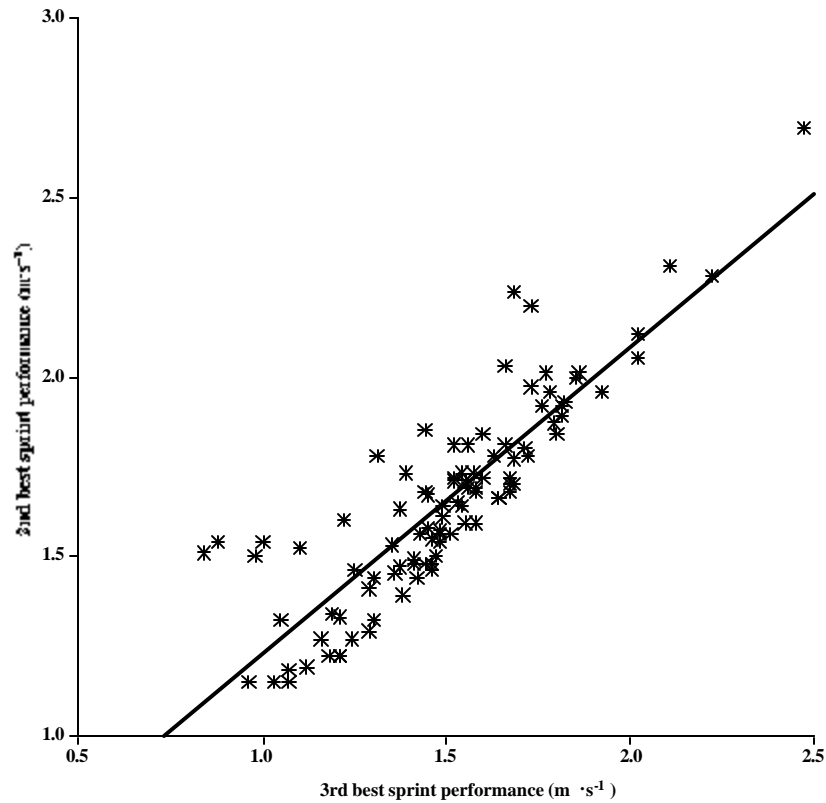
Some degree of swimming performance is undoubtedly critical to the success of individuals of the species we chose to examine these issues with, the European sea bass (*Dicentrarchus labrax*). This economically important species develops in estuaries where it is subject to substantial predation, migrates as both a juvenile and an adult and is a pelagic predator of other active species throughout its adult life. Thus, various swimming styles are required by sea bass to complete their life cycle and understanding the factors that control different types of swimming could be important for predicting an individual sea bass' chances for success. Fishery models generally use a single number for all

members of a species when it concerns factors such as swimming speed and metabolic rate. However, we have found that laboratory-raised sea bass of similar size can vary 300% in their rates of oxygen consumption at the same swimming speed; likewise the significantly repeatable sprinting ability of similar-sized, laboratory-raised sea bass can vary by over 200% (Figure 1). Thus, the first criterion for demonstrating natural selection on locomotion (and metabolism) exists in sea bass, namely, measurable variation in the traits (Endler 1986). The work described here was designed to ascertain whether a second criterion for natural selection, a link with survivorship exists. The purpose of this experiment was to: 1) develop swimming tests for sea bass that assessed their performance capacities on different temporal scales; 2) measure the performances of 120 individual sea bass with multiple performance tests and establish the inter-relationship of the various tests with each other, morphology and metabolic rate, and, 3) release the animals to man-made estuaries without predators at densities that assured competition for the natural forage base.

Methods

European sea bass of both sexes were obtained from a local hatchery in March 2002 and brought to the Centre de Recherche sur les Ecosystèmes Marins et Aquacoles (CREMA) in L'Houmeau France. Upon arrival at the laboratory, they were transferred to 500 l tanks supplied with recirculated and filtered natural seawater and had a Personal Identification Transponder (PIT) tag inserted subcutaneously. Endurance performance was measured with a modified critical swimming speed protocol (Brett 1964) in a Brett-type swim-tunnel respirometer. After overnight acclimation to a 15cm/s current, the fish were raised to 50cm/s and then subjected to successive increments of 10 cm/s every 30 min until exhausted. Sprint performance was measured with a laser detection "drag strip" as described by Nelson et al. (2002), but the chamber was modified for the dimensions of juvenile sea bass and employed numerous advances in electronic technology. Each fish was sprinted between four and seven times in a single day (Figure 1). Mass, length, width and height of each fish were measured upon arrival in the laboratory. These measurements were repeated immediately prior to release into the estuaries and after 60, 110, and 160 days of residence in the ponds. Detailed morphological measurements were made on all fish from digital images taken both prior to release into the earthen ponds and on all survivors of 160 days in the ponds. Morphometric analyses was performed with the public domain "National Institutes of Health Image" program. The program was used to make a truss network consisting of 22 measurements modeled after Bookstein *et al.* (1985).

Figure 1. Relationship between sprint trials of 125 sea bass. The highest velocity reached by each individual in its second fastest of four-six trials run over a period of one-two hours is graphed against the highest velocity reached in that same individual's third fastest trial $P < 0.00001$ (Spearman rank).



To assess whether resource acquisition and survival are functions of locomotor capacity, the sea bass were randomly divided between two identical 200 m² by 1 m deep earthen ponds that connect to the Atlantic Ocean via a canal. The tidal earthen ponds are designed to allow a natural forage base to arrive with the incoming tide while standpipes and meshing prevent the experimental fish from escaping. Sea bass were initially stocked at a density of 60 fish per pond to

ensure intense competition for resources. However, following 110 days in the ponds and 50% mortality, the experimental ponds were supplemented with live food seined from neighboring ponds to prevent further mortality. After 160 days, surviving animals (53) were removed to the laboratory for assessment of active metabolic rate and re-assessment of morphology and performance.

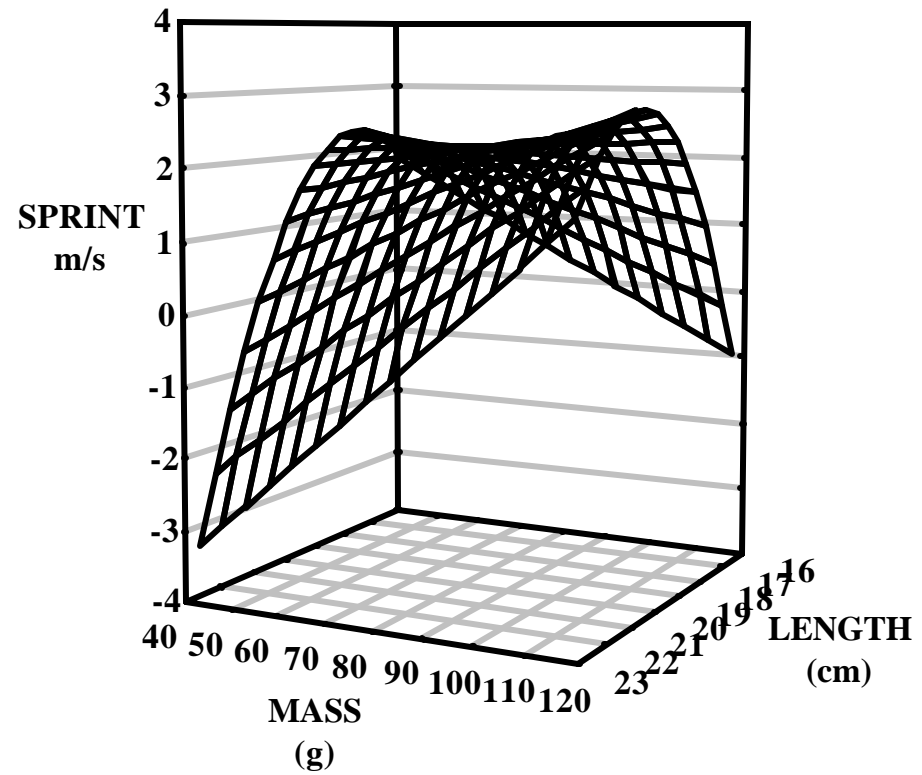
Results

While there was no simple univariate relationship between any initial measurement of laboratory-raised fish and subsequent survival in the estuaries, definitive selection occurred in the ponds and several other interesting results emerged. Growth rate from a previous interval (laboratory or field) always predicted survival in a subsequent interval ($P < 0.01$). Intense intraspecific competition for resources led to a significant narrowing of variance in morphology towards a body shape that was also optimal for sprinting ability among survivors (Figure 2). Related to this finding was a significant tradeoff between growth performance in the estuaries and subsequent sprint performance ($P < 0.01$). Finally, non-parametric rank tests suggest that the U_{crit} test was significantly repeatable after 160 days in a simulated field environment ($P < 0.05$) and the sprint test nearly so ($P = 0.056$). These experiments are currently being expanded to include the role of predation.

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Figure 2. Maximal sprint performance as a function of fish condition factor (length and mass) in 53 juvenile seabass that survived 5 months in man-made estuaries.



DISPERSAL ACROSS OXYGEN GRADIENTS

FITNESS TRADE-OFFS FOR AN AFRICAN CYPRINID

Lauren J. Chapman

Department of Zoology, University of Florida, Gainesville, FL, 32611, USA
& Wildlife Conservation Society, 2300 Southern Blvd., Bronx, NY, 10460,
USA

Phone: (352) 392-7474

FAX: (352) 392-3704

E-mail: lchapman@zoo.ufl.edu

Sarah Schaack

Department of Biology, Indiana University, 1001 E. Third St., Bloomington, IN
47405-3700, USA

Colin A. Chapman

Department of Zoology, University of Florida, Gainesville, FL, 32611, USA
& Wildlife Conservation Society, 2300 Southern Blvd., Bronx, NY, 10460,
USA

EXTENDED ABSTRACT ONLY – DO NOT CITE

We are exploring the role of dissolved oxygen (DO) as a divergent selective factor contributing to inter- and intrademic variation in freshwater fishes. Given the widespread occurrence of hypoxia in aquatic systems, and increasing levels associated with anthropogenic influence, diversification in response to low-oxygen stress may be a frequent phenomenon in aquatic habitats.

In the African cyprinid *Barbus neumayeri*, interademic variation in respiratory traits occurs across DO gradients. *Barbus neumayeri* from hypoxic swamp waters are characterized by larger gill size than conspecifics from well-oxygenated waters (Chapman et al., 1999; Schaack and Chapman, 2003). In addition, swamp-dwelling *B. neumayeri* have a lower critical oxygen tension (Chapman unpublished data) and a lower threshold for aquatic surface respiration (Olowo and Chapman, 1996) than stream-dwelling fish indicating higher respiratory performance in swamp fish. However, large-gilled swamp

fish are also characterized by reduction in the size of key trophic muscles and a lower feeding performance relative to stream-dwelling conspecifics (Schaack and Chapman, 2003). These differences in performance between large- and small-gilled fish create the potential for a fitness trade-off between high- and low-oxygen environments, and this may contribute to the maintenance of local phenotypes.

We carried out a field dispersal study of *B. neumayeri* in Kibale National Park, Uganda to quantify the degree to which respiratory phenotypes of *B. neumayeri* (large- and small-gilled fish) mix across the ecotone of the papyrus-dominated Rwembaita Swamp and the Njuguta River, into which the swamp feeds. Chapman et al. (1999) reported mean DO levels (3 years of monthly samples) of 1.6 mg l⁻¹ and 6.2 mg l⁻¹ for the dense interior of the Rwembaita Swamp and the open waters of the Njuguta River, respectively. Water leaving the swamp flows through a forested channel for ~80 m before meeting the well-oxygenated waters of the river. Although, the outflow picks up some DO prior to meeting the river, there is clear transition between the dark, clear, hypoxic waters of the swamp stream and the turbid, well-oxygenated waters of the river. Fish were captured in minnow traps at four sites in swamp outflow (20, 40, 60, and 80 m upstream of the ecotone), at the meeting of the waters, and at four sites in the Njuguta River (20 and 40 m upstream and downstream of the ecotone). Fish were measured and marked with tattoo ink according to the site of capture and released. This sampling regime was carried out approximately every 2 weeks over a 4.5-yr period. A total of 259 fish were recaptured of the >7000 fish marked; 58 of these fish were recaptured at a site different than their origin. Of the movers, 25 were recaptured in the same habitat as their original capture (swamp or stream); 7 had moved to the ecotone, and 17 had crossed into a different habitat (from swamp to stream or visa versa, Figure 1). There was no difference in the proportion of movers (within), movers (across), and stayers between the fish of swamp and river origin ($X^2=0.21$, $P>0.90$).

