Environmental influences on the development of lordosis and musculo-skeletal tissues in Sea bass (*Dicentrarchus labrax*): the ORCIS project

The ORCIS Partnership

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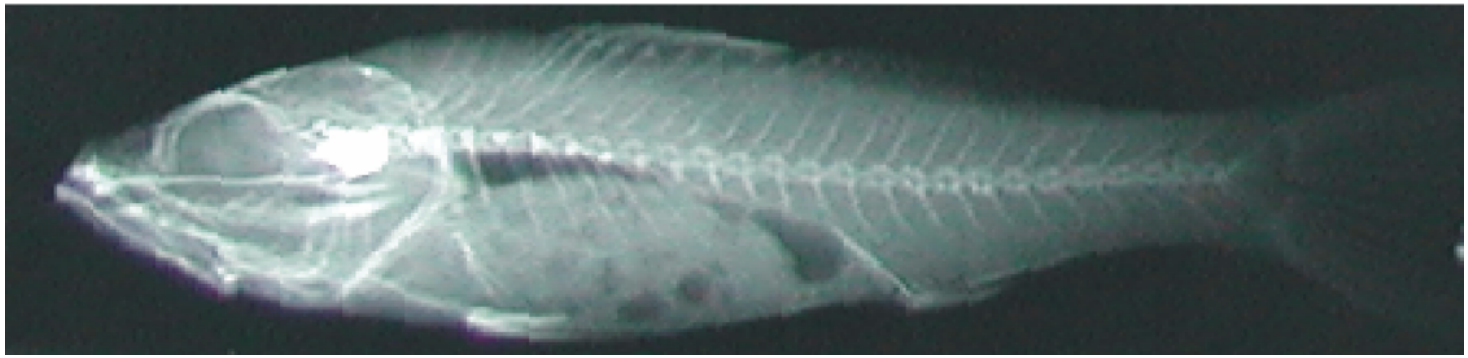
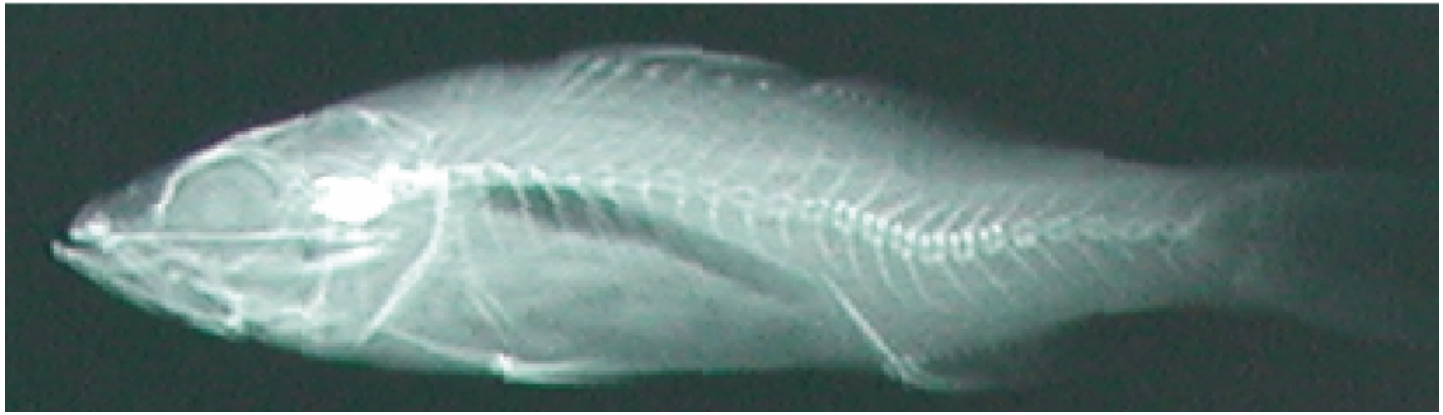
Lordosis is a significant problem in aquaculture of several species, including sea bass.



