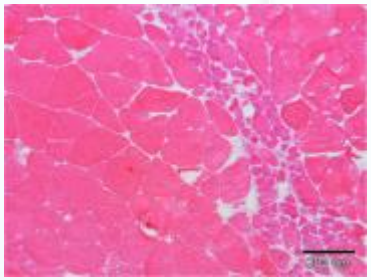
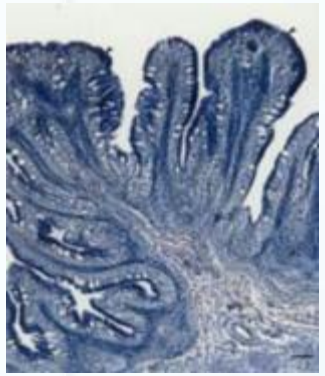




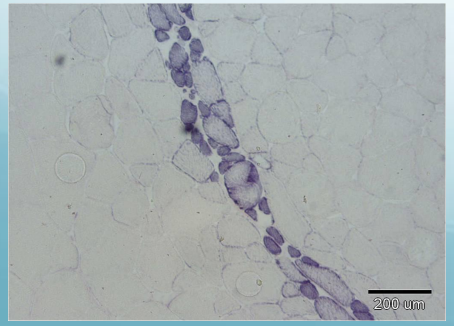
Histological and histochemical methods in the analysis of the locomotor and digestive system of fish



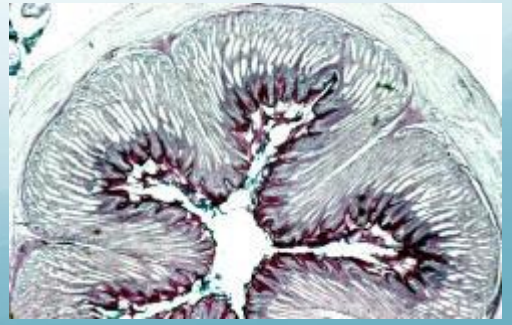


Srebrenka Nejedli, Zvonimir Kozarić, Ivana Tlak Gajger

Faculty of Veterinary Medicine, University of Zagreb, Croatia





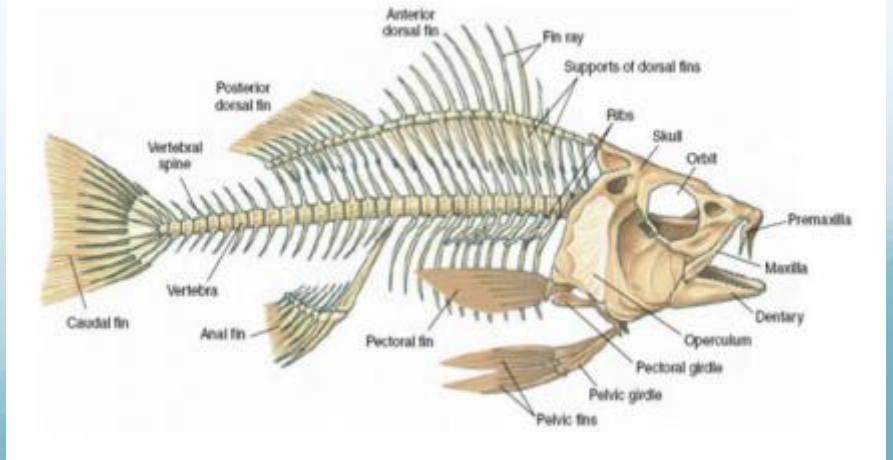


LOCOMOTOR SYSTEM

- **The locomotor system of fish is one of the most important systems because it includes the muscular system, which is commercially important, and the skeletal system that has importance in the initiation for survival.**

- **The skeleton of a fish includes the bones of the head, spine, ribs and supporting elements of fins. This system develops from the mesoderm.**

- **Teleostei fish have completely ossified skeleton. During metamorphosis, larval fish undergo different stages of development of skeletal system. By monitoring the process of ossification of certain bones or groups of bones from hatching, it is possible to follow the order of ossification of bones as well as the completion of ossification and the emergence of some pathological changes in the skeleton significant for the further development of the individual.**