The degree of ecological mixing observed across the ecotone is interesting given documented morphological differences between these populations (Schaack and Chapman 2003); this suggests the possibility of habitat-specific selection pressures on dispersers. To explore potential fitness costs of moving across the physico-chemical gradient, we used a reciprocal transplant cage experiment. Sixteen cylindrical cages (1 m long x 0.5 m diameter) were constructed from 4-mm plastic mesh that permitted free flow of small aquatic invertebrates, detritus, and other food. Eight cages were placed in the Njuguta River between 20 and 40 m upstream of the swamp-river ecotone, and eight cages were placed in the

swamp stream 80 m upstream of the ecotone. One swamp fish and one river fish of equivalent total length were held for ~10 weeks in each cage, and we quantified growth rate over the trial. The experiment was repeated sequentially over a 2-year period to produce an adequate sample size and to represent seasonal variation. Fish of river origin grew faster when held in river habitat than in swamp habitat (Figure 2). Thus, one potential cost of dispersal for a river fish is growth depression in the swamp habitat. This may relate to the lower respiratory performance of river fish under hypoxia that has been demonstrated in earlier studies, and this may minimize mixing of low- and high-oxygen phenotypes. Interestingly, the growth rate for fish of swamp origin did not differ significantly between the cage habitats. These findings suggest that swamp fish would not be compromised by moving into well-oxygenated river waters with respect to growth (at least on the short-term); however, there may be other costs related to lower feeding performance (Schaack and Chapman 2003) or higher mortality that may contribute to the maintenance of these phenotypes in the field.

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Acknowledgements

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from the National Council for Science and Technology. We also thank the field assistants of the Kibale Fish Project.

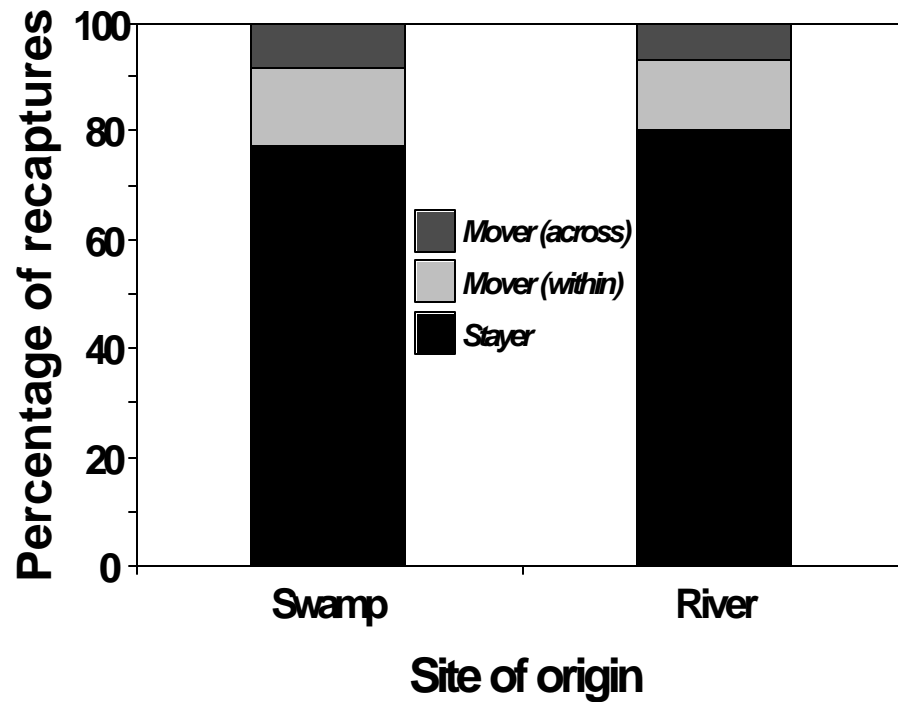


Figure 1. The percentage of *Barbus neumayeri* that were recaptured at the site of origin, moved within their original habitat (hypoxic swamp stream or well-oxygenated river), or moved across the swamp-river ecotone. Data were derived from a mark and recapture study in Rwembaita Swamp/Njuguta River system of Kibale National Park, Uganda.

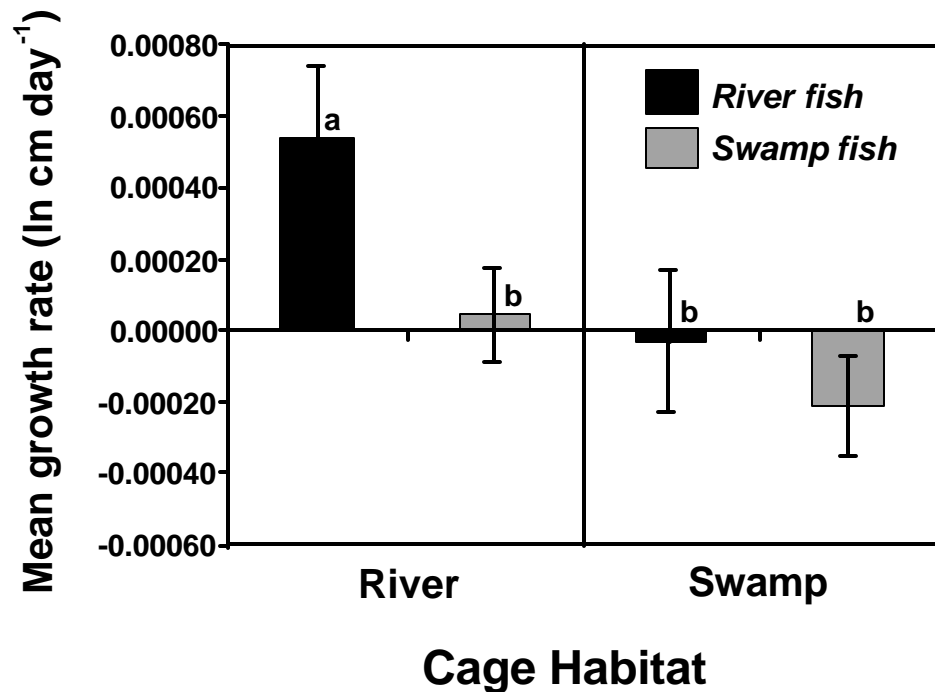


Figure 2. Mean growth rate (ln cm day⁻¹ ± SE) of *Barbus neumayeri* collected from the outflow of the Rwembaita Swamp and open waters of the Njuguta River of Kibale National Park, Uganda. Fish were held in cages for ~10 weeks in both habitats (reciprocal transplant design). Shared letters indicate no significant difference at P<0.05 (LSD).

ENZYME ACTIVITIES
IN THE GULF KILLIFISH, FUNDULUS GRANDIS,
DURING LONG-TERM HYPOXIC EXPOSURE

Mery L. Martínez
Department of Biological Sciences, University of New Orleans, New Orleans,
Louisiana 70148, USA
Telephone: (504) 280-2769. FAX: (504) 280-6121
E-mail: mmartine@uno.edu

Christie Landry², Ryan Boehm¹, Steve Manning³,
Ann Cheek², and Bernard B. Rees¹

¹ Department of Biological Sciences, University of New Orleans ²Department of
Biological Sciences, Southeastern Louisiana University, Hammond, Louisiana
70402, USA ³Gulf Coast Research Laboratory, University of Southern
Mississippi, Ocean Springs, MS 39566, USA

EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Low dissolved oxygen, or hypoxia, has become a major concern in aquatic habitats (Díaz and Rosenberg, 1995). Fish respond to hypoxia via behavioral, physiological, and biochemical adjustments (Hochachka, 1980). With regard to biochemical adjustments, previous studies with mammalian cell and tissues suggest that they respond to hypoxia by a coordinated increase in the capacity for anaerobic metabolism and a decrease in aerobic capacity (Webster, 2003). However, less is known about the metabolic responses in organisms that encounter fluctuations in oxygen in their natural environment. We used the *Fundulus grandis* to examine the effects of long-term hypoxic exposure on the activities of glycolytic and gluconeogenic enzymes. These experiments test the hypothesis that this fish responds to aquatic hypoxia via a coordinated regulation of enzymes of carbohydrate metabolism.

Methods

Gulf Killifish, *Fundulus grandis*, were purchased in Pascagoula Mississippi, U.S.A., and kept at the Gulf Coast Research Lab, (University of Southern Mississippi) at Ocean Springs, Mississippi, U.S.A. Fish were held under normoxia (>80% saturation) and hypoxia (about 15% saturation) for one month at 27°C and ~15‰ salinity. Photoperiod was 16 h light-8 h dark.

Fish were killed and liver, white skeletal muscle, heart and brain were dissected. Hexokinase (HK), phosphoglucose isomerase (PGI), phosphofructokinase (PFK), aldolase (ALD), triosphosphate isomerase (TPI), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerokinase (PGK), phosphoglyceromutase (PGM), enolase (ENO), pyruvate kinase (PK), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), fructose 1,6-bisphosphatase (FBPase), and glucose 6-phosphatase (GPase) were measured. Protein was measured with the bicinchoninic acid assay. Statistical analyses were performed with SYSTAT 10.

Results and Discussion

General Morphology. Fish in the two groups had equal body masses at the beginning of the experiment (Table 1). After one month, normoxic fish were significantly heavier than hypoxic fish, indicating a higher growth rate in normoxia. The condition factor of normoxic fish was also significantly higher than hypoxic fish. The effect of hypoxia was likely due to a decrease in food intake (Chabot and Dutil, 1999).

Table 1. Morphological characteristics of *Fundulus grandis* subjected hypoxia and normoxia for four weeks at 27 °C.

Parameters	Hypoxia	Normoxia
Initial Standard length (mm)	80 ± 4.6	81 ± 6.7
Initial mass (g)	10.0 ± 2.3	10.8 ± 3.2
Initial Fulton Index (mass * length ⁻³)* 100	1.94 ± 0.2	1.97 ± 0.1
Final Standard Length (mm)	81 ± 5.3*	85 ± 6.9
Final mass (g)	11.7 ± 05**	15.2 ± 3.6
Final Fulton Index (mass * length ⁻³)* 100	2.19 ± 0.17**	2.46 ± 0.18
Number	22	19

Values are means ± S.D; * $P < 0.05$; ** $P < 0.005$.

Effects of Hypoxia on Enzyme Activities. Webster (2003) suggested that all glycolytic enzymes increase in a coordinated fashion during low oxygen, whereas enzymes involved in aerobic processes decrease. Our results do not support this view. The effects of hypoxia upon enzymes of carbohydrate metabolism were tissue-specific and varied among enzymes (Table 2).

Liver showed the most changes during hypoxia. Compared to normoxic controls, four enzyme activities were higher in hypoxia (PFK, GAPDH, PGK and LDH), while two were lower (HK and ALD). In addition, three of five gluconeogenic enzymes were higher during hypoxia than during normoxia (MDH, PC, and FBPase; $P < 0.005$). The increase in gluconeogenic enzymes may support the formation and export of glucose. Our observations suggest that liver responds to hypoxia by increasing the capacity for both anaerobic and aerobic carbohydrate metabolism, rather than a coordinated up-regulation of one and down-regulation of the other.

Table 2. Summary of changes observed on the glycolytic enzymes activities in tissues of *Fundulus grandis* subjected to hypoxia.

Enzyme	Tissue			
	Liver	Muscle	Heart	Brain
HK	↓*	n.d.	↑**	↑**
PGI	-	↓*	-	-
PFK	↑**	-	-	-
ALD	↓*	↓*	-	-
TPI	-	-	↑*	↑*
GAPDH	↑**	-	-	-
PGK	↑**	-	-	↑*
PGM	-	-	-	↓*
ENO	-	-	-	-
PYK	-	-	↑**	-
LDH	↑**	-	-	↑*

↓ Decreased relative to normoxia; ↑ Increased relative to normoxia, - No change; n.d. not determined.

* $P < 0.05$; ** $P < 0.005$.

In white skeletal muscle, no glycolytic enzymes increased during hypoxia. This tissue is endowed with high concentrations of glycolytic enzymes, and may not require further increases during low oxygen exposure. In contrast, PGI and ALD decreased. This decrease may be related to the lower growth of fish in hypoxia (see above). In other fish, growth rate is positively correlated with levels of glycolytic enzymes in muscle (Martínez *et al.*, 2003).

In heart, the activities of three enzymes increased during hypoxia (HK, TPI and PYK). In brain, the activities of four enzymes increased (HK, TPI, PGK and LDH) and one enzyme decreased during hypoxia (PGM). In both tissues, HK increased during hypoxia, which may reflect the reliance of these tissues on blood glucose.

In summary, rather than a coordinated regulation of all pathway enzymes, our results demonstrate enzyme-specific adjustments to environmental hypoxia. Among the glycolytic enzymes that showed the most significant increases during hypoxia were some enzymes traditionally considered to be rate-limiting

(HK, PFK, PYK). These observations support the idea that increased glycolytic flux may occur via changes in only selected pathway enzymes.

