Centro de Ciencias do Mar

Skeletal development and bone-related gene expression in fish larvae

P Gavaia and ML Cancela

The fish skeleton

Composed of: cartilage, bone, chondroid bone, notochord, connective tissues, scales, enamel and dentin

Functions

Movement and muscle attachment

Body shape

Protection (*e.g.* scales, spines)

Reproduction

Feeding habits

Calcium and phosphate reservoir (Salmonids)

The fish skeleton

Cartilage: composed by an ECM rich in water, collagens (type II, X), proteoglycans and chondrocytes that differentiate from mesenchymal cells

Bone: composed by a mineralized ECM rich in minerals, collagen type I, and by two types of cells: osteoblasts, that differentiate from mesenchymal cells, and osteoclasts, originating from the macrophage monocyte lineage

- Cellular - Osteocytes included in the calcified ECM
- Acellular (anosteocytic)- Does not contain osteocytes and has a vascularized woven structure

Fish bone contains collagen type II and chondroitin sulphate

The fish skeleton

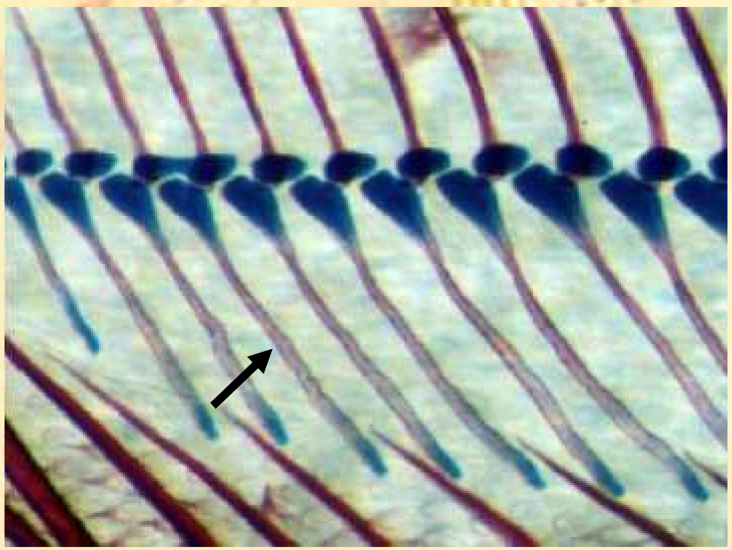
<u>Cartilage replacement:</u> **Cartilage gradually replaced by bone**

<u>Endochondral ossification</u> when calcification begins within the cartilage

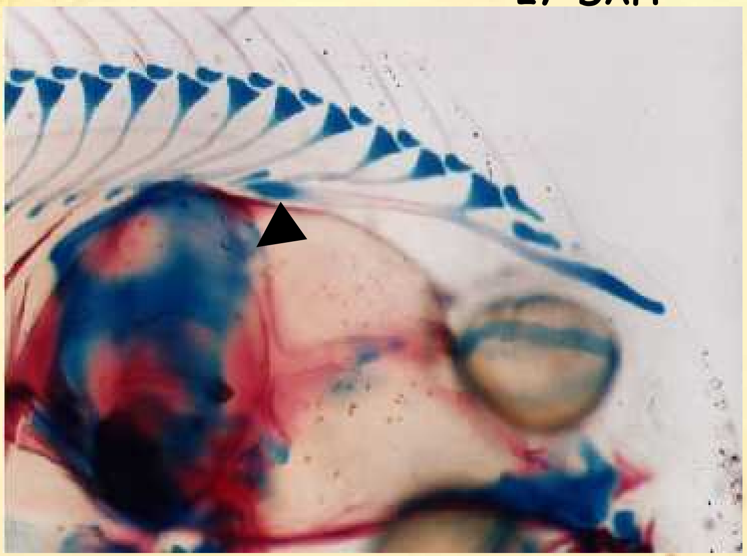
<u>Perichondral ossification</u> when bone begins to form around the periphery of the cartilage

(Cranial unpaired bones, pterigophores, mandibula, hypuralia, ….)

S. senegalensis
27 DAH



S. senegalensis
27 DAH



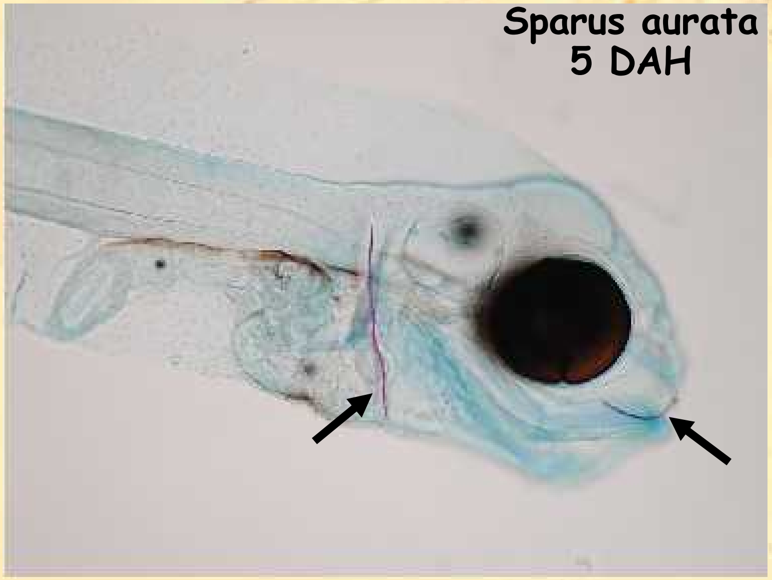
P.J. Gavaia (2004)

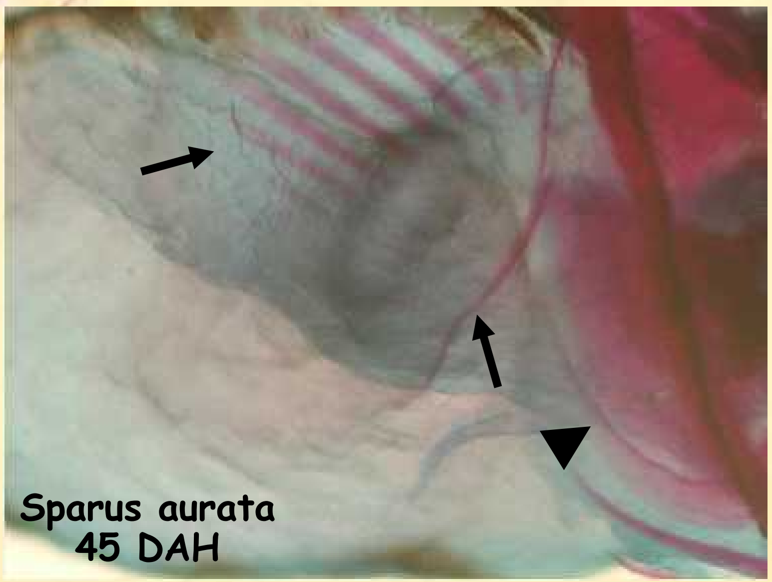
The fish skeleton

<u>Dermal or intramembranous ossification:</u> Bone develops directly from the connective tissue

> **Mesenchymal cells condense, the tissue vascularizes, and cells differentiate directly into osteoblasts**

(Cleithrum, cranial paired bones, dermatotrichia e lepidotrichia, supra mandibular, opercula, ….)





P.J. Gavaia (2003)

Skeletal malformations

- Uncommon in nature

Madriaga & Cendrero (1973) Allue (1984)

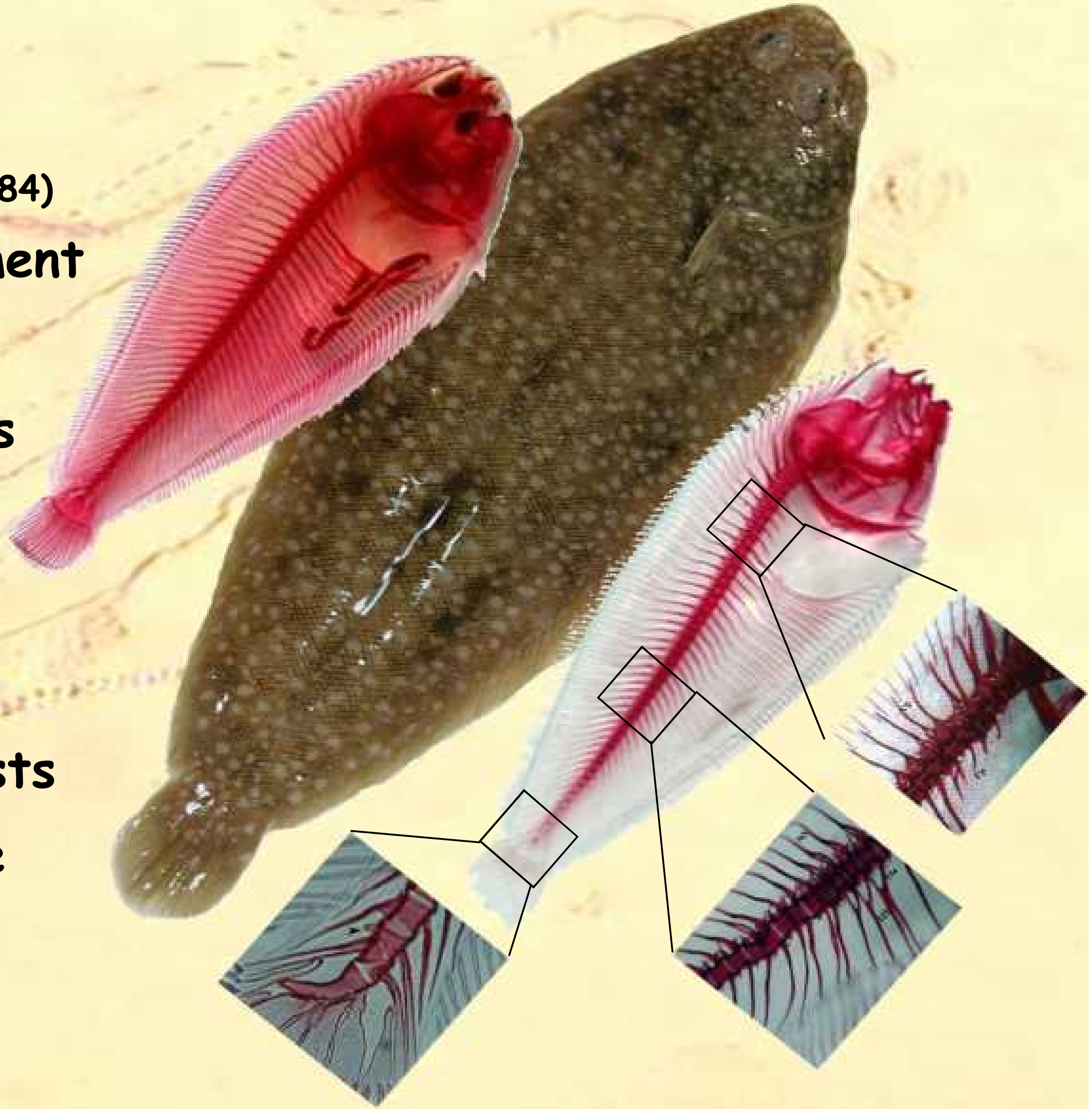
- Appear early in development
- Frequent in reared larva
- Reflect culture conditions
- Leads to:

-Decreased growth rate

-Increased mortality

-Increased prodution costs

-Decreased market price



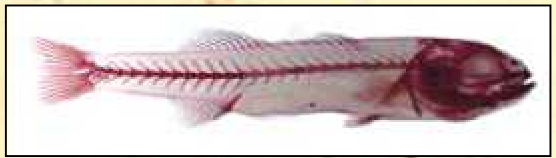
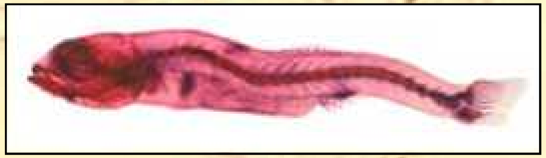
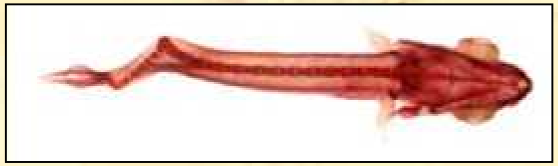
P.J. Gavaia (2004)

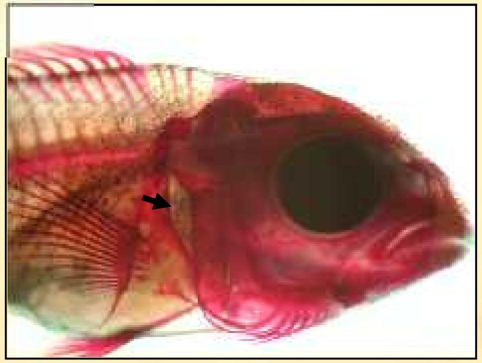
Skeletal malformation in mediterranean species

P.J. Gavaia (2003)

