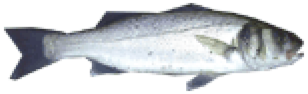
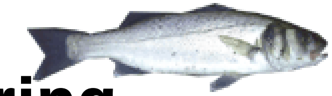


Environmental influences on the development of musculo-skeletal tissues in sea bass

Dr. Clare Ashton

Prof. Neil Stickland

The Royal Veterinary College, London, U.K.



Acknowledgements – Optimisation of Rearing Conditions in Sea Bass (ORCIS) Team

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Prof. Geoffrey Goldspink & Dr. Jenny Weaden, Royal Free & University College Medical School, London, U. K.

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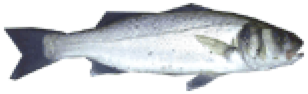
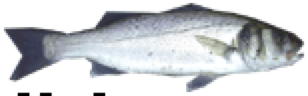


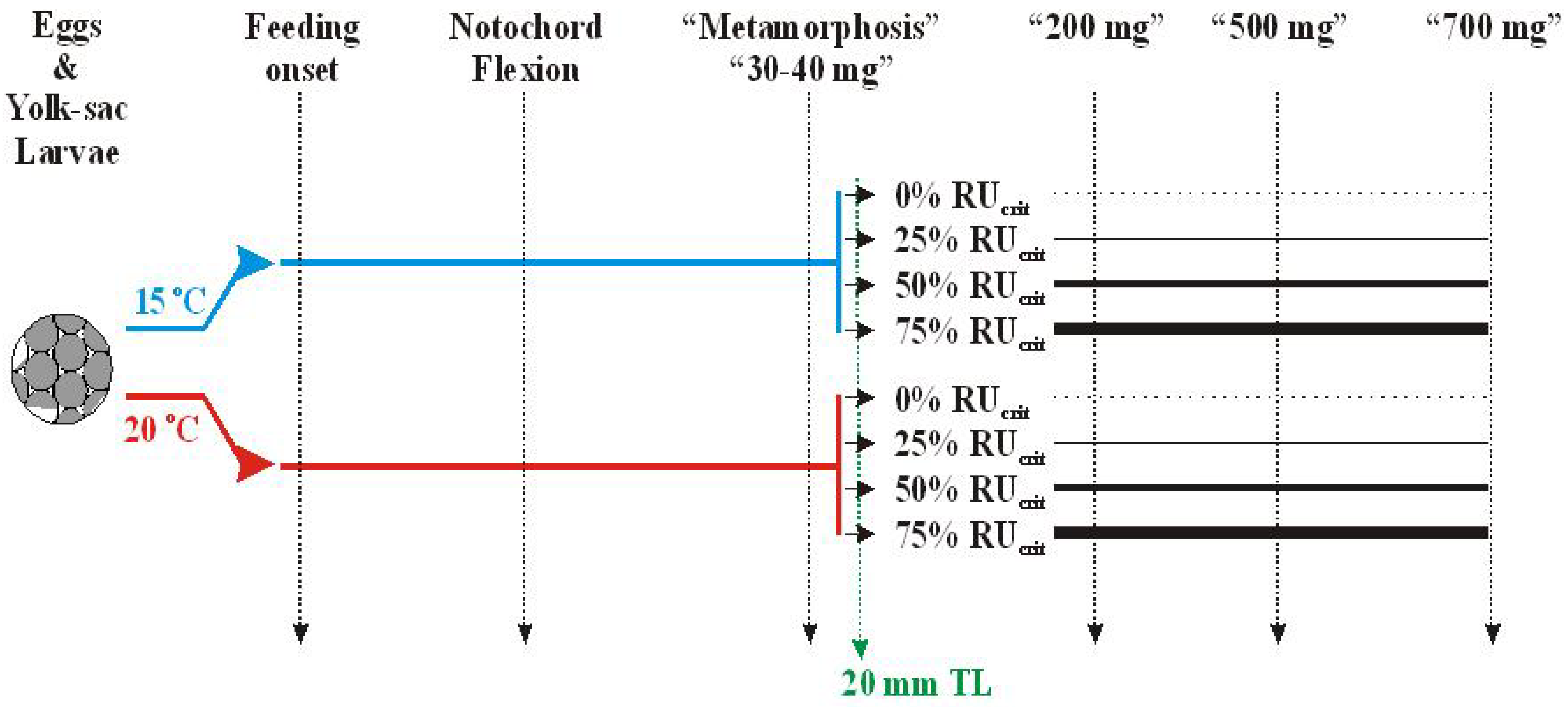
ORCIS Objectives

1. To assess the influence of rearing temperature, current velocities and nutrition (Vitamin C, E & Selenium), and their interactions on the incidence of lordosis

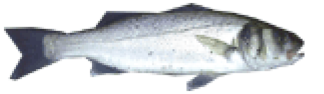
2. To assess, for the same factors and groups of fish, the influence on musculoskeletal growth and development (at tissue, cell, protein and molecular level), as an informed basis for reducing the problem of lordosis and for optimising musculoskeletal growth.



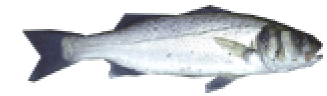
Experimental Plan – Environmental Conditions



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.