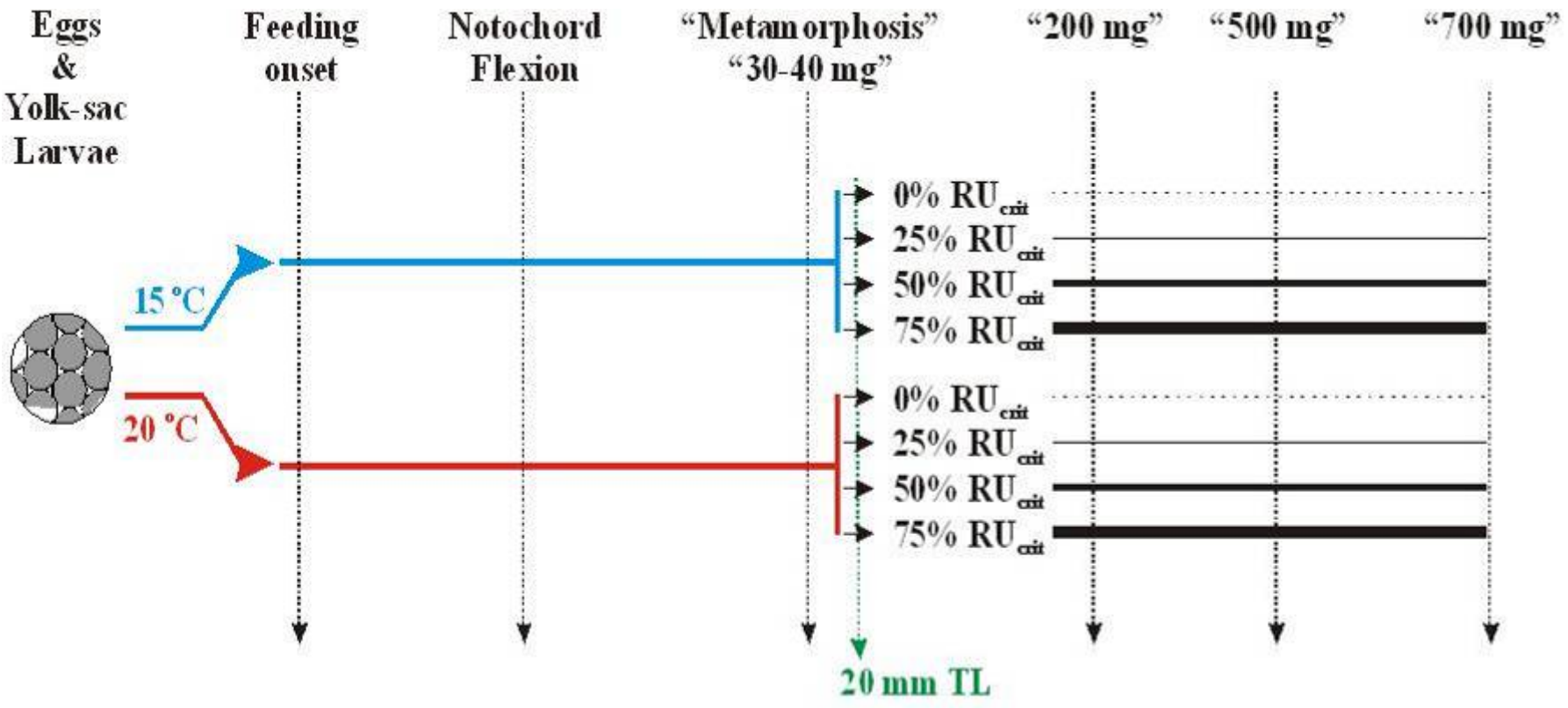


ORCIS Objectives

1. To assess the influence of rearing temperature, current velocities and their interactions on the incidence of lordosis

2. To assess, for the same factors and groups of fish, the influence on musculoskeletal growth and development (at tissue, cell, protein and molecular level), as an informed basis for reducing the problem of lordosis and for optimising musculoskeletal growth.

Experimental Plan – Environmental Conditions



Eggs & larvae were reared at 15 & 20ºC up to 18-20mm total length (TL). The juveniles (200, 500, 700-1000mg) were then reared at different current speeds (0, 25, 50, 75%) at an ambient temperature.

ORCIS: Key Results

Environmental influences on:

- Incidence of lordosis
- Biomechanics of lordosis
- Differential allometric growth of bone and muscle
- Musculoskeletal gene expression
- Muscle development

Incidence of Lordosis

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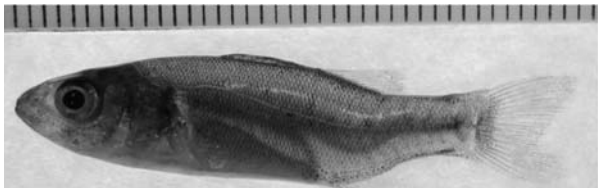
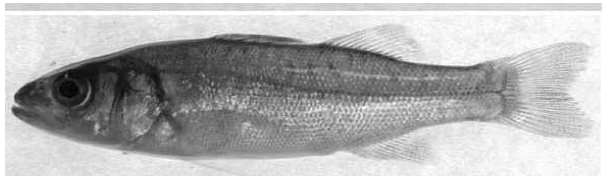




- Lordosis was more prevalent in fish reared at 20^{o}C than at 15^{o}C

- Lordosis frequency increased with water-current velocity

- The growth of the fish was better in fish originally at 15^{o}C but not affected by the current speed, but the highest current speed (75%) significantly increased mortality

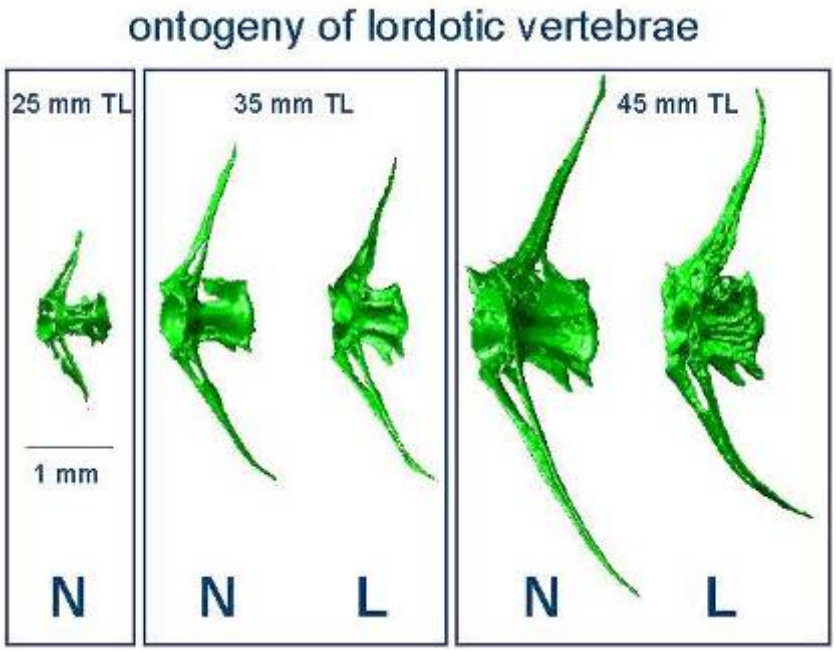
- Swimming performance significantly correlated with the total length of the sea bass and was better in fish originally at 15^{o}C than at 20^{o}C. Therefore, thermal history had a significant influence on swimming performance

