

Expression of Gla proteins during fish skeletal development

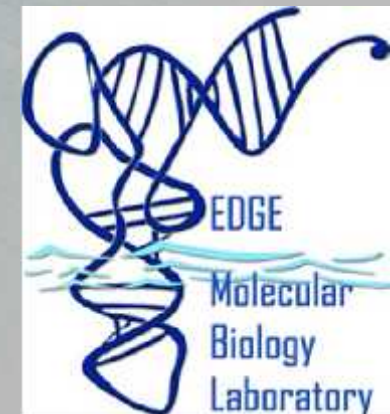
Paulo Gavaia



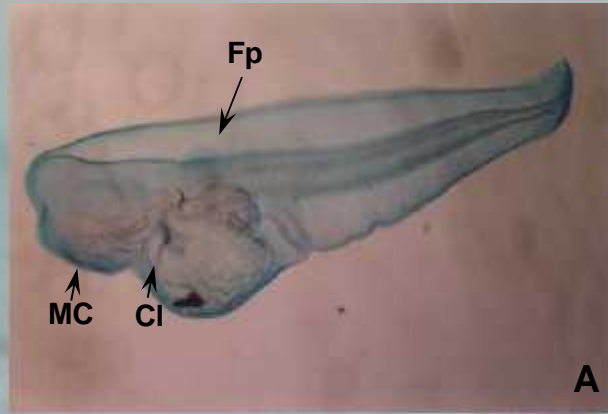
University of Algarve



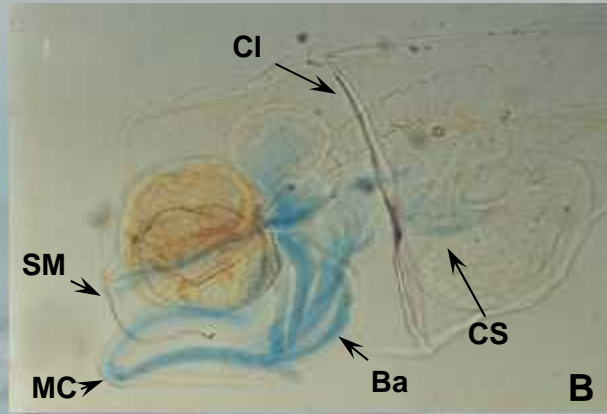
Center for Marine Sciences



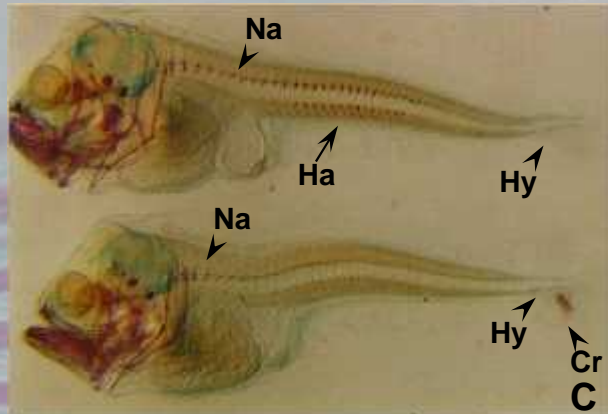
Senegal sole skeletal development