Types of skeletal malformations

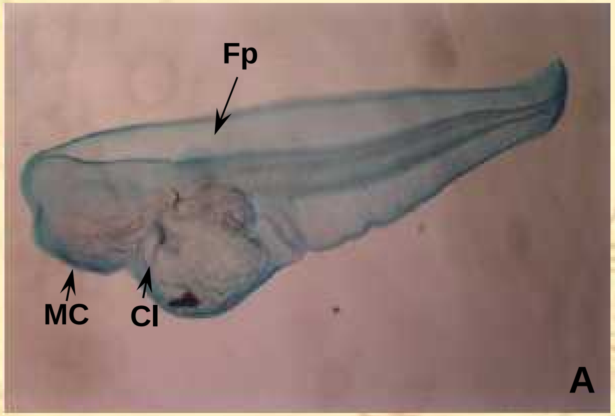
- Vertebral column :
  - Lordosis
  - Kifosis
- Vertebrae :
  - vertebral fusion
  - vertebral malformation
- Fins :
  - supra/subnumerary rays
  - fused rays/pterigophores)
- Mandibula
- Opercula

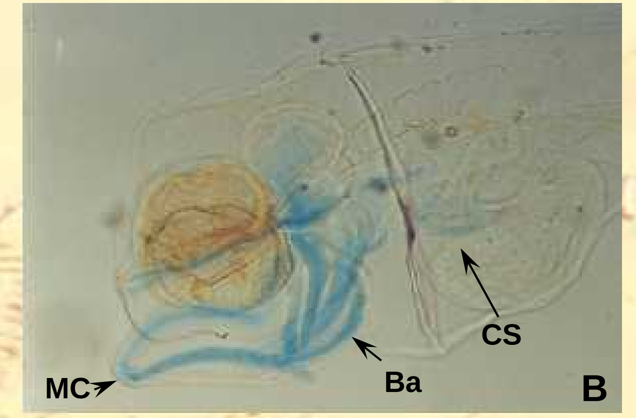


P.J. Gavaia (Unpublished)

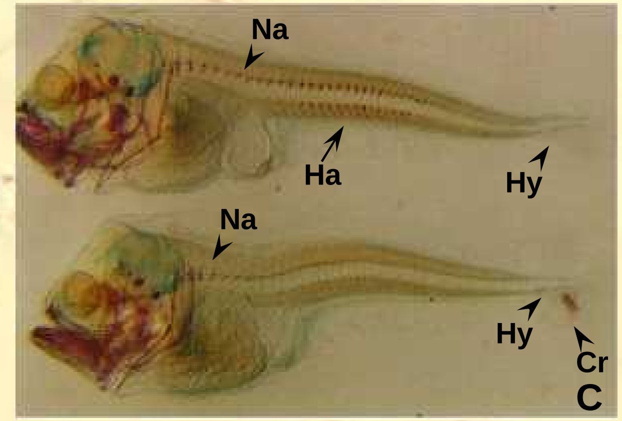
Senegal sole skeletal development



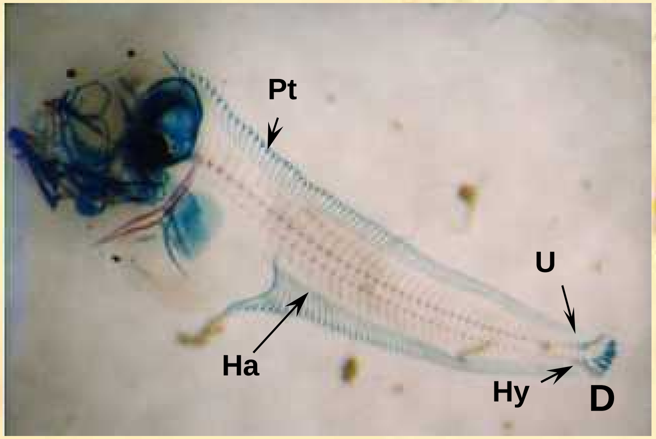
2 DPF



7 DPF



15 DPF



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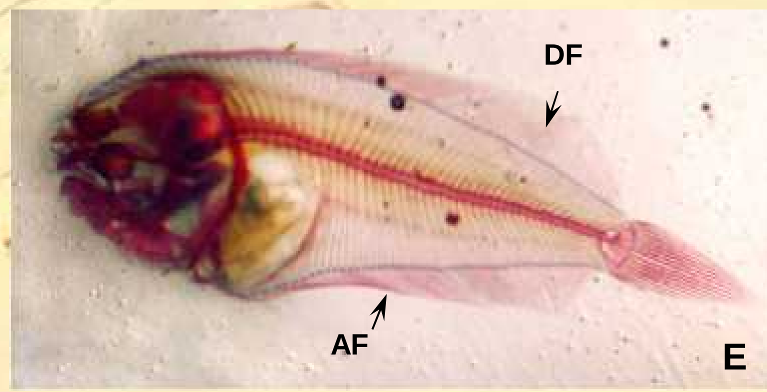
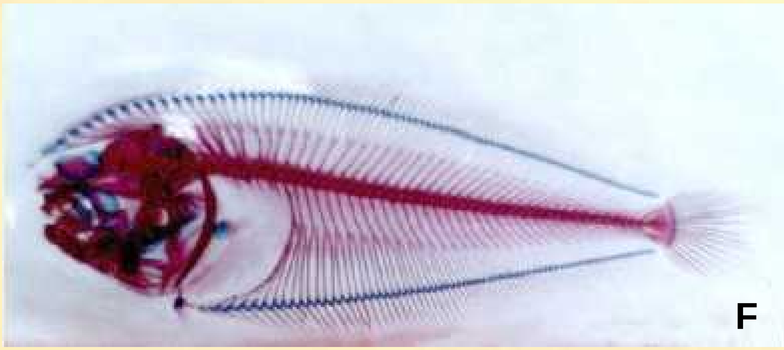


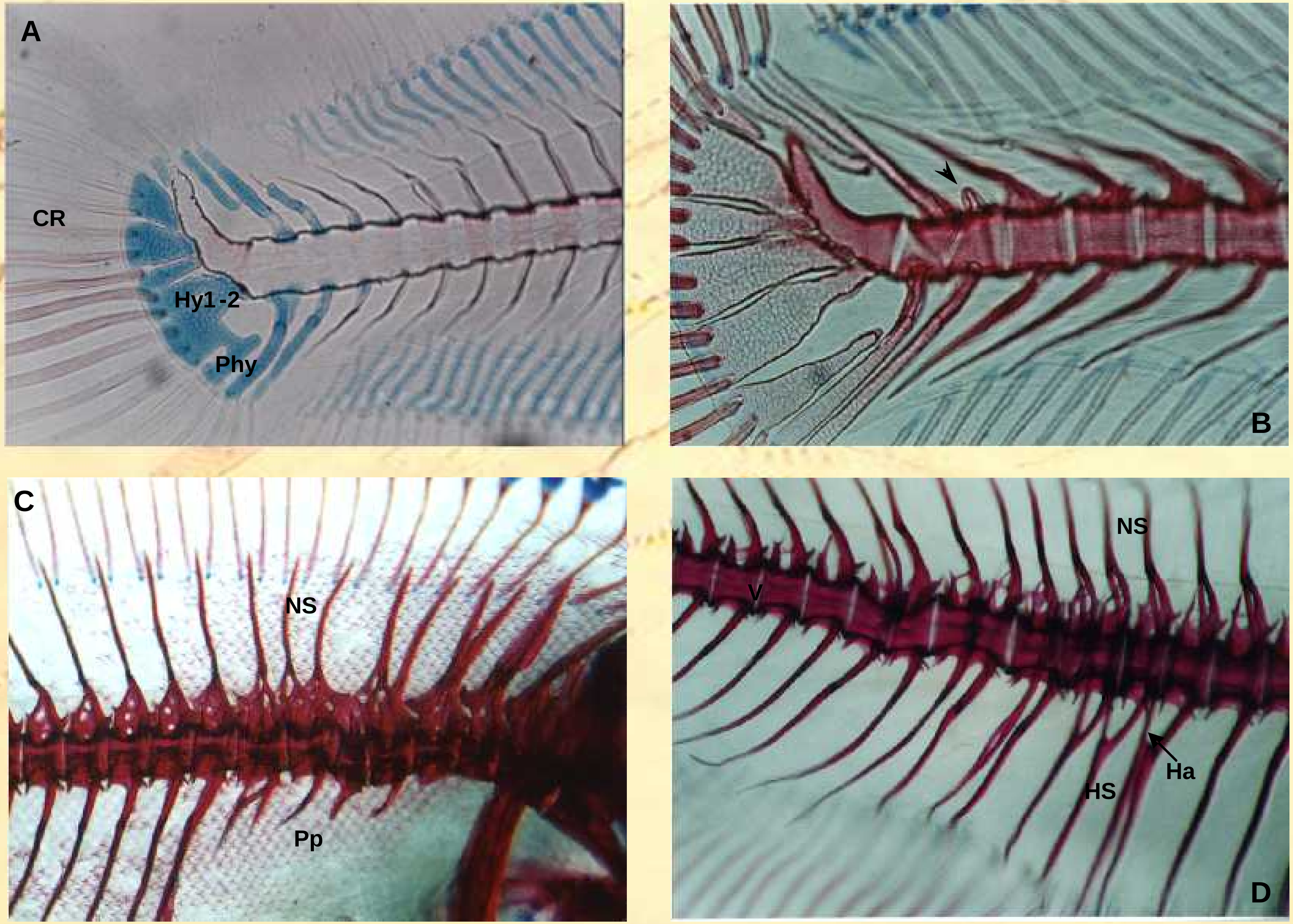
25 DPF



75 DPF

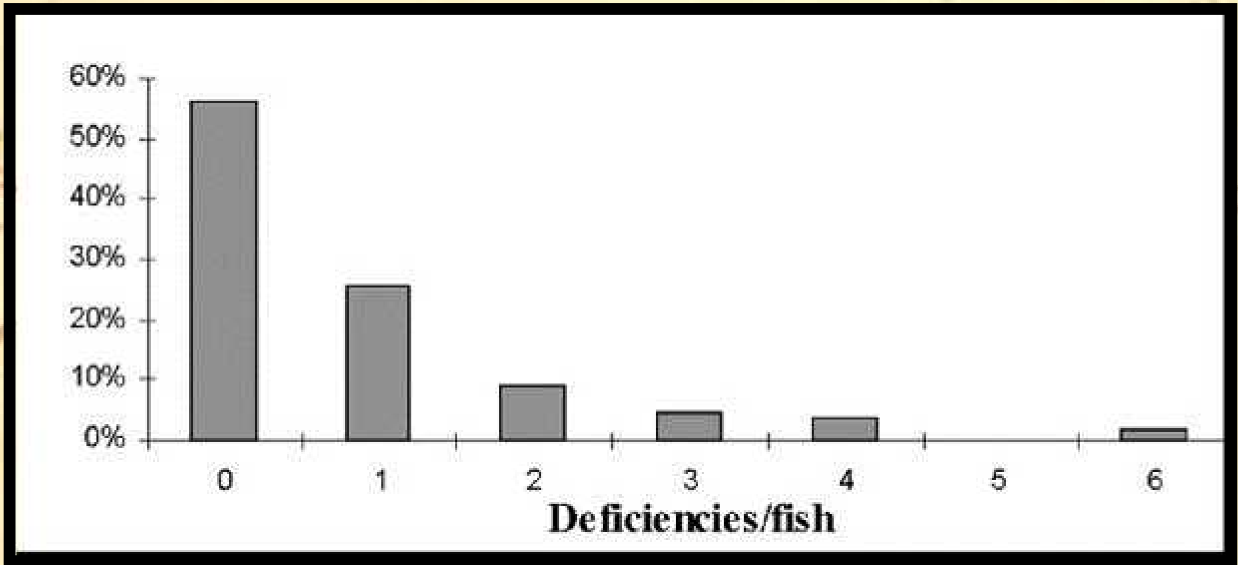
P.J. Gavaia et al. (2002). Aquaculture .