Biomechanics of lordosis

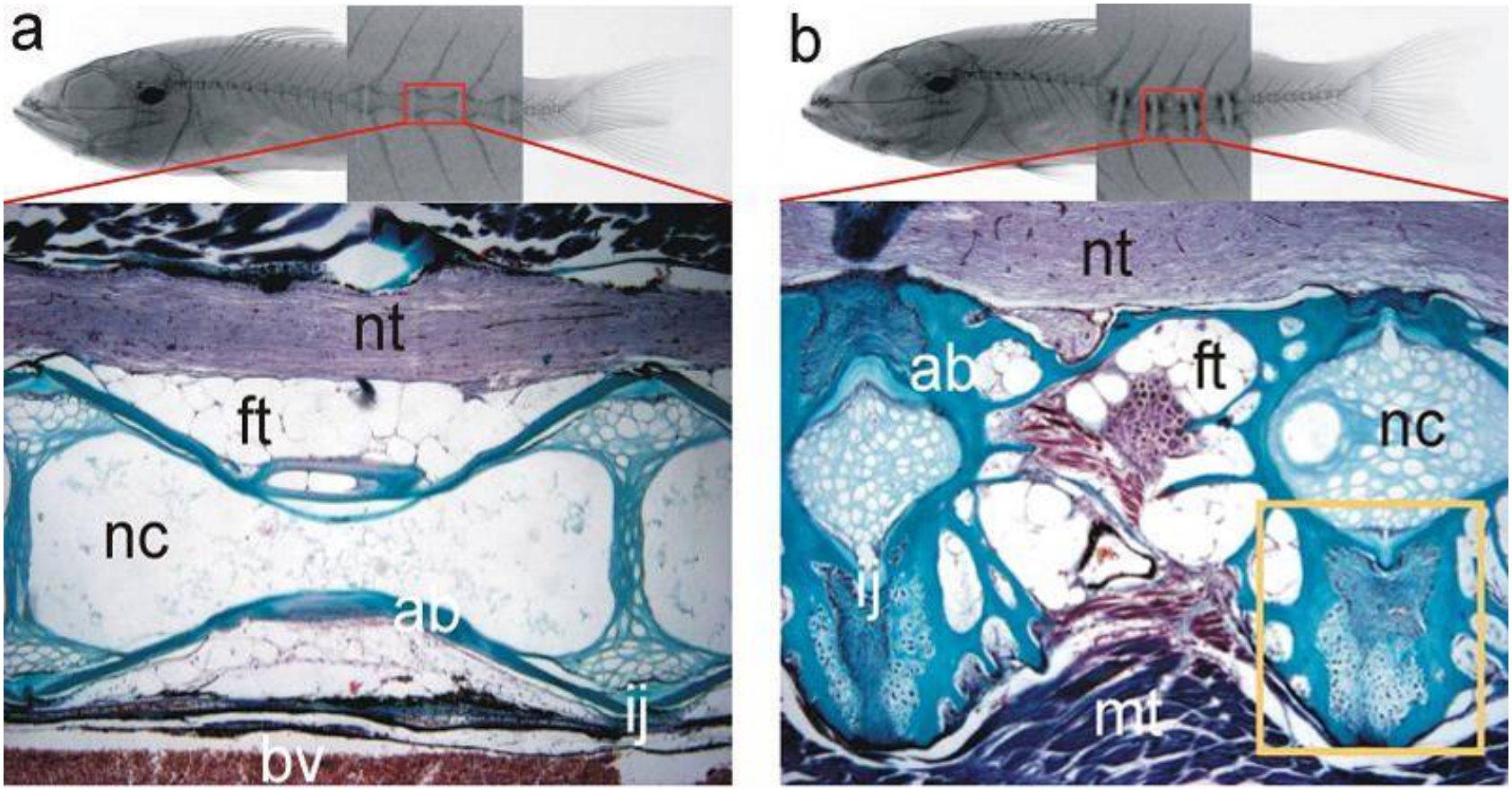
Prof. Dr. Ir. Johan Van Leeuwen
Dr. Sander Kranenbarg,
Wageningen University,
Wageningen, The Netherlands

Lordosis – A Buckling Problem

- Lordosis is a buckling problem of the vertebral column
- There is a sensitive period of around 15mm notochord length
- It involves local adaptation of the tissue, particularly at the articular surfaces



The normal vertebra (a) shows a small amount of bone matrix around the notochord (nc). The lordotic vertebra (b) shows large amounts of bone matrix.



Sagittal sections through a) a normal vertebra and b) a lordotic vertebra.
ab: acellular bone, bv: blood vessel, ft: fat tissue, ij: intervertebral joint, mt: muscle tissue, nc: notochord, nt: neural tube.

Biomechanics - Summary

- Lordosis comprises: buckling and adaptation
- Lordotic vertebrae contain more bone matrix and are stronger than normal vertebrae.
- Articular surfaces (zygapophyses) are major sites of remodelling.
- Remodelling is probably an adaptation to increased external loading and thus a beneficial response to an adverse situation (water velocity, high temperature).
- **Lordotic vertebrae are shorter, not malformed, but well adapted to increased loads**

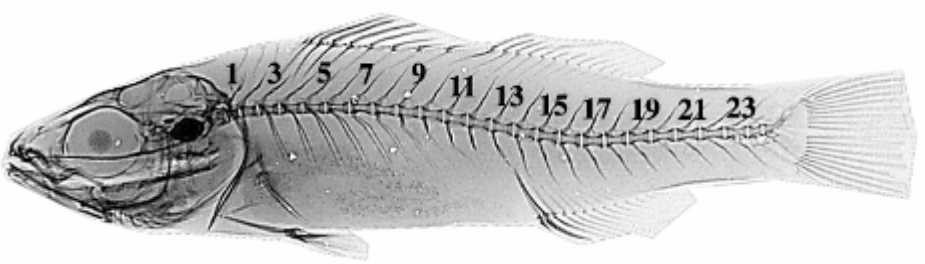
Differential allometric growth of bone and muscle under different environmental conditions

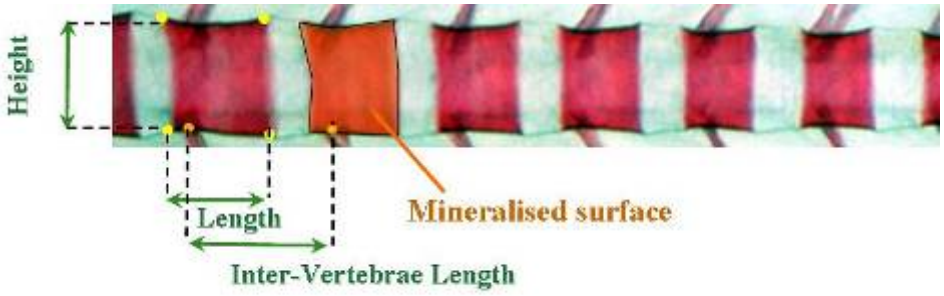
Dr. Benoit Fauconneau
Dr. Adnane Kacem
INRA, Rennes, France