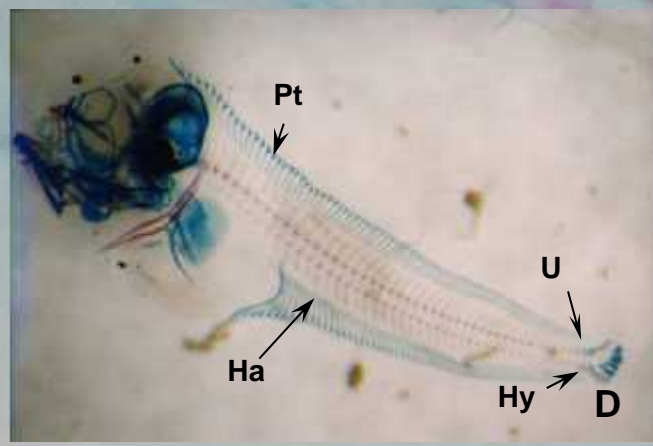
2 DPF



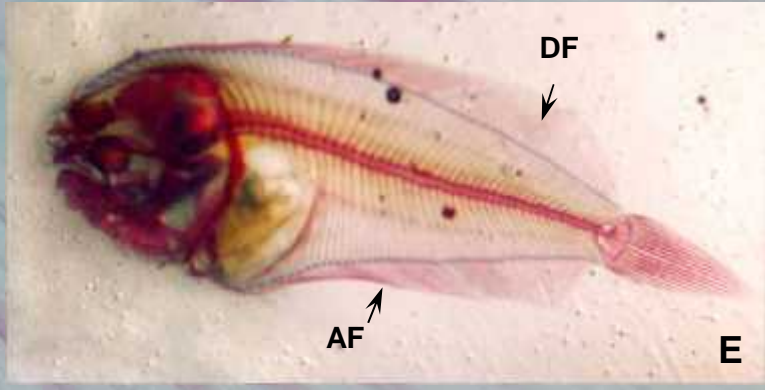
7 DPF



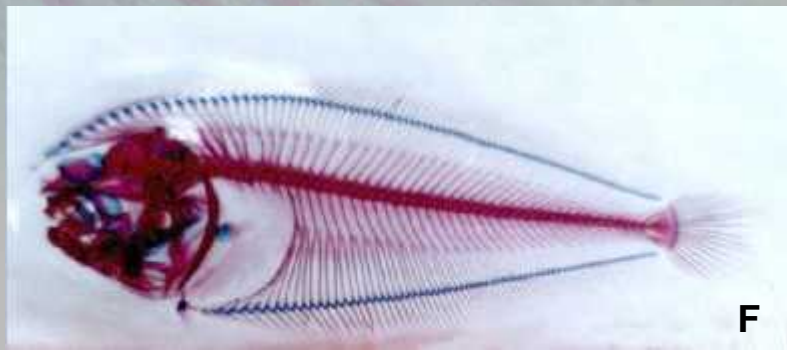
15 DPF



17 DPF



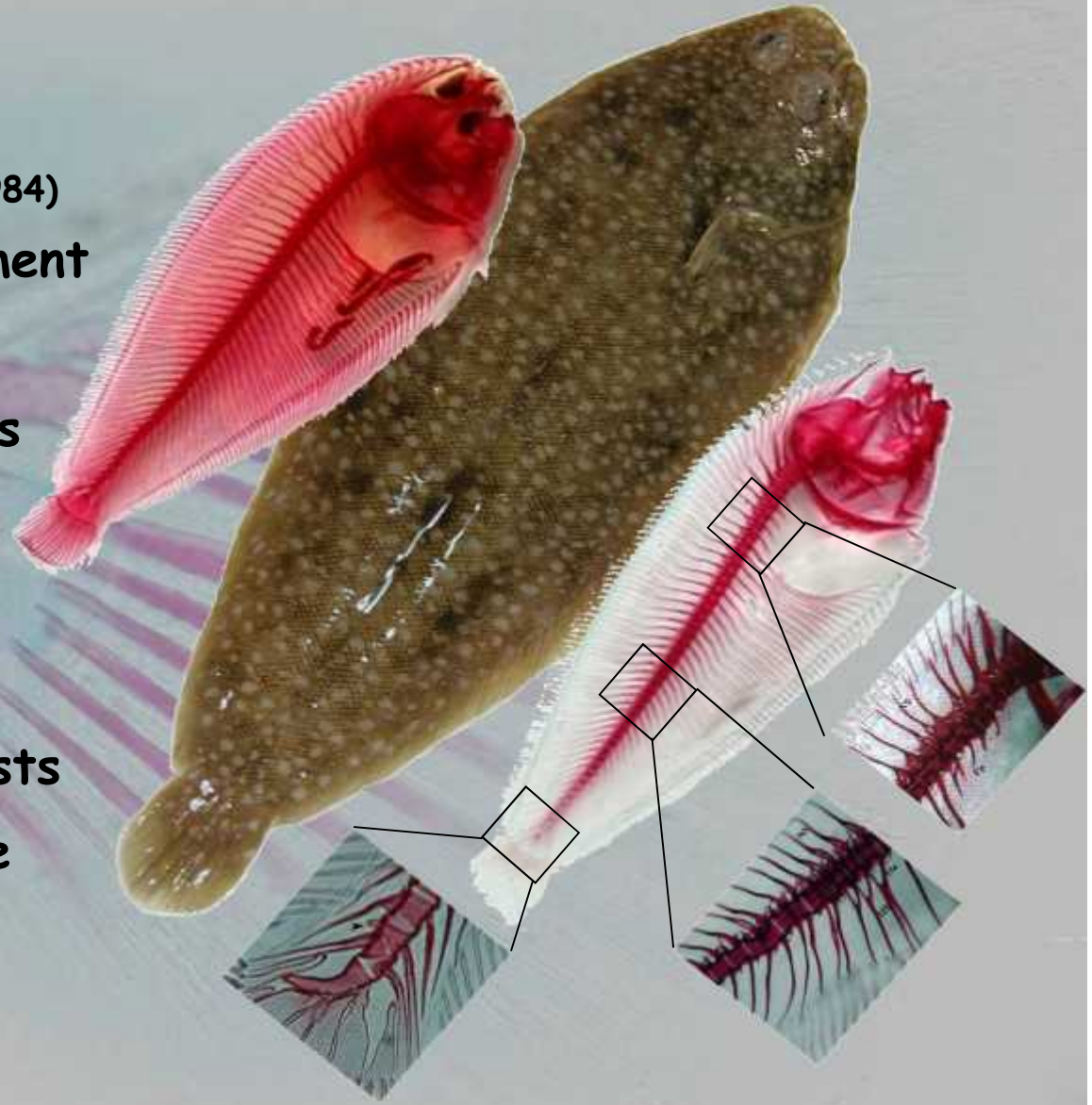
25 DPF



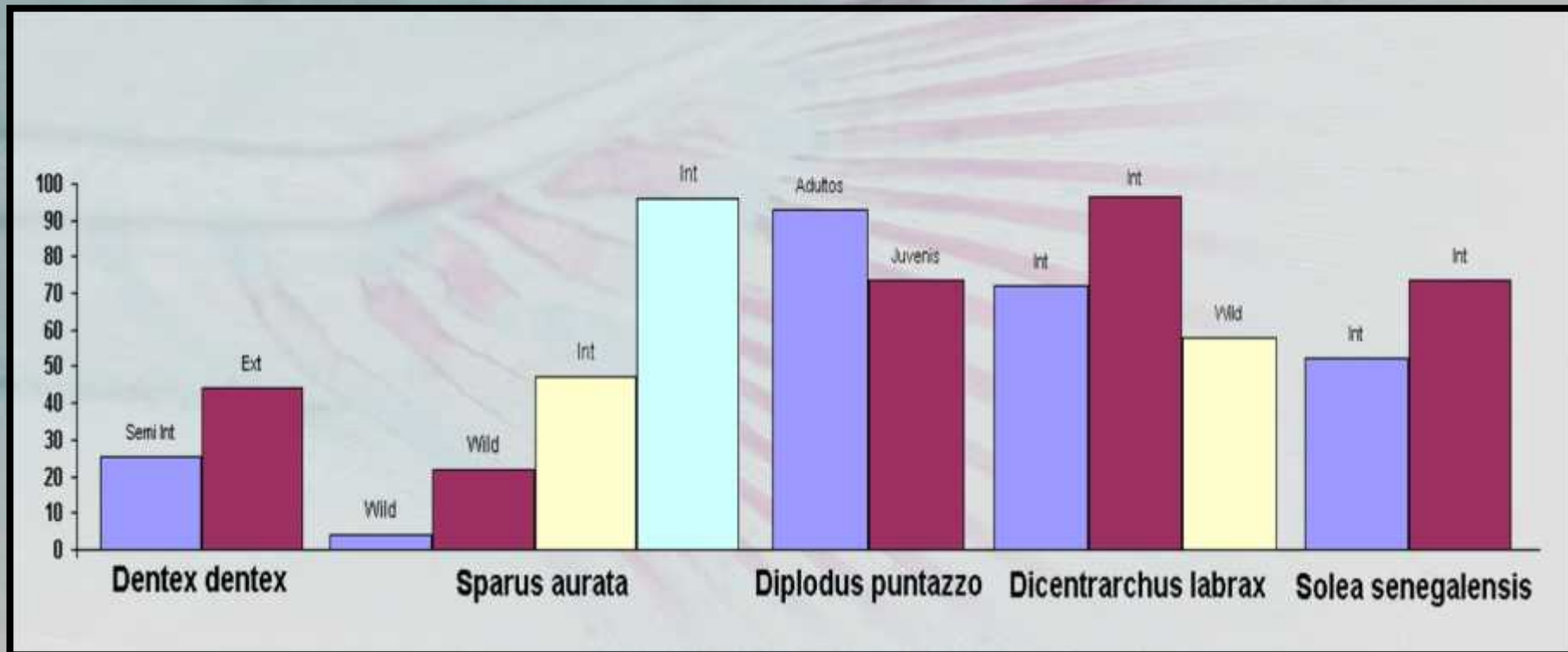
75 DPF

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 production costs
 - Decreased market price