Senegal sole skeletal deformities

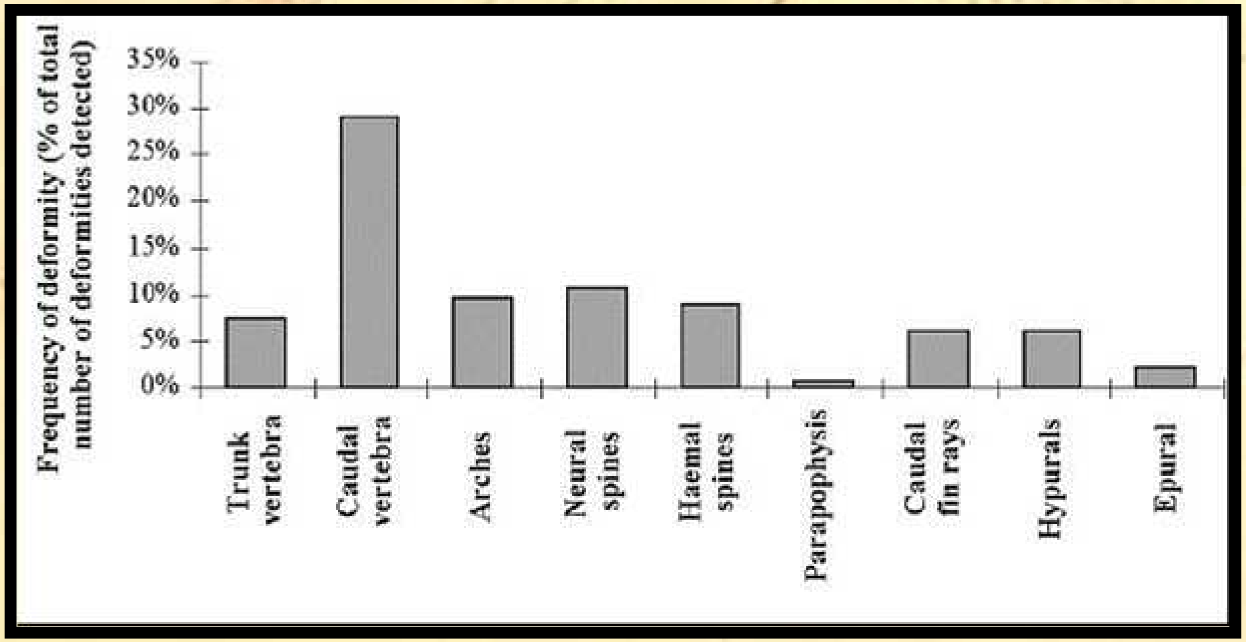


P.J. Gavaia et al. (2002). *Aquaculture* .

Senegal sole skeletal deformities



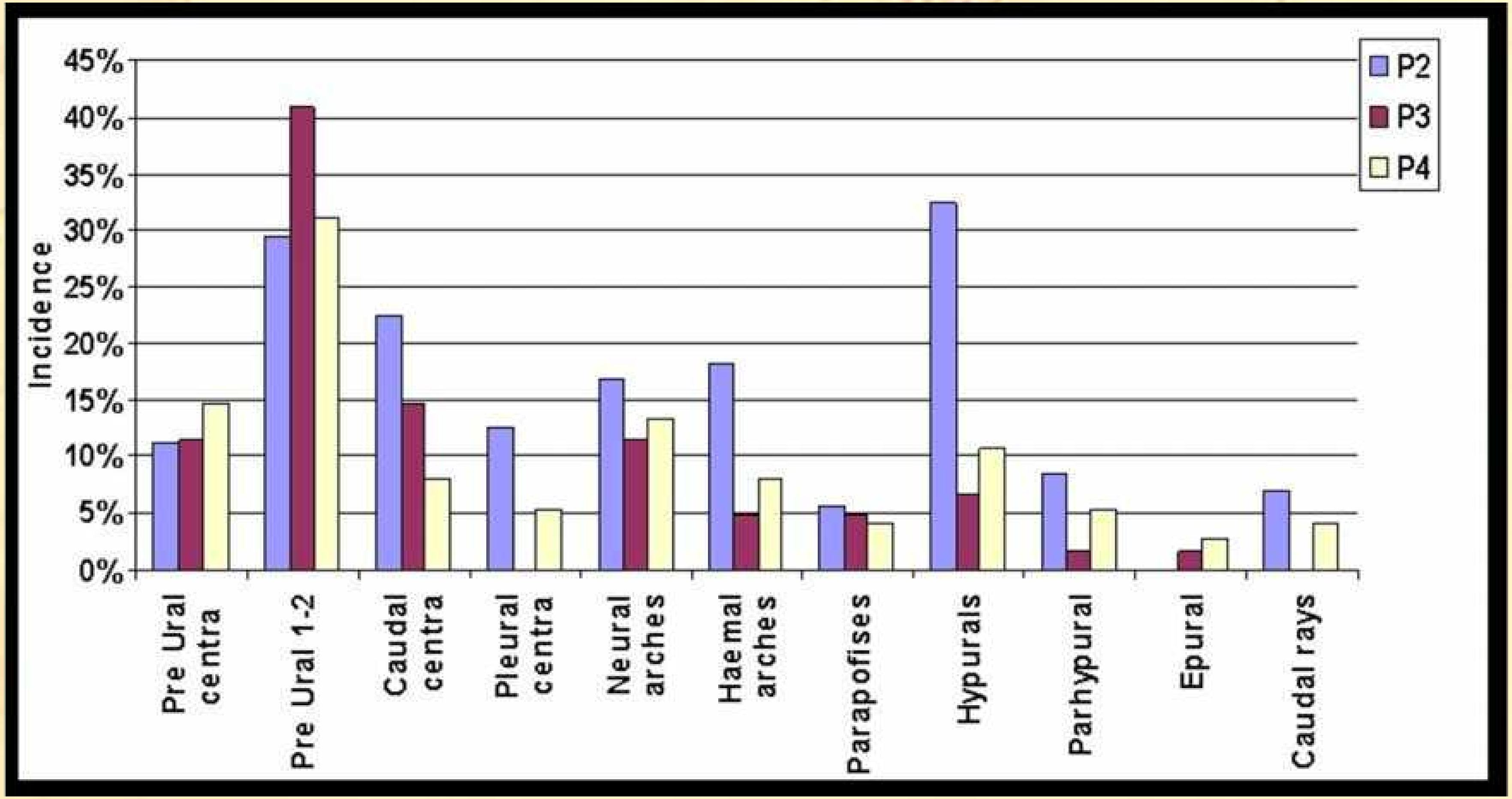
44% deformed fish



28% affect caudal vertebrae

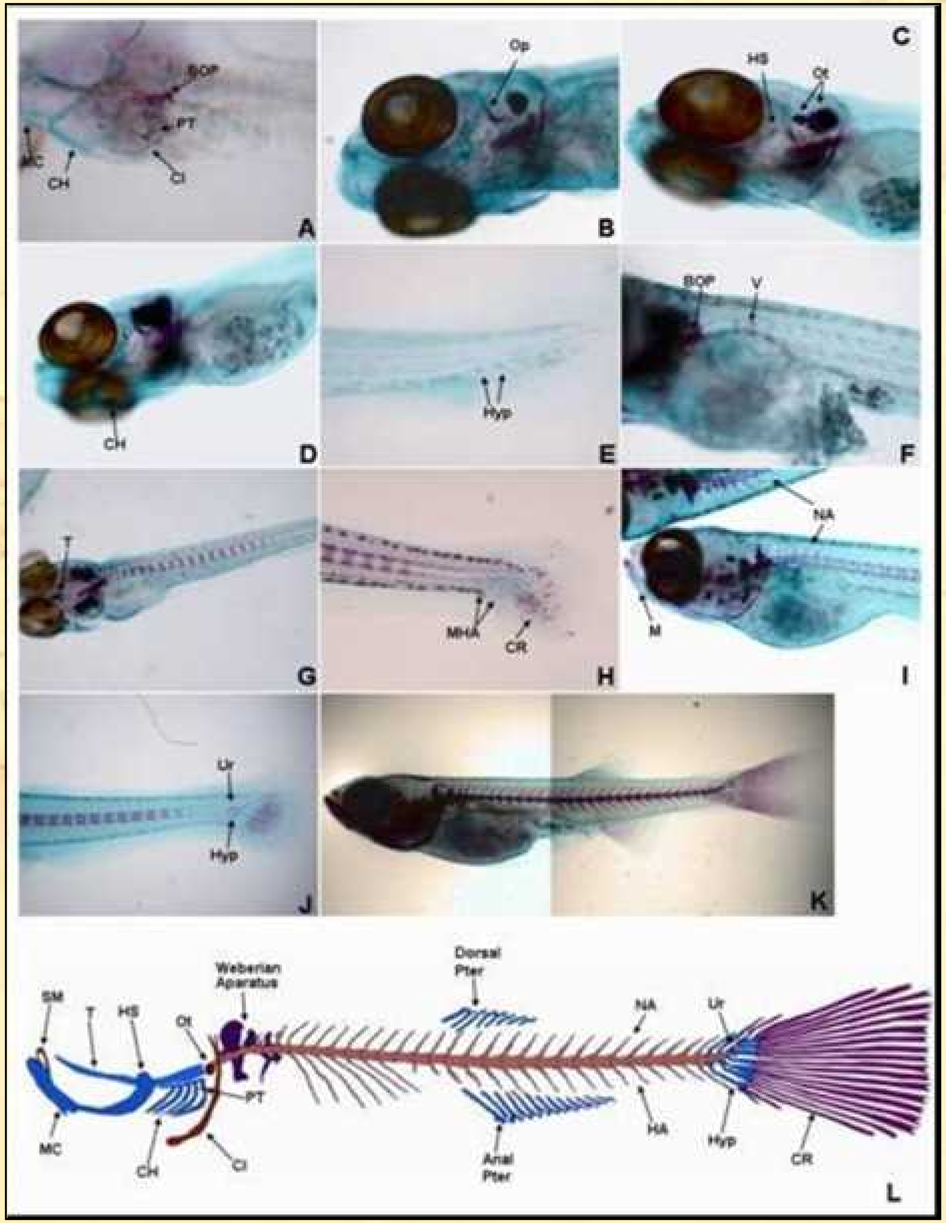
P.J. Gavaia et al. (2002). Aquaculture .

Senegal sole skeletal deformities



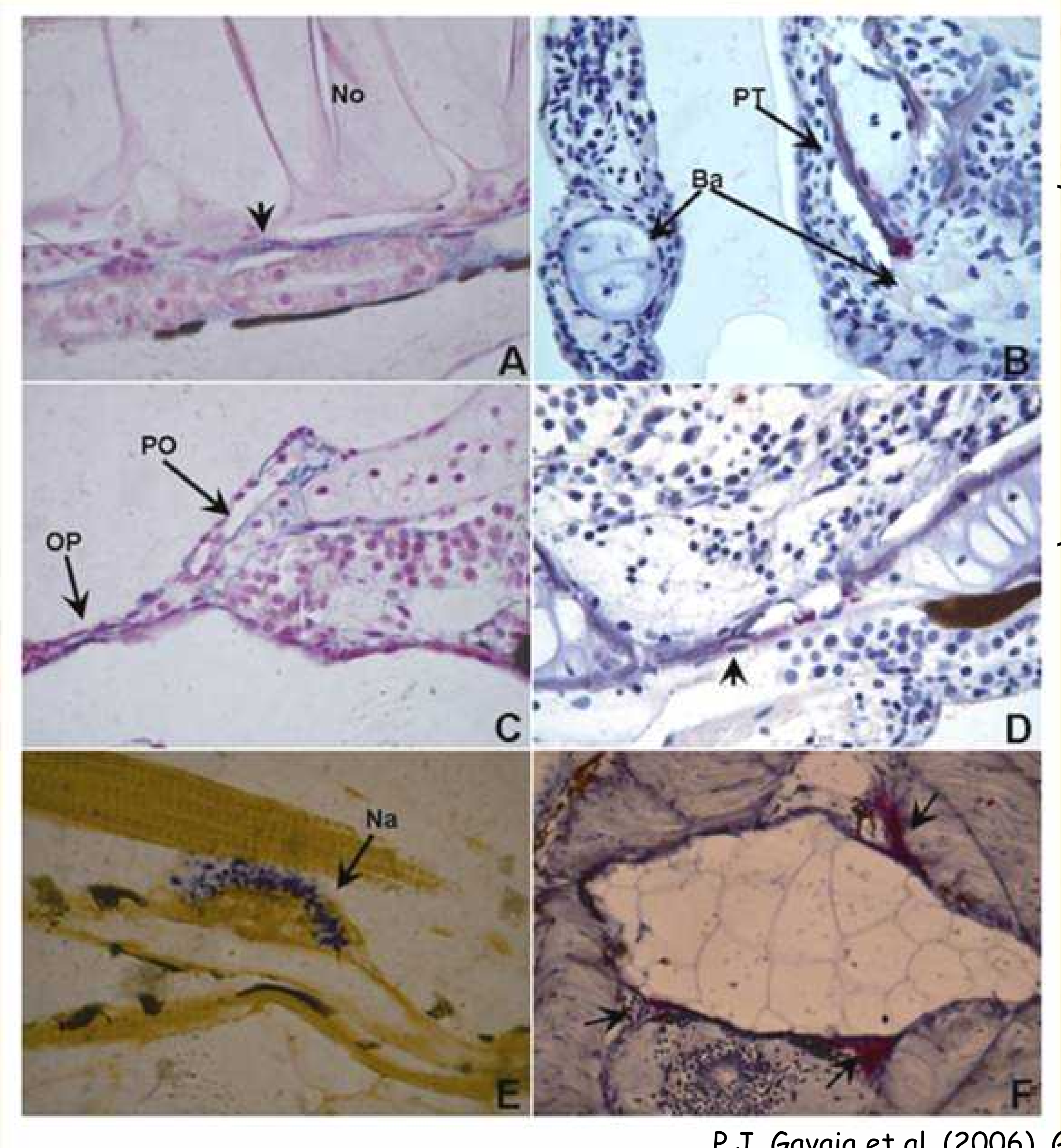
P.J. Gavaia et al. (2002). Aquaculture .

Skeletal development in *Danio rerio*



P.J. Gavaia et al. (2006). Gene Expression Patterns .