Acknowledgements

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**IMPACTS OF HYPOXIA ON JUVENILE FISH GROWTH:
EVIDENCE FROM LABORATORY AND FIELD STUDIES**

Kevin L. Stierhoff
University of Delaware - Graduate College of Marine Studies
700 Pilottown Rd, Lewes, DE 19958 USA
stierhof@udel.edu /
(Phone) 302.645.4378 / (Fax) 302.645.4378

Timothy E. Targett
University of Delaware - Graduate College of Marine Studies
700 Pilottown Rd, Lewes, DE 19958 USA
ttargett@udel.edu /
(Phone) 302.645.4396 / (Fax) 302.645.4378

EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Shallow coastal ecosystems, such as salt marsh creeks and coastal bays, provide important nursery habitats during the larval and juveniles stages for a variety of fishes (Weinstein 1979). The recent degradation of water quality, however, seriously threatens the value of these habitats as nurseries for estuarine-dependent fishes.

Physicochemical factors such as temperature, salinity, and dissolved oxygen (DO), which vary temporally and spatially, can have substantial effects on growth rates of young-of-the-year (YOY) fishes and consequently nursery habitat quality. Of particular concern for estuarine nursery habitats is the increased occurrence of hypoxic events (Diaz 2001). Chronic and diel-cycling hypoxia frequently occurs in shallow coastal ecosystems during summer months. Although fishes vary in their sensitivity to changes in ambient DO concentration, sub-lethal hypoxia typically results in reduced growth rates. Since mortality during the larval and juvenile stages is largely a size-dependent phenomenon (Anderson 1988, Sogard 1997), it is clear that chronic and severe hypoxia could have substantial impacts on the quality of estuarine habitats as nurseries.

Recent advances in molecular techniques, particularly the fluorometric quantification of nucleic acids (Caldarone et al. 2001), have made it possible to reliably measure near real-time growth rates of fishes in response to short-term (1-4 d) changes in environmental conditions. The RNA/DNA ratio (R/D) has been used in several studies to estimate recent growth in fishes. While the DNA content of fish tissue remains relatively stable and provides an index of cell number, RNA (primarily rRNA) content changes in response to transcription-dependent protein synthesis that is directly correlated with ribosomal activity, and thus growth rate.

The objectives of this study were to investigate the impacts of chronic and diel-cycling DO on the growth rates of the YOY of two estuarine-dependent fishes: summer flounder, *Paralichthys dentatus*, and weakfish, *Cynoscion regalis*. Both species are abundant in eastern U.S. estuaries, and their tributaries, during the summer when severe diel fluctuations (0-200% saturation) in DO can occur. We will present the results from laboratory-based growth experiments and growth rates (estimated from R/D) of both species from areas of Pepper Creek, a mesohaline tributary of the Delaware Inland Bays.

Methods

Laboratory growth experiments

Juvenile *P. dentatus* and *C. regalis* were fed *ad libitum* under constant (2.0, 3.5, 5.0 and 7.0 mg O₂ l⁻¹) and diel-cycling (2.0-11.0 mg O₂ l⁻¹) DO conditions at 20°C, 25°C and 30°C for 7-14 d. Growth rates were measured as specific growth rate (SGR, % body mass d⁻¹) and compared between DO treatments using ANCOVA (initial fish mass as the covariate) and the Tukey's multiple comparison test.

Laboratory experiments were conducted to empirically establish 1) the relationship between R/D, growth rate, and temperature and 2) the temporal latency of the R/D to changes in feeding status for juvenile *P. dentatus* and *C. regalis*. RNA and DNA content of muscle samples were measured by the fluorescence of ethidium bromide after sequential addition of RNase and DNase (Caldarone et al. 2001).

In situ growth rates

Juvenile *P. dentatus* and *C. regalis* were collected from three fixed sampling sites in Pepper Creek, DE from late-April to late-August in 2002 and 2003. During the sampling period, temperature, salinity, and DO data were recorded at each location every 15 min. The temperature and salinity was similar between sites, while the degree and duration of diel-cycling hypoxia varied temporally within sites, among sites, and between years (Figure 1). R/D-based growth rates of wild fish will be compared between sampling sites and with ambient DO conditions prior to capture.

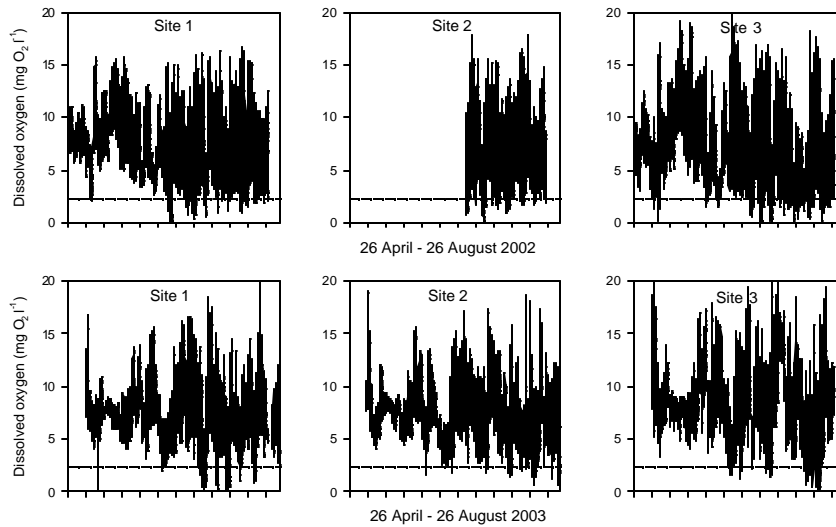


Figure 1. Daily dissolved oxygen (DO) data from Pepper Creek every 15 min from 26 April to 26 August in 2002 (top panels) and 2003 (bottom panels). The dashed line represents the 2.3 mg O₂ l⁻¹ survival-protective DO criteria established by the USEPA.

Results and Conclusions

At 20°C and 25°C, *SGR* of *P. dentatus* was significantly reduced at 3.5 mg O₂ l⁻¹ (~25% less than maximum) and at 2.0 mg O₂ l⁻¹ (50-60% less than maximum) ($p < 0.05$) (Figure 2). Growth rate of *P. dentatus* was most affected by low DO at 30°C where *SGR* was significantly reduced at 5.0 mg O₂ l⁻¹ and was ~90% less than maximum at 2.0 mg O₂ l⁻¹. *SGR* was also significantly reduced (~35% less than maximum) in the diel-cycling treatment at 25° C and 30° C. These data suggest that growth of *P. dentatus* is substantially impacted by DO conditions similar to those typically observed in Pepper Creek and other coastal bays during summer nursery periods, particularly at higher temperatures.

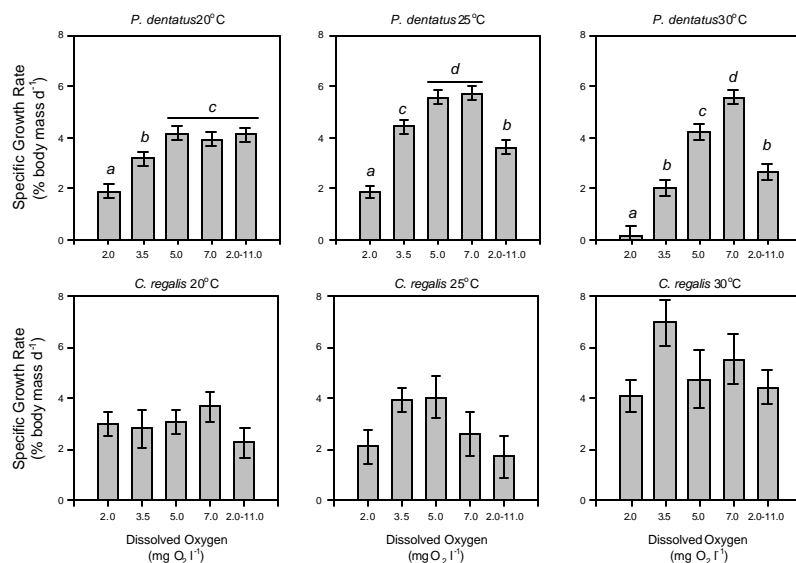


Figure 2. Mean (\pm SE) specific growth rates of juvenile summer flounder, *Paralichthys dentatus* (top panels), and weakfish, *Cynoscion regalis* (bottom panels) exposed to chronic and diel-cycling dissolved oxygen at three temperatures. Bars sharing superscripts in the same panel are not statistically different ($p > 0.05$).

Growth rates of *C. regalis* were highly variable within all DO and temperature treatments, and thus no significant differences were observed between DO treatments at any temperature (Figure 2) ($p > 0.05$). It is unclear from these data that DO (within the range tested) affects the growth of juvenile *C. regalis* in these systems.

The R/D data from field-collected fishes will provide further insight into the impacts of low DO on growth of YOY *P. dentatus*, and will hopefully provide a clearer understanding of the effects of low DO on YOY *C. regalis*. Results from the laboratory R/D experiments on each species, as well as comparisons between laboratory and *in situ* growth rates of *P. dentatus* and *C. regalis* will be presented.

Acknowledgements

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**GUT LENGTH AND MASS IN PRICKLEBACK FISHES:
ONTOGENY, DIETARY, AND PHYLOGENETIC EFFECTS**

Michael H. Horn
Department of Biological Science, California State University,
Fullerton CA 92834-6850 U.S.A.
Phone (714) 278-3707, Fax (714) 278-3426,

E-mail agawlicka@fullerton.edu

Donovan P. German
Department of Zoology, University of Florida,
Gainesville FL 32611-8525 U.S.A.

EXTENDED ABSTRACT ONLY - DO NOT CITE

We measured relative gut length and mass and calculated Zihler's index (relating gut length to body mass) for four species of prickleback fishes and determined the effects of ontogeny, diet, and phylogeny on these gut dimensions. Of the four species, *Cebidichthys violaceus* and *Xiphister mucosus* shift to herbivory with growth (>45 mm SL), whereas *X. atropurpureus* and *Anoplarchus purpureus* remain carnivores. *A. purpureus* belongs to a carnivorous clade, and the three other species belong to an adjacent, herbivorous clade.

Gut dimensions were compared in three categories of the four species: (1) small, wild-caught juveniles representing the natural diet condition of the two carnivores and the two species that have shifted to herbivory; (2) larger, wild-caught juveniles representing the natural diet condition of the two carnivores and the two species that have shifted to herbivory; and (3) laboratory-raised juveniles produced by feeding a high-protein animal diet to small juveniles until they had reached the size of the larger, wild-caught juveniles. Comparisons of gut dimensions in categories (1) vs. (2) tested for an ontogenetic effect, in (2) vs. (3) for a dietary effect, and within each of the categories (1), (2), and (3) for a phylogenetic effect.

All three members of the herbivorous clade, *C. violaceus*, *X. atropurpureus*, and *X. mucosus*, significantly increased gut dimensions with increase in body size (Table 1). In contrast, *A. purpureus*, the sole representative of the carnivorous clade, showed only one significant change, a decrease in ZI, with increase in body size (Table 1). These results demonstrate a strong, clade-specific ontogenetic effect suggesting that members of each clade are genetically programmed to develop relatively large guts if in the herbivorous clade and smaller guts if in the carnivorous clade. The strength of the phylogenetic signal is indicated further in that the sister taxa, *X. atropurpureus* and *X. mucosus*, responded similarly despite their different natural diets.

Table 1. Within-species comparisons for relative gut length (RGL), Zihler's index (ZI), and relative gut mass (RGM) from the ontogenetic (W_{30-40} vs. W_{60-75}) and dietary (W_{60-75} vs. L_{60-75}) perspectives in the four species of prickleback fishes. Values (means \pm SEM, $n = 20$) were compared with a two-tailed t test with family error rate of $P = 0.05$. $DF = 18$ for all comparisons. S = significant, NS = non-significant. Positive sign (+) indicates an increase in the gut dimension, negative (-) indicates a decrease. See Table 2 for the RGL, ZI, and RGM values.

Species	RGL	ZI	RGM
Ontogenetic effect (W_{30-40} vs. W_{60-75})			
<i>C. violaceus</i>	S (+)	S (+)	S (+)
<i>X. mucosus</i>	S (+)	S (+)	NS
<i>X. atropurpureus</i>	S (+)	S (+)	S (+)
<i>A. purpureus</i>	NS	S (-)	NS
Dietary effect (W_{60-75} vs. L_{60-75})			
<i>C. violaceus</i>	S (-)	S (-)	S (-)
<i>X. mucosus</i>	NS	S (+)	NS
<i>X. atropurpureus</i>	S (-)	S (-)	S (-)
<i>A. purpureus</i>	S (+)	S (+)	NS

The two clades differed in the majority of gut size measures and, in each case, the members of the herbivorous clade contained significantly larger guts than did *A. purpureus* in the carnivorous clade (Table 2). None of the differences was seen in the small wild (W_{30-40}) category. These results provide evidence for a

strong phylogenetic effect, with members of the herbivorous clade increasing in gut size with ontogeny more than *A. purpurescens*, which showed a slight decrease in gut size with increase in body size. Nevertheless, marked divergence also was detected in that the herbivorous *X. mucosus* had a larger gut in most instances than the carnivorous *X. atropurpureus*.