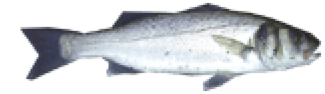


ORCIS: Key Results
– Musculoskeletal Growth



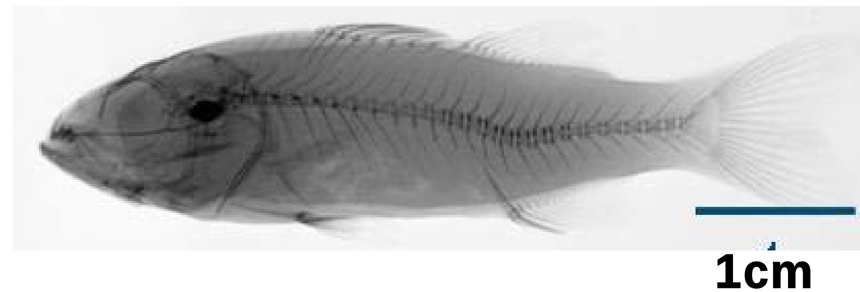
Biomechanics of lordosis

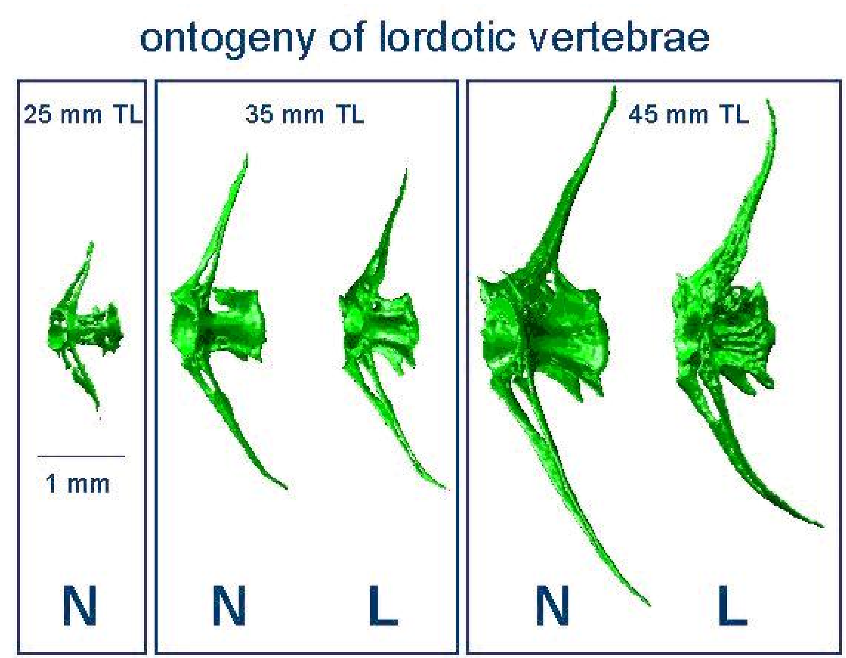
Prof. Dr. Ir. Johan Van Leeuwen
Dr. Sander Kranenbarg,
Wageningen University,
Wageningen, The Netherlands

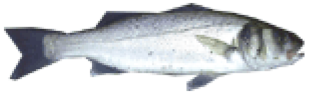


Lordosis – A Buckling Problem

- 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

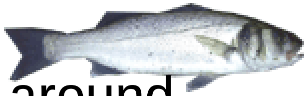




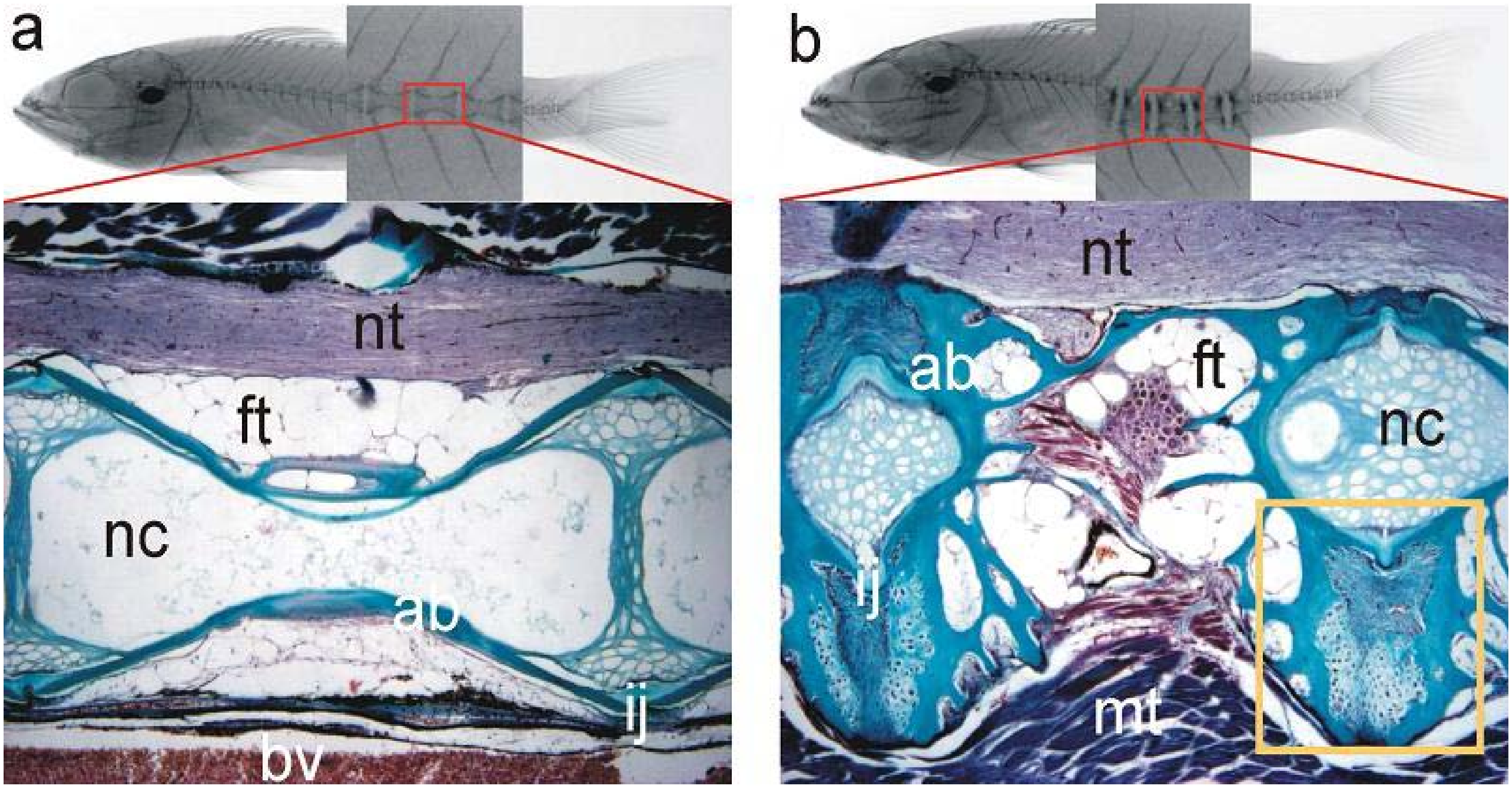


What causes buckling?

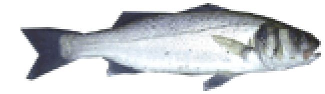
- muscle activity
  - non-inflated swim bladder
  - high temperature
  - high current velocity
  - ...
- axial skeleton failure
  - nutrition
  - heavy metals
  - pathogens
  - ...