ALP and TRAP in skeletal development

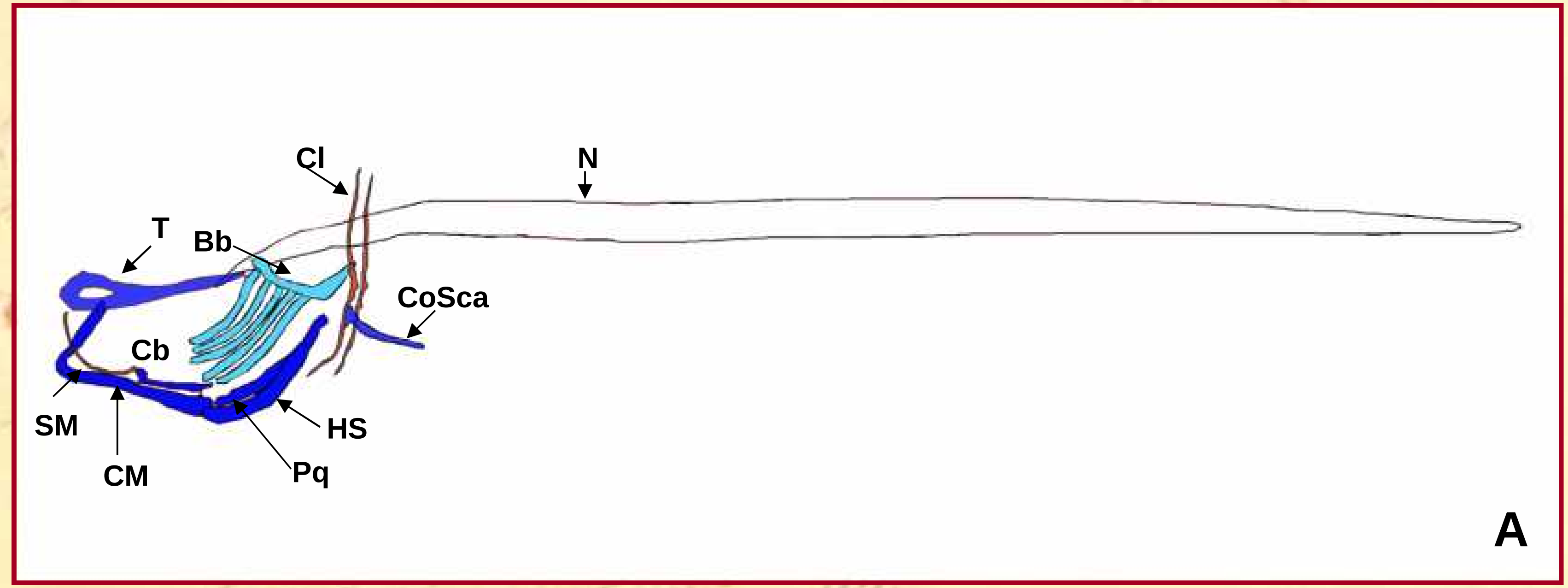


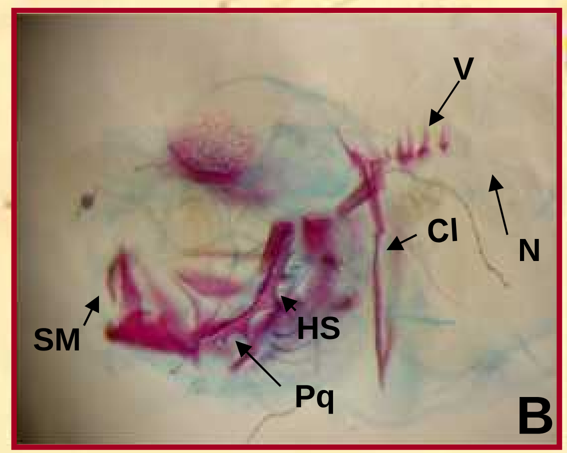
Zebrafish

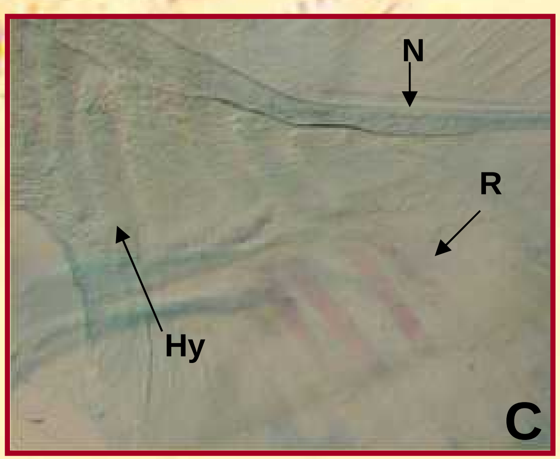
Senegal sole

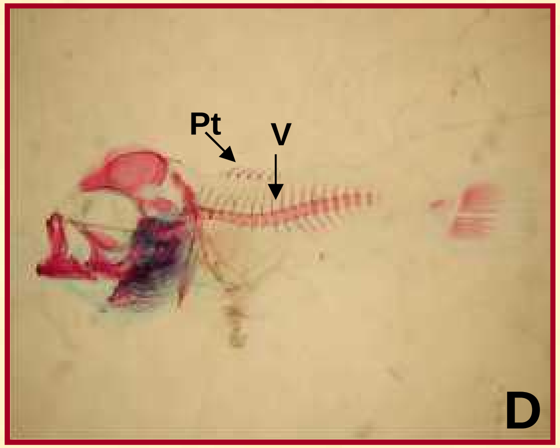
P.J. Gavaia et al. (2006). *Gene Expression Patterns* .

Skeletal development in *Pagrus auriga*



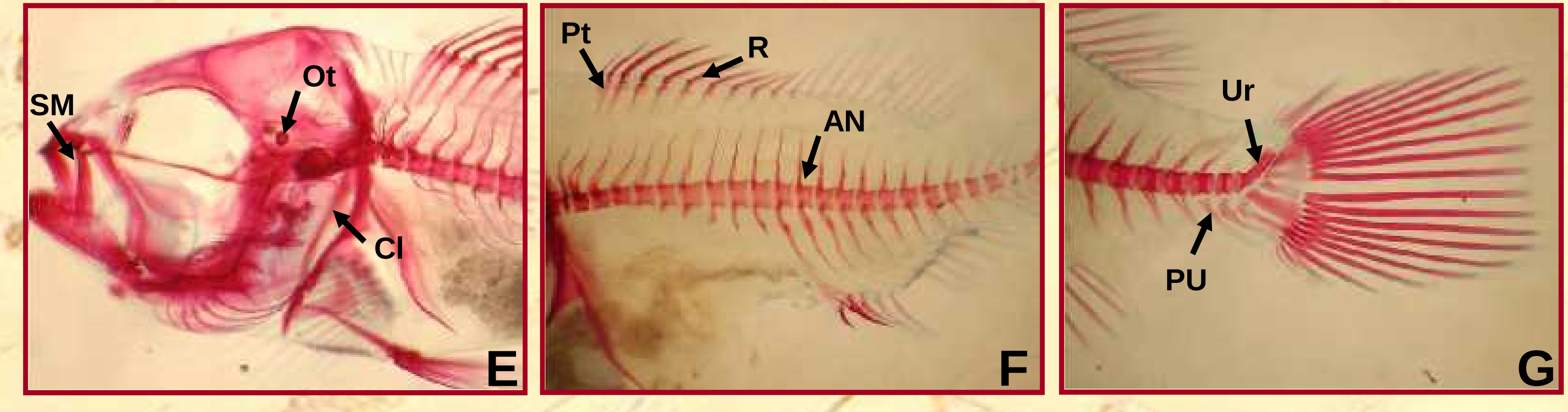


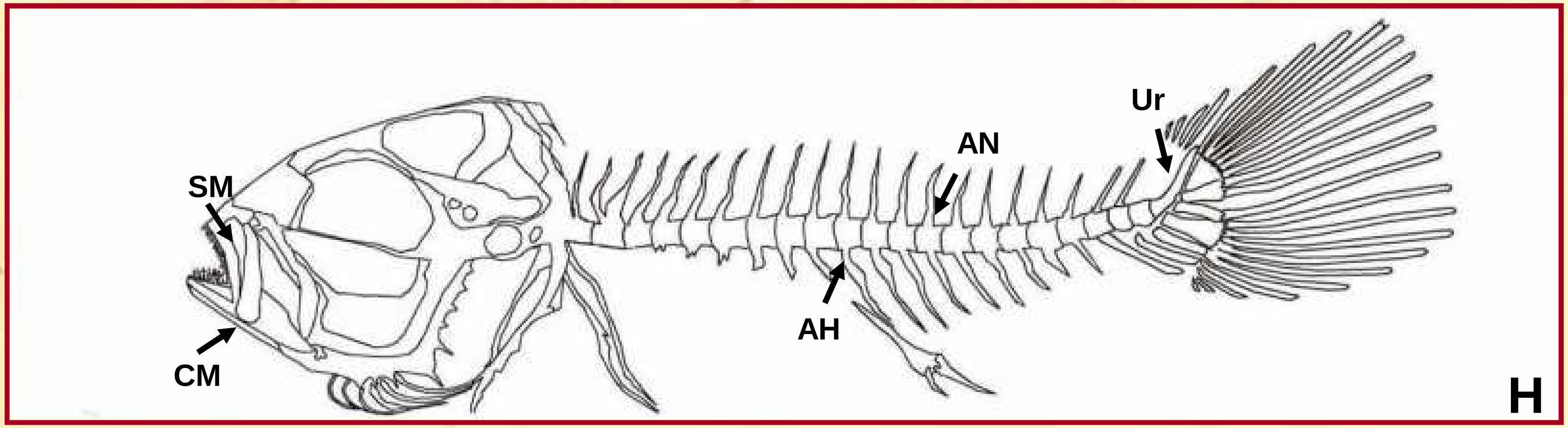




P.J. Gavaia et al. (2005).

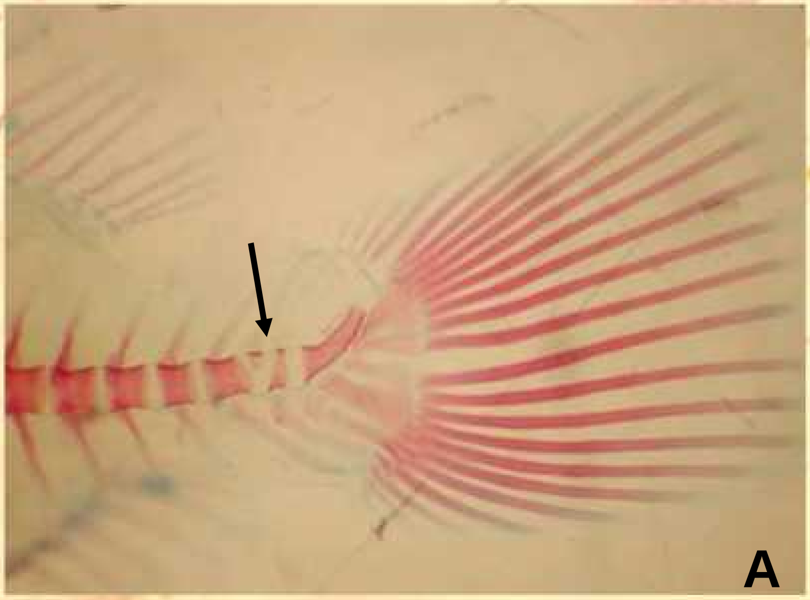
Skeletal development in *Pagrus auriga*

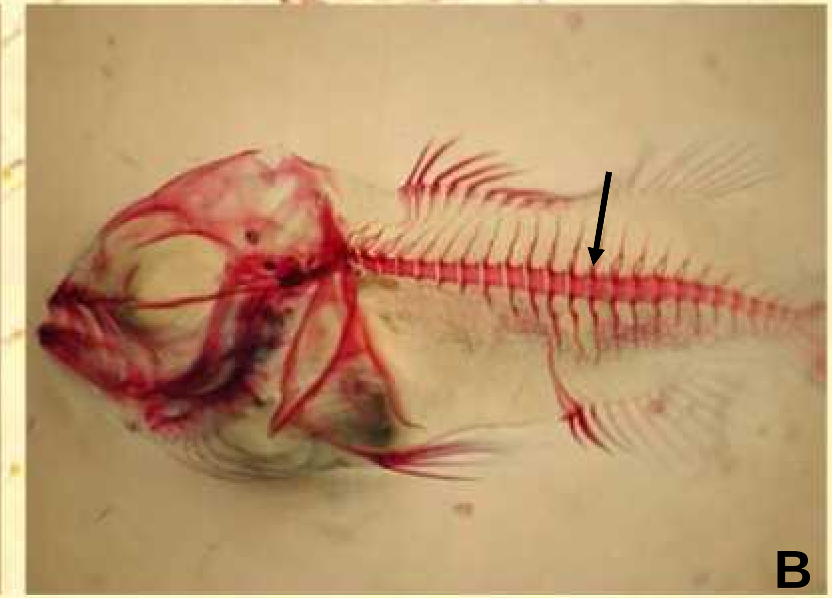




P.J. Gavaia et al. (2005).