Overall, despite mixed dietary effects suggesting some degree of phenotypic plasticity, our study showed a clear, clade-specific ontogenetic effect and a phylogenetic effect supporting the hypothesis that *X. atropurpureus* evolved in an herbivorous clade despite its carnivorous diet.

Acknowledgements

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Table 2. Among-species comparisons of body mass (BM), relative gut length (RGL), Zihler's index (ZI), and relative gut mass (RGM) in each of the three feeding categories (w_{30-40} , W_{60-75} , and L_{60-75} ,) in the four species of prickleback fishes. Standard lengths (SL) are provided for reference and were not analyzed. Values (mean \pm SEM, $n = 20$) within each category were compared with one-way ANOVA and Tukey's pairwise comparison test with a family error rate of $P = 0.05$. $P < 0.001$ in all cases. Values that share the same letter (in each column) are not significantly different.

Species	SL	BM	RGL	ZI	RGM
Small wild-caught (w_{30-40})					
<i>C. violaceus</i>	37.90 ± 0.35	0.321 ± 0.010 c	0.77 ± 0.01 b	4.27 ± 0.06 c	0.056 ± 0.005 b
<i>X. mucosus</i>	34.90 ± 0.47	0.169 ± 0.009 a	0.59 ± 0.01 a	3.75 ± 0.10 b	0.062 ± 0.003 c
<i>X. atropurpureus</i>	38.60 ± 0.35	0.266 ± 0.007 b	0.55 ± 0.01 a	3.33 ± 0.04 a	0.043 ± 0.004 a
<i>A. purpurescens</i>	37.60 ± 0.41	0.323 ± 0.011 c	0.57 ± 0.01 a	3.11 ± 0.05 a	0.035 ± 0.002 a
		$F_{3, 76} = 59.57$	$F_{3, 76} = 61.47$	$F_{3, 76} = 56.49$	$F_{3, 76} = 18.50$
Large wild-caught (W_{60-75})					
<i>C. violaceus</i>	69.93 ± 0.75	1.982 ± 0.064 c	0.98 ± 0.02 c	5.46 ± 0.10 c	0.065 ± 0.003 c
<i>X. mucosus</i>	69.73 ± 0.61	1.308 ± 0.034 a	0.73 ± 0.01 b	4.63 ± 0.09 b	0.064 ± 0.003 c
<i>X. atropurpureus</i>	71.78 ± 0.86	1.337 ± 0.052 a	0.71 ± 0.01 b	4.66 ± 0.05 b	0.050 ± 0.002 b
<i>A. purpurescens</i>	66.30 ± 1.07	1.690 ± 0.087 b	0.53 ± 0.01 a	2.95 ± 0.05 a	0.037 ± 0.002 a
		$F_{3, 76} = 26.43$	$F_{3, 76} = 168.65$	$F_{3, 76} = 191.26$	$F_{3, 76} = 36.77$
Large laboratory-fed (L_{60-75})					
<i>C. violaceus</i>	68.70 ? 0.41	2.016 ? 0.050 b	0.95 ? 0.01 c	5.16 ? 0.05 d	0.054 ? 0.004 c
<i>X. mucosus</i>	67.25 ? 0.48	1.401 ? 0.030 a	0.77 ? 0.02 b	4.89 ? 0.09 c	0.066 ? 0.002 d
<i>X. atropurpureus</i>	70.23 ? 0.41	1.137 ? 0.027 a	0.60 ? 0.01 a	4.04 ? 0.05 b	0.043 ? 0.002 b
<i>A. purpurescens</i>	60.50 ? 0.82	1.388 ? 0.141 a	0.58 ? 0.02 a	3.20 ? 0.08 a	0.039 ? 0.003 a
		$F_{3, 76} = 23.16$	$F_{3, 76} = 122.94$	$F_{3, 76} = 159.86$	$F_{3, 76} = 42.58$

**GENE EXPRESSION OF AMYLASE AND TRYPSIN
IN PRICKLEBACK FISHES:
ONTOGENETIC, DIETARY, AND PHYLOGENETIC EFFECTS**

Anna Gawlicka
Department of Biological Science, California State University,
Fullerton CA 92834-6850 U.S.A.
Phone (714) 278-3675, Fax (714) 278-3426,
E-mail agawlicka@fullerton.edu

Kelly H. Kim and Michael H. Horn
Department of Biological Science, California State University,
Fullerton CA 92834-6850 U.S.A.
Phone (714) 278-3707, Fax (714) 278-3426,
E-mail mhorn@fullerton.edu

EXTENDED ABSTRACT ONLY - DO NOT CITE

We measured the intensities of amylase and trypsin gene expression in four closely related species of carnivorous and herbivorous prickleback fishes (family Stichaeidae) and determined the effects of ontogeny, diet, and phylogeny on these intensities. Of the four species, *Cebidichthys violaceus* and *Xiphister mucosus* shift to a more herbivorous diet as they grow (>45 mm SL), whereas *X. atropurpureus* and *Anoplarchus purpureus* remain carnivores throughout life. *A. purpureus* belongs to the Aletriini, a carnivorous clade, and the three other species belong to the Xiphisterini, an adjacent, mostly herbivorous clade. *X. atropurpureus* and *X. mucosus* are recognized as sister taxa.

Gene expression was determined on sections of pancreatic tissue using *in situ* hybridization with cRNA probes prepared from the cDNAs of winter flounder (Douglas and Gallant, 1998; Douglas et al., 2000), and the intensities of expression were quantified using image analysis. Intensities were compared in three feeding categories of the four species: (1) small, wild-caught juveniles (30-40 mm SL, abbreviated w₃₀₋₄₀) representing the carnivorous condition before

two of the species shift to herbivory; (2) larger, wild-caught juveniles (60-75 mm SL, abbreviated W_{60-75}) representing the natural diet condition of the two carnivorous species and the two that have shifted to herbivory; and (3) larger, laboratory-raised juveniles (60-75 mm SL, abbreviated L_{60-75}) produced by feeding a high-protein animal diet to small juveniles until they reached the size of the larger wild-caught juveniles. Comparison of intensities between w_{30-40} and W_{60-75} allowed us to test for an ontogenetic effect, between W_{60-75} and L_{60-75} for a dietary effect, and within each of the three categories (w_{30-40} , W_{60-75} , L_{60-75}) for a phylogenetic effect (alectriine vs. xiphisterine, *X. atropurpureus* vs. *X. mucosus*).

Our results showed a significant ontogenetic effect for the expression of the trypsin gene in the four species, but for expression of the amylase gene in only three species (Table 2). A significant dietary effect was found only for the expression of the trypsin gene in the two carnivorous species. Significant phylogenetic effects in gene expression were seen with the two clades being different for amylase and the sister taxa being indistinguishable for both enzymes (Table 2).

Table 1. Within -species comparisons (two-tailed *t*-tests) of the intensities of amylase and trypsin gene expression from the ontogenetic (w_{30-40} vs. W_{60-75}) and dietary (W_{60-75} vs. L_{60-75}) perspectives in the four species of prickleback fishes as determined by quantitative *in situ* hybridization.

Species	Amylase		Trypsin	
	ontogenetic	dietary	ontogenetic	dietary
<i>C. violaceus</i>	S	NS	S	NS
<i>X. mucosus</i>	S	NS	S	NS
<i>X. atropurpureus</i>	S	NS	S	S
<i>A. purpurescens</i>	NS	NS	S	S

Note: n = 3 fish for each species and category. DF = 4 for all comparisons. S = significant (P = 0.05), NS = non-significant (P > 0.05). See Table 2 for the intensity values.

Our results on gene expression together with those obtained on the biochemical activity of amylase and trypsin in the same experimental design (German et al., 2004) suggest that both enzymes are transcriptionally regulated, trypsin more phenotypically plastic in the carnivorous pricklebacks and amylase more phylogenetically constrained in the herbivores.

Acknowledgements

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Table 2. Among-species comparisons of the intensities of amylase and trypsin gene expression from the phylogenetic perspective within each of the three feeding categories (w_{30-40} , W_{60-75} , and L_{60-75}) of the four species of prickleback fishes as determined by quantitative *in situ* hybridization.

Species	Amylase - phylogenetic			Trypsin - phylogenetic		
	w_{30-40}	W_{60-75}	L_{60-75}	w_{30-40}	W_{60-75}	L_{60-75}
<i>C. violaceus</i>	104.3 ± 6.5 c	134.0 ± 1.2 b	136.7 ± 8.4 b	189.3 ± 2.9 b	212.7 ± 2.0 b	211.3 ± 4.8 b
<i>X. mucosus</i>	98.3 ± 1.2 bc	138.0 ± 1.5 b	130.3 ± 2.3 b	144.7 ± 6.7 a	173.0 ± 4.7 a	185.0 ± 1.5 a
<i>X. atropurpureus</i>	85.7 ± 3.3 b	132.7 ± 6.2 b	146.0 ± 4.0 b	136.7 ± 7.9 a	171.3 ± 5.4 a	192.0 ± 2.5 a
<i>A. purpurescens</i>	64.0 ± 2.6 a	65.7 ± 3.4 a	71.0 ± 7.4 a	139.7 ± 4.3 a	164.7 ± 4.2 a	182.3 ± 2.0 a
ANOVA	$F_{3,8} = 20.65$ $P < 0.01$	$F_{3,8} = 90.42$ $P < 0.01$	$F_{3,8} = 31.38$ $P < 0.01$	$F_{3,8} = 18.14$ $P < 0.01$	$F_{3,8} = 26.16$ $P < 0.01$	$F_{3,8} = 19.11$ $P < 0.01$

Note: Values are mean (\pm 1SE, n = 3) intensities of all pixels of expression. The intensities were evaluated using a reverse gray scale from 0 = white to 255 = black. Values within each category were compared with one-way ANOVA and Tukey's pairwise comparison test with a family error rate of $P = 0.05$. Values that share the same letter (in each column) are not significantly different.

**EFFECTS OF ACCLIMATION PERIOD TO DIFFERENT SALINITIES
ON THE BIOENERGETIC BUDGET OF JUVENILES
OF *CENTROPOMUS PARALLELUS* (POEY)**

Arthur José da Silva Rocha

“ Instituto Oceanográfico, Universidade de São Paulo, Praça do Oceanográfico,
191 Cidade Universitária São Paulo SP, Brazil CEP: 05508-900”

Phone.: +55- 11-3091-6561; fax.: +55-11-3091-6607

E-mail address: arrocha@usp.br

Vicente Gomes, Phan Van Ngan, Maria José de Arruda Campos Rocha Passos,
Rosaria Rios Furia

“ Instituto Oceanográfico, Universidade de São Paulo”

Abstract

The bioenergetic budget regarding the time of acclimation to different salinities was established for juveniles of *Centropomus parallelus*. The energy of food consumed (C), faecal losses (F), routine metabolic rate (Rr) and energy incorporated as mass production (P), were determined for groups of fishes acclimated for 15 and 30 days to 5‰, 20‰ and 30‰ salinities. The energy of food consumed was significantly lower at 30‰ for both acclimation periods, in comparison to results of the correspondent periods at other salinities. Energy lost as faeces decreased with salinity in both periods. After 15 days, the routine metabolic energy was lowest at 20‰. On the other hand, after 30 days the highest metabolic demand occurred at this same salinity. Energy incorporation was significantly lower at 30‰ in both periods, in comparison to results of the correspondent periods at other salinities. For 30 days of acclimation, the highest energy incorporation was at 5‰. Similar results were obtained for 15-day period at the same salinity. At 20‰, incorporation decreased significantly in function of periods. Results indicated that 5‰ was the better and stable condition for juvenile of *C. parallelus* to efficiently drive the energy of food to growth.

Keywords: energetic budget; *Centropomus parallelus*; fat-snoek; salinity, acclimation period.

Introduction

The fat-snook, *Centropomus parallelus*, is a fish species widely distributed in coastal zones from south Florida to Southeast Brazil (Rivas, 1986), living in estuaries and lagoons, where they feed on small invertebrates and fishes (Figueiredo and Menezes, 1980). Fat-snook have an elevated commercial value, and their high growth rate makes them a suitable species to aquaculture, since fishes can reach up to 500-700 mm of body length (Tucker, 1987; Neidig, *et al.*, 2000). Snook usually spawns near shore and, after hatching, larvae drifts with the currents to the protected estuarine areas (Gilmore *et al.*, 1983; McMichael and Parsons, 1989). Depending on the life stage, fat-snook explores different estuarine habitats, whose salinity ranges from 0 to 30‰, what certainly implies in metabolic constraints (Peterson and Gilmore Jr., 1991; Aliaume *et al.*, 1997; Peters *et al.*, 1998). Many estuarine fishes can cope with the strong salinity gradient, provided that part of metabolic energy is expended with osmoregulatory processes (Moser and Miller, 1994).