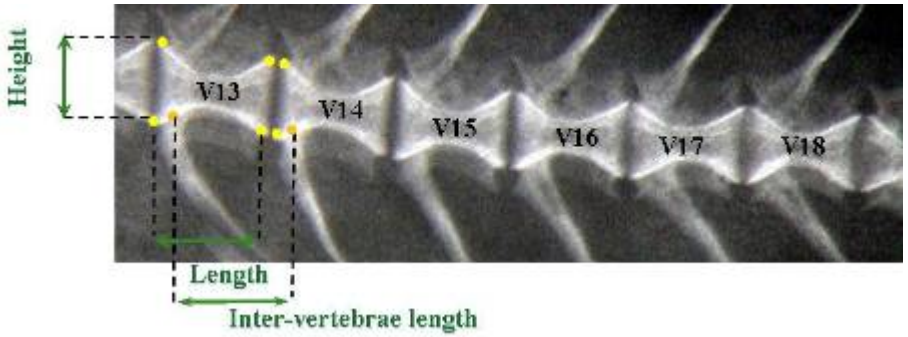
Vertebrae morphometry

Cartilage and bone staining with
alcian blue & alizarin red

X Ray Picture of sea bass juvenile

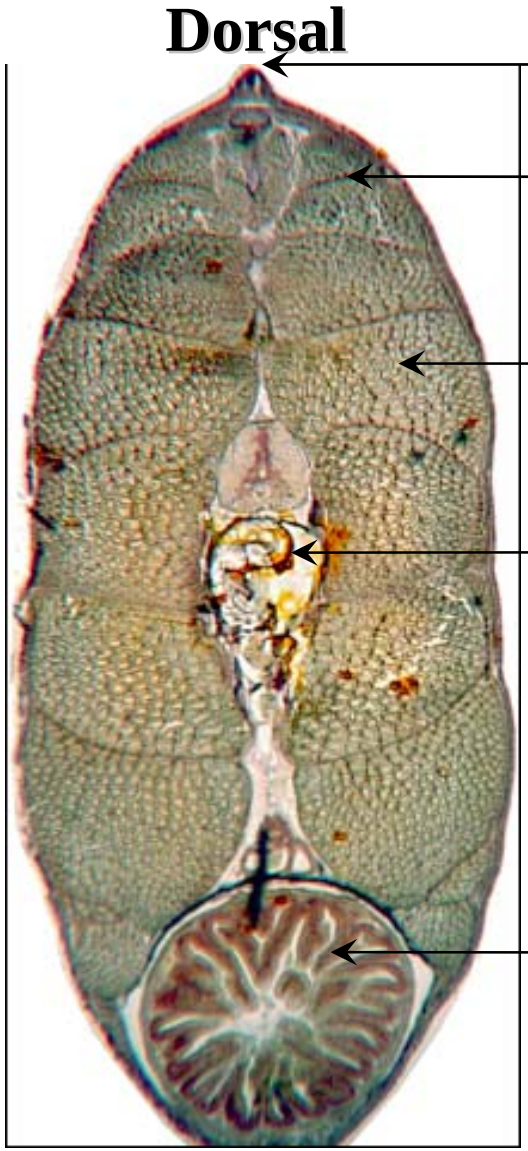




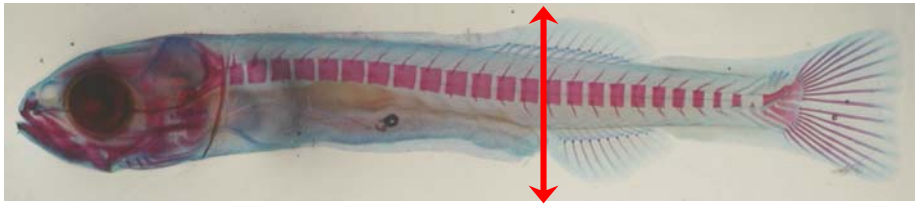


morphometric traits of vertebrae in lordosis area

Myomere Morphometry: larval and juvenile



Landmark points were used to study myomere appearance and disappearance



Transverse section of larva (18mm TL)

Differential allometry - Summary

Effect of high temperature (20°C) during larval stages:

- Lower relative growth of vertebrae (length, height) in lordotic area
- Higher relative growth of myomere (height, width) in lordotic area
- Still observed in juvenile (500mg) on vertebrae & myomeres

Current velocity during juvenile stages:

- Stimulated myomere development but not vertebrae development
- Higher force and stress of muscle on vertebrae at high swim speeds

Interaction between high temperature and high current velocity:

- → **Increasing force and stress of muscle on vertebrae**
- → **Higher susceptibility to deformity such as lordosis**

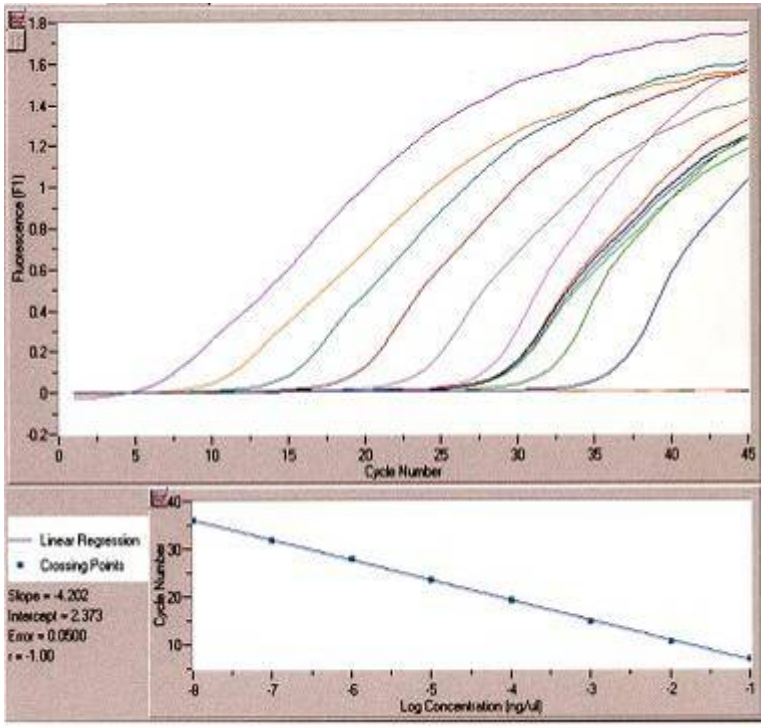
Environmental Influences on Musculo-skeletal Gene Expression

Dr Jenny Weaden
Prof Geoff Goldspink
Royal Free Hospital, London, U.K.