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.

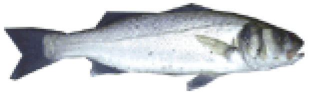


Sagittal sections through a) a normal vertebra and b) a lordotic vertebra.
ab: acellular bone, bv: blood vessel, ft: fat tissue, ij: intervertebral joint, mt: muscle tissue, nc: notochord, nt: neural tube.



Summary

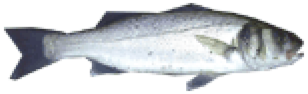
- Lordosis comprises: buckling and adaptation
- Lordotic vertebrae contain more bone matrix and are stronger than normal vertebrae.
- Articular surfaces (zygapophyses) are major sites of remodelling.
- Remodelling is probably an adaptation to increased external loading and thus a beneficial response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



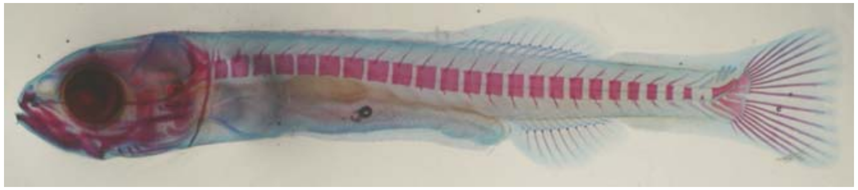
Differential allometric growth of bone and muscle under different environmental conditions

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

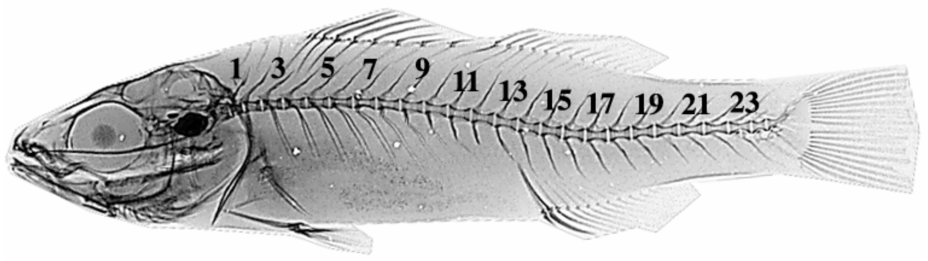
Vertebrae morphometry

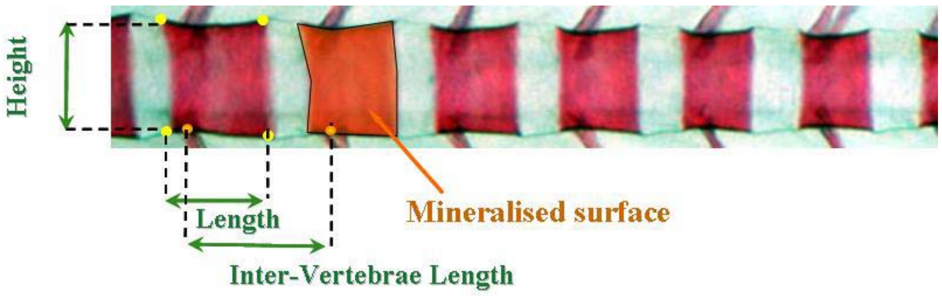


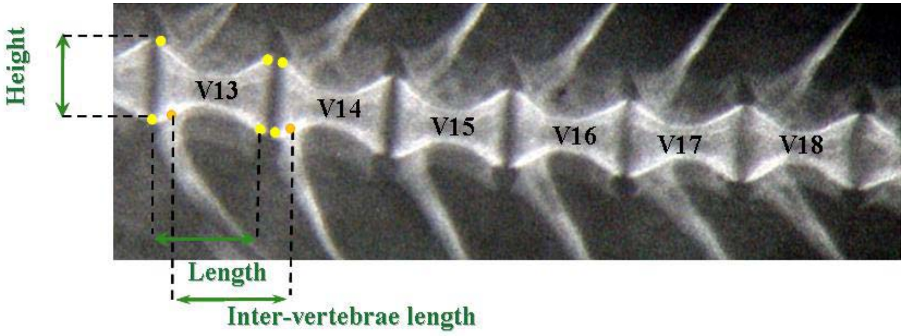
Cartilage and bone staining with
alcian blue & alizarin red