Bioenergetic studies are suitable for the assessment of environmental factors that affect most of the biological functions. Energetic budgets are broadly employed to the management of living marine resources, environmental protection and aquaculture (Karas and Thoreson, 1992; Lesser *et al.*, 1996; MacIsaac, *et al.*, 1997; Lemos *et al.* 2001). Up to 40% of the energy demands of fishes are spent with metabolism (Du Preez *et al.*, 1990).

Despite the euryhalinity of fat-snook, data concerning bioenergetic aspects of salinity acclimation are still lacking. The objective of this study was to investigate the energetic budget of juveniles of *C. parallelus*, at different salinities, according to the model presented by Beamish *et al.* (1975), $C = F + U + R + P$, where $R = R_a + R_d + R_r$ (R_a : active metabolism; R_d : Specific Dynamic Action metabolism; R_r : routine metabolism). This indicates that the energy of food consumed by fish (C) is lost as faeces (F), excretion of nitrogenous compound (U), metabolic heat (R), and the remainder retained for growth and reproduction (P).

Materials and Methods

Juveniles of *C. parallelus* were collected in Cananéia estuary, South Coast of São Paulo State, Brazil (25°05'S - 47°55'W). The animals were brought to the laboratory of "Instituto Oceanográfico-USP", where they were placed in 500L outdoor tanks, and acclimated to the salinities of 5‰, 20‰ and 30‰ for one

month. After that, 30 individuals per salinity were weighted (± 2 g initial weight) and placed in 50L plastic boxes for the acclimation periods of 15 and 30 days, at controlled temperature of $22^{\circ}\text{C} \pm 1$. Water was renewed daily to prevent the toxicity of metabolites.

Food was supplied once a day and consisted of living juveniles of Palemonidae shrimp *Macrobrachium sp.* Shrimps were offered up to 15% of initial mean weight of fishes, and the remainder, not eaten shrimp, was removed and weighted. Total daily ingestion rate was calculated by differences between weights of shrimp offered and removed. Faeces were also collected and weighted daily. The growth rate of *C. parallelus* was assessed by differences of the initial and final weight averages of fishes, obtained for both acclimation periods. The energy amount of food consumption (C), faecal losses (F) and incorporated as mass production (P) was measured by the wet combustion method (Karzinkin and Tarkovskaya, 1964) for small samples of shrimp, faeces and fish, which were previously dried at 50°C and weighted. The amount of metabolic energy was estimated by applying the energetic equivalent 4.64 cal/mlO_2 (Brett, 1985) on the routine oxygen consumption rate (Rr) of 48 hours starved juveniles of *C. parallelus*, measured in sealed respirometric chamber (Rocha *et al.*, in prep.). The remainder energy of the model was considered as a group of the non-measured parameters U, Ra and Rd. The energy of food consumption, faecal losses, metabolic heat and mass production was calculated as calories per day per gram of wet weight of fish (cal/day/g-fish).

Data of each parameter was tested by two-way ANOVA after the confirmation of normality and homogeneity of variance by the *t*-test. Differences among the salinities were compared by the Newman-Keuls test (Zar, 1996).

Results

The averages of food ingested, faeces produced and weight increment of juveniles of *C. parallelus* in different salinities for both acclimation periods are shown on Table 1. The oxygen consumption rate employed to estimate Rr of *C. parallelus*, is shown on Table 2.

Table 1. Mean values (mg dry-weight/day/g-fish) of food consumption, faeces produced and weight increment of juveniles of *C. parallelus*, at different salinities and acclimation periods.

Salinity	Period (days)	Food consumption	Faeces produced	Weight increment
5‰	15	20.29 ±4.22	4.17 ±0.74	2.42 ±1.81
	30	18.87 ±3.09	4.34 ±0.85	2.01 ±0.39
20‰	15	22.86 ±5.22	3.36 ±0.91	3.80 ±2.19
	30	19.52 ±3.84	3.44 ±0.60	1.02 ±0.12
30‰	15	13.80 ±3.40	2.42 ±0.74	1.21 ±0.65
	30	12.10 ±3.30	2.80 ±0.80	0.43 ±0.20

Table 2. Oxygen consumption rate of juveniles of *C. parallelus*, at different salinities and acclimation period.

Salinity	Period (days)	Oxygen consumption mgO ₂ /g/h
5‰	15	0.308 ±0.06
	30	0.261 ±0.02
20‰	15	0.222 ±0.02
	30	0.316 ±0.05
30‰	15	0.282 ±0.05
	30	0.245 ±0.05

Differences on daily energy consumption (C) of fishes were significant between 5‰ and 30‰, and between 20‰ and 30‰, for both acclimation periods (Fig. 1A). No differences occurred between the acclimation periods. The amount of energy lost as faeces (F) decreased with salinity (Fig. 1B), being significantly lower at 30‰ in both acclimation periods, with no differences between them. The metabolic energy demand (R_r) was significant different between 5‰ and 20‰, and between 20‰ and 30‰, for both periods (Fig. 1C). Significant difference on the metabolic energy demand between the acclimation periods was found at 20‰. After 15 days, the lowest metabolic demand was at 20‰. On the other hand, after 30 days an opposite trend occurred, with the highest metabolic demand at the same salinity.

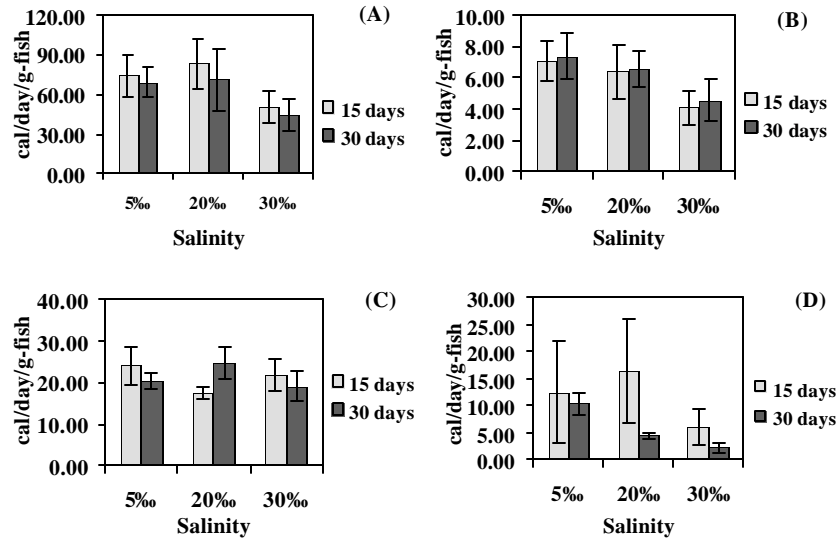


Figure 1. (A) Energy of food consumption C; (B) lost as faeces F; (C) routine metabolic demand Rr and (D) mass incorporation P of juveniles of *C. parallelus* as function of acclimation period and salinity.

Differences of energy incorporated at different salinities (P) were also significant, being the lower values at 30‰, on both acclimation periods (Fig. 1D). The energy incorporated at 20‰ was significantly different between the acclimation periods. After 30 days, P was significantly higher at 5‰, in comparison to the same period at other salinities. The bioenergetic budget established for juveniles of *C. parallelus* is shown on Table 3.

Table 3. Bioenergetic budget of juveniles of *C. parallelus*, as the percentage (%) of energy consumed (%cal/day/g-fish) lost as faeces (F), routine metabolism (Rr), mass incorporated (P) and the remainder estimated as active metabolism Ra, Specific Dynamic Action (Rd) and excretion of nitrogen compound (U).

Salinity	Period (days)	F	Rr	P	Ra; Rd; U
5‰	15	9.55	32.46	16.90	41.09
	30	10.69	29.55	15.05	44.71
20‰	15	7.66	20.74	19.76	51.84
	30	9.19	34.58	6.20	50.04
30‰	15	8.15	43.56	12.26	36.03
	30	10.31	43.16	4.93	41.59

Discussion

Many juvenile fish species choose intermediary water salinities of estuaries and coastal lagoons where they find advantageous conditions for their development. Accordingly, substantial energy saved in a favourable isosmotic environment may be driven to growth (Morgan and Iwama, 1991; Soengas *et al.*, 1995; Altinok and Grizzle, 2001). This seems to have occurred to *C. parallelus*, as the salinity exerted significant and interactive effects on the energy utilization of fishes. At 20‰, the relative high energy incorporated by juvenile fat-snook at the 15 days acclimation period coincided with the low metabolic energy expended. Results obtained by Cardona (2000) showed a better growth performance of juveniles of flathead grey mullet *Mugil cephalus* associated to the low metabolic rate of fishes acclimated in 5‰ oligohaline water. Physiological responses occur on the osmoregulatory adaptation through the involvement of an active process of Na⁺ – K⁺ ATPase enzyme, whose energy expenditure is not well understood, but does not seem to be negligible (Jobling, 1993). An opposite trend occurred for 30 days acclimation period at 20‰ salinity. The elevated metabolic demand may be responsible for the low energy incorporated as mass production that was similar to the correspondent values at 30‰, in both periods. Sampaio and Bianchini (2002) reported a lower growth rate of flounder *Paralichthys orbignianus*, related to the energetic costs of ionosmotic imbalance of fishes long-term exposed to freshwater. At 30‰, the additive effects of the lowest food energy intake and the elevated metabolic demand resulted in the lowest growth of juveniles of *C. parallelus* for both acclimation periods. The costs of ionic regulation may compromise the energy available for growth unless the fish can compensate by increasing its feeding rate (Wootton, 1990). It has been hypothesised that, if the external environment were manipulated to ensure a reduction of metabolic costs of iono-osmoregulation to a minimum, the food utilisation and growth of fish would be

improved (Jobling, 1994). In this sense, the relatively high metabolic rate of fishes submitted to 5‰ salinity seems to be compensated by food intake (Tab. 1), resulting in suitable energy incorporation in both acclimation periods.

Du Preez *et al.* (1990) proposed a model where: $C(100\%) = F(10\% \pm 6) + U(4\% \pm 1) + Rr(23\% \pm 13) + Rd(21\% \pm 3) + P(42\% \pm 11)$. Present results showed that **F** and **Rr** of *C. parallelus* at different acclimation periods and salinities were in accordance to the model above (Tab. 3). On the other hand, **P** represented 1/3 or less than the 42% proposed by Du Preez for different surf-zone fish species ranging from 400-1500mm body-length. Fishes of the present study were small (50mm body-length) and around 2 or 3 month old, a long time before the first maturation. For these animals, at this life stage, **P** represents only the somatic growth, so that the high energetic compounds such as lipid reserves related to gonad maturation are not present yet. The remainder 36.03% to 51.84% of food energy consumed in the budget can be attributed to the not measured energy utilized for SDA metabolism, active metabolism and nitrogen excretion. For instance, according to Jobling (1994) the energy lost as excretion after feeding may account up to 15% of the food energy consumed.

These results indicate that 5‰ salinity seems to be the better condition for juveniles of *C. parallelus* to be rearing, as fishes, at this life stage and at the experimental conditions, always exhibited a stable and efficient physiological performance. The present study contributes to understand the role of a major environmental factor of estuarine ecosystems that governs the physiology of juvenile *C. parallelus*. In addition, better conditions can be achieved to have good results in commercial aquaculture of fat-snook.

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**THE INFLUENCE
OF ENVIRONMENTAL VARIABLES
ON SQUATINA GUGGENHEIM DISTRIBUTION
IN THE ARGENTINE-URUGUAYAN
COMMON FISHING ZONE (AUCFZ)**

Rodolfo Vögler
Departamento de Oceanografía, Facultad de Ciencias Naturales y
Oceanográficas, Universidad de Concepción
Cabina 9, Casilla 160-C. Concepción, Chile
Tel.: (5641) 20-42-39/ Fax: (5641) 25-65-71/ rvogler@udec.cl

Andrés C. Milessi
Departamento de Oceanografía, Facultad de Ciencias Naturales y
Oceanográficas, Universidad de Concepción

Renato A. Quiñones
Centro de Investigación Oceanográfica en el Pacífico Sur Oriental (COPAS),
Departamento de Oceanografía, Facultad de Ciencias Naturales y
Oceanográficas, Universidad de Concepción

Ciro Oyarzún
Departamento de Oceanografía, Facultad de Ciencias Naturales y
Oceanográficas, Universidad de Concepción

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Introduction

The role of environmental factors in modulating the distribution of fish in the ocean still remains as an open question. Despite several decades of research, there are few demonstrable generalizations with respect to the influence of abiotic variables on fish ecology and physiology.

Temperature and salinity have been mentioned as possible important factors in determining the distribution of elasmobranchs (Snelson and Williams, 1981). However, only recently a strong quantitative approach has been used to evaluate environmental effects on elasmobranchs distribution (e.g. Hopkins and Cech, 2003; Campana and Joyce, 2004).