Materials and methods

- **Studies have been conducted on the larvae: sea bass (Dicentrarchus labrax L.), rainbow trout (Oncorthynchus mikiss), catfish (Silurus glanis), carp (Cyprinus carpio) and garfish (Belone belone) of different ages.**
- **Larvae were fixed in 10% formalin (also alcohol) and stained with alcian blue and alizarin red staining (WASSERUNG, 1976).**
- **With alcian blue and alizarin red staining cartilage is stained blue (alcian blue) and bones tissue is stained red (alizarin red) (WASSERUNG, 1976).**
- **This method is very acceptable for the study of fish larvae skeleton and small adult fish whose skeletons cannot be prepared with other methods. This method can help us in the study of post-hatching development of fish skeletons and skeletal pathology, which is very important in fish breeding.**

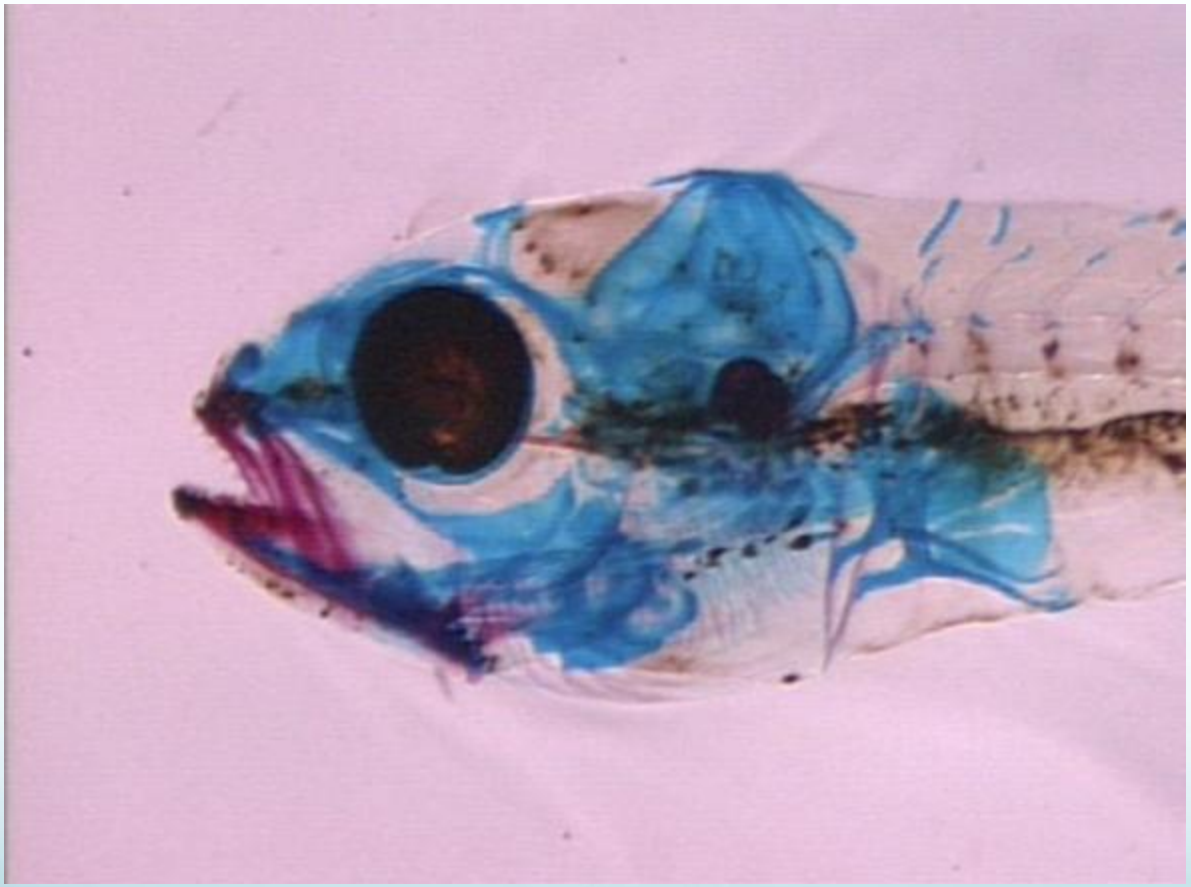
Alcian blue and alizarin red staining

- **After fixation, the samples were washed in distilled water, with a larger shell removed.**
- **Alcian blue staining (24-48 hours) cartilage tissue turns blue and after that the samples are transferred through the drop-down percentage alcohols and washed in distilled water for 2-3 hours. The compositions are then placed in a solution of sodium barbiturate, and trypsin distilled water until the tissue becomes transparent. This process takes 2-3 weeks depending on the size of the larvae.**
- **After that, the bone tissue is staind alizarin red dissolved in 0.5% aqueous solution of KOH. This takes about 24 hours, and the excess paint is washed with 0.5% KOH-glycerine which was added in 3% hydrogen peroxide. This procedure takes several days until a dark pigment loss in the tissue, after which the products are placed in pure glycerol with addition of a few crystals of thymol.**
- **The said method of staining tissue has become transparent and under the scrutiny, the blue stained cartilage tissue and a dark red-colored bone tissue could easily be spotted. Samples stained method alcian blue and alizarin red staining are quite stable and do not lose their color even after one year.**

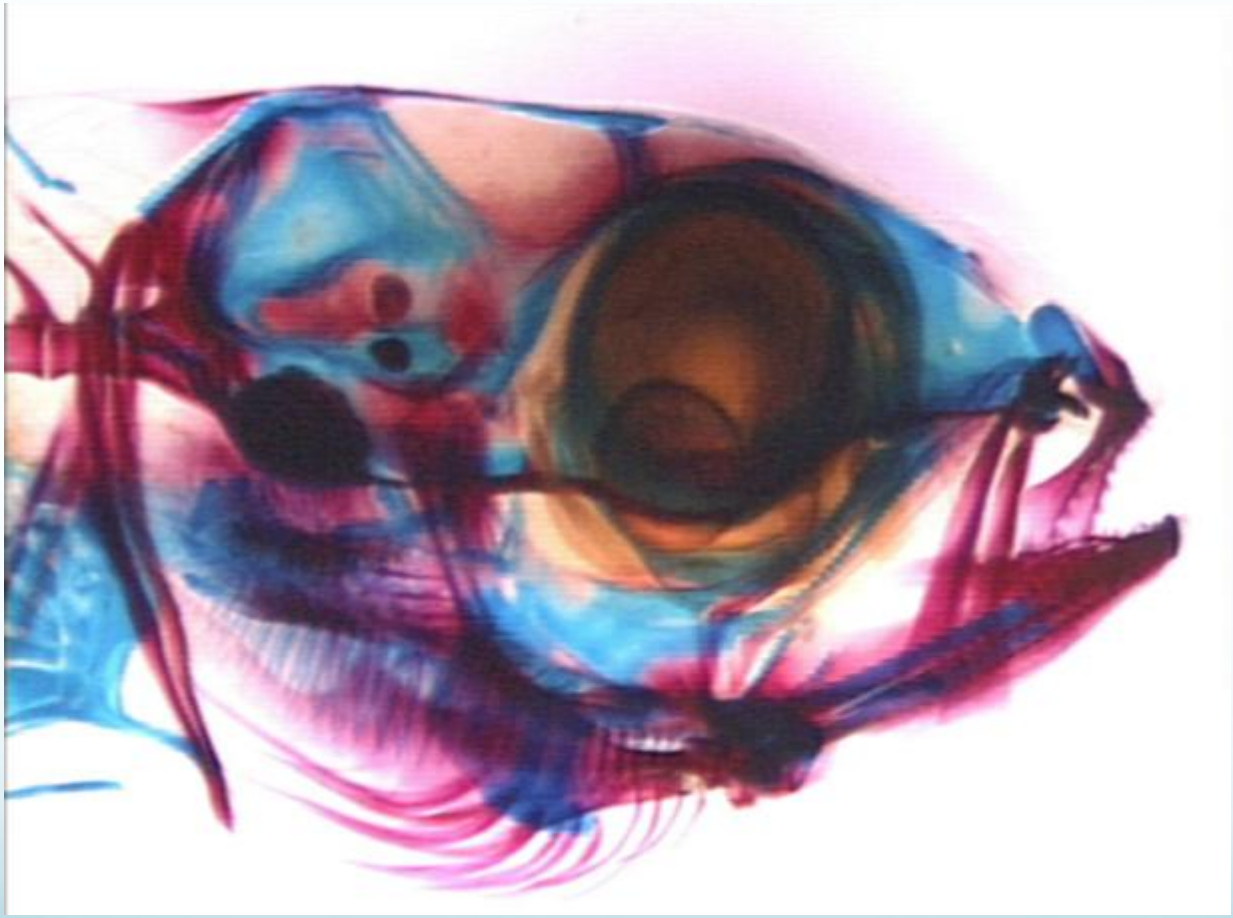
Sea bass (*Dicentrarchus labrax*) larvae at the age of 35 days post-hatching (dph), cartilaginous elements of head are blue, 5x.



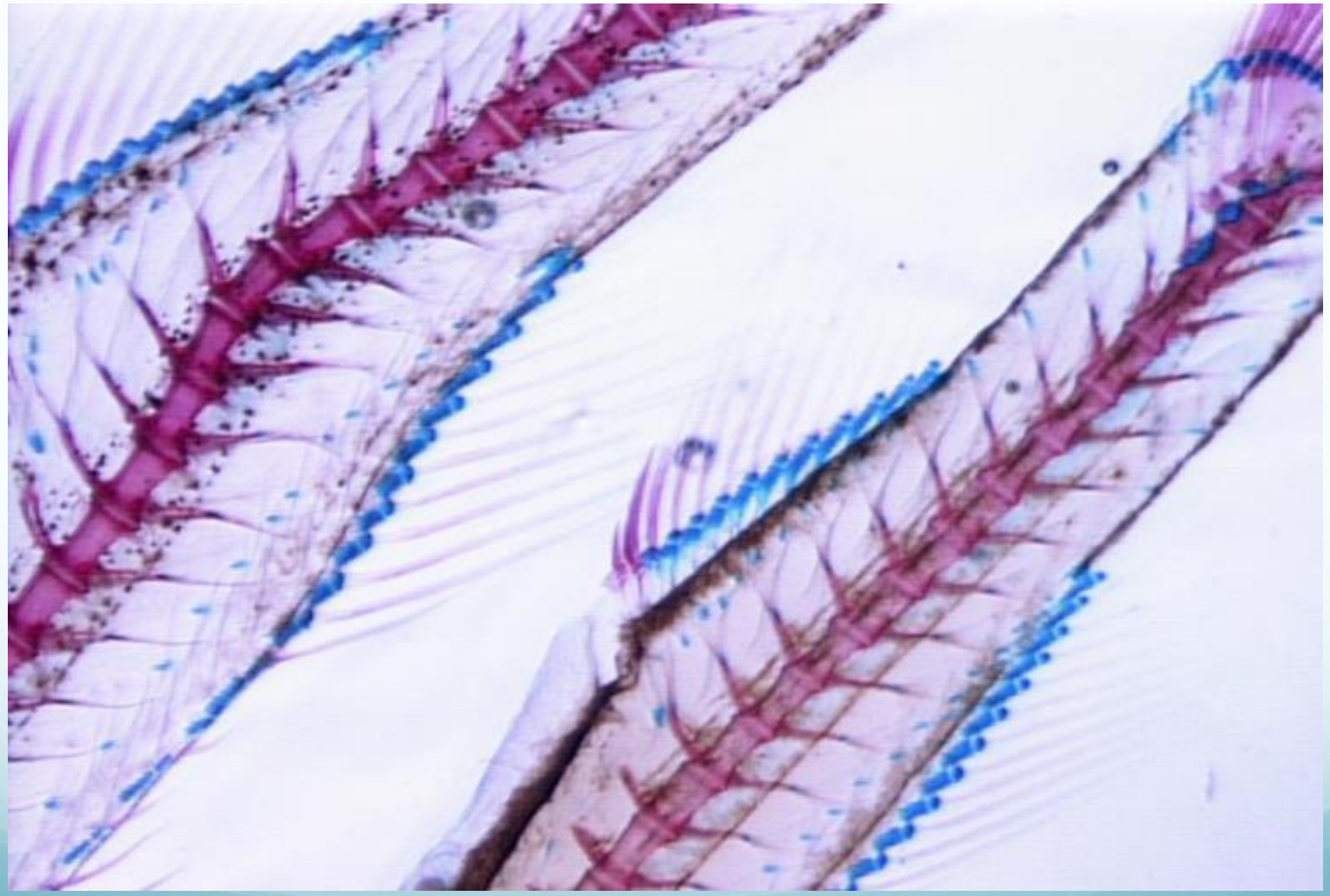
Sea bass (*Dicentrarchus labrax*) larvae at the age of 40 dph, bone tissue on dental and maxillary bones are red.9x,



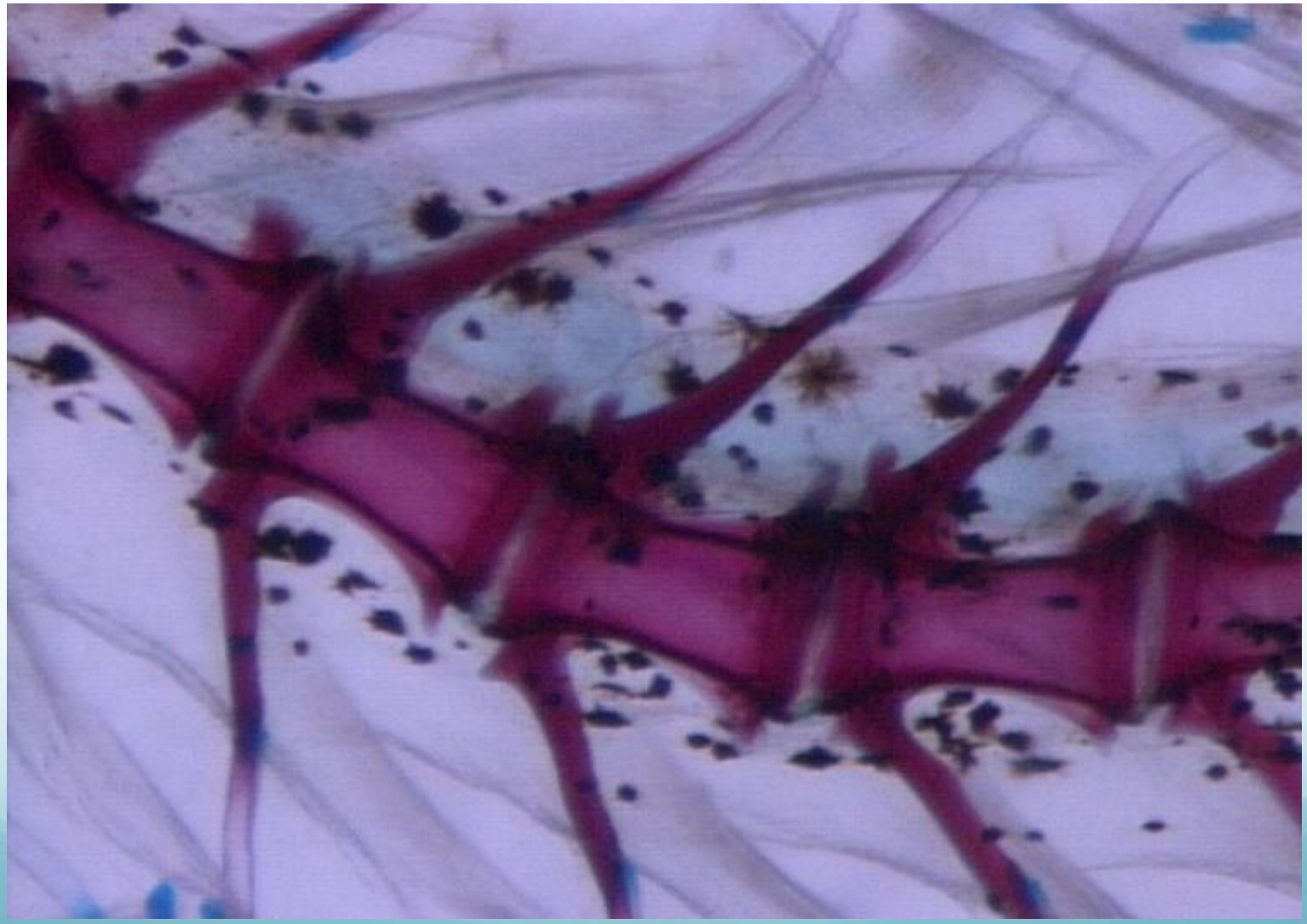
Sea bass (*Dicentrarchus labrax*) larvae at the age of 50 dph, ossification process progresses and osseous elements of temporomandibular port and brachiostegal endings are visible, 1x.



Sea bass (*Dicentrarchus labrax*) larvae at the age of 40 dph, visible deformed spine and spine without pathological changes 2x.



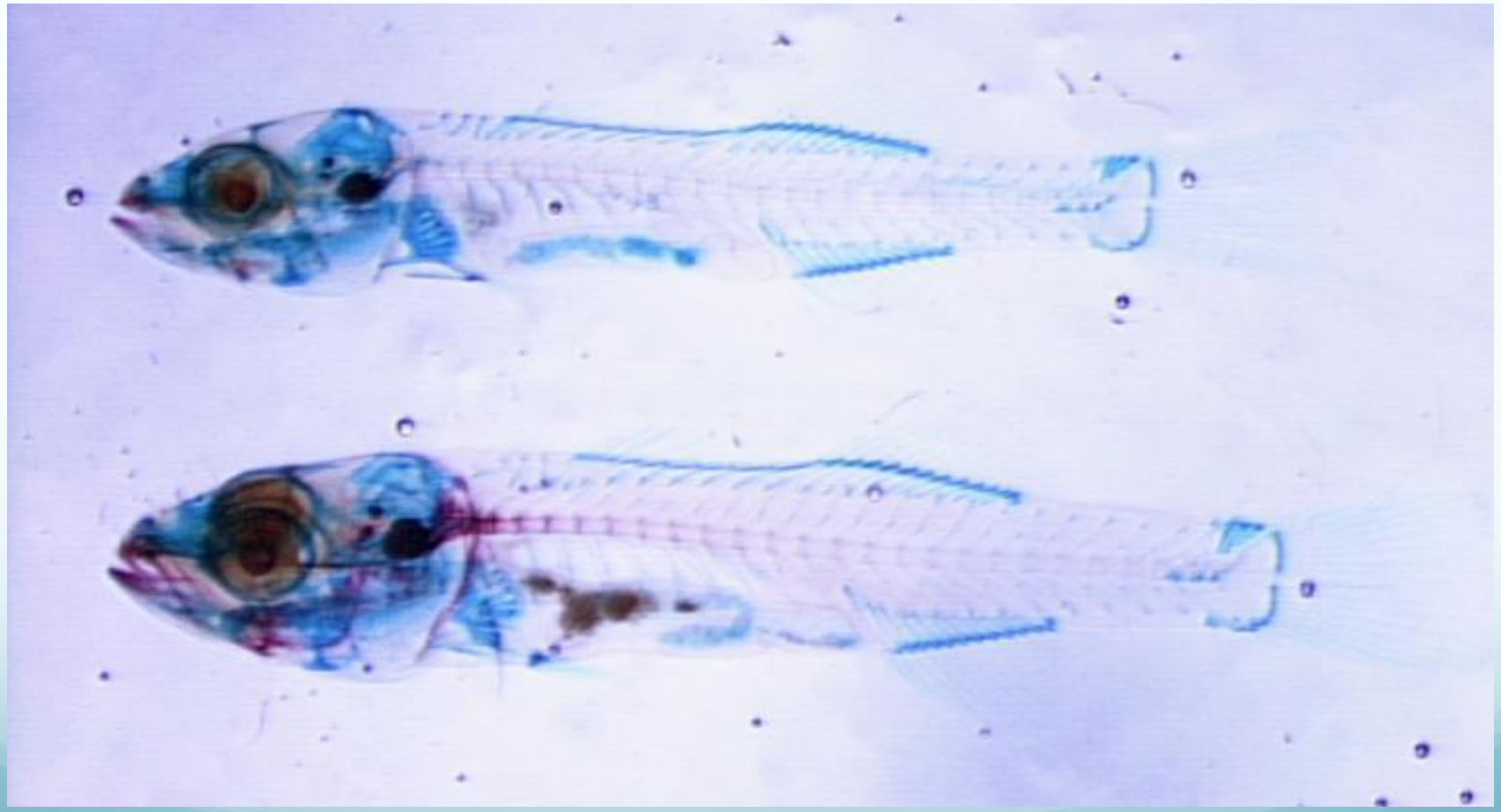
Sea bass (*Dicentrarchus labrax*) larvae at the age of 40 dph, pathological process on vertebres are visible 7x.



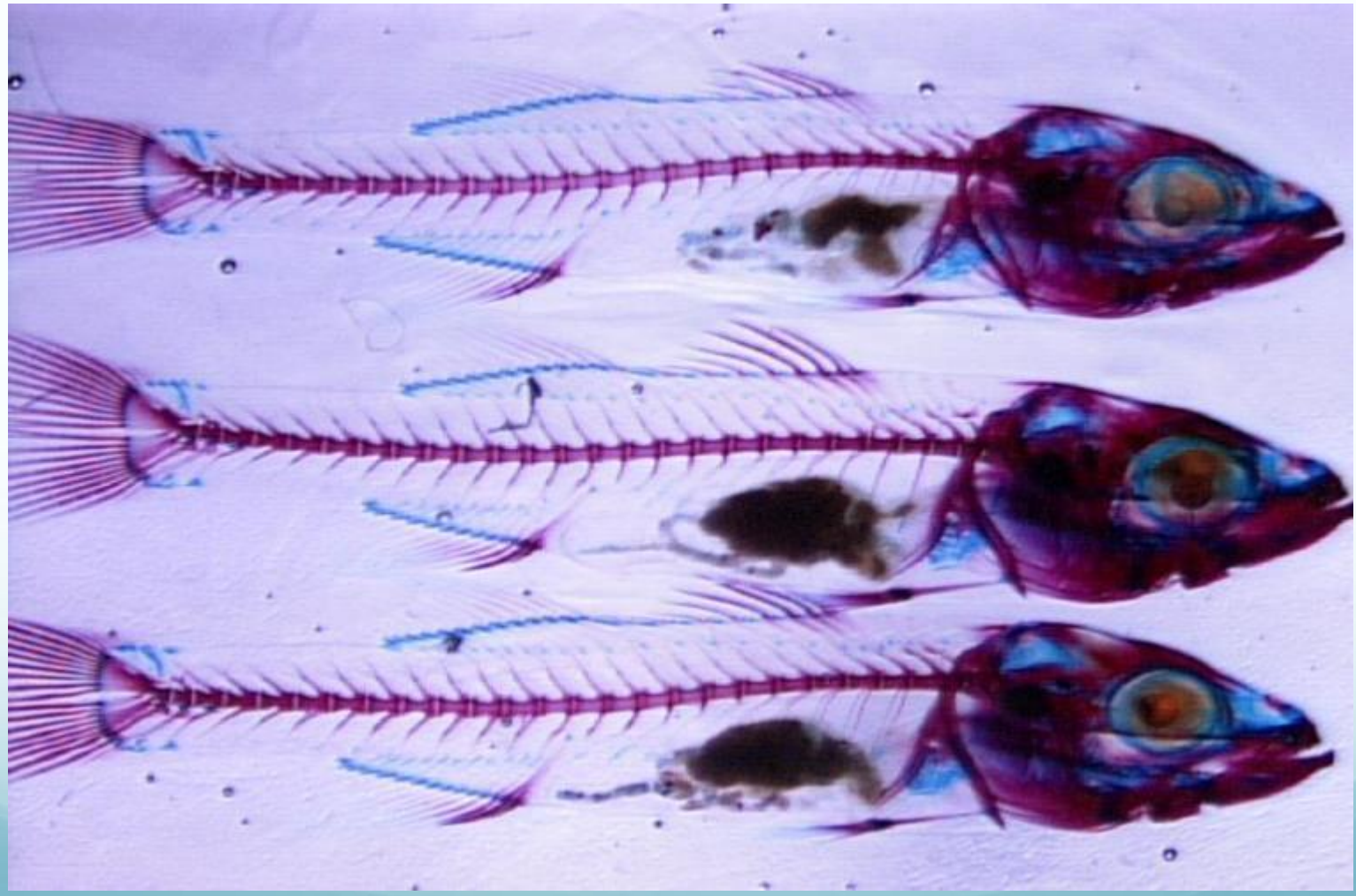
Sea bass (*Dicentrarchus labrax*) larvae at the age of 50 dph, the second larva has an extremely visible strain on the spine, 1x.