Molecular Techniques

Cloned Sea Bass Sequences

qRT-PCR



Muscle

- IGF-I Ea-2 and Ea-4
- IGF-I Receptor
- Others

Bone

- Osteocalcin

(is the most abundant non-collagenous matrix protein present in bone and is necessary for the correct formation of hydroxyapatite crystal).

Temperature Trial

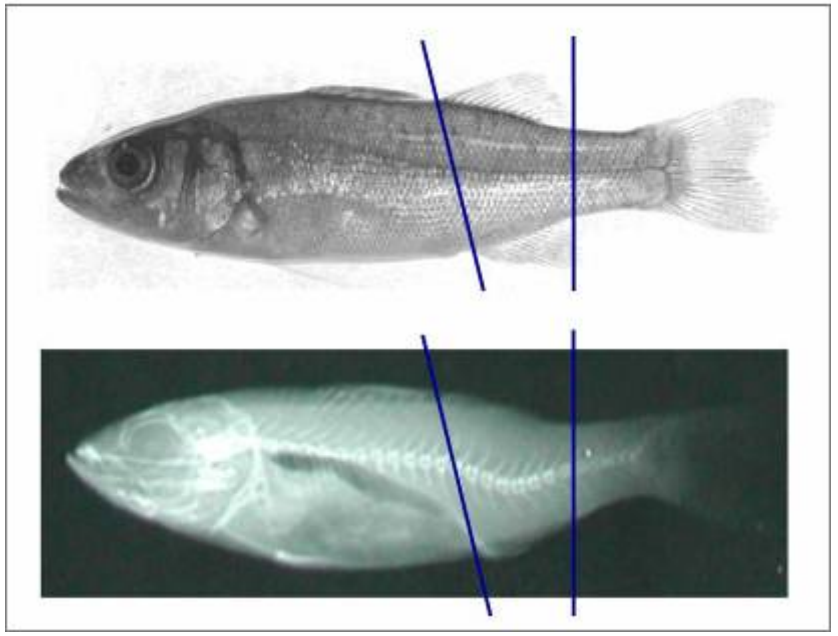
Samples

- First feeding
- Notochord Flexion
- Metamorphosis
- 200, 500, & 1000mg fish

- Reared at 15^{o}C or 20^{o}C

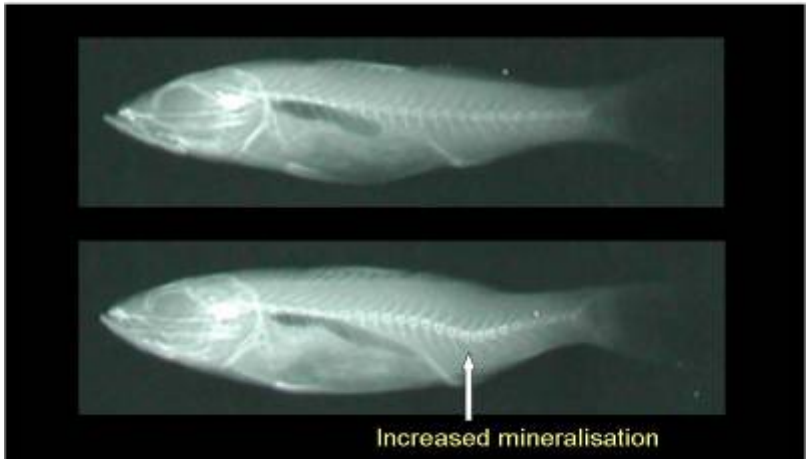
- RNA samples derived from whole fish (larvae) & lordosis-prone region (juvenile)