X Ray Picture of sea bass juvenile

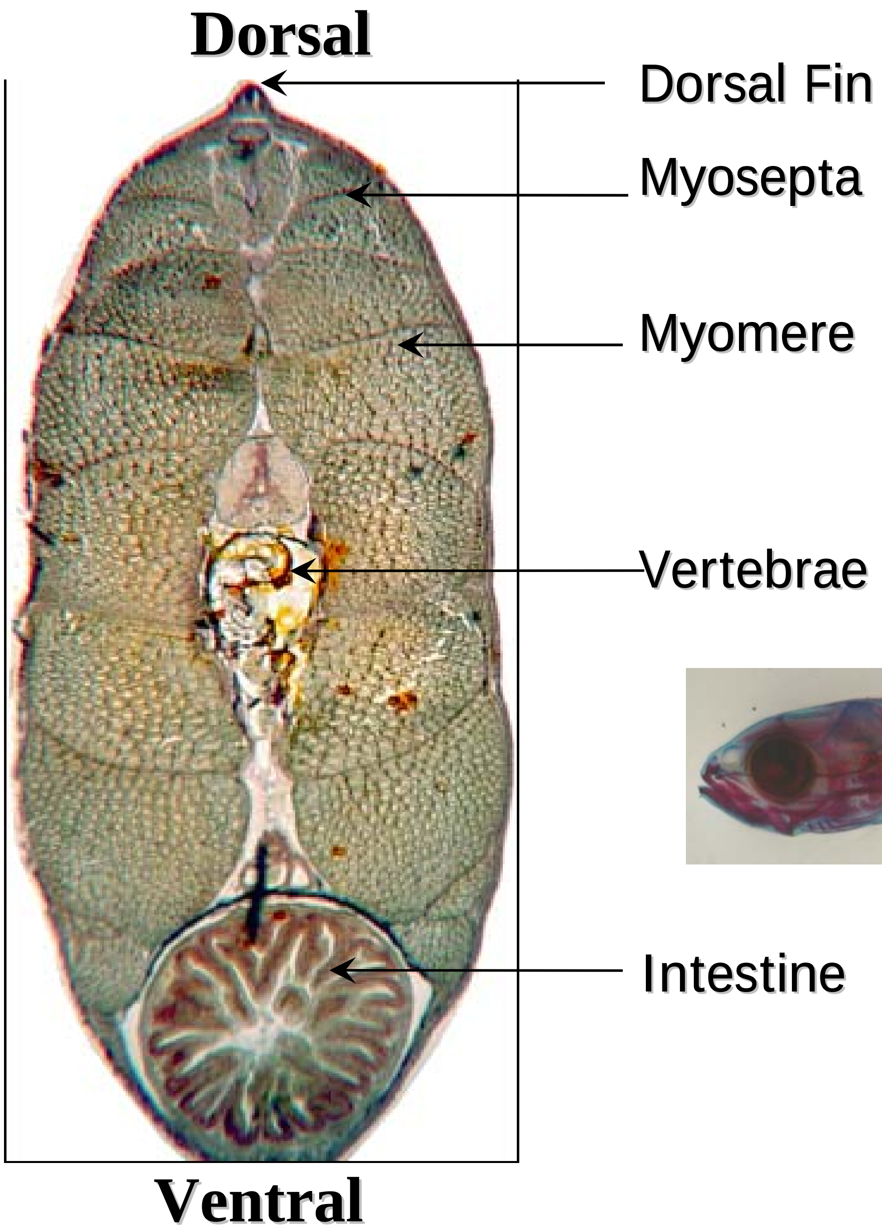




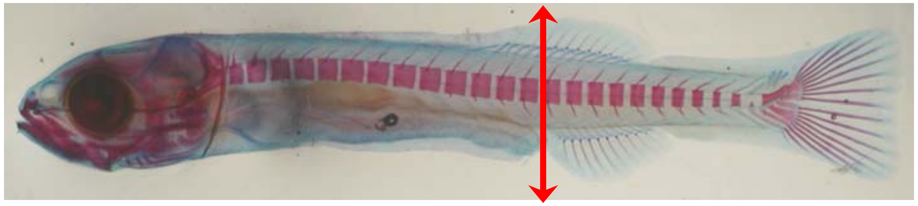


morphometric traits of vertebrae in lordosis area

Myomere Morphometry: larval and juvenile



Landmark points were used to study myomere appearance and disappearance



Transverse section of larvae (18mm TL)

Effect of environmental factors on muscle & vertebrae allometry during larval & juvenile development - Summary

Effect of high temperature (20ºC) during larval stages:

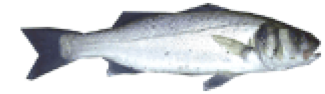
- Lower relative growth of vertebrae (length, height) in lordotic area
- Higher relative growth of myomere (height, width) in lordotic area
- Still observed in juvenile (500mg) on vertebrae & myomeres

Current velocity during juvenile stages:

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

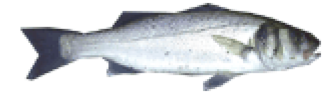
Interaction between high temperature and high current velocity:

- → ***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

Muscle

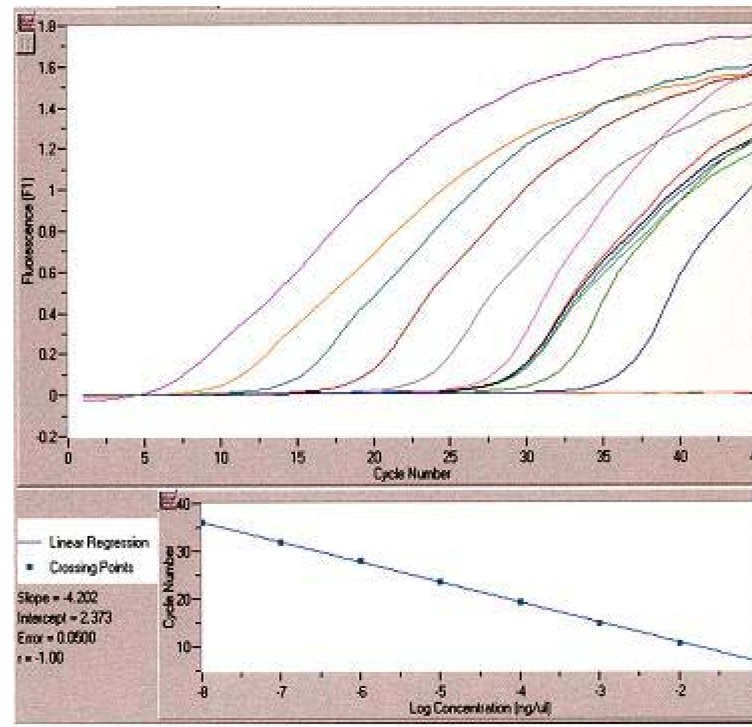
- **IGF-I Ea-2 and Ea-4**
- **IGF-I Receptor**