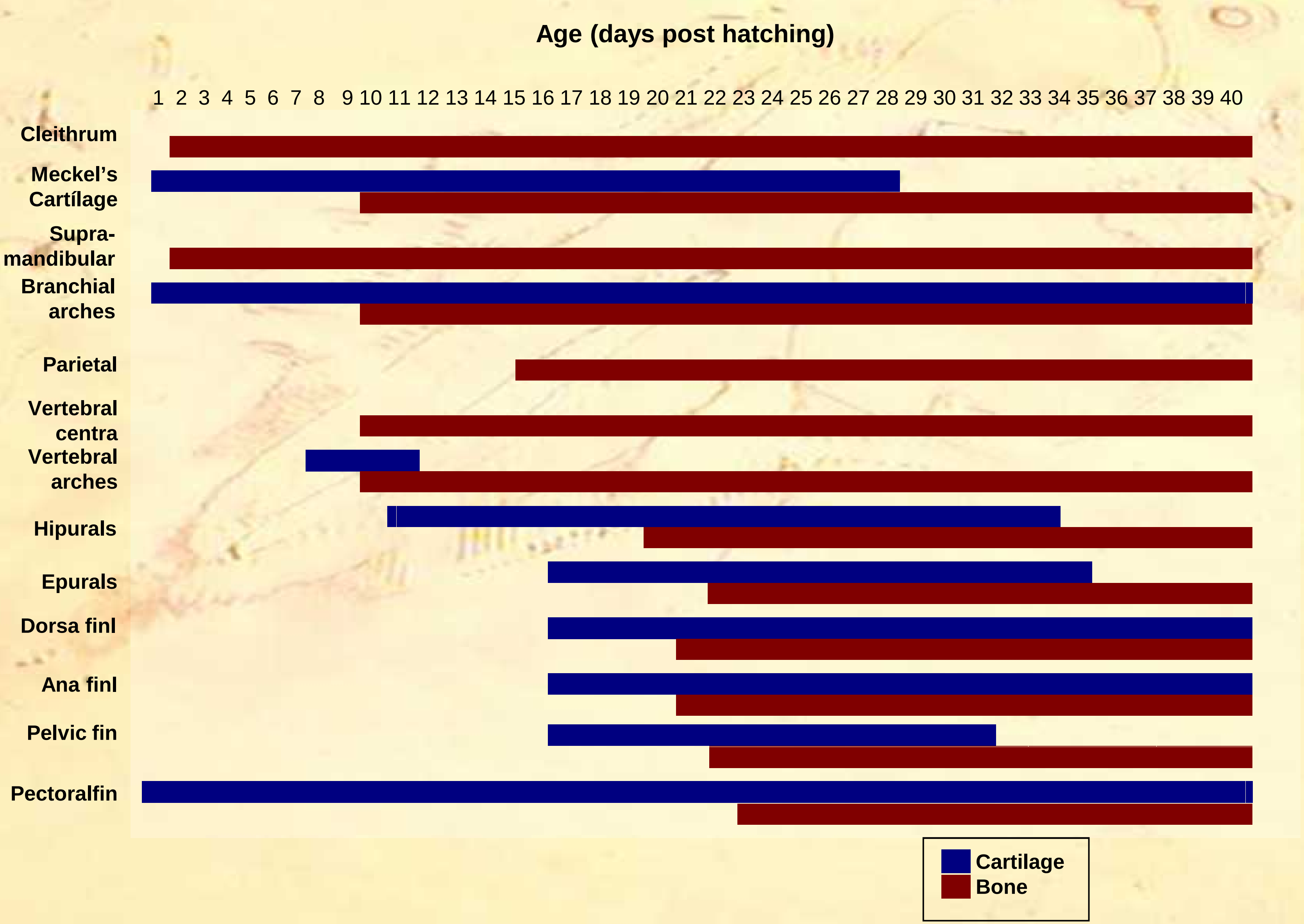
Skeletal deformities in *Pagrus auriga*





P.J. Gavaia et al. (2005).

Time course of skeletal development in *Pagrus auriga*

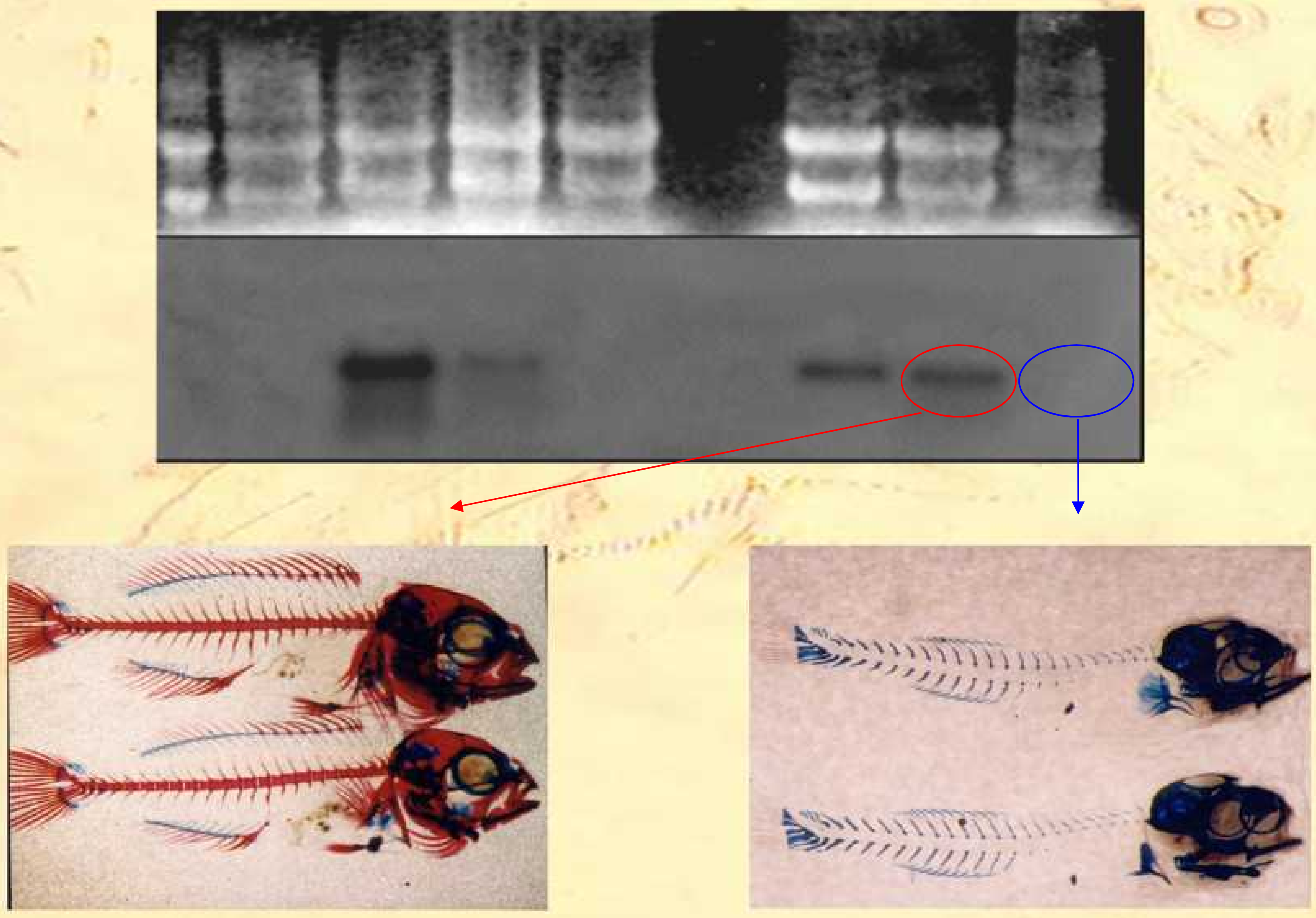


P.J. Gavaia et al. (2005).

BGP vs. MGP

	BGP	MGP
Tissue distribution	Bone, teeth	Cartilage, kidney, lung, aorta, tooth
Cellular expression	Osteoblasts, odontoblasts	Immature and hypertrophic chondrocytes, vascular smooth muscle cells (VSMC), endothelial cells, pneumocytes, kidney cells, fibroblasts, cementoblasts
Sites of accumulation	Calcified extracellular matrix (ECM)	Calcified ECM of cartilage, bone, dentin and pathological calcifications
Time of appearance	After onset of mineralization	Early development
Marker gene	Osteoblastic function and differentiation, bone formation	Chondrogenic differentiation

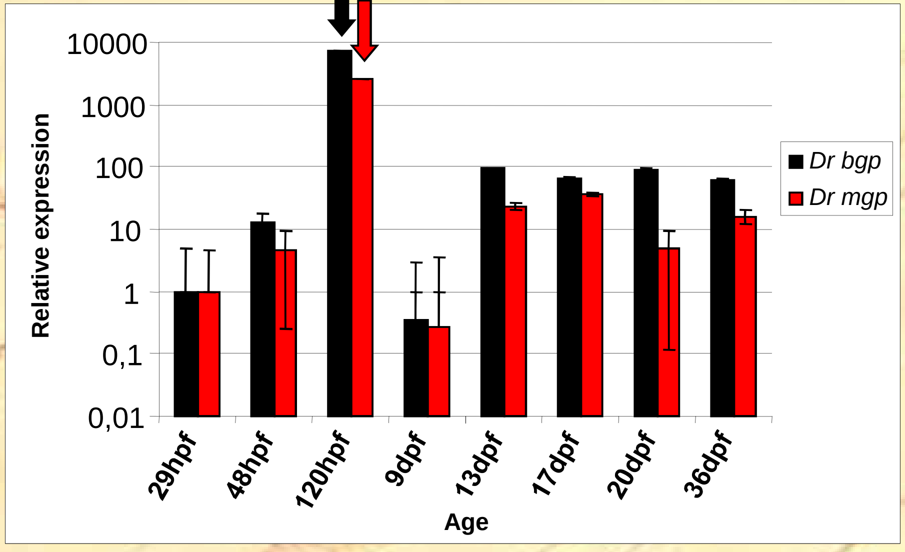
Spatio-temporal pattern of *bgp* expression

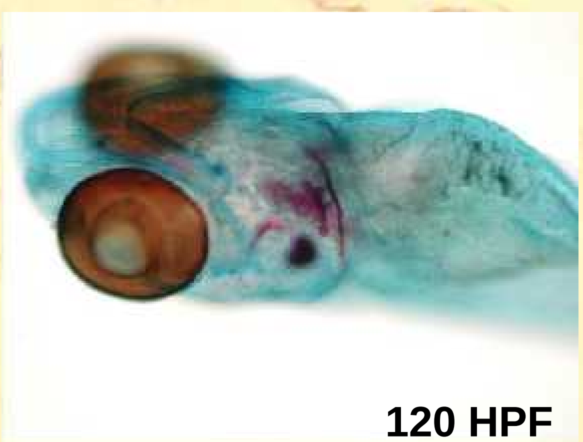


Pinto et al. (2001). Gene.