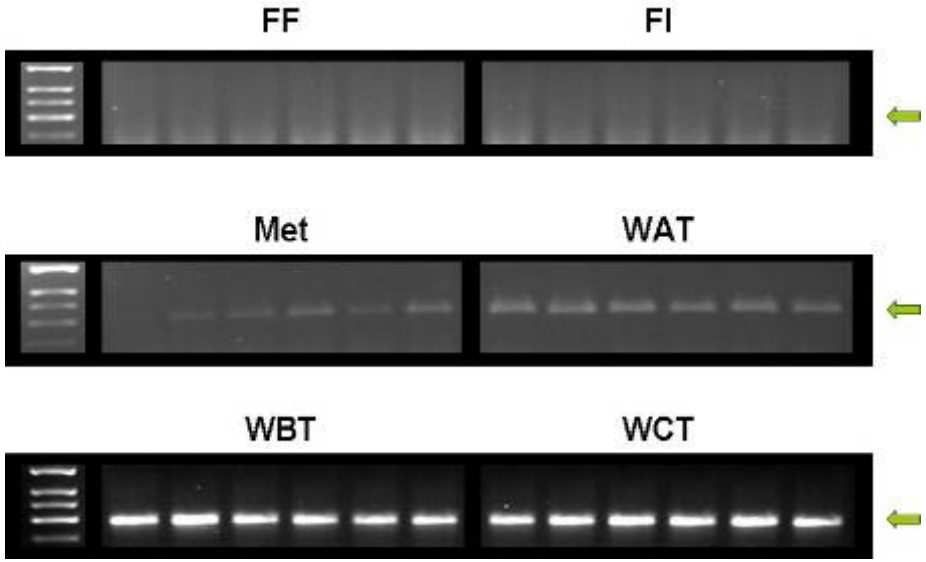
Tissue dissection

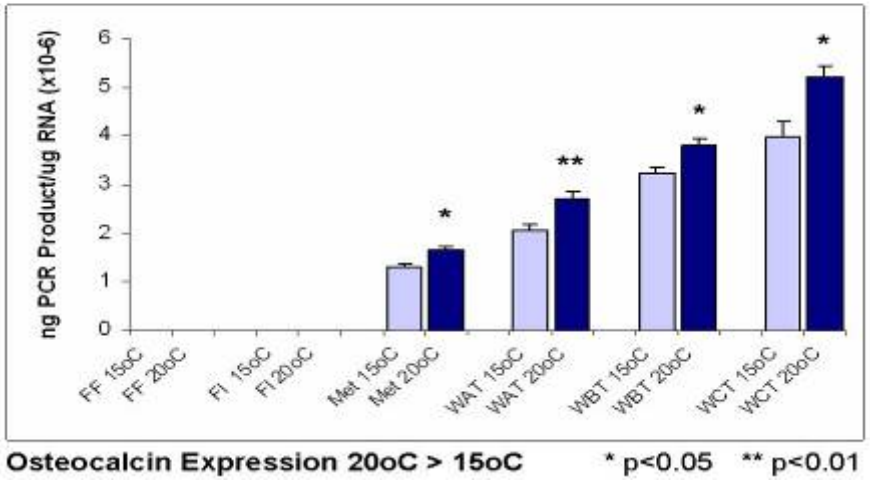


Osteocalcin expression

Osteocalcin expression is elevated at 20^{o}C possibly leading to over-mineralization of the vertebrae







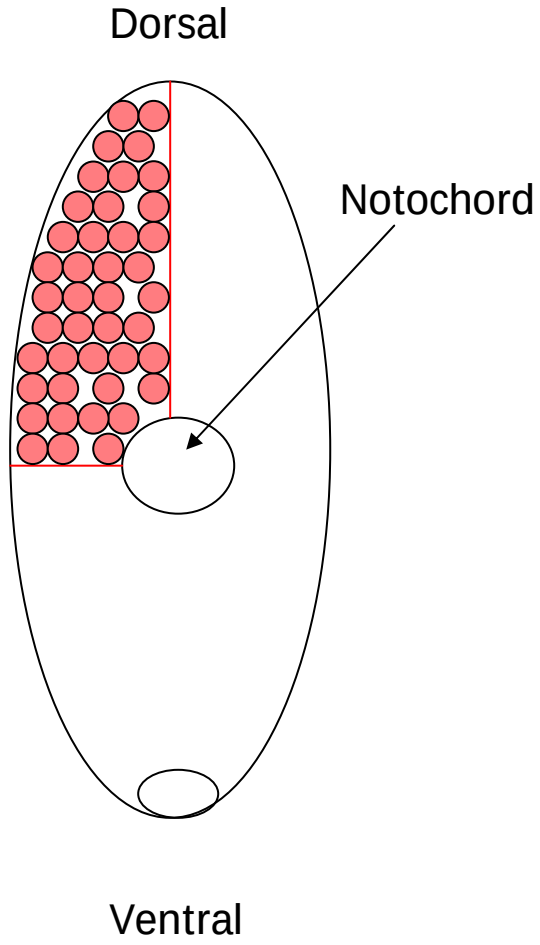
Environmental Influences on Muscle Development

Dr. Clare Ashton
Prof. Neil Stickland
The Royal Veterinary College,
London, U.K.

Analysis of muscle cellularity

Objective

To analyse the effects of early temperature and swim speed on the ontogeny of sea bass muscle.



Samples

- First feeding
- Notochord Flexion
- Metamorphosis
- 1000mg fish
- current speeds (0, 25, 50, 75%).

Muscle cellularity - Summary

- The incubation temperature of sea bass eggs affected muscle growth in embryos.

- Lower temperature delayed differentiation of muscle but caused the formation of greater numbers of myofibres at later stages.

- This may help to explain the faster growth rate in the 15^{o}C reared fish - in other species an increase in muscle fibre number is reflected in an enhanced post-hatch growth rate.

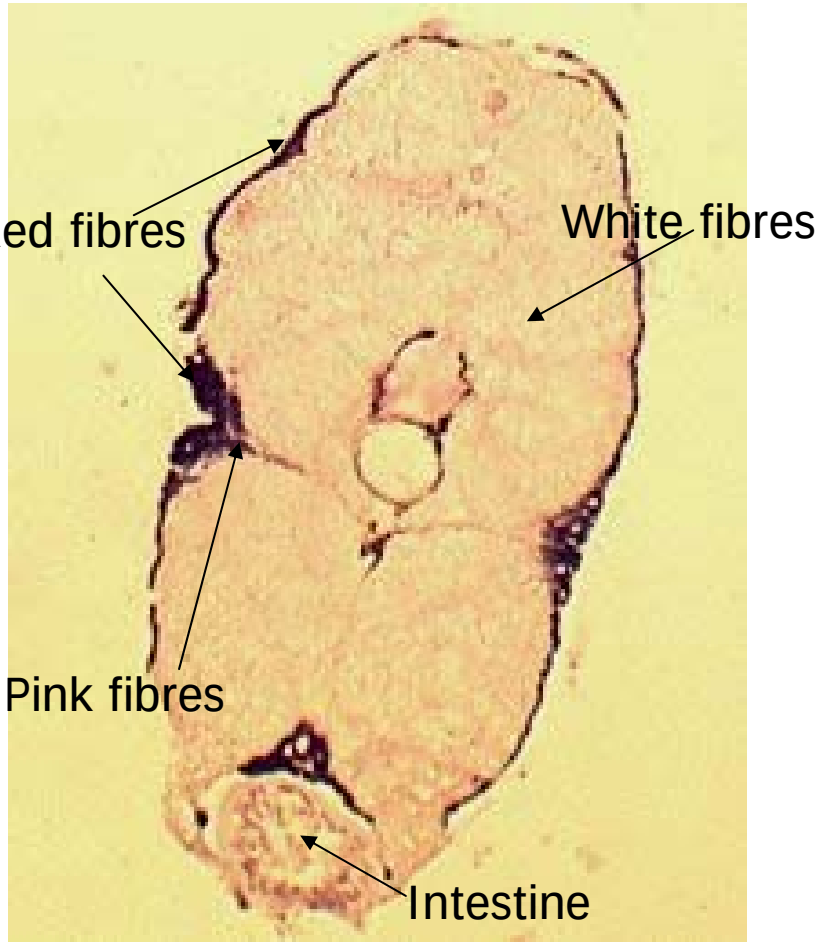
Muscle/Swimming Performance Relationship

- Swimming performance (ambient ^{o}C) of the weaned sea bass reared at 15^{o}C was better than of those fish reared at 20^{o}C.