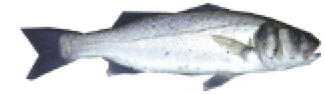
Bone

- **Osteocalcin**

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

qRT-PCR



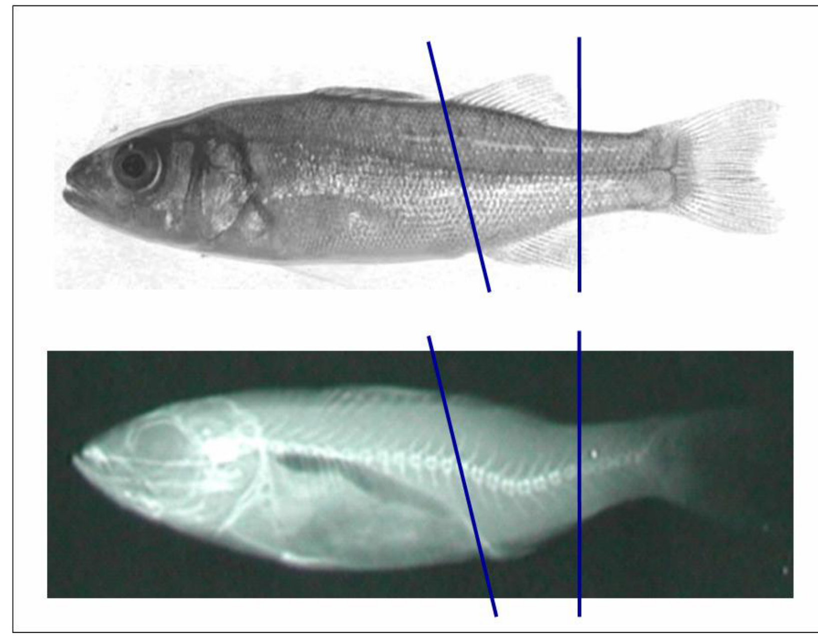


Temperature Trial

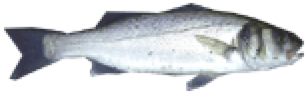
Samples

- 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

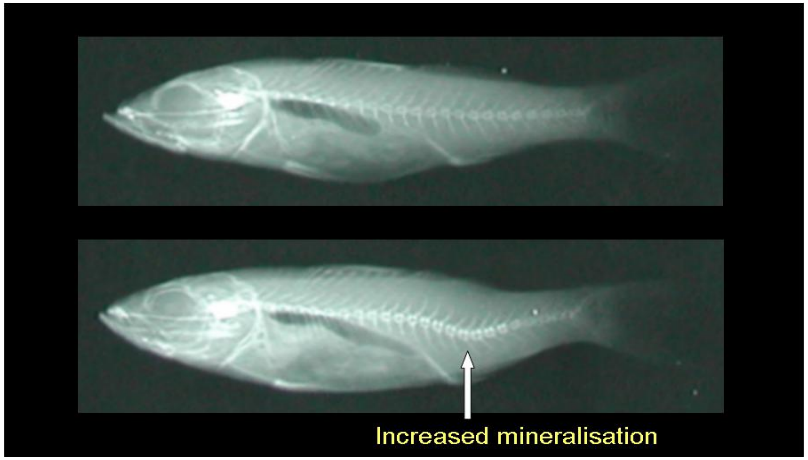


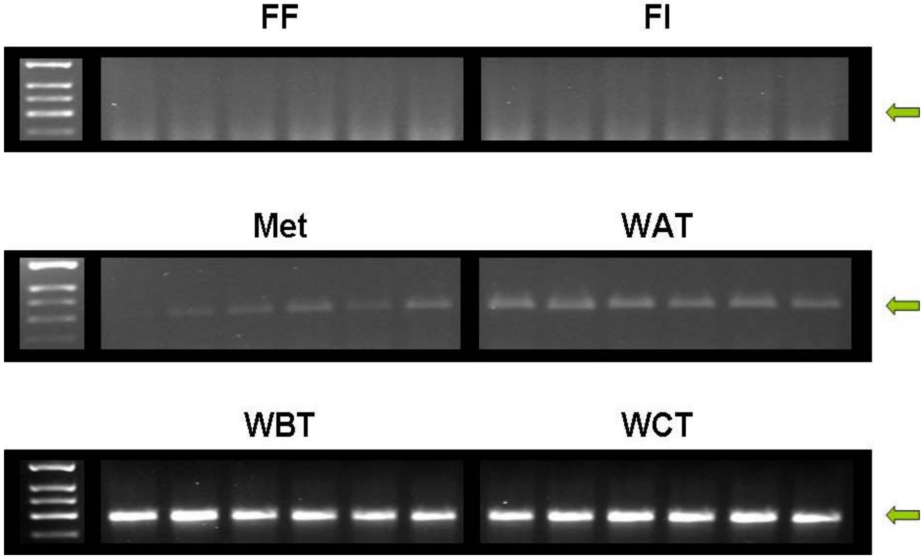
Osteocalcin expression



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

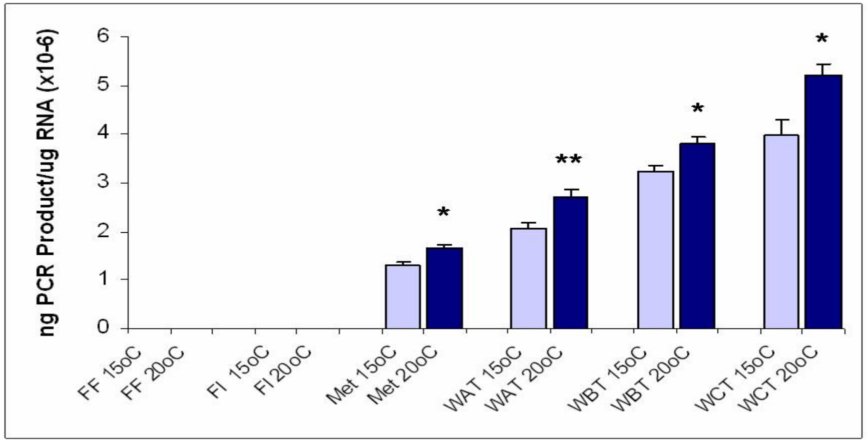
Skeletal Effect of Lordosis



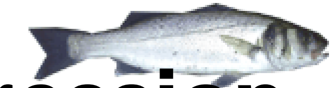


Temperature Trial

Osteocalcin mRNA Quantitation



Osteocalcin Expression 20oC > 15oC * p<0.05 ** p<0.01

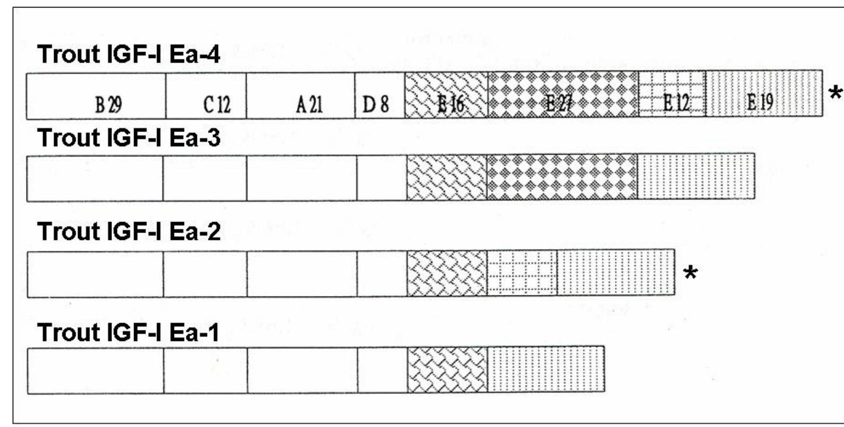


Insulin Growth Factor (IGF-I) expression

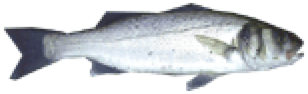
- Promotes growth of an organism
- Produced by Liver and locally by muscle
- Induced by exercise and damage

- IGF-1 Ea2 and Ea4 were elevated at 20ºC in the 1000mg juveniles
- IGF-IR showed no consistent change due to temperature

Fish IGF-I Splice Variants



Fish Physiology, NY Acad. Press: p179



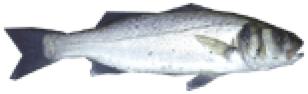
Environmental Influences on Muscle Development

Dr. Clare Ashton

Prof. Neil Stickland

The Royal Veterinary College,

London, U.K.