Angel sharks are demersal elasmobranchs belong to the genus *Squatina* (Duméril). *Squatina guggenheim* Marini is a coastal species that is widespread distributed from Espírito Santo (Brazil, 18° S) to northern Patagonia (Argentina, 43° S) (Vooren and Silva, 1991). The influence of environmental factors in determining the distribution of this angel shark in the southwest Atlantic is unknown.

Here, we determine the influence of depth, bottom temperature, and bottom salinity on the distribution of *S. guggenheim* population in the Argentine-Uruguayan Common Fishing Zone (AUCFZ).

Materials and Methods

Research area

The research area was located within the AUCFZ (34°00'-39°30' S; 51°10'-59°10' W) covering the continental shelf and part of the continental slope.

Sample collection

Individuals were collected from bottom trawl surveys conducted during five research cruises in spring and fall of 1995, 1997 and 1998 onboard the R/V Aldebaran. The cruise C9512 covered only the coastal area (3.5-60.0 m) while the four remaining cruises covered most of the AUCFZ (21.0-312.0 m). The sampling stations were selected based on a random stratified sampling design.

Following each trawl, all angel sharks were identified, counted, and weighed (± 0.1 kg). The hydrographic information recorded in each sampling station was surface and bottom temperature (°C), surface and bottom salinity, and depth (m). Only bottom temperature, bottom salinity, and depth were the abiotic variables considered in the analyses since angel sharks present demersal habits.

Identification of habitat associations

Comparison between the distribution of angel shark population and concurrent environmental factors were made using the catch-weighted cumulative distribution function approach developed by Perry and Smith (1994). CPUE corresponded to the weight of the captured angel sharks in 30 minutes of towing ($CPUE_{\text{angshark}} = \text{kg} / 30 \text{ min}; N=1536$).

The univariate analysis between each of the environmental variables considered and the $CPUE_{\text{angshark}}$ was established for each cruise separately. The angel shark's ranges of environmental preferences and rejection were identified.

Results

Bottom temperature

The distribution of the angel shark inside the AUCFZ presented a significant relationship with bottom temperature both in spring and autumn (Table 1). However, the temperature ranges where the highest angel shark densities were found varied widely among cruises (Table 2). The differences in the preference temperature ranges observed are not only due to seasonality but also to the geographical locations of the cruises, especially in terms of the distance from the shore (e.g. spring cruises C9512 vs C9704; Table 2). The species rejected waters with temperatures lower than 7.0 °C, demonstrating a preference for bottom temperatures greater than 8.0 °C in the majority of the cruises. A maximum thermal limit of rejection was not found in any cruise (Table 2).

Table 1. Probability values of the random univariate relationship tests between abiotic variables (bottom temperature, bottom salinity, depth) and the CPUE_{angshark} (kg / 30 min) for *Squatina guggenheim* population, separated by cruise. *P≤0.05, **P≤0.01.

<i>Angel shark population</i>				
Cruise	Season	Temperature	Salinity	Depth
C9512	Spring	0.02*	0.001**	0.07
C9702	Autumn	0.003**	0.65	0.34
C9704	Spring	0.004**	0.01**	0.55
C9801-02	Autumn	0.05*	0.05*	0.06
C9803	Autumn	0.05*	////	0.65

//// Salinity data is unavailable for the cruise C9803.

Bottom salinity

The association between the distribution of angel shark population and bottom salinity was statistically significant ($P \leq 0.05$) with the exception of the autumn cruise C9702 (Table 1). The preferred salinity range and the rejection values were similar among offshore cruises of different seasons and years. However, these ranges were different than those found during the coastal cruise (C9512) (Table 2).

Depth

The distribution of *S. guggenheim* population was distributed independently of depth in the study area both in spring and autumn (Table 1).

Table 2. Thermal (°C) and saline ranges of preference and rejection for *Squatina guggenheim* population, separated by cruise. Values obtained from the significant relationship tests ($P \leq 0.05$) between CPUE_{angshark} (kg / 30 min) and each environmental variable.

<i>Angel shark population</i>					
Cruise	Season	Principal distribution range		Rejection values	
		Temperature	Salinity	Temperature	Salinity
C9512	Spring	17.5-20.5	29.5-33.2	<12.0	<25.0
C9704	Spring	8.0-9.5	33.5-33.6	<7.0	<33.4
C9702	Autumn	14.0-21.0		<12.0	
C9801-02	Autumn	16.0-18.0	33.5-33.6	<7.0	<33.4
C9803	Autumn	12.5-14.5		<7.0	

Discussion

The environmental variables affecting the distribution of *S. guggenheim* population as a whole in the AUCFZ were bottom temperature and bottom salinity.

The only previous information regarding the relationship between the distribution of *S. guggenheim* and environmental variables is the contribution of Vooren and Silva (1991). They suggested that the distribution of angel sharks is affected by depth and temperature. Our results, in contrast, show that salinity is an important factor in determining the distribution of the angel shark. In addition, we also demonstrate that depth is not an important factor when the population of *S. guggenheim* is considered as a whole (*i.e.* disregarding body size and sex). The disagreement between our results and those of Vooren and Silva (1991) rely in the fact that they did not use a specifically designed methodology to detect quantitatively preference and rejection ranges for environmental factors such as the Perry and Smith (1994)'s method used here.

Finally, the ranges of preference and rejection of the angel shark to bottom temperature and salinity varied among the spring and autumn cruises. This is likely due to different water masses and coast-ocean gradients take place

seasonally in the AUCFZ generating different environmental conditions in terms of temperature and salinity in each of the cruises.

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EFFECTS OF PRESSURE ON EEL PHYSIOLOGY:

HOW ARE EELS PRE-ADAPTED

TO MIGRATE AT DEPTH?

Sebert Philippe

Unité Haute Pression et Métabolisme
Faculté de Médecine, 22, Avenue C. Desmoulins
CS 93837, 29238 Brest Cedex – France
Phone : 33 2 98016462
Fax : 33 2 98016313
E-mail : philippe.sebert@univ-brest.fr

Vettier Aurélie

Unité Haute Pression et Métabolisme
Faculté de Médecine 22, Avenue C. Desmoulins
CS 93837 29238 Brest Cedex – France

Eel is a particular shallow-water fish in that it undergoes two metamorphoses and a very long migration. Briefly (because eel biology is extensively described in Tesch, 2003) eels live in the river at the yellow stage: it can be more than 1 meter long and weigh several kilograms. Life at this stage ranges from 7-15 years to several decades. Normally, if the environmental conditions are optimum, the yellow eel undergoes a first metamorphosis which corresponds to anatomical (increase in eye size, changes in body color and head shape etc.) and physiological changes which mainly concern muscle biochemistry (changes in enzyme activities involved in energy production; Lewander et al., 1974; Egginton, 1986). After this metamorphosis, the eel becomes a silver eel and begins its migration towards the Sargasso sea which is the presumed breeding area. The migration has been shown to represent about 6000 km of swimming activity at a speed of about half body length per second. Energy cost of swimming activity has been estimated by Van Ginneken and Van den Thillart (2000) who have shown that fat stores were sufficient to ensure energy expenditure, bearing in mind that migration is performed without feeding. However, this is not the only surprising feature of migration. In fact, as it is performed from river to ocean, it corresponds to a transfer from freshwater to

seawater with changes in water temperature and absence of light and more especially, migration is performed at depth. The exact depth is not known but is probably between 200 and 1000 meters (perhaps even more). Thus, when the eel reaches the Sargasso sea, it reproduces (we do not know the depth: it could range from 200 to perhaps 2000m or more) then the larvae return towards the rivers. When they reach the continental plateau, they undergo a second metamorphosis from larvae to small eels (glass eels) which go up the rivers thus closing the loop. The reader who is not familiar with high pressure biology must understand that even if the depth is “only” 200 m, this corresponds to 20 times the atmospheric pressure which is a huge change in regards to what is observed in temperature and/or salinity. This remark justifies the interest concerning the effects of pressure on eel biology. To be more precise, as migration corresponds to a high energy demand from the muscle, we will focus our paper on the energy metabolism of eel under pressure.

The title raises the question “how are eels pre-adapted to migrate at depth?”, a question which must be extended to the following: “Is the metamorphosis from the yellow stage to the silver stage necessary to ensure pressure adaptation?”. In 1993, Sébert et al., showed that short term pressure exposure induces a state resembling histotoxic hypoxia in fish i.e. an alteration in aerobic energy metabolism. This was based on the observation of decreases in ATP production and cytochrome oxydase activity (the last complex of the respiratory chain), together with an increase in lactate production. However, these pressure effects appeared much more deleterious in fish which do not experience pressure during their life cycle (like the trout) as in the eel does even at the yellow stage. These experiments highlighted an unusual pressure resistance in the eel and supposed pre-adaptation. In order to test this possibility, further experiments were carried out, exposing yellow eels to high pressure (101 ATA- 1000m depth) for a long time. After one month under pressure (see Sébert, 2003 for review), ATP production, enzyme activities involved in the Krebs’ cycle and/or in the respiratory chain were restored to normal levels. These facts are sufficient to evoke pressure acclimatisation but they are accompanied by other changes. For example, modifications in muscle fibre sizes are observed. However, the changes observed at the gill level are even more interesting. Firstly evidence proving homeoviscous adaptation described in deep sea fishes: the yellow eel under pressure restores to normal level membrane fluidity which had previously been altered by compression. Secondly long term pressure exposure induces the proliferation of chloride cells (involved in Cl⁻ extrusion) despite the fact that the eel is studied in freshwater. It is important to note that the increase in chloride cell numbers also happens in normal conditions during the metamorphosis from

the yellow to the silver stage (Thomson and Sargent, 1977). In other words, it appears that in the non-migratory yellow eel, pressure triggers numerous events in order to cope with the environmental constraints linked to migration: salinity change and high energy demand. Recent results from our team have shown that pressure acclimatisation in the yellow eel is accompanied by an increase in ADP/O ratio (figure 1) i.e. an improvement in oxidative phosphorylation efficiency (Theron et al, 2000). It has been hypothesised that the yellow eel

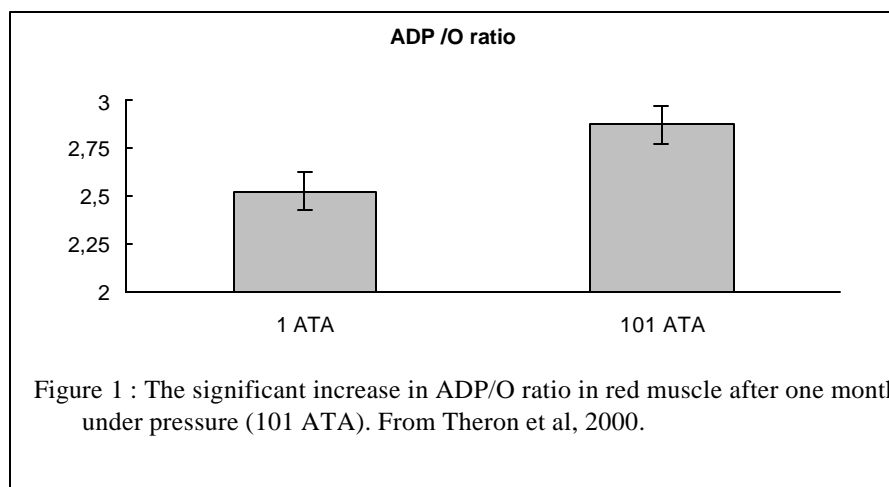
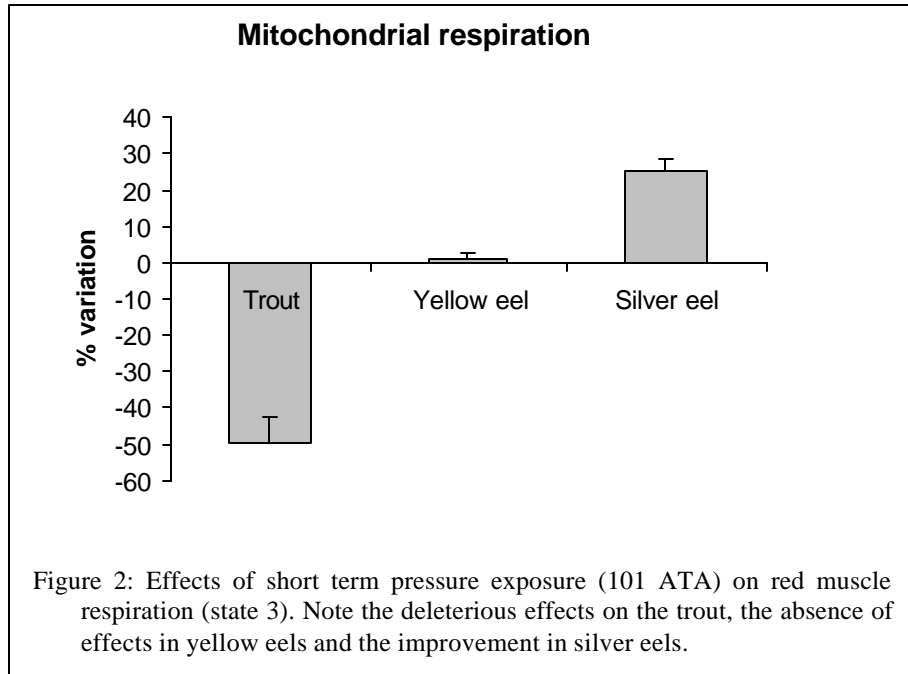