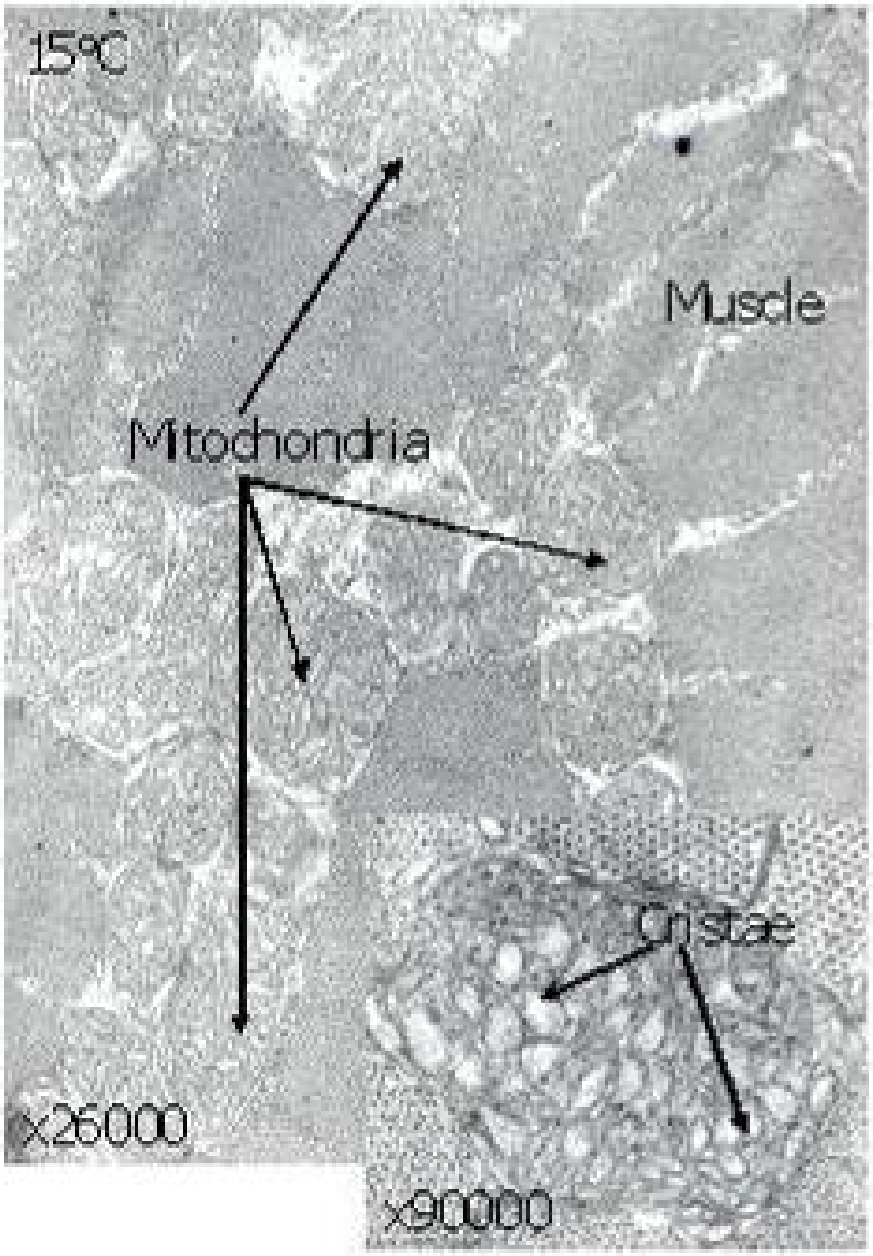
- Hypothesis: The difference in the swimming performance between the two populations of sea bass (reared at 15 v. 20^{o}C) is due to a difference in aerobic potential and/or muscle contractile proteins.