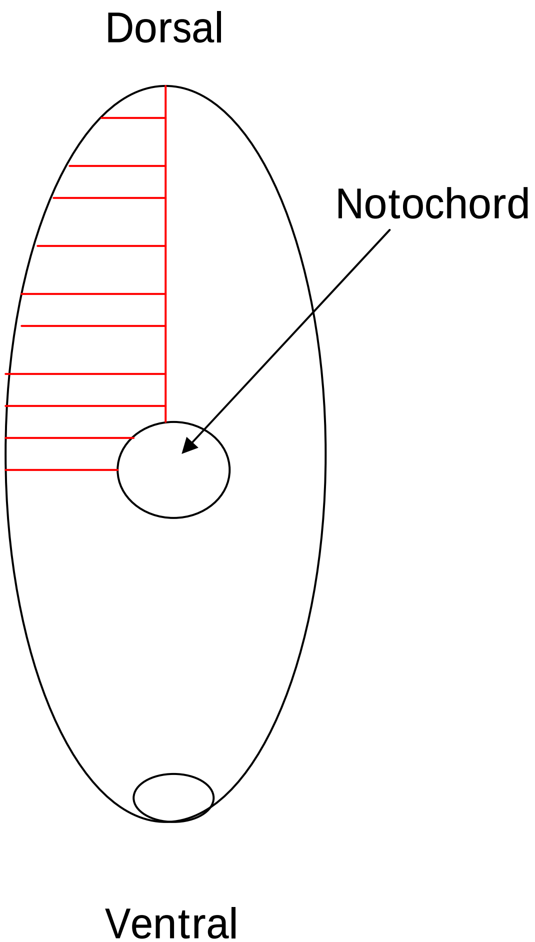
Analysis of muscle cellularity

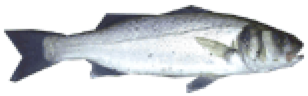
<u>Objective</u>

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

<u>Samples</u>

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



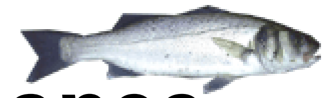


Analysis of muscle cellularity - Summary

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

- Lower temperature caused the formation of greater numbers of myofibres, hence this may help to explain the faster growth rate in the 15^{o}C reared fish.

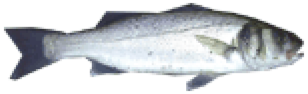
- In other species an increase in muscle fibre number is reflected in an enhanced post-hatch growth rate.



Mitochondria/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.

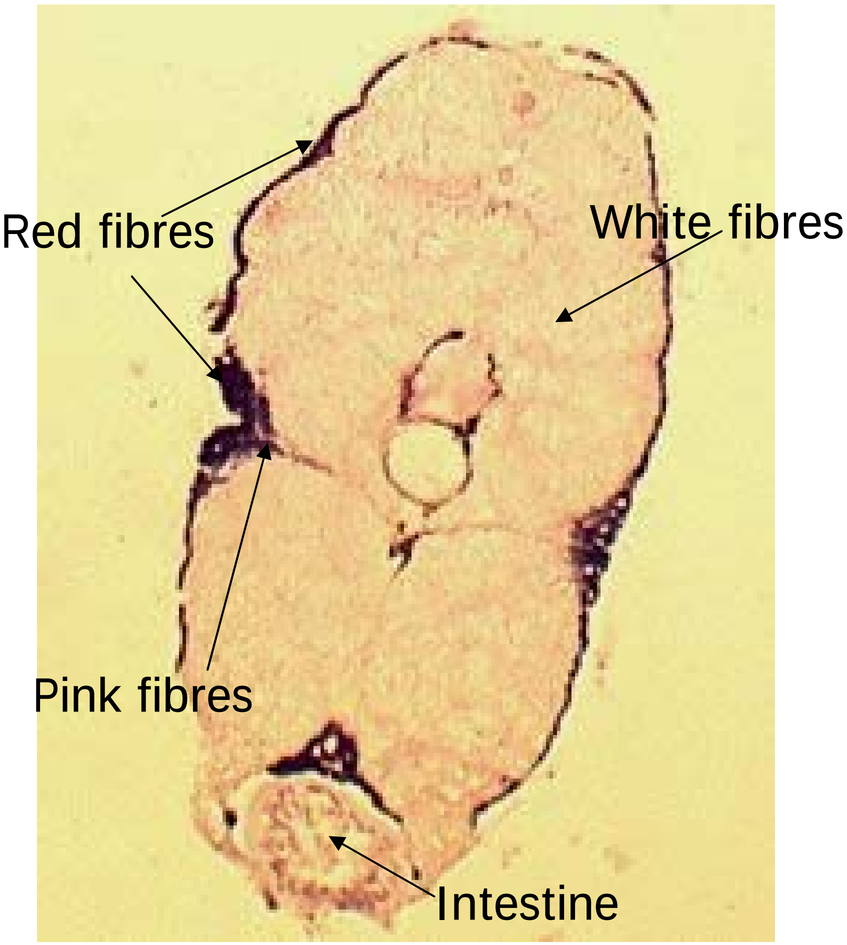
- Hypothesis: The difference in the swimming performance between the two populations of sea bass (reared at 15^{o}C v. 20^{o}C) is due to a greater mitochondrial enzyme activity within the red muscle