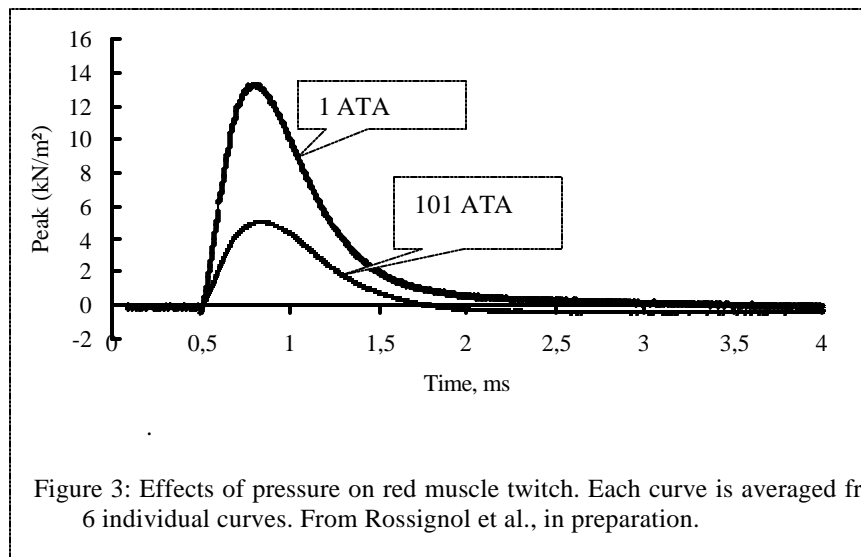
Figure 1 : The significant increase in ADP/O ratio in red muscle after one month under pressure (101 ATA). From Theron et al, 2000.

has a supranormal mitochondria functioning (low efficiency) at atmospheric pressure in order to cope with the high pressure environment encountered during its migration. Maintaining such a supra functioning state does probably mean high energy cost but it takes place during a period of food intake. In contrast, migration is performed under pressure without feeding and requires a great amount of energy. We have thus put forward the idea that despite the induced energy loss, the eel has “chosen” to maintain a supra normal functioning state at atmospheric pressure in order to have normal functioning at pressure instead of triggering a hypothetical and long regulatory process when facing pressure at the start of migration (Sébert and Theron, 2001).



If the yellow eel is able to acclimatise to high pressure, what are the advantages of metamorphosis i.e. of the silvering process in terms of energy metabolism? The biochemical changes observed in muscle after metamorphosis from the yellow to the silver stage (myoglobin content, carbohydrate and lipid metabolisms, enzyme activities; see Boström and Johansson, 1972; Lewander et al, 1974; Egginton, 1986 for example) leave no doubt concerning the fact that the silvering process prepares the eel for spawning migration. All these changes play a role in improving muscle performance prior to migration (Ellerby et al, 2001). When pressure resistance is studied in terms of mitochondrial respiration, it appears clearly that pressure improves mitochondria functioning depending on the eel's origin (Vettier and Sébert, 2004). Figure 2 shows how the silvering process is involved not only in pressure resistance but also in improving the respiratory process. The question remains whether the improvement in energy metabolism with pressure is accompanied by an increase in muscle performance (for swimming activity) or compensates its possible alteration. We have seen that the silvering process improves red muscle performance. However, recent

data from our team seem to show muscle contraction alteration under pressure (Rossignol et al, unpublished results; figure 3). This alteration can be balanced by the observed improvement in energy metabolism. We cannot exclude the hypothesis that muscle performance, during the silvering process, increases at atmospheric pressure in order to become “normal” under pressure i.e. it could well be the same mechanism that for energy production (see above).



As a conclusion (Figure 4), we consider that compared to teleosts which do not experience high pressure, the eel probably has an over capacity for energy production at the yellow stage at atmospheric pressure. The silvering process affords, among other processes, an improvement in pressure resistance and muscle functioning efficiency (energetics and mechanics) in order to cope with the pressure effects. However, as pressure improves most of the functions, a final question must be raised: is the eel a shallow water fish which can acclimatise to the pressure effects or is the eel a deep water fish which adapts to atmospheric pressure?

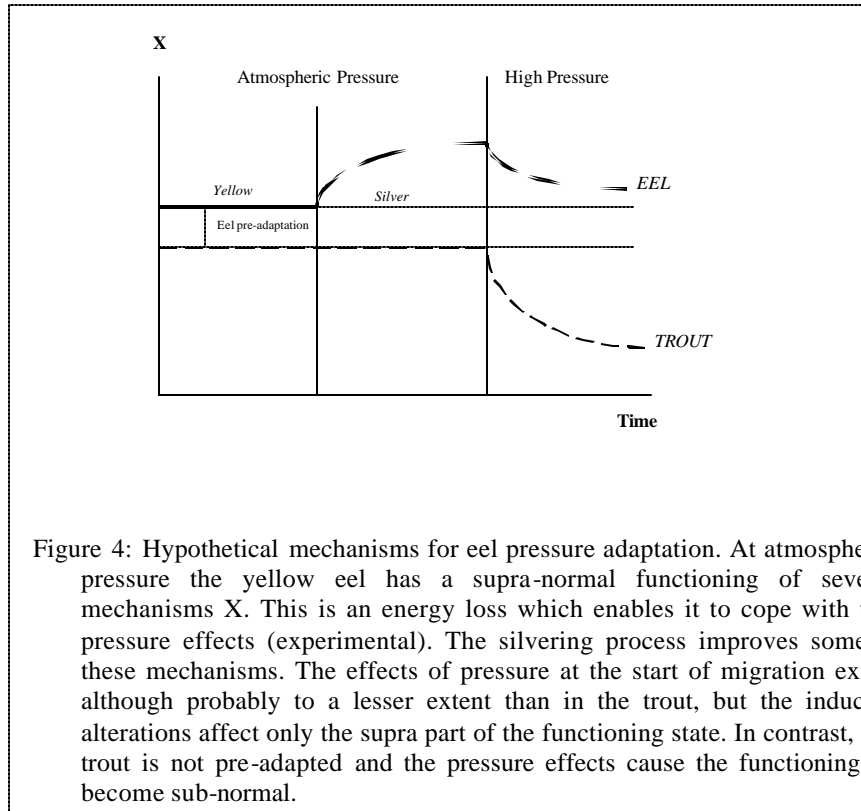


Figure 4: Hypothetical mechanisms for eel pressure adaptation. At atmospheric pressure the yellow eel has a supra-normal functioning of several mechanisms X. This is an energy loss which enables it to cope with the pressure effects (experimental). The silvering process improves some of these mechanisms. The effects of pressure at the start of migration exist, although probably to a lesser extent than in the trout, but the induced alterations affect only the supra part of the functioning state. In contrast, the trout is not pre-adapted and the pressure effects cause the functioning to become sub-normal.

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**TRIMETHYLAMINE OXIDE AS AN ORGANIC OSMOLYTE
IN DEEP-SEA FISHES:
CORRELATIONS WITH DEPTH AND
STABILIZATION OF PROTEINS UNDER PRESSURE**

Paul H. Yancey, Athena Samerotte, Garth Brand
Biology Department, Whitman College
Walla Walla, WA 99362 USA
Phone: 001-509-522-1637/Fax 001-509-527-5904
Email: yancey@whitman.edu

K.M. Kemp and D.M. Bailey
Department of Zoology, University of Aberdeen, Scotland

EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Most marine organisms are osmoconformers, having internal osmotic pressures equal to seawater at about 1000 mOsm. However, their cells do not have high salt concentrations; instead their cells accumulate intracellular organic osmolytes to about 600 mOsm (with 400 mOsm from basic inorganic and organic solutes). Marine invertebrates and algae use primarily neutral amino acids (e.g., glycine, taurine) and polyols (e.g., inositol). Invertebrates may also have methylamines, especially glycine betaine (hereafter called betaine) and trimethylamine N-oxide (TMAO). Vertebrate osmoconformers include cartilaginous fishes, which use urea and TMAO. Except for urea, organic osmolytes are used because, unlike common inorganic ions, most do not perturb proteins, i.e., they are "compatible" solutes. Moreover, many osmolytes—especially methylamines—enhance the function and structure of proteins and can counteract effects of perturbants such as urea (a protein destabilizer) and NaCl. For example, shallow-water cartilaginous fishes have urea typically at 300-400 mM as an osmolyte, accompanied by TMAO at 150-200 mM in muscle cells (Yancey, 2001). At this ratio (about 2:1), urea's inhibitory effects are often fully offset by TMAO's stabilizing effects.

Most other marine vertebrates are osmoregulators, having internal fluids less than 1000 mOsm. In shallow marine teleosts, TMAO can be a significant cellular solute, but at less than 70 mmol/kg, leaving the animals hypo-osmotic—typically at about 350-400 mOsm. However, our recent studies have revealed an approximately linear increase in TMAO contents of white muscle with depth in several families of teleosts, rising to 288 mmol/kg wet wt in a morid cod from 2900 m (Gillett et al., 1997; Kelly and Yancey, 1999). In this study, we examine a fish from a much greater depth to determine whether this trend continues.

We also continue testing our hypothesis that TMAO (as a general protein stabilizer) counteracts the inhibitory effects of hydrostatic pressure on proteins (Gillett et al., 1997). While some deep-sea proteins have evolved resistance to pressure effects, some exhibit significant pressure sensitivities and thus seem incompletely adapted (Siebenaller and Somero, 1978). We have found that TMAO can counteract these effects. Two examples are the ADP K_m of pyruvate kinase (PK) and the NADH K_m of lactate dehydrogenase (LDH). Both K_m s are pressure-sensitive for deep-sea as well as shallow PK and LDH homologues, but TMAO can offset most or all inhibition. Here, we examine other common osmolytes (betaine, glycine, and myo-inositol) to determine whether TMAO is unique as a stabilizer against pressure.

Materials and Methods

Atlantic grenadiers (*Coryphaenoides armatus*) were collected by net from 4.8 km on the Porcupine Abyssal Plain (North Atlantic) and frozen at -80°C . White muscle tissue was analyzed for TMAO according to Kelly and Yancey (1999). LDH from this species was purified (Yancey et al., 2001) and PK from rabbit muscle and other chemicals were purchased from Sigma Chemicals. Kinetics were analyzed as described by Yancey et al. (2001).

Results and Discussion

Activity of LDH from *C. armatus* was tested at 30 μM NADH. TMAO, but not betaine, glycine or myo-inositol, was able to counteract inhibition at 500 atm (50 MPa) completely (Table 1). The APD K_m of rabbit PK (selected as a model system) was increased by 250 atm from 1.8 to 2.2 mM; this inhibitory effect was completely counteracted by TMAO, partly counteracted by betaine, and not

altered by glycine and myo-inositol (Table 1). These results support the hypothesis that TMAO is unique among common osmolytes as an adaptation for offsetting pressure effects in the deep.

Table 1. Effect of 250 mM solutes on activity of grenadier LDH and ADP K_m of rabbit PK. Data are expressed as percentages of control activity at 1 atm (0.1 MPa) with no osmolyte; * indicates significant difference from control. Note that an increase in K_m indicates inhibition.

No osmolyte	TMAO	Betaine	Glycine	Inositol
Grenadier LDH activity at 500 atm (50 MPa)				
86% \pm 2*	99% \pm 3	88% \pm 3*	86% \pm 4*	84% \pm 3*
Rabbit PK ADP K_m at 250 atm (25 MPa)				
121% \pm 6*	95% \pm 4	105% \pm 3*	120% \pm 5*	115% \pm 4*

Muscle tissue of Atlantic grenadiers (*C. armatus*) from 4.8 km contained TMAO at 261 ± 12 mmol/kg (n=5). This precisely fits a linear depth trend extrapolated from previous data on Pacific grenadiers (various species including *C. armatus*) and cods from shallower depths (Fig. 1). This linear depth correlation lends further support to the hypothesis that TMAO accumulation is related to pressure, the only environmental factor that is also linear with depth.