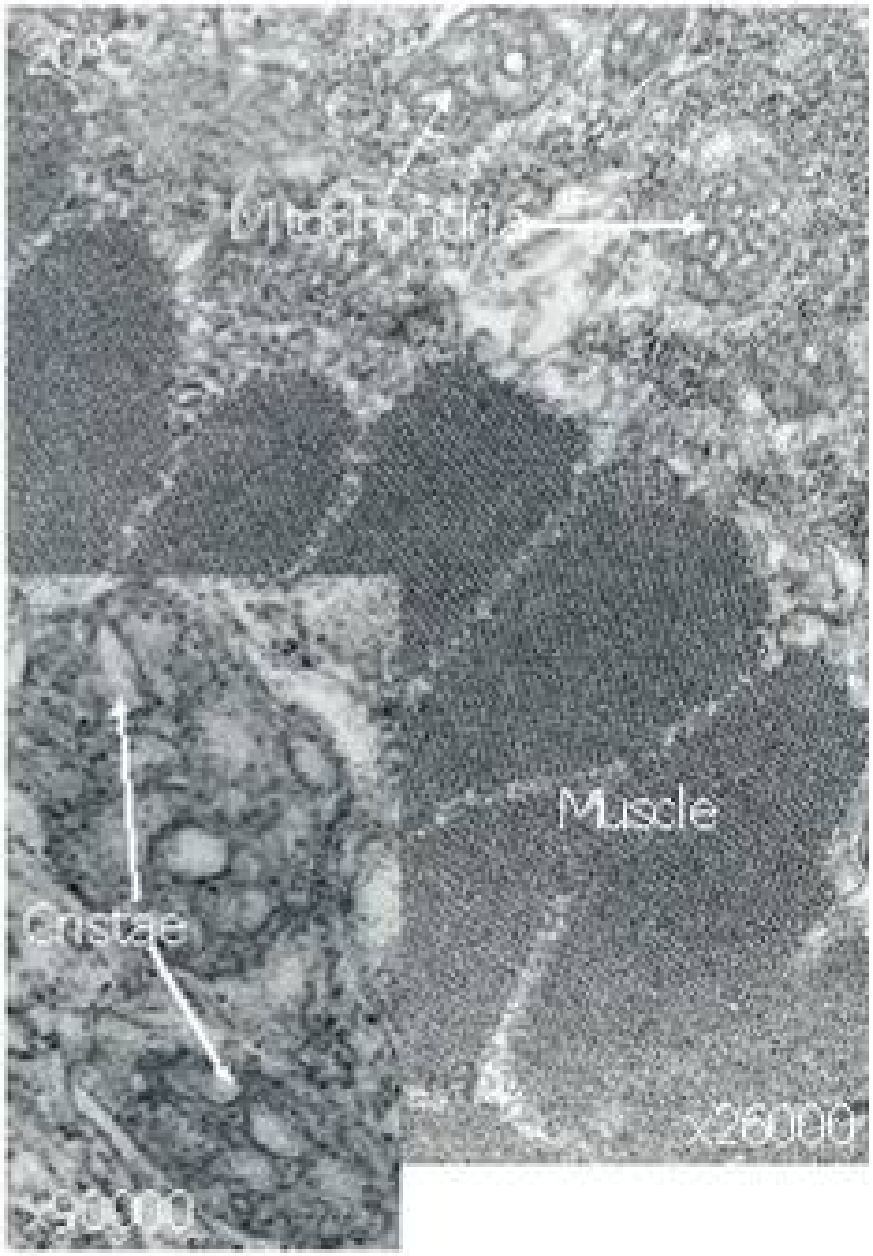
Aerobic potential

- Measuring the activity of the mitochondrial enzyme Succinate dehydrogenase (SDH) shows the oxidative capacity of muscle fibres.
- More red muscle and more stain in fish reared at 15^{o}C

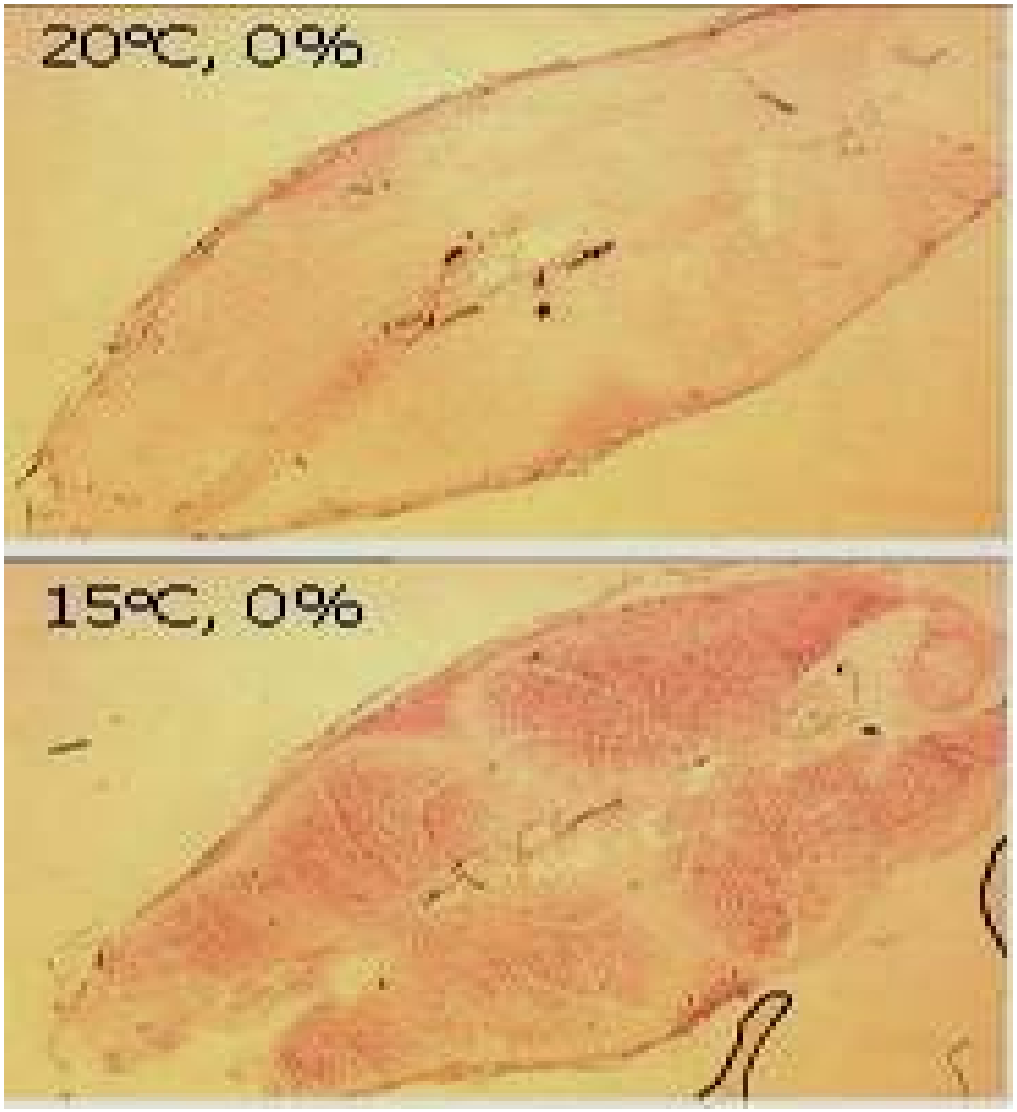


TEM Images of Mitochondria





Muscle contractile proteins



Slow myosin antibody used.

There appears to be more staining in red and white muscle in the 15°C reared fish at later stages.

This suggests the early thermal history appears to affect the rate of muscle protein differentiation. This may affect the muscle function at critical stages.

Muscle/Swimming Performance Relationship - Summary

- The fish reared at 15^{o}C exhibited an increased aerobic potential at all the weaned stages.

- This was shown in differences in red muscle area, mitochondrial number, mitochondrial cristae number and SDH activity.

- Differences in myosins were observed between the two temperature groups

ORCIS – Key Results

- Incidence of lordosis greater in fish reared at 20°C (in contrast to 15°C). This is exacerbated by increased current speeds.
- Reduced vertebrae growth and increased myomere growth (lordotic area) in 20°C reared fish and at high swim speed
- Lordotic vertebrae were shorter and well adapted to increased loads.
- Osteocalcin expression was less in fish reared at 15°C.
- There were more muscle fibres in fish reared at 15°C.
- Fish reared at 15°C had a better swimming performance, more red muscle and muscle mitochondria as well as differences in muscle contractile proteins at later juvenile stages.