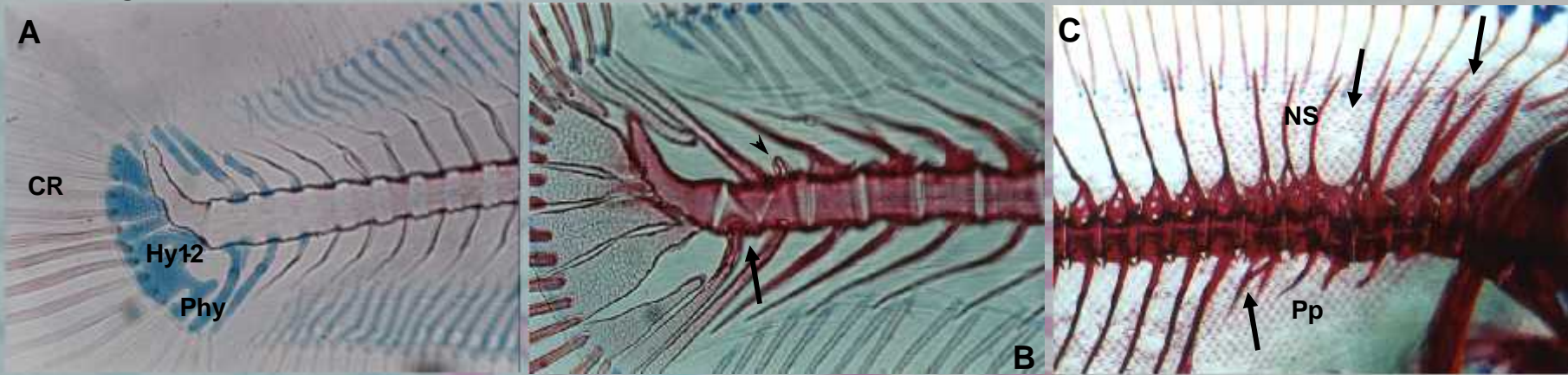


Skeletal malformation in mediterranean species



Skeletal malformations

Senegal sole

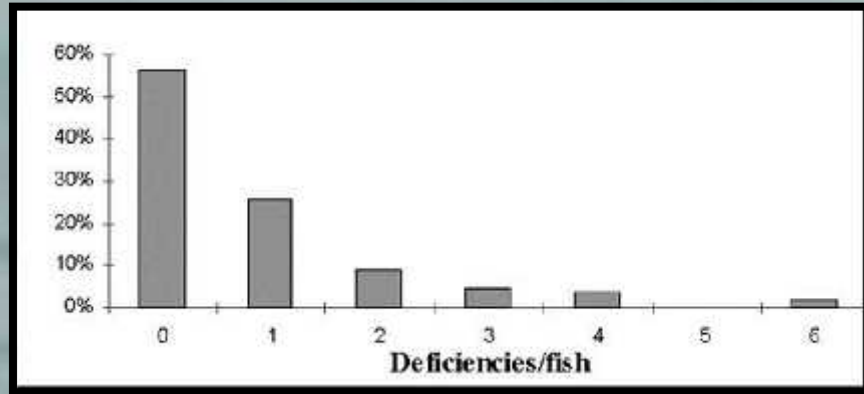


Sparids

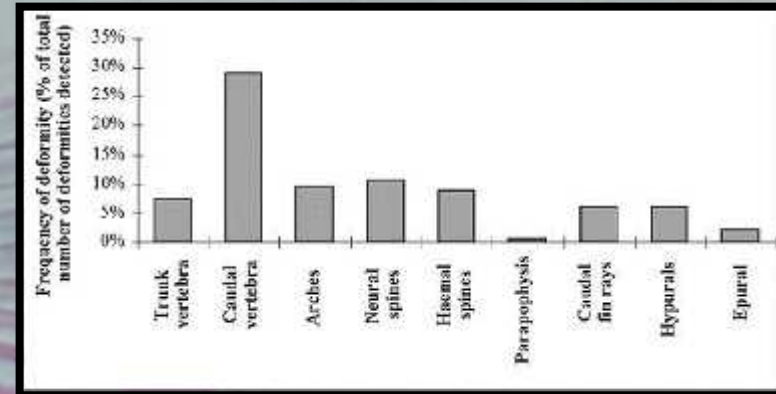


Senegal sole skeletal deformities

44% deformed

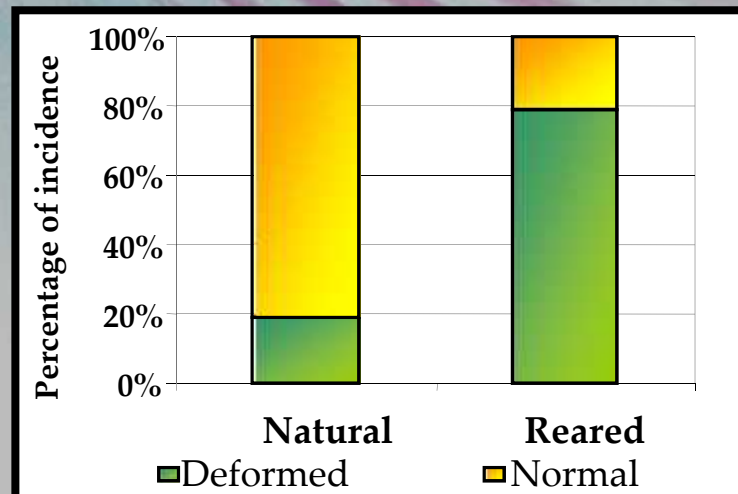


Caudal vertebrae 28%



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

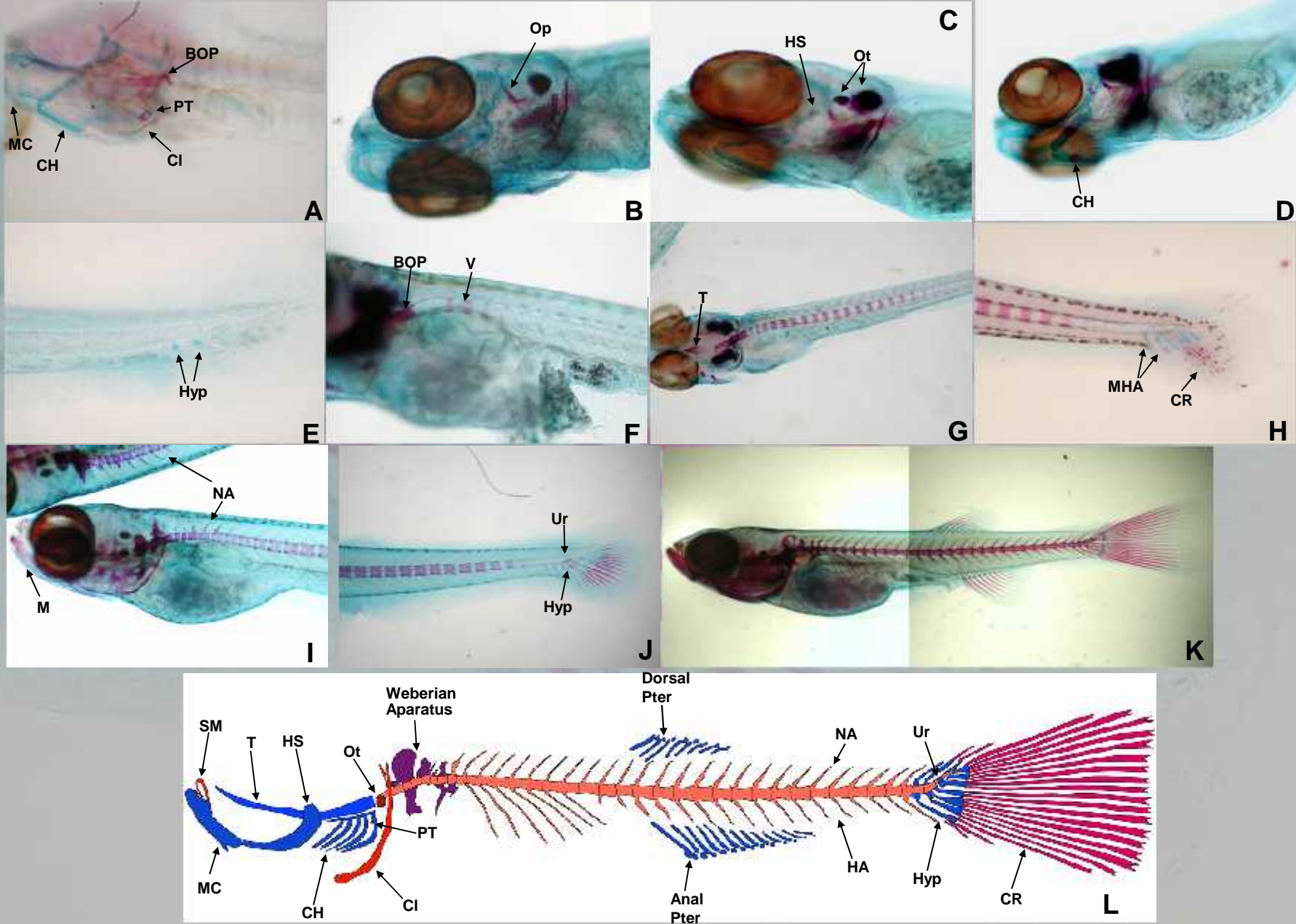
Deformations are rare in nature



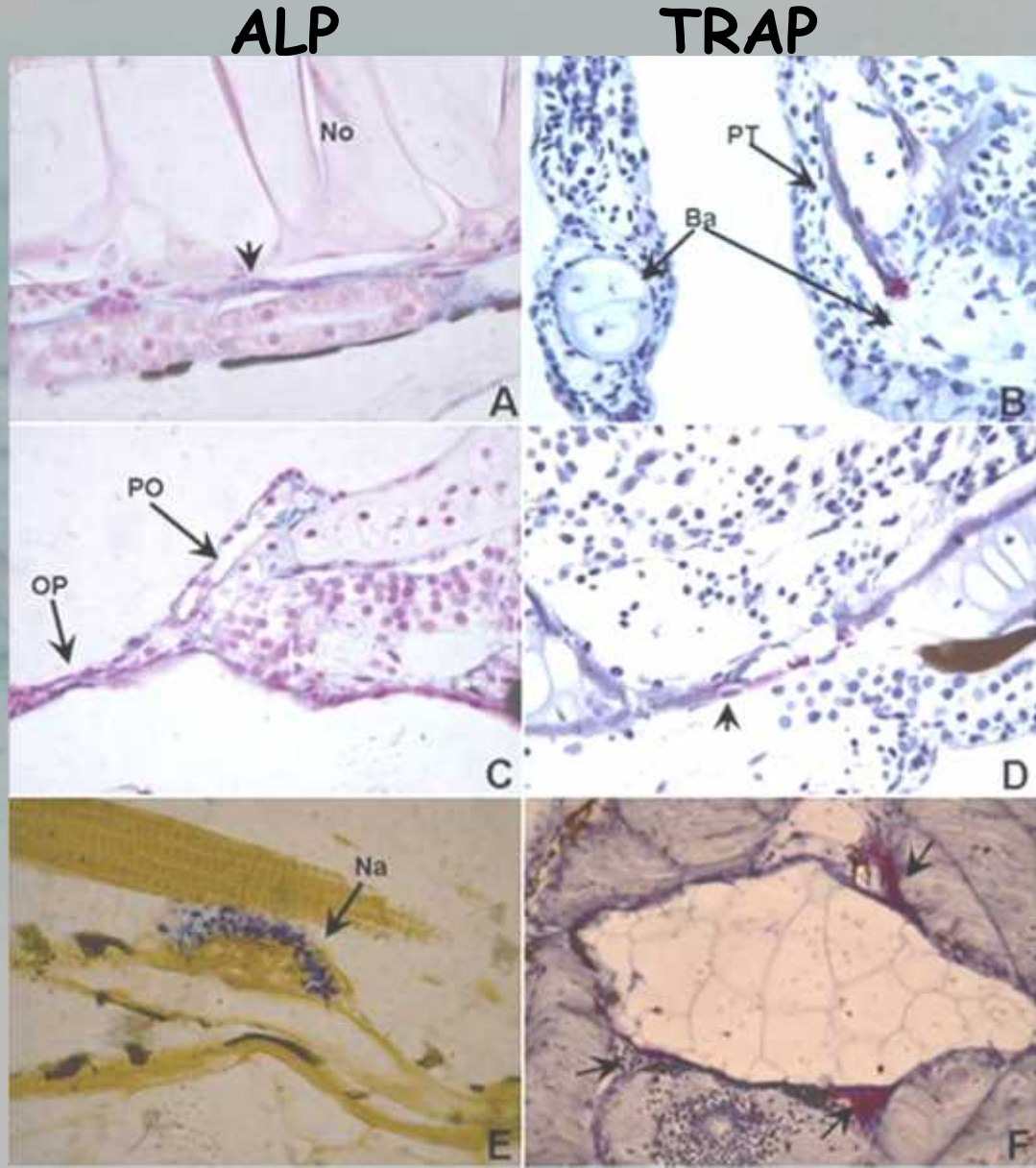
Malformed fish suffer negative selection in nature