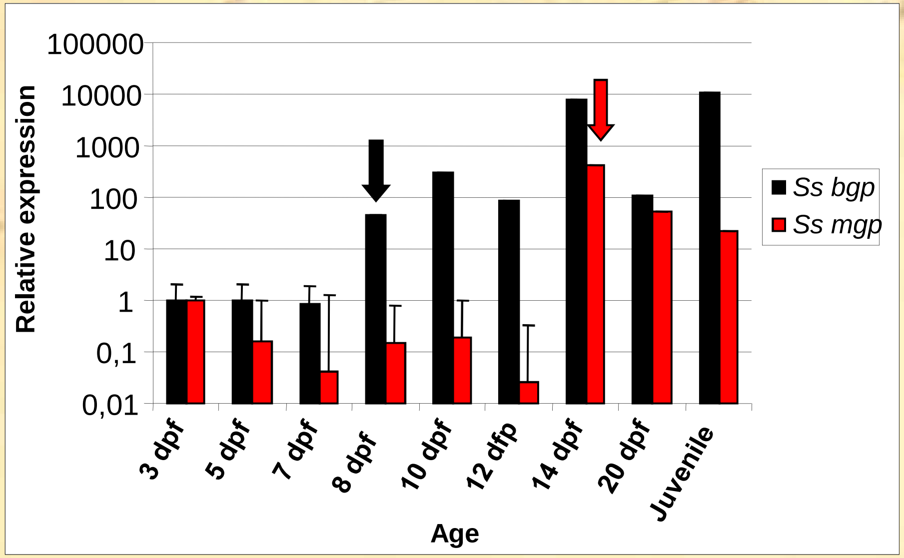
bgp / mgp expression during development

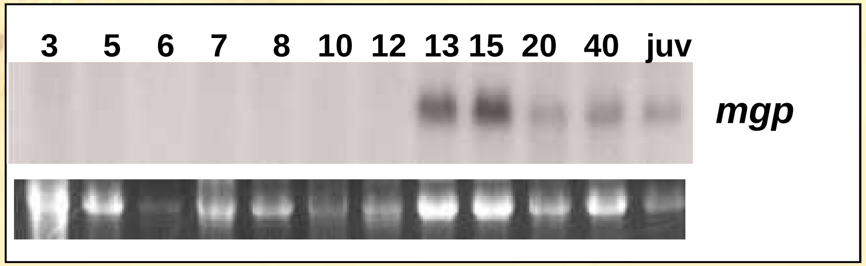
Zebrafish

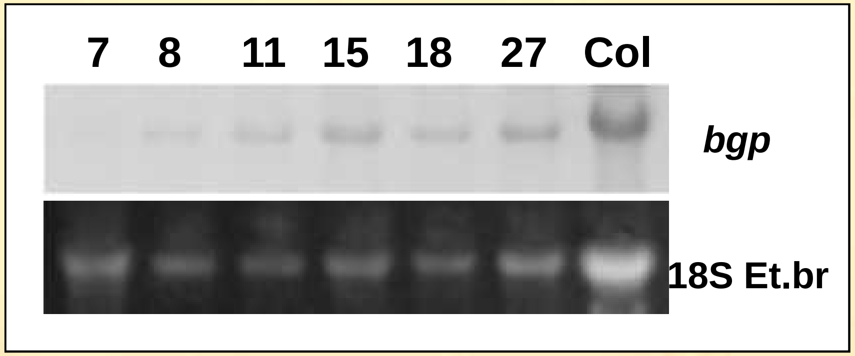




Senegal sole

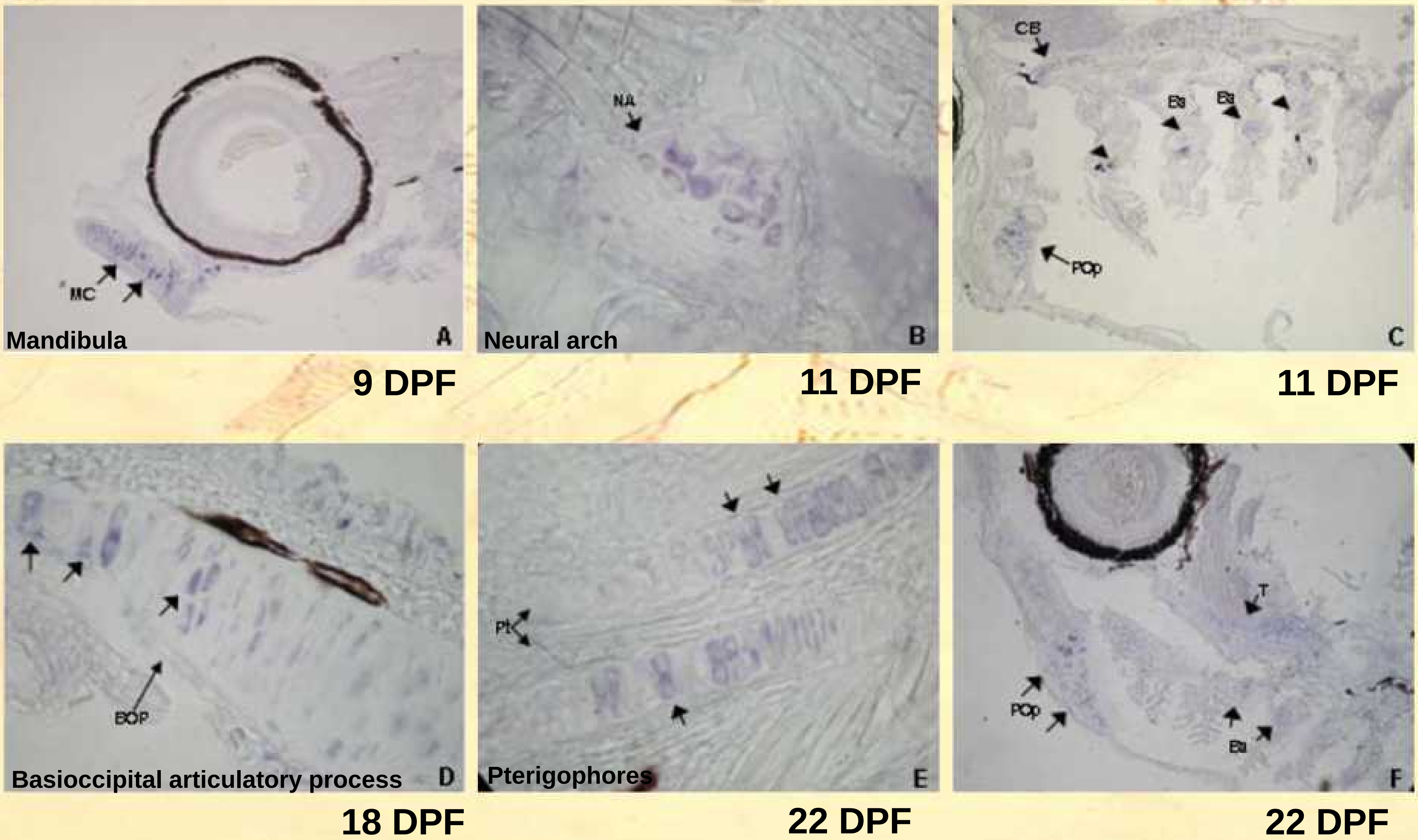






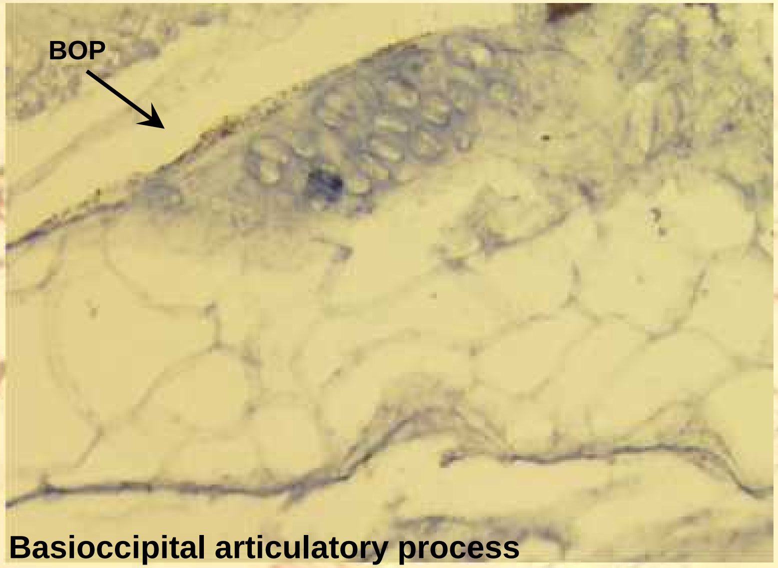
P.J. Gavaia et al. (2006). Gene Expression Patterns .

In situ localization of zebrafish *bgp* mRNA

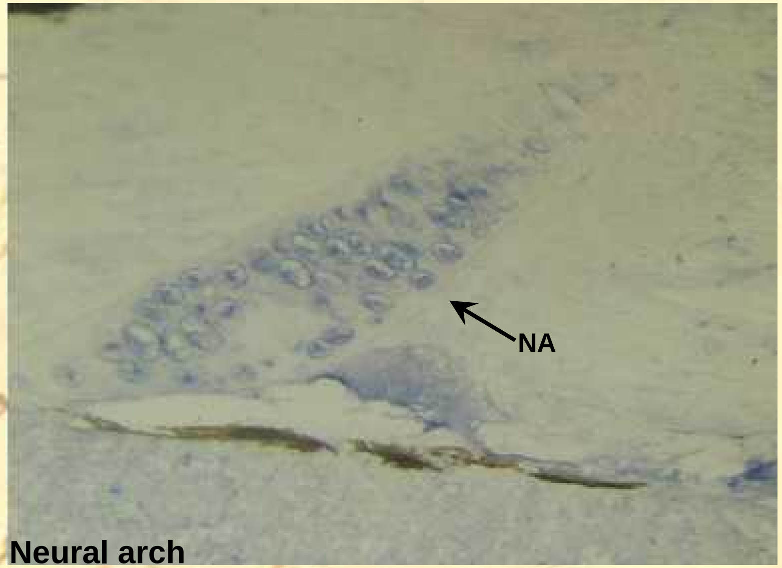


P.J. Gavaia et al. (2006). Gene Expression Patterns .

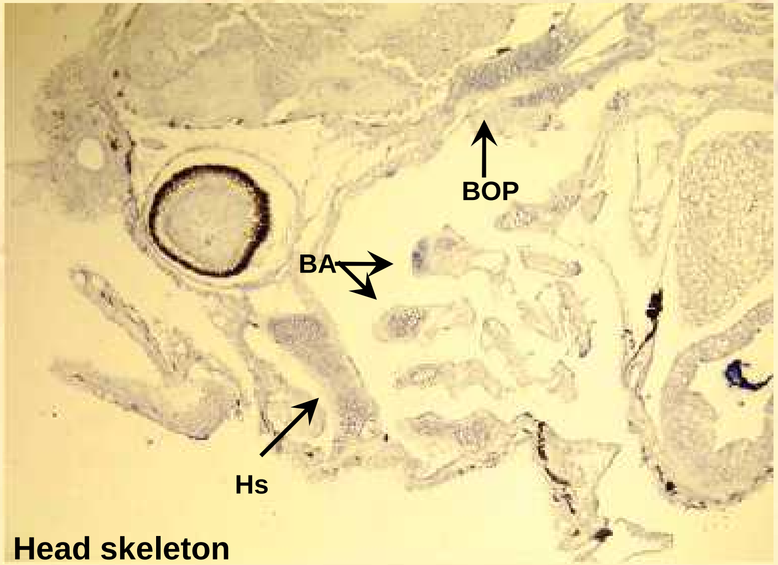
In situ localization of sole *bgp* mRNA



15 DPF



17 DPF



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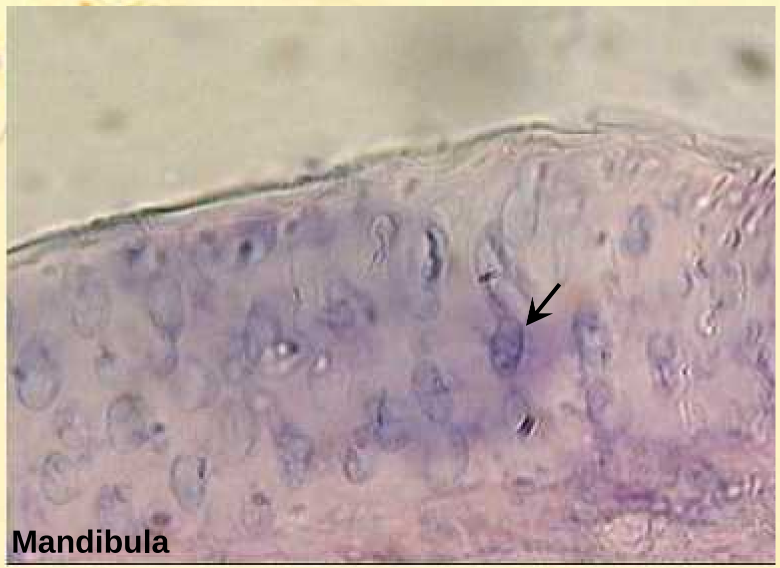
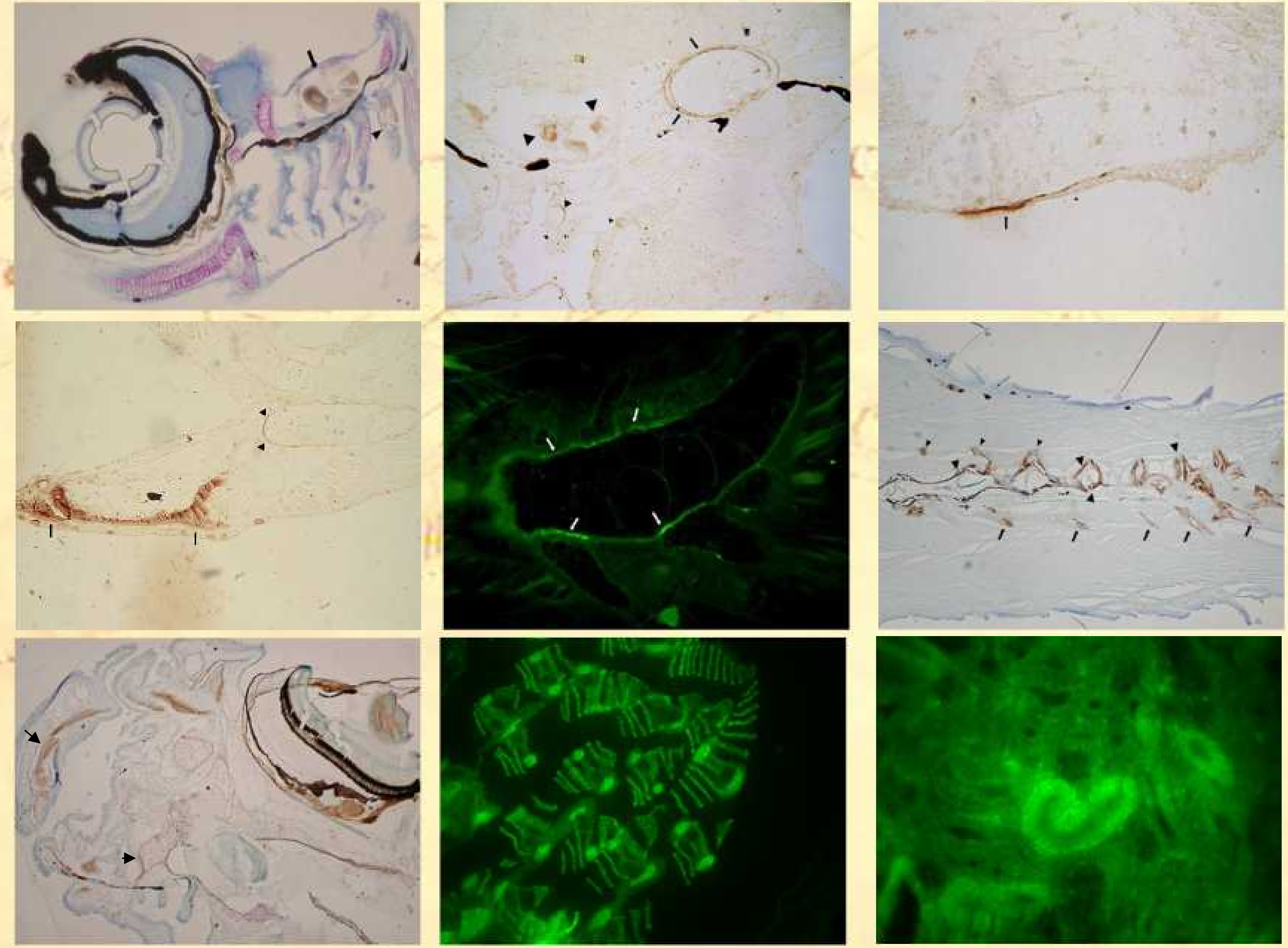