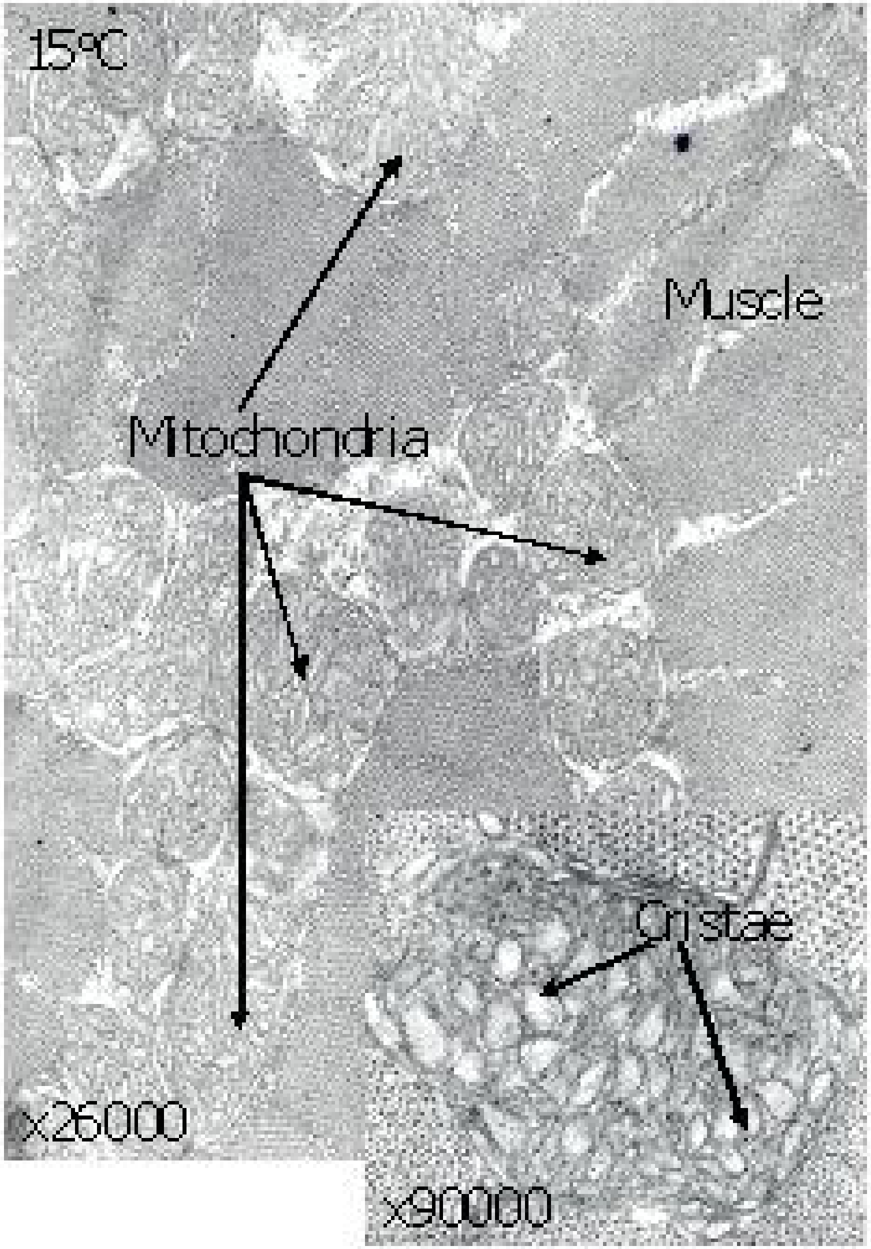
Histological Staining (SDH)

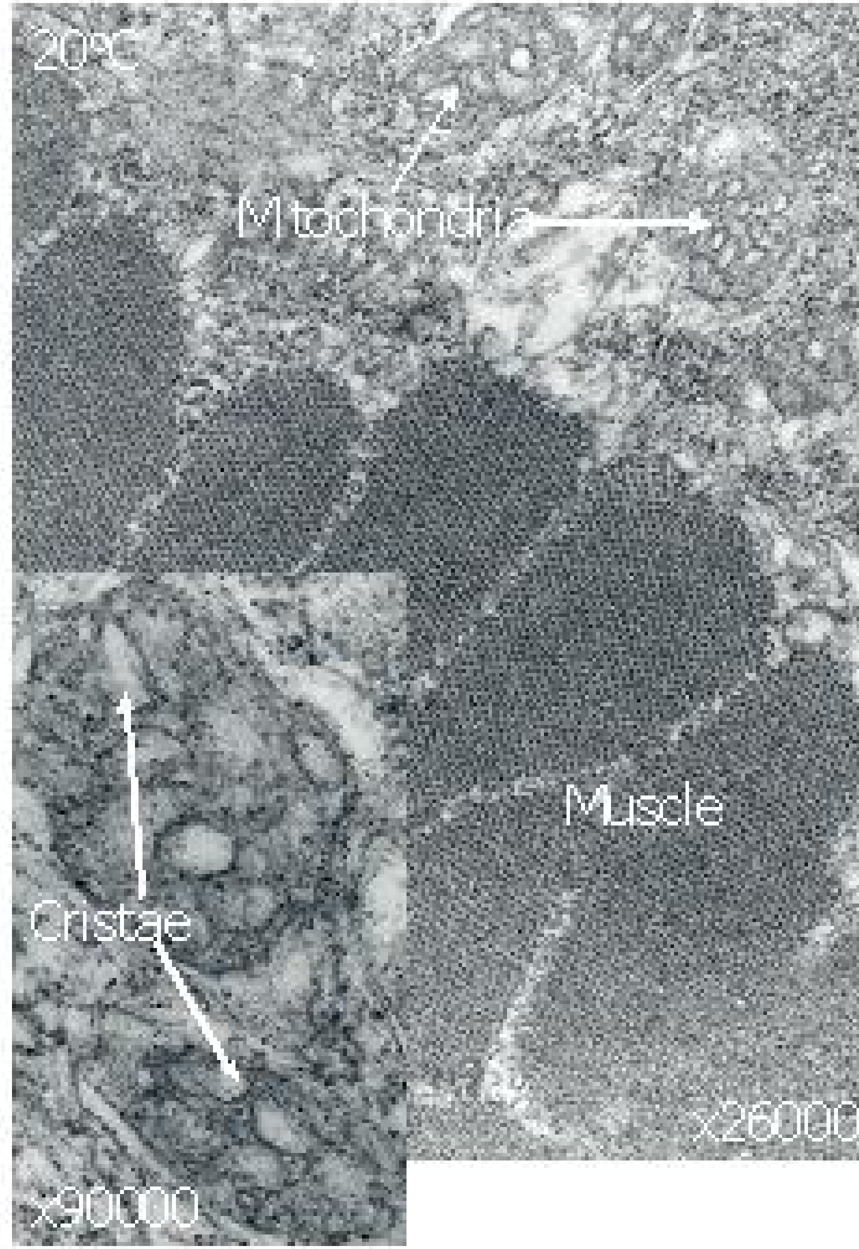
- Measuring the activity of the mitochondrial enzyme Succinate dehydrogenase (SDH) shows the oxidative differentiation of muscle fibres.