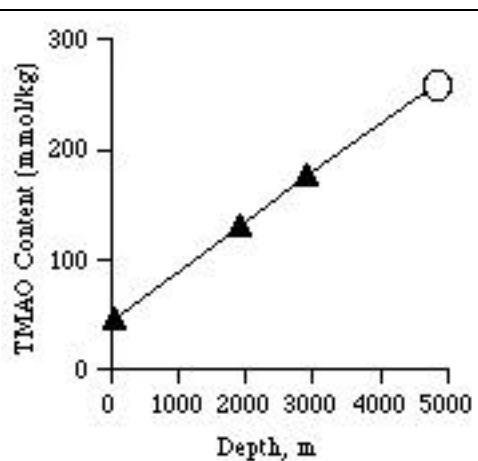


Figure 1. TMAO contents in muscles of cods from shallow water and grenadiers from deep waters. Triangles are from Kelly and Yancey (1999); the circle is new data.

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**EFFECTS OF HYDROSTATIC PRESSURE
ON G PROTEIN SIGNALING
IN DEEP-SEA FISHES**

Joseph F. Siebenaller
Department of Biological Sciences
Louisiana State University
Baton Rouge, Louisiana 70803 USA
Phone: 225-578-1746 Fax: 225-578-2597
e-mail: zjose@lsu.edu

EXTENDED ABSTRACT - DO NOT CITE

The physical environment has long been regarded as a critical factor for the life forms of the deep ocean. The deep sea is characterized by low temperatures and high hydrostatic pressures. Membrane-associated processes may be particularly susceptible to perturbation by the environment of the deep ocean. Among the membrane-associated processes that may be affected are those involved in cell communication. This ubiquitous physiological process involves the reception and transduction of extracellular signals into intracellular metabolic responses. One major class of signal transduction involves guanyl nucleotide binding protein (G protein)-coupled receptors (GPCRs). In mammals, more than a 1000 GPCRs have been identified (Gether, 2000). The effects of low temperature and high hydrostatic pressures on transmembrane signaling by the A₁ adenosine receptor - inhibitory G protein - adenylyl cyclase (AC) complex were examined in brain membranes of 12 teleost fishes from three families (Macrouridae, Moridae and Scorpaenidae). Pressure acts at four loci identified as potential sites of perturbation, namely, (1) agonist activation of the GPCR, (2) the interaction of the receptor with the G protein, (3) the G protein GTPase cycle, and (4) the activation and function of the effector element, AC (Siebenaller and Garrett, 2002). Increased pressure decreases agonist efficacy and in several species alters G protein interactions with receptors, GTP binding to G protein α subunits and the intrinsic GTPase activity of α subunits (Siebenaller, 2003). AC activity and modulation are affected by increased pressure (Siebenaller, 2000). However, at pressures approximating those the species experience *in situ*, AC

activity retains its sensitivity to modulators. Among the species examined there are a range of responses to increased hydrostatic pressure. Only A₁ adenosine receptor modulation of AC in deep-sea morid *Antimora rostrata* displayed complete adaptation. Pressure has no effect on basal AC activity and does not decrease the efficacy of agonists in this deep-sea teleost. The function of this system is not impaired at any of the pressures encountered over the depth range of *A. rostrata* (Siebenaller and Garrett, 2002).

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**INTEGRATED *IN SITU* AND *IN VITRO* STUDIES OF
DEEP-SEA FISH PHYSIOLOGY**

David M. Bailey
Marine Biology Research Division, Scripps Institution of Oceanography,
University of California, San Diego, La Jolla, CA, 92093-0202, USA.
Tel. +1 858 534 4858, Fax. +1 858 534 7313, e-mail dmbailey@ucsd.edu

Bertrand Genard & Jean-François Rees
Laboratoire de Biologie Cellulaire, Institut des Sciences de la Vie, Université
Catholique de Louvain, Croix du Sud 5, B-1348,
Louvain-La-Neuve, Belgium.

Martin A. Collins, Philip M. Bagley, Alan J. Jamieson & Imants G. Priede.
Oceanlab, University of Aberdeen, Newburgh,
Aberdeenshire, AB41 6AA, UK.

EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

The floor of the deep-sea (>200 m) is the largest habitat on Earth, and is home to a diverse assemblage of fish and other mobile megafauna. These animals rapidly consume carrion falling from the waters above, and utilise a wide range of behavioural strategies and sensory mechanisms to scavenge and hunt. Due to the practical and economic difficulties involved in working with deep-sea animals, our understanding of the physiological characteristics of deep-sea fish is poor.

Three main options exist for studying deep-sea fish physiology: 1) to work from measurements or samples of dead animals (e.g. *in vitro* assays), 2) to bring animals to the surface and either work on them under pressure or depressurise them, 3) work *in situ* using submersibles, remotely-operated or autonomous vehicles. In addition certain deductions can be made from modelling the performances (e.g. swimming speeds) necessary to support observed activities. There are clear advantages and disadvantages to each approach, in terms of the cost, numbers of animals that can be studied, and ease of interpretation. For the

purposes of this abstract the use of autonomous vehicles, samples of captured animals and mathematical models will be described.

Materials and Methods

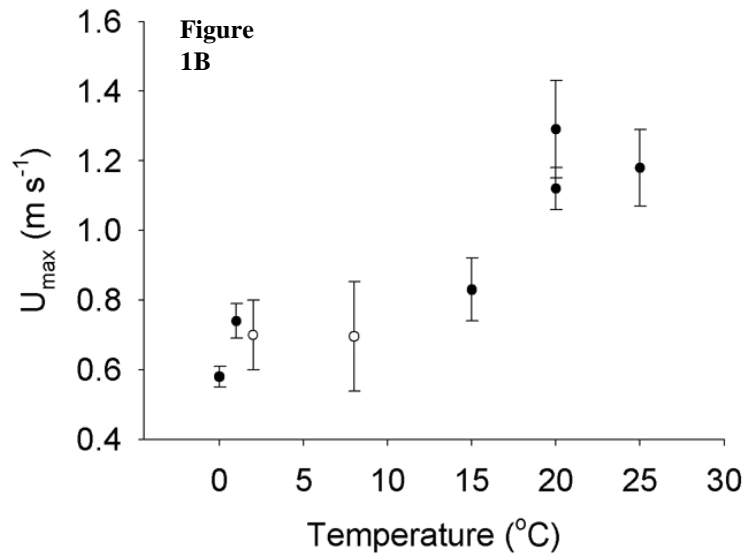
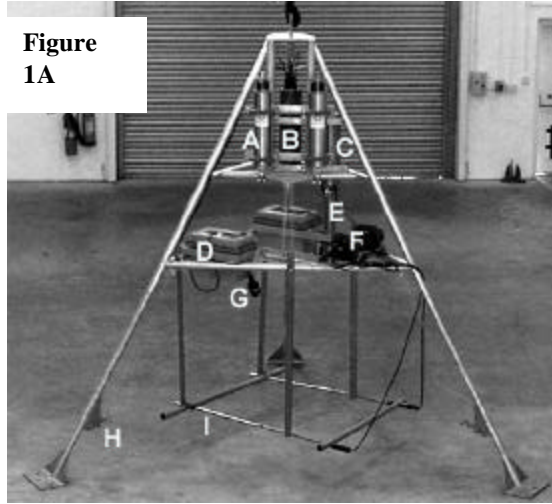
Experiments using autonomous landers were used to video and capture fish for studies of locomotion and metabolic rate. Current and temperature measurements were taken by the landers. Figure 1A shows the “Sprint” lander used to collect video recordings of burst and routine swimming performance. Full details of the landers used are provided by Bailey et al. (2002; 2003). Fish captured at nearby sites were used to determine the enzyme activities and sensory orientations of conspecifics. Mathematical models were also used to estimate the effect of swimming speed and foraging strategy on success in finding carrion (Bailey and Priede 2002).

Results

Analysis of video recordings of the deep-water fish *Antimora rostrata* (2500 m) showed that its burst swimming performances were low, but not significantly lower than expected for a large, cold-water fish (Bailey et al. 2003). More recent studies have provided similar data on the eel *Synaphobranchus kaupi*. Peak burst swimming speeds for these species are presented in figure 1B. Experiment using a respirometer lander revealed metabolic rates in the grenadier *Coryphaenoides armatus* (4000 m) one tenth of those expected of a similar sized cod at the same temperature (Bailey et al. 2002), while the eel *Synaphobranchus kaupi* (1500 m) exhibited oxygen consumptions similar to, or higher than, shallow-water eels (Figure 2).

Figure legends. *Figure 1A* – Sprint lander system. Parts indicated by letters are A - acoustic release, B - video camera, C – controller, D – battery, E – current meter, F – shock unit, G – lamp, H – ballast clamp and I – electrode. *Figure 1B.* Peak burst swimming velocities for deep-sea fish (hollow points), *A. rostrata* at 2°C and *S. kaupi* at 8°C compared to published values for shallow-water fish (Wakeling and Johnston 1998). *Figure 2.* Metabolic rates of *C. armatus* and *S. kaupi* (hollow points) obtained by the respirometer lander. Compared to published values for shallow water species (Johnston et al. 1991).

Figure 1A



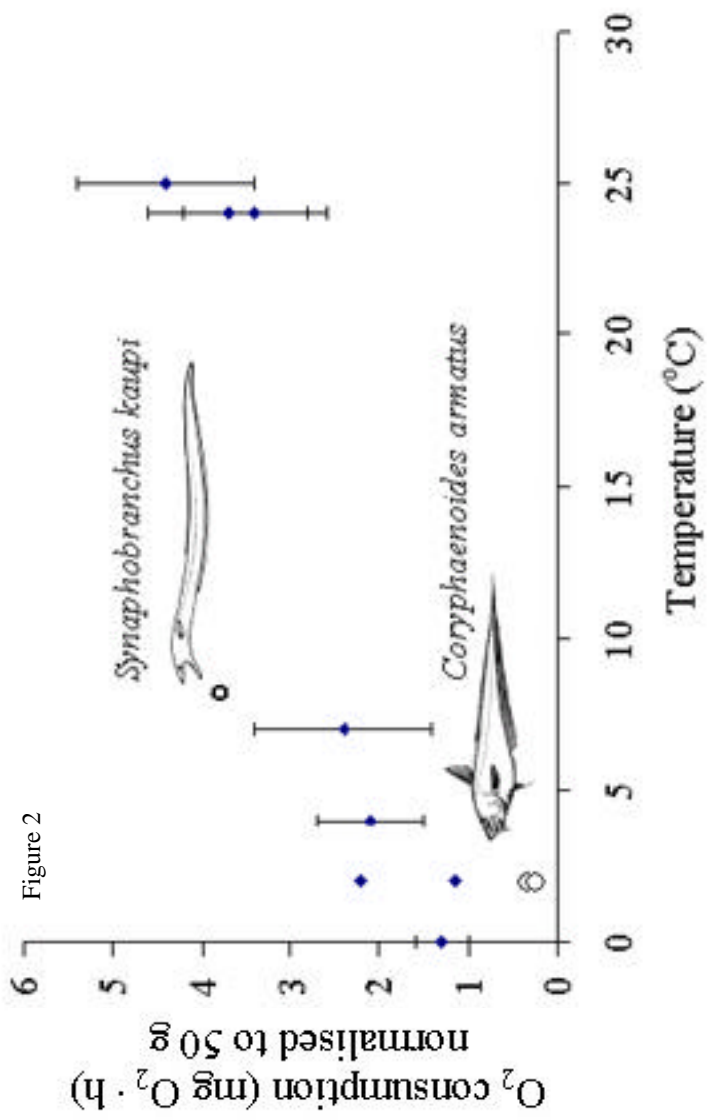


Figure 2

Discussion

Autonomous vehicles can successfully undertake simple experimental tasks such as fast-start and respirometry experiments that would be extremely expensive to achieve using submersibles or ROVs. Results collected to date indicate high variability in metabolic rate between species, and that burst swimming speeds are similar to those of shallow-water species.

The ability of landers to take measurements of water temperature and current velocity at the experimental station is extremely useful in understanding the environment of the fish at the moment at which it is observed or captured. Models using data on routine swimming velocity and currents collected by the landers have provided useful information on the importance of fish swimming performance and foraging strategy on foraging success. The observed differences in routine swimming speeds between species is correlated with the light level, current regime, and energy supply to different deep-sea environments.

Although landers use ship-time extremely efficiently (the ship need not be present during the experiments and can do other work) these methods can only examine a small number of animals and/or experimental conditions. Using trawl-caught conspecifics for stomach content analyses, anatomical measurements and *in vitro assays* provides essential information on the ecological and physiological mechanisms underpinning the observed performances and behaviours. These studies have shown scaling relationships, inter-specific and seasonal changes in muscle metabolism. Inter-specific differences and ontogenetic changes in sensory anatomy have also been established. An example of these interactions is the finding that changes in habitat use and dietary shifts in predatory fish can be linked to changes in muscle energy supply.

Integrated approaches using *in situ* observations and respirometry in fish, environmental measurement, behavioural models, *in vitro* assays and anatomical measurements are essential to understanding the biology of deep-sea fish.

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