56 DPF

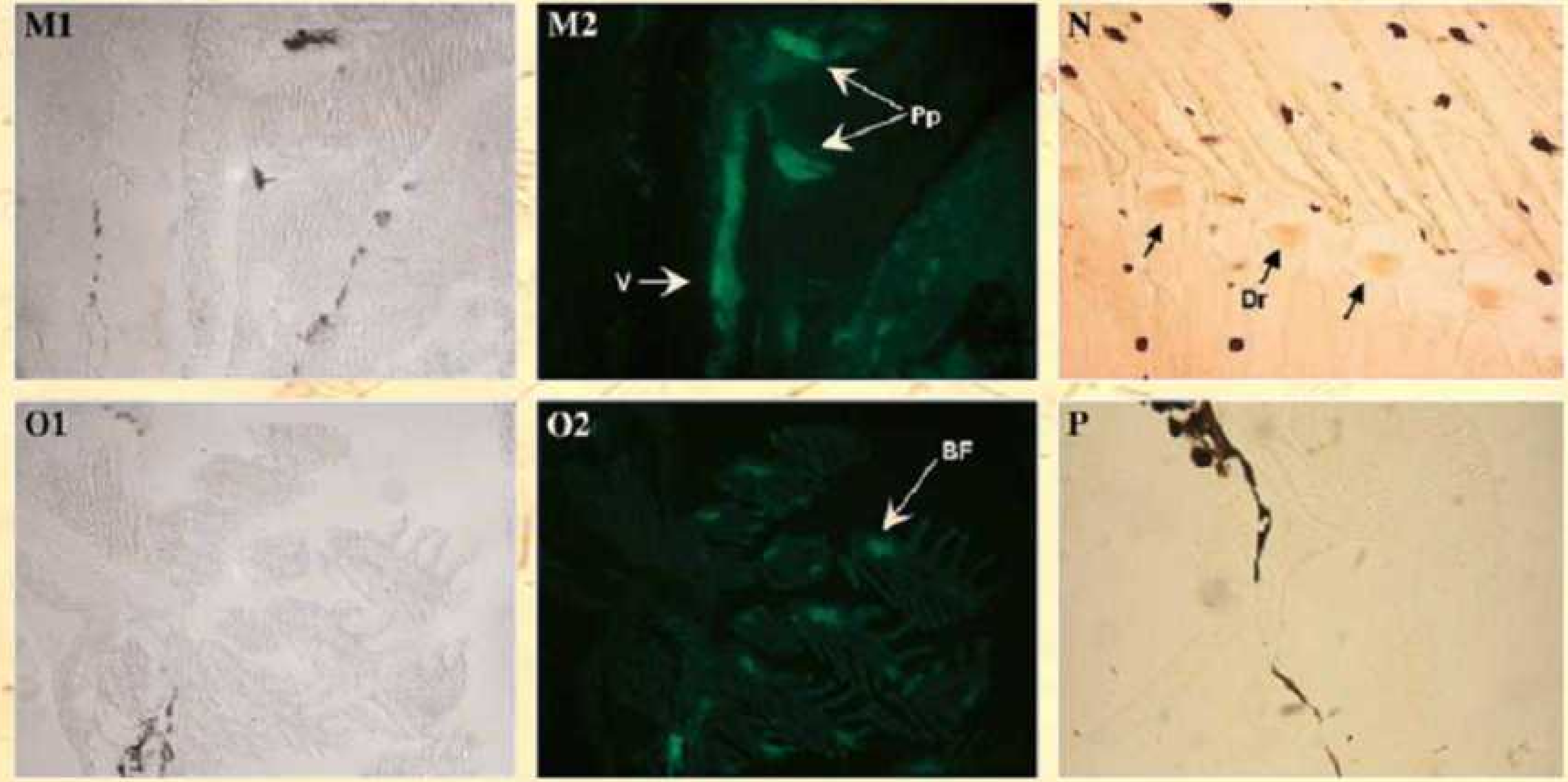
P.J. Gavaia et al. (2006). *Gene Expression Patterns* .

Immuno-detection of Bgp accumulation in zebrafish



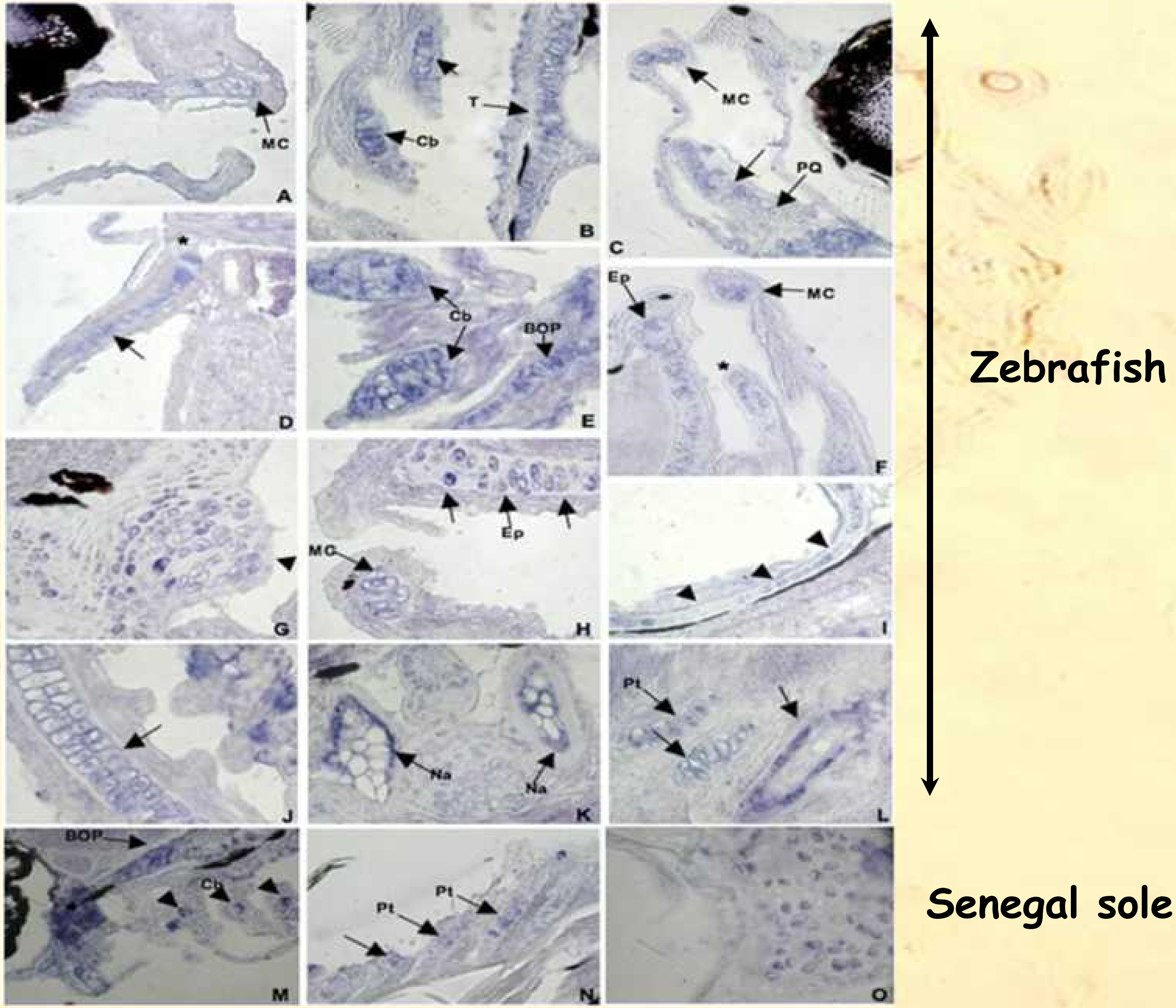
P.J. Gavaia et al. (2006). *Gene Expression Patterns*.

Immuno-detection of Bgp accumulation in sole



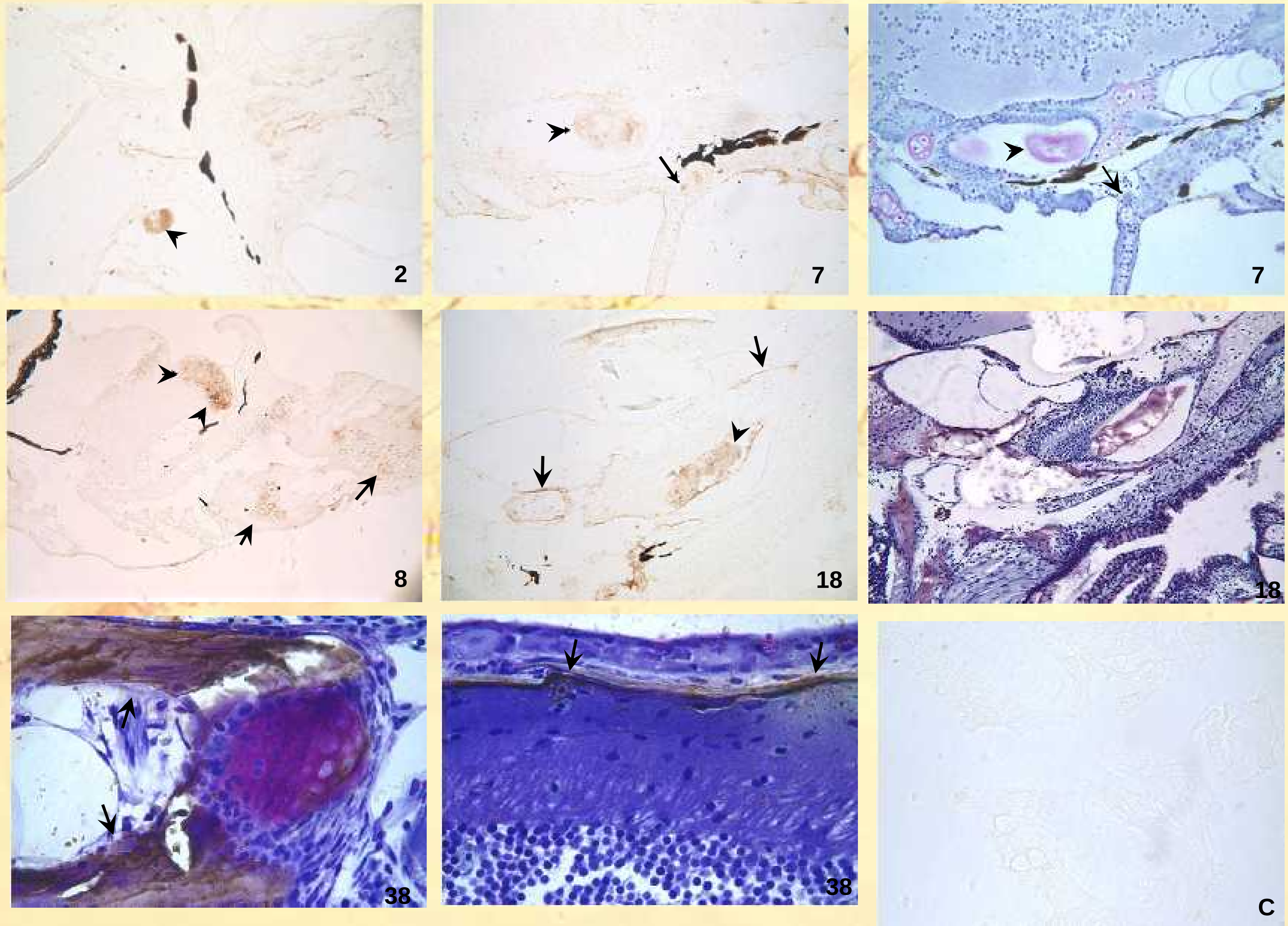
P.J. Gavaia et al. (2006). Gene Expression Patterns .