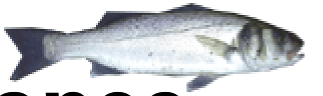
- **SDH demonstrates the relative proportions of mitochondria within the muscle fibres.**



TEM Images of Mitochondria

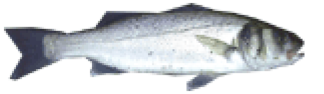






Mitochondria/Swimming Performance Relationship - Summary

- 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.
- This may help to explain the increased swimming performance of the fish reared at 15^{o}C.

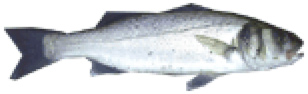


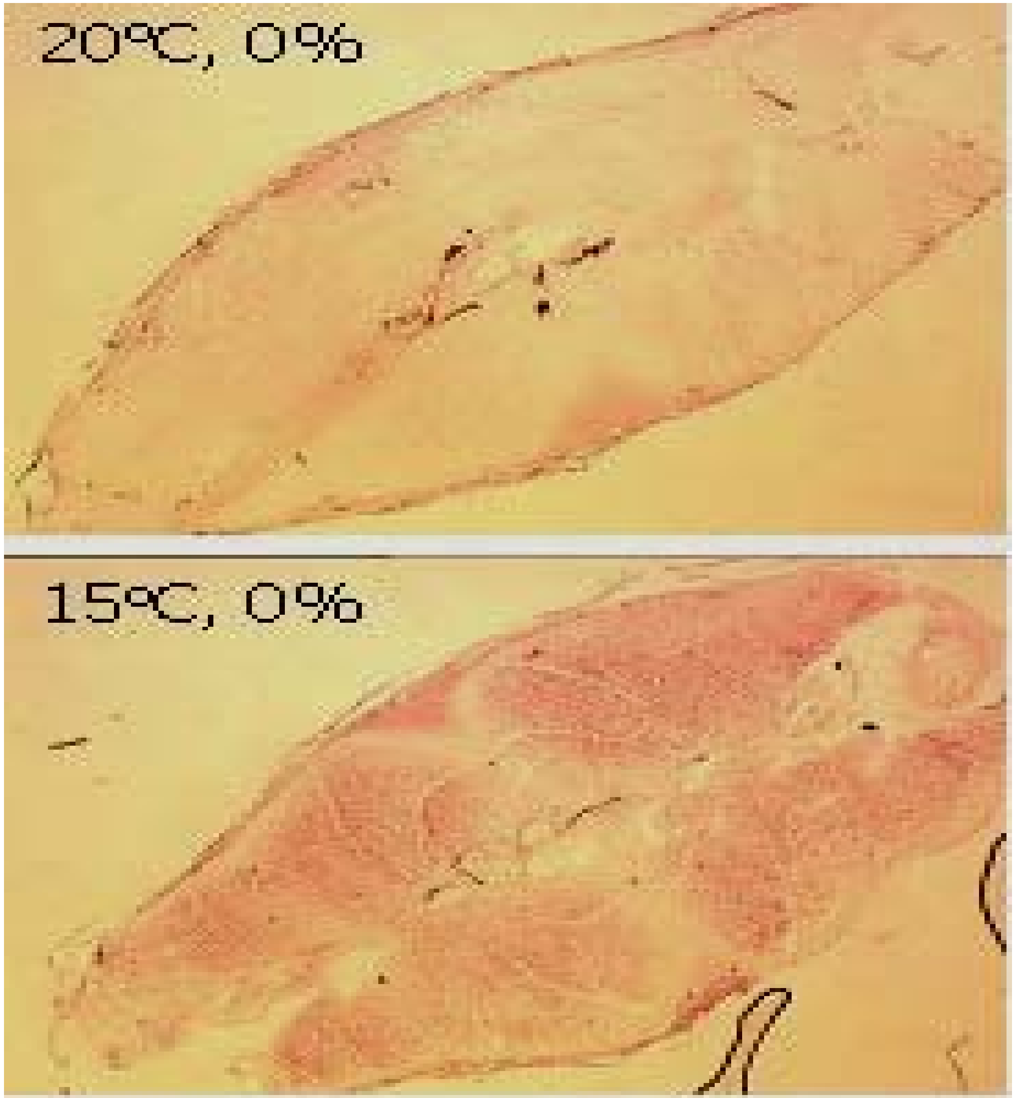
Immunocytochemistry

Objective

This study was performed to determine if differences were occurring in the development and expression of myosin isoforms between the two differently reared sea bass groups (15ºC versus 20ºC).

Myosins are the major proteins of muscle necessary for muscle function.

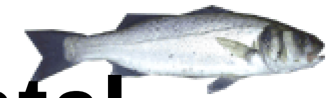
Immunocytochemistry - Results 



Slow myosin is found in the red muscle regions of fish reared at 15ºC & 20ºC.

There appears to be more staining in red and white muscle in the 15ºC reared fish.

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



Overall Summary – Environmental Influences

Muscle Cellularity: more muscle fibres in fish reared at 15^{o}C, which will convey better muscle growth performance

Mitochondria/Swimming Performance Relationship: Fish reared at 15°C have a better swimming performance, more red muscle, and more muscle mitochondria.

Immunocytochemistry: Larval rearing temperature may alter muscle protein differentiation in 15°C fish. This may have consequences on the function of the muscle.

ORCIS – Key Results – Musculoskeletal Growth

- Reduced vertebrae growth and increased myomere growth (lordotic area) in 20°C reared fish & at high swim speed
- Lordotic vertebrae were shorter & well adapted to increased loads.
- Osteocalcin expression was less in fish reared at 15°C and less in lordotic vertebrae even though lordotic vertebrae were more mineralised.
- There were more muscle fibres in fish reared at 15°C.
- Fish reared at 15°C had a better swimming performance, more red muscle & muscle mitochondria at later juvenile stages.
- Larval rearing temperature appeared to influence muscle proteins at later stages possibly due to slower protein differentiation at 15°C.