P.J. Gavaia et al. (2006). Submitted

Zebra fish as model system: skeletal development



Identification of bone cells / skeletal development



Zebrafish

Senegal sole

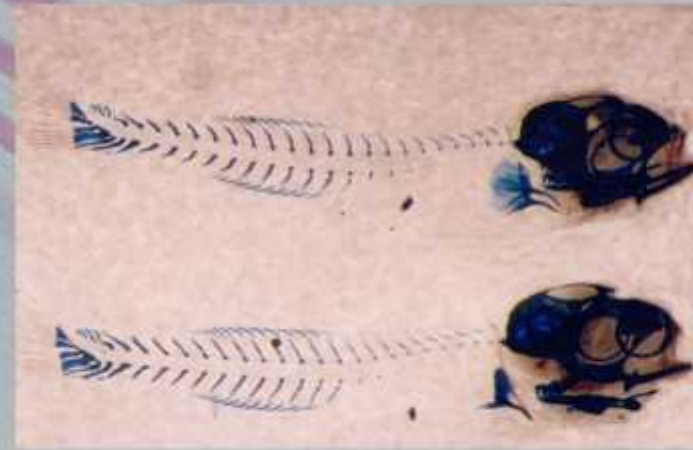
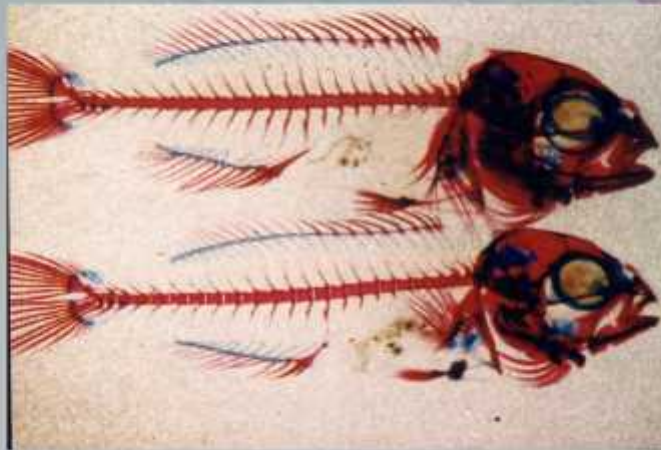
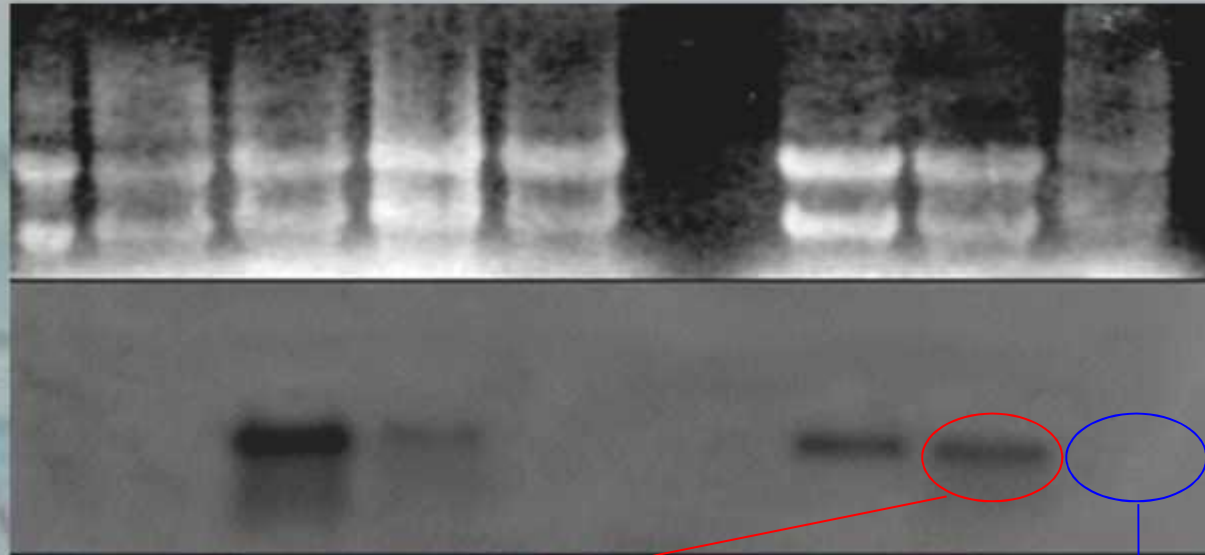
BGP vs. MGP

BGP

MGP

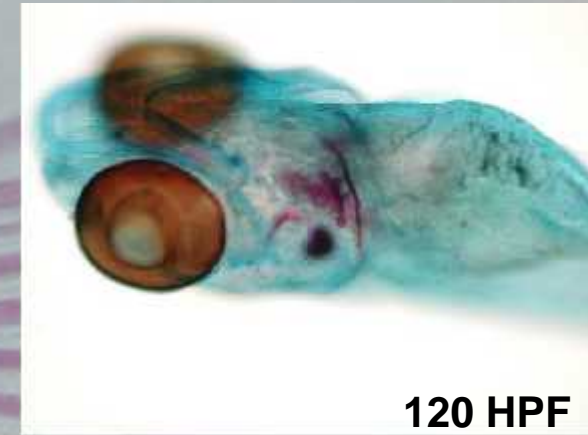
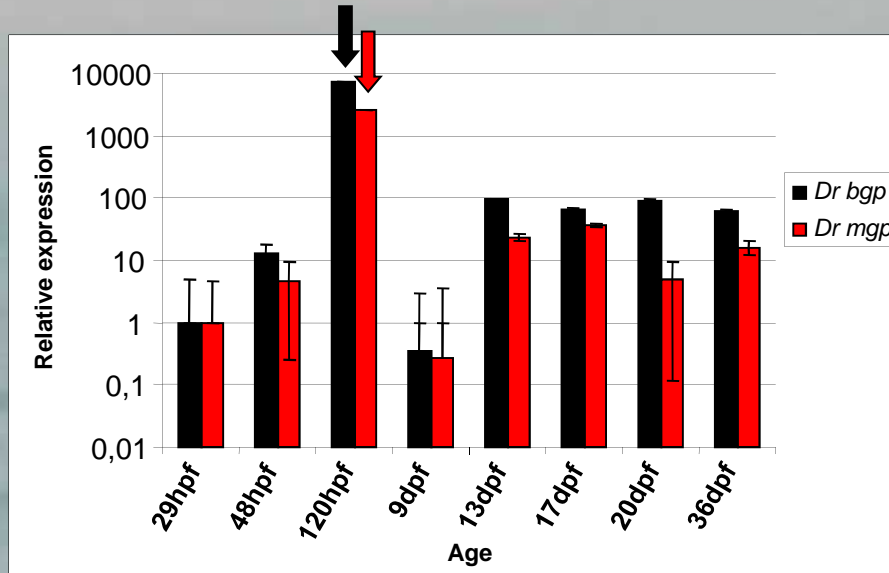
	BGP	MGP
Tissue distribution	Bone, teeth	Cartilage, kidney, lung, aorta, tooth
Cellular expression	Osteoblasts, odontoblasts	Immature and hypertrophic chondrocytes, vascular smooth muscle cells (VSMC), endothelial cells, pneumocytes, kidney cells, fibroblasts, cementoblasts
Sites of accumulation	Calcified 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

Spatial - temporal pattern of *bgp* expression

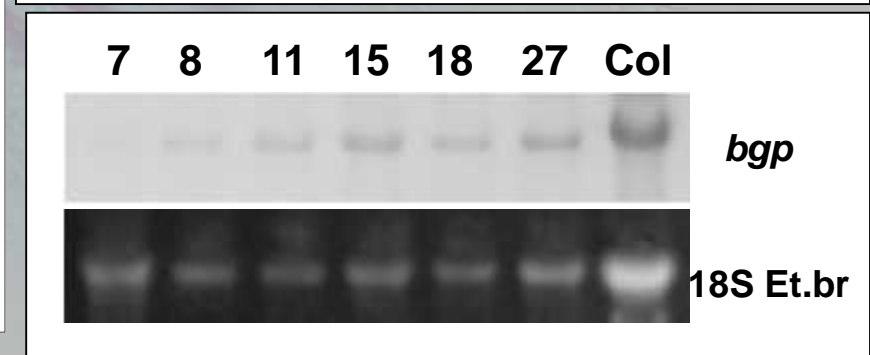
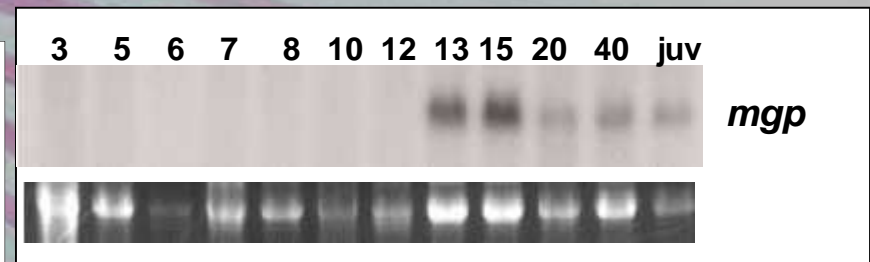
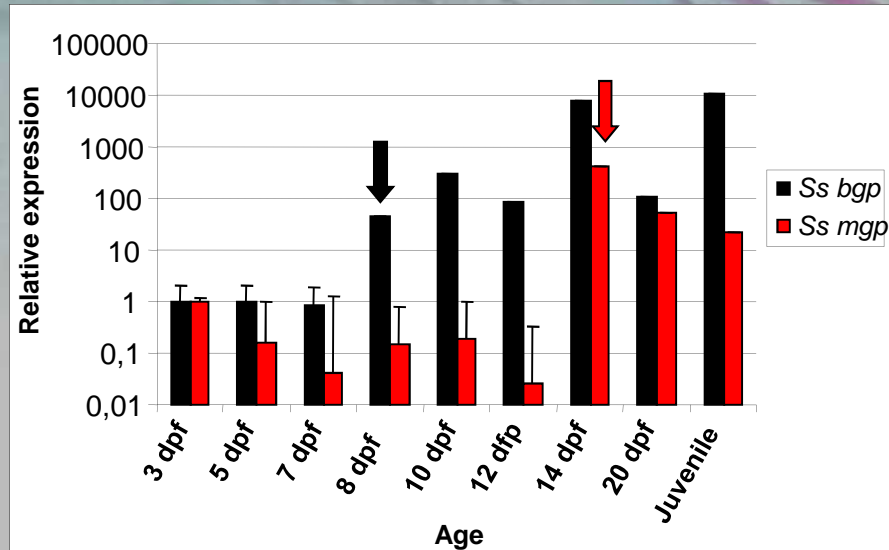


Spatial - temporal pattern of *bgp* / *mgp* expression

Zebrafish



Senegal sole

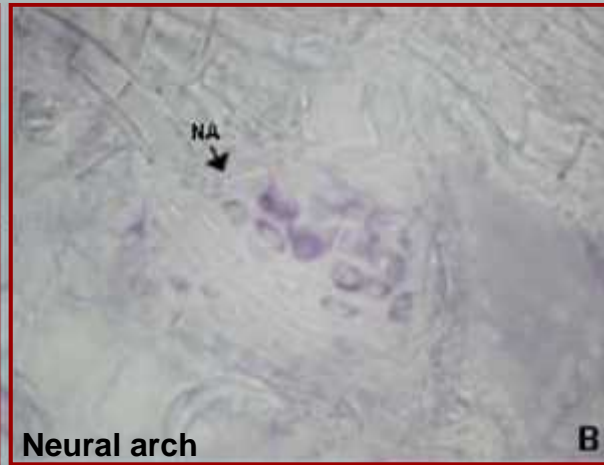


Single cell resolution: localization of *bgp* mRNA

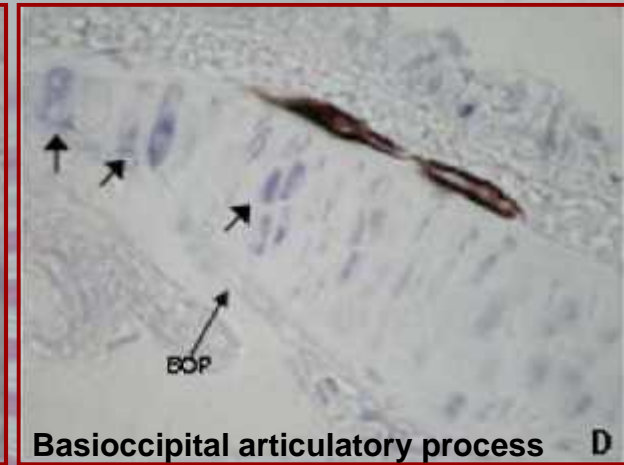
Zebrafish



9 DPF

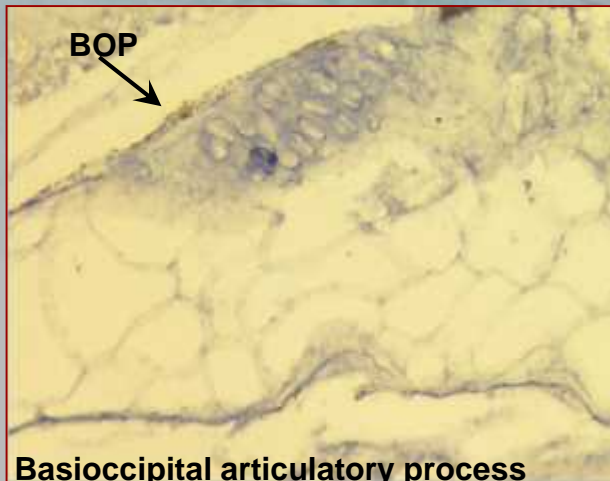


11 DPF

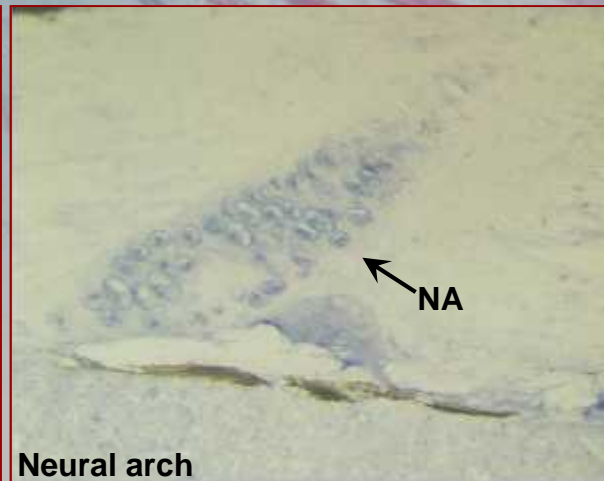


18 DPF

Senegal sole



15 DPF



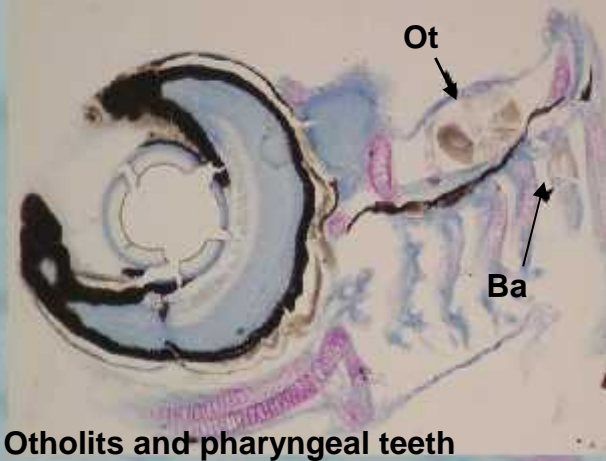
17 DPF



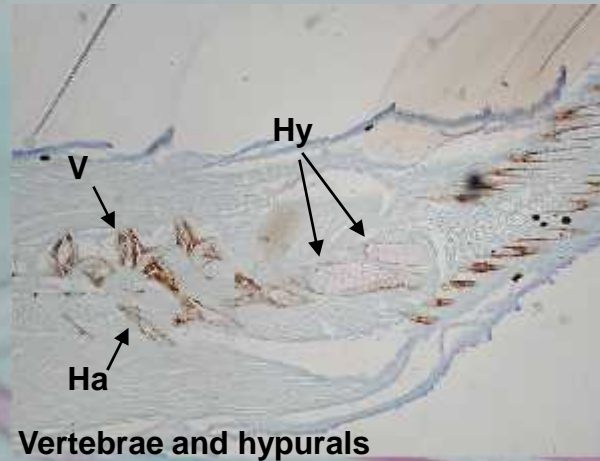
56 DPF

Single cell resolution: Immunolocalization of *Bgp*

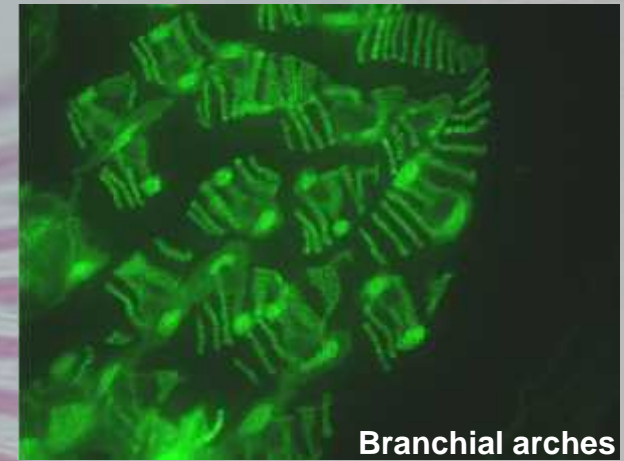
Zebrafish



8 DPF



20 DPF

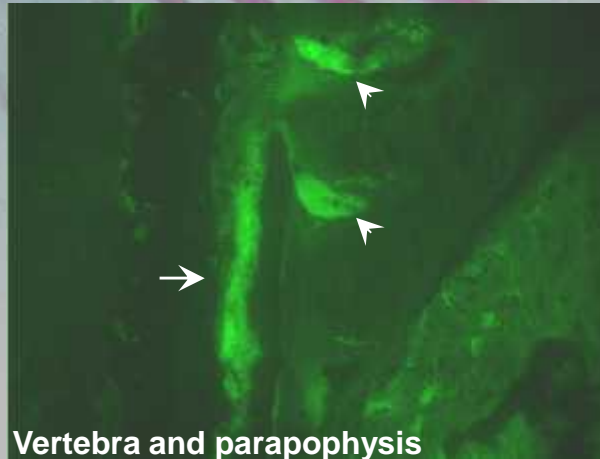


26 DPF

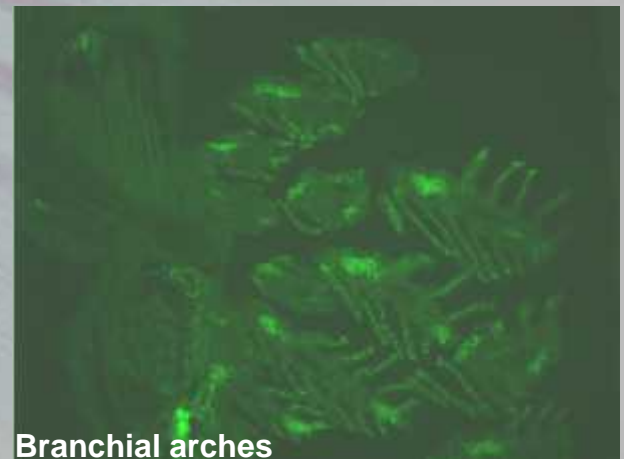
Senegal sole



11 DPF

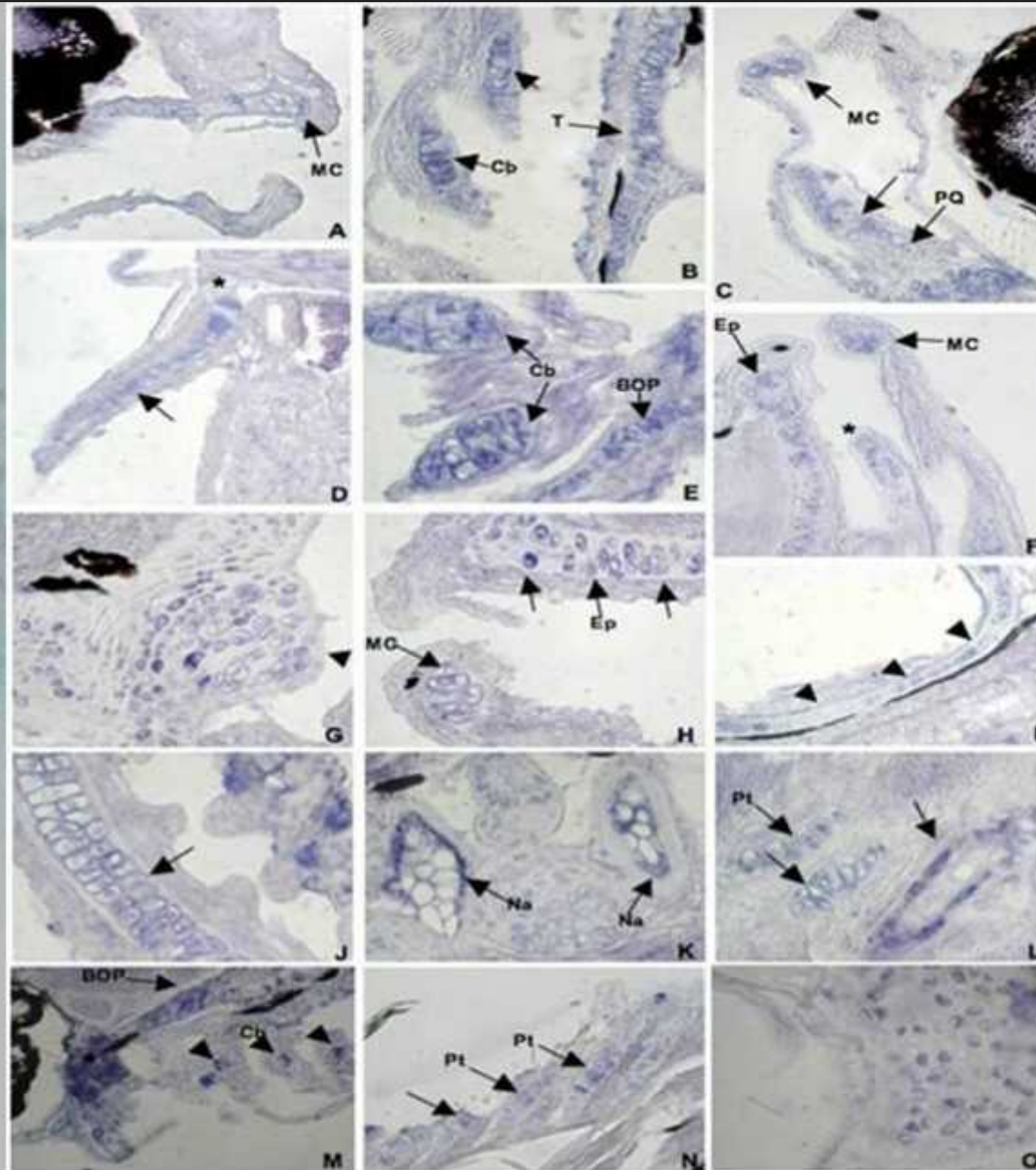


15 DPF



45 DPF

Single cell resolution: localization of *mgp* mRNA

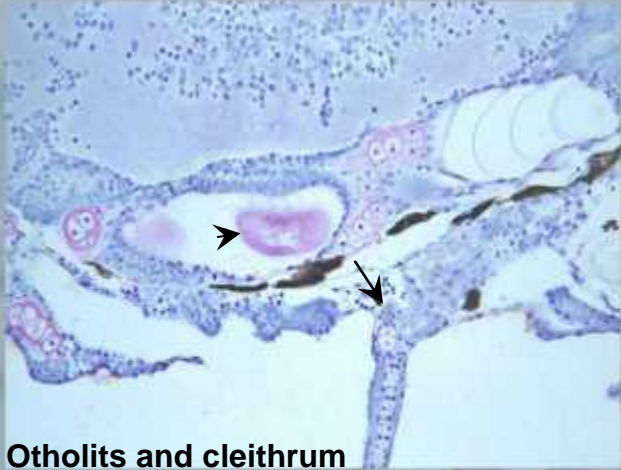


Zebrafish

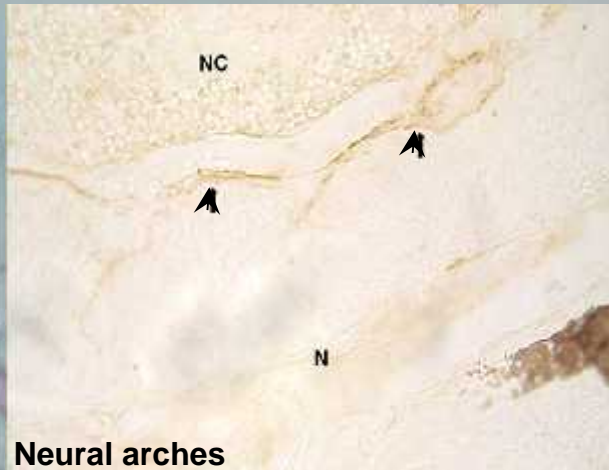
Senegal sole

Single cell resolution: Immunolocalization of *Mgp*

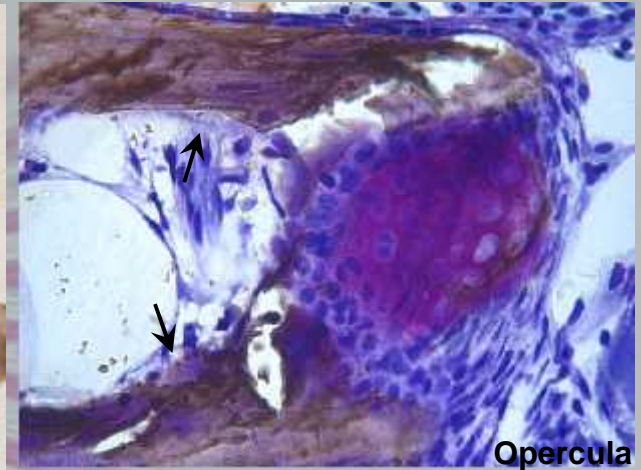
Zebrafish



Otholits and cleithrum
9 DPF



Neural arches
13 DPF



Opercula
38 DPF

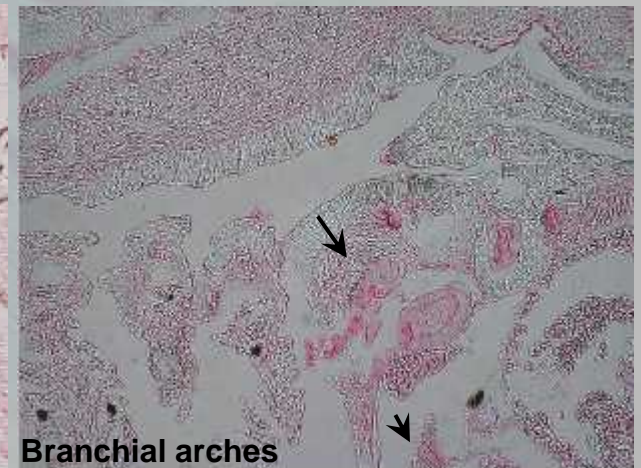
Senegal sole



Othic capsule
8 DPF

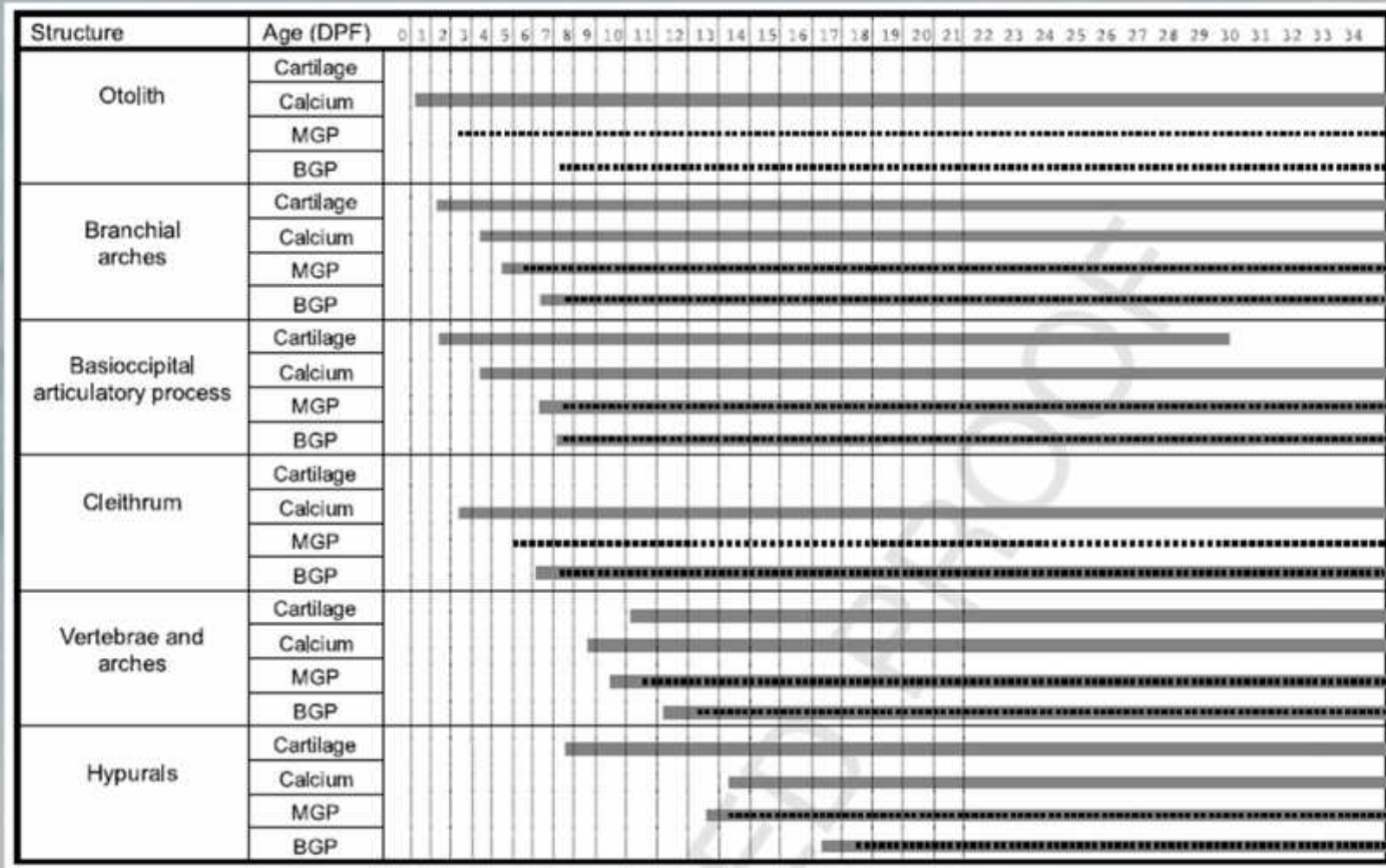


Trabeculae
17 DPF



Branchial arches
26 DPF

Summary of zebrafish skeletogenesis



In situ hybridization (—) and immunolocalization (---)

An in vivo system to uncover MGP and BGP function

- ▶ Over-expression of *bgp/mgp* cDNA
- ▶ Silencing of OC/MGP gene expression
- ⊕ Effect on fish skeletogenesis/calcification and other bone-related gene expression
- ▶ Regulation of *bgp/mgp* gene expression by agents affecting bone formation
- ▶ Functional knock-down using warfarin

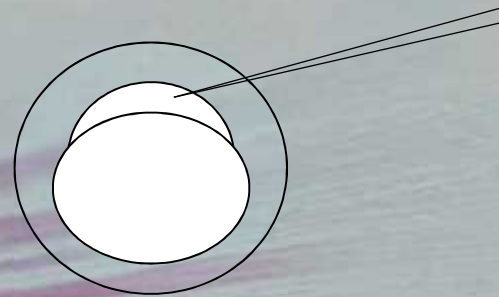
An in vivo system to uncover MGP and BGP function



An in vivo system to uncover MGP and BGP function

Microinjection

- Inject as fast as you can

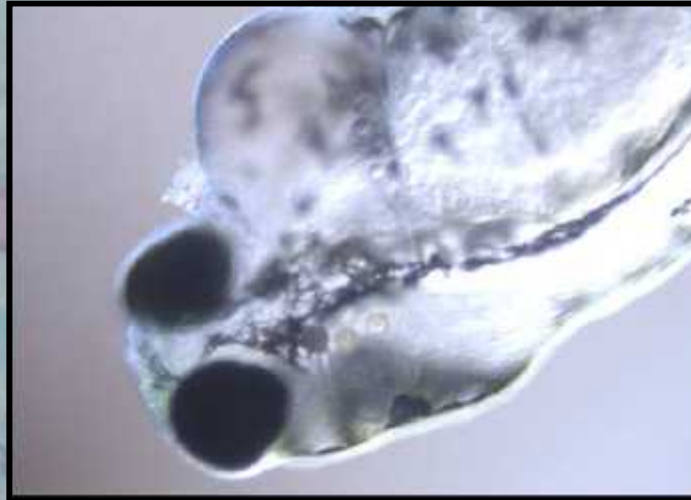
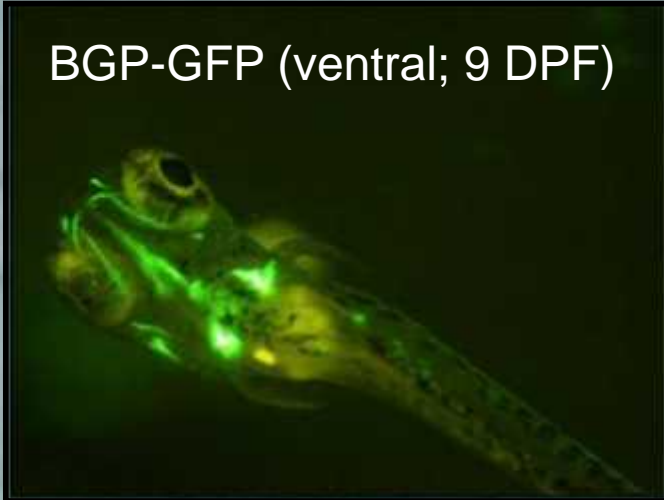


Transfection

- Liposomes

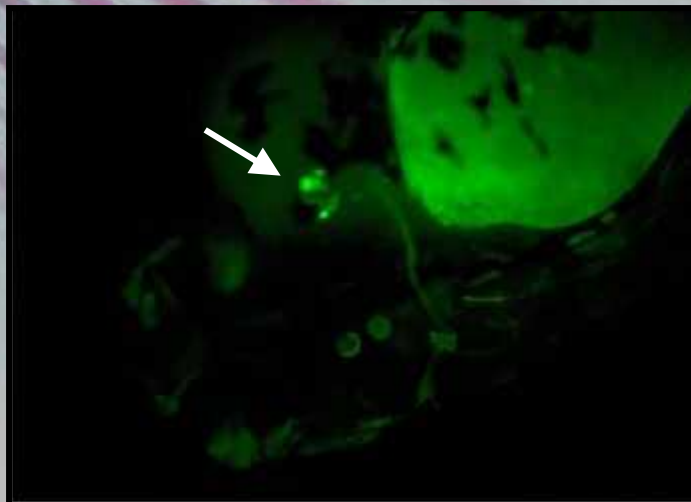
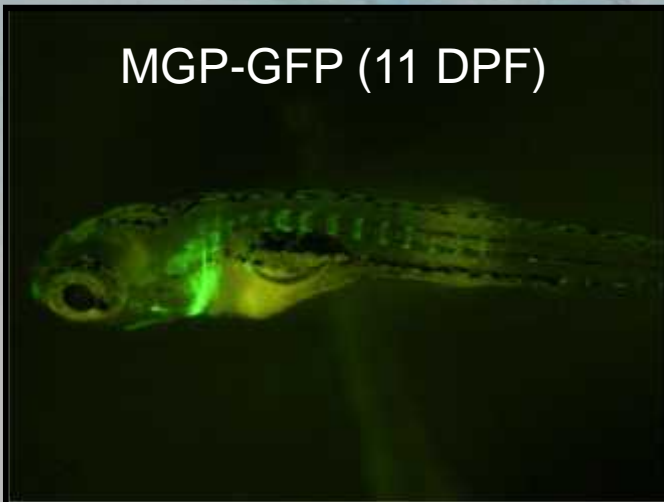
An in vivo system to uncover MGP and BGP function

BGP-GFP (ventral; 9 DPF)



MGP-GFP
6 DPH
Bright field

MGP-GFP (11 DPF)

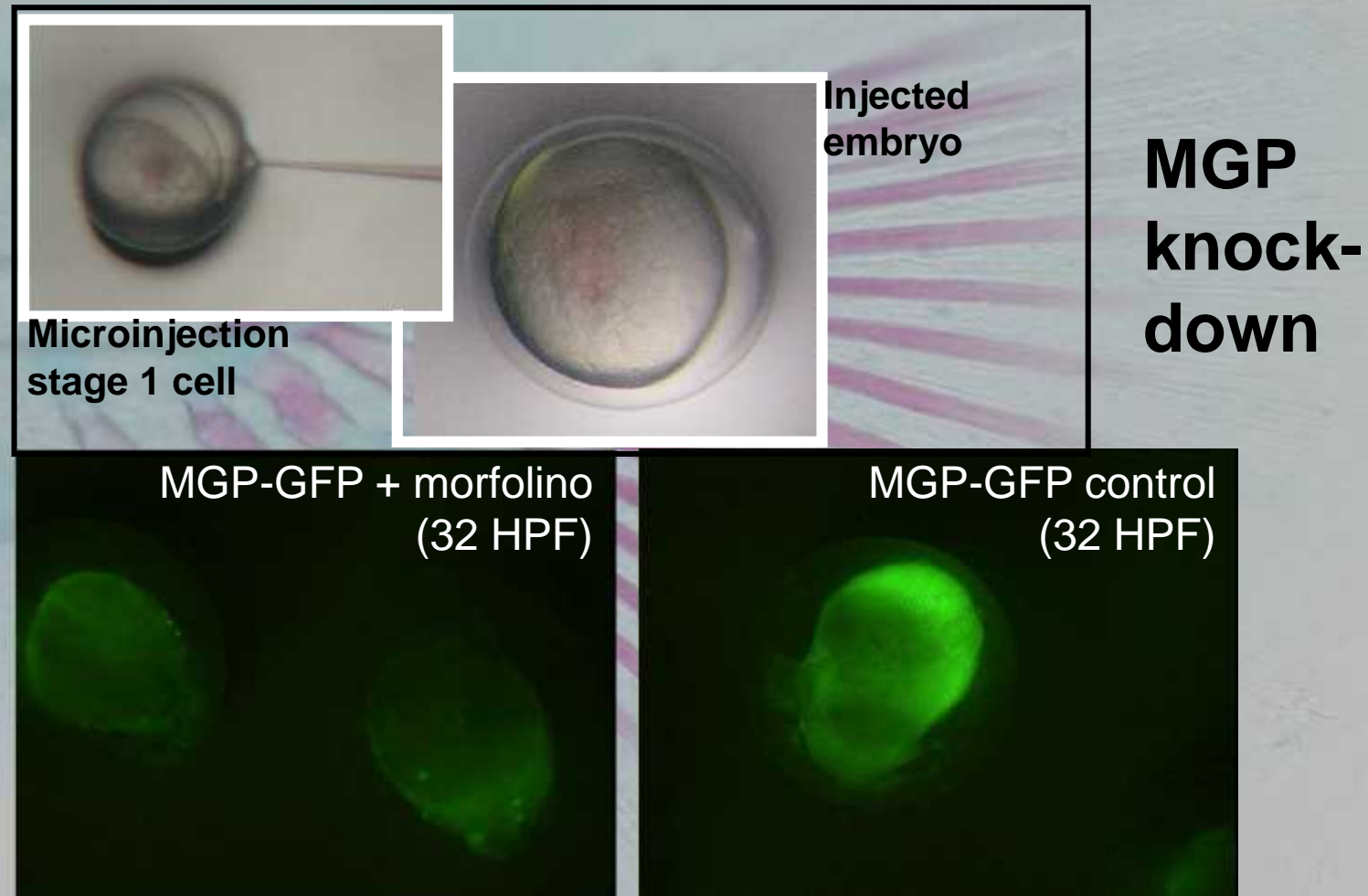


MGP-GFP
6 DPH
Dark field

(Gavaia et al.,
unpublished)

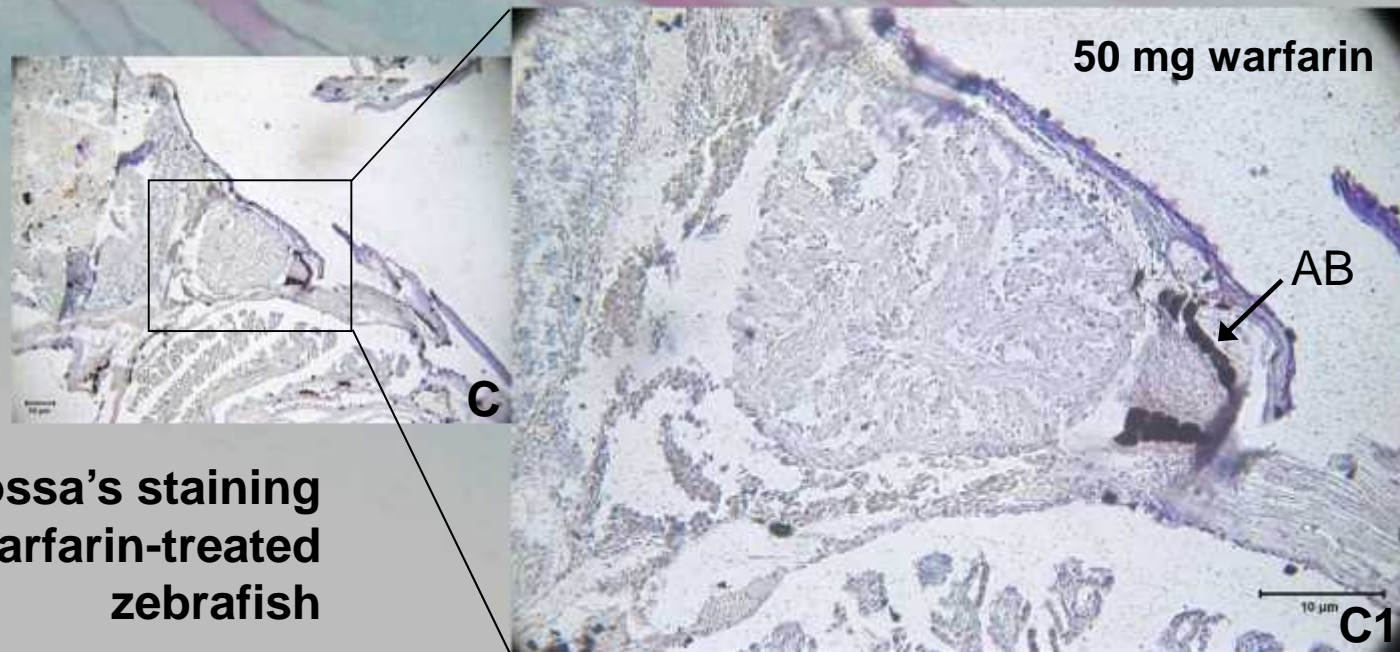
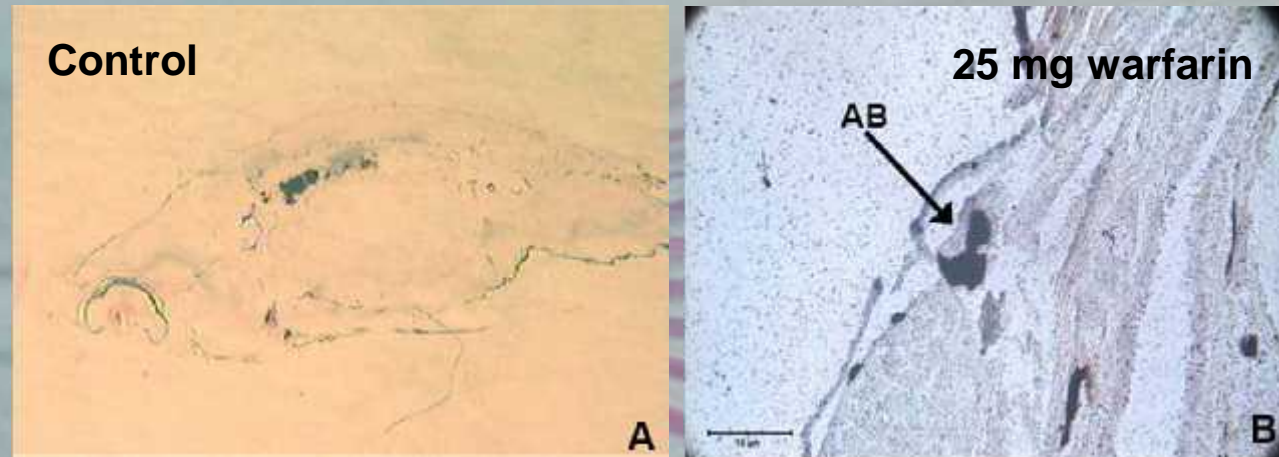
An in vivo system to uncover MGP and BGP function

(Gavaia et al., unpublished)



Morfolino = oligonucleotide binds to mRNA and prevents translation >> decreased protein production

An in vivo system to uncover MGP and BGP function

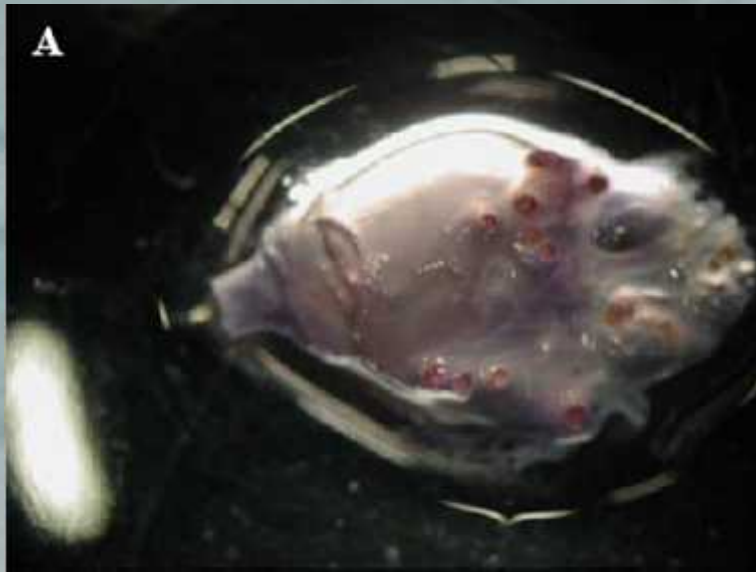


von Kossa's staining
of warfarin-treated
zebrafish

(Gavaia et al., submitted)

Other results: Effects of warfarin on toadfish

Toadfish heart



Control



15 mg warfarin/kg

Alizarin red staining

(Gavaia et al., submitted)

Contributions and collaborations

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Marta Rafael
Vania Roberto

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