In situ localization of zebrafish and sole *mgp* mRNA



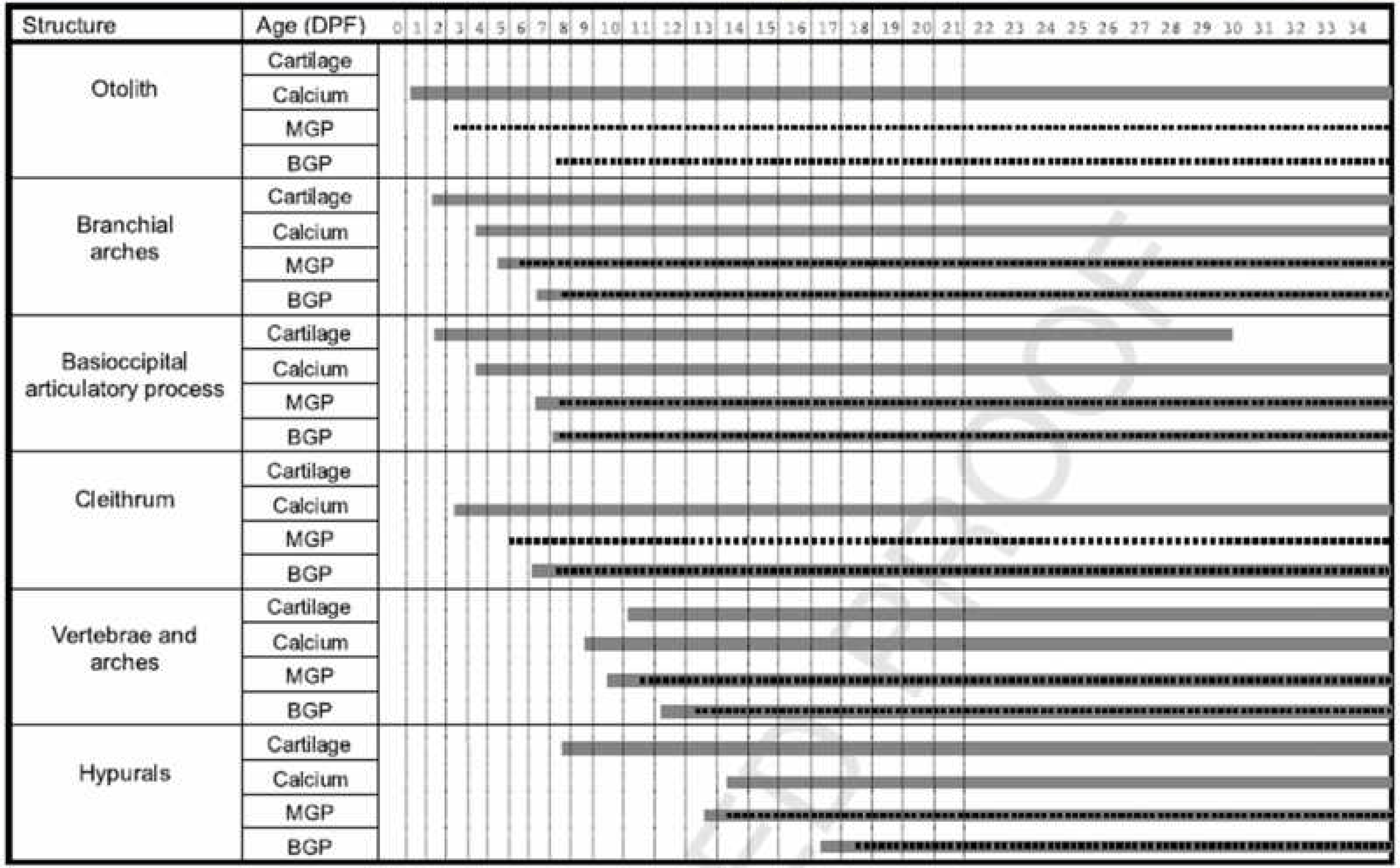
P.J. Gavaia et al. (2006). Gene Expression Patterns

Immuno-detection of MGP accumulation in zebrafish



P.J. Gavaia et al. (2006). Gene Expression Patterns .

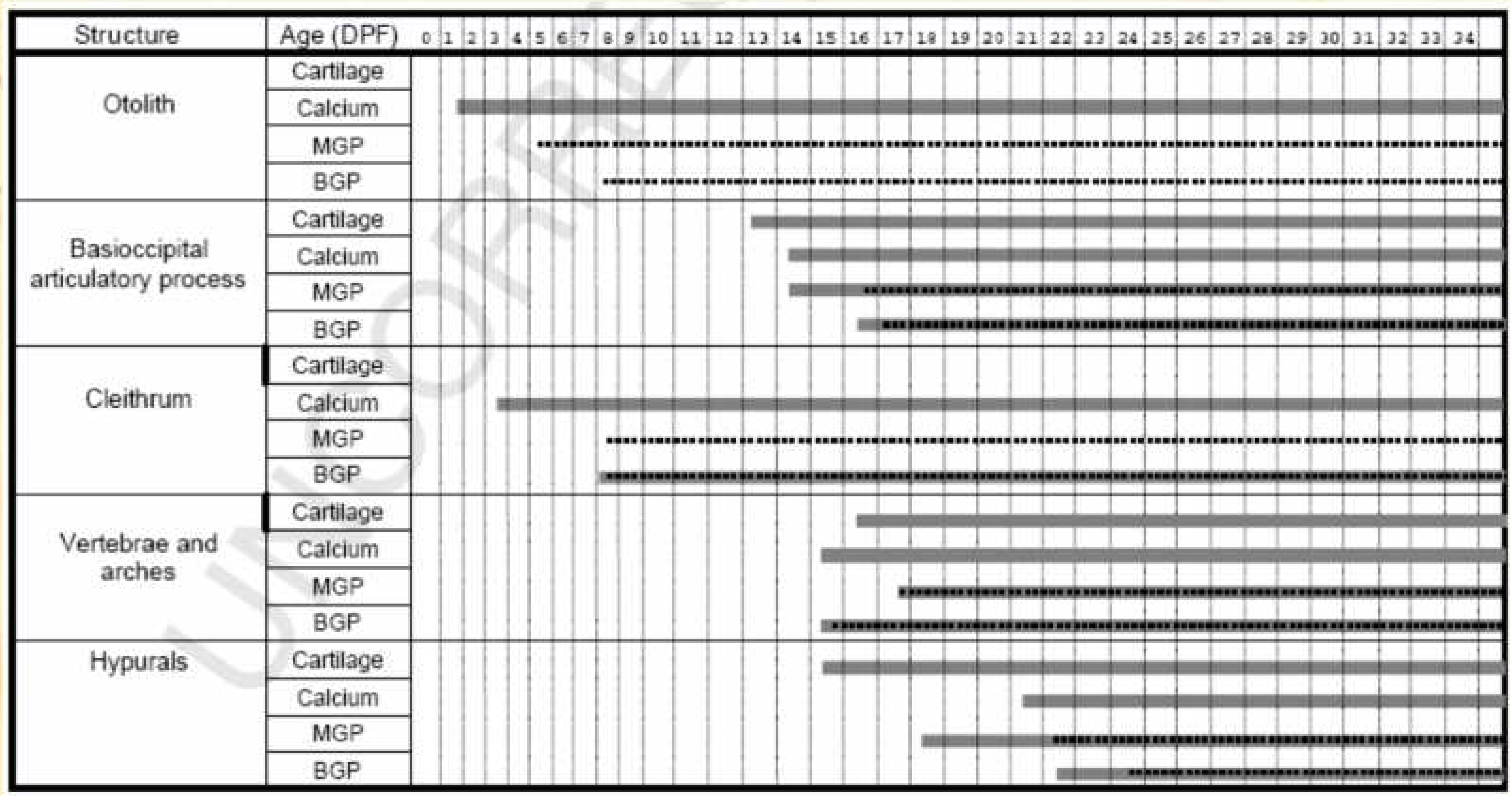
Summary of zebrafish skeletogenesis



In situ hybridization (▬) and immunolocalization (···)

P.J. Gavaia et al. (2006). Gene Expression Patterns .

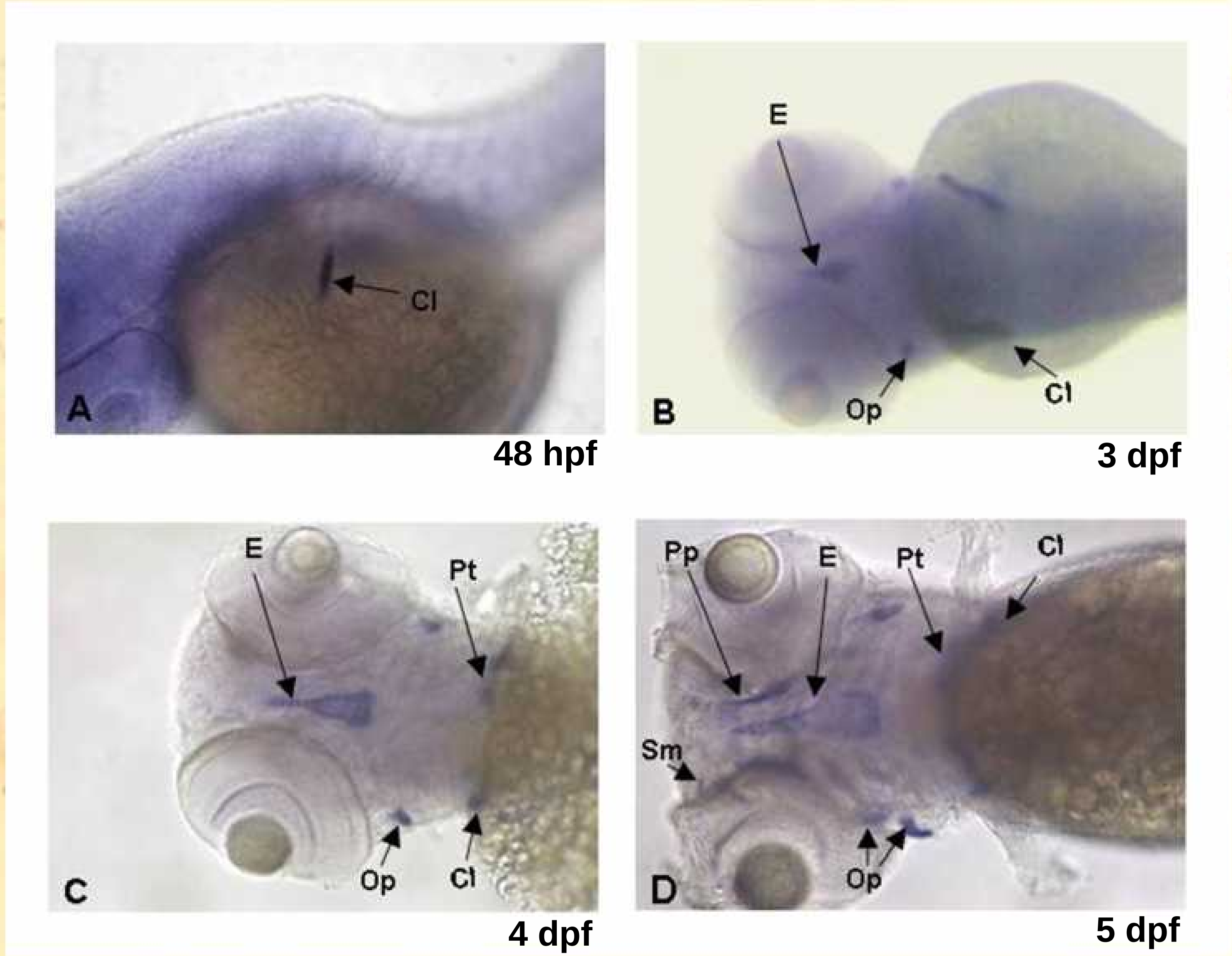
Summary of Senegal sole skeletogenesis



In situ hybridization (▬) and immunolocalization (···)

P.J. Gavaia et al. (2006). Gene Expression Patterns .

In situ localization of zebrafish CollX~1 mRNA



Simões et al. (2006). Under publication

Conclusions

- Skeletogenesis in fish starts early in embrionic development
- First calcified structures are normally associated to feeding and movement
- Formation of bony skeletal structures occurs by both endochondral and intramembranous ossification
- The formation of the axial skeleton begins later in larval life and parallels the formation of unpaired fins

FISH BONE HAS DIFFERENT CHARACTERISTICS FROM OTHER VERTEBRATES

Conclusions

- High rates of deformities are observed in cultured fish
- Future work should focus on understanding the mechanisms responsible for the incidence of malformations on early larval stages, in order to reduce their appearance
- Marker *gene* expression can be used to follow normal development and characterize the developing stuctures
- Abnormal *gene* expression and/or protein accumulation may reflect early malformations and therefore be used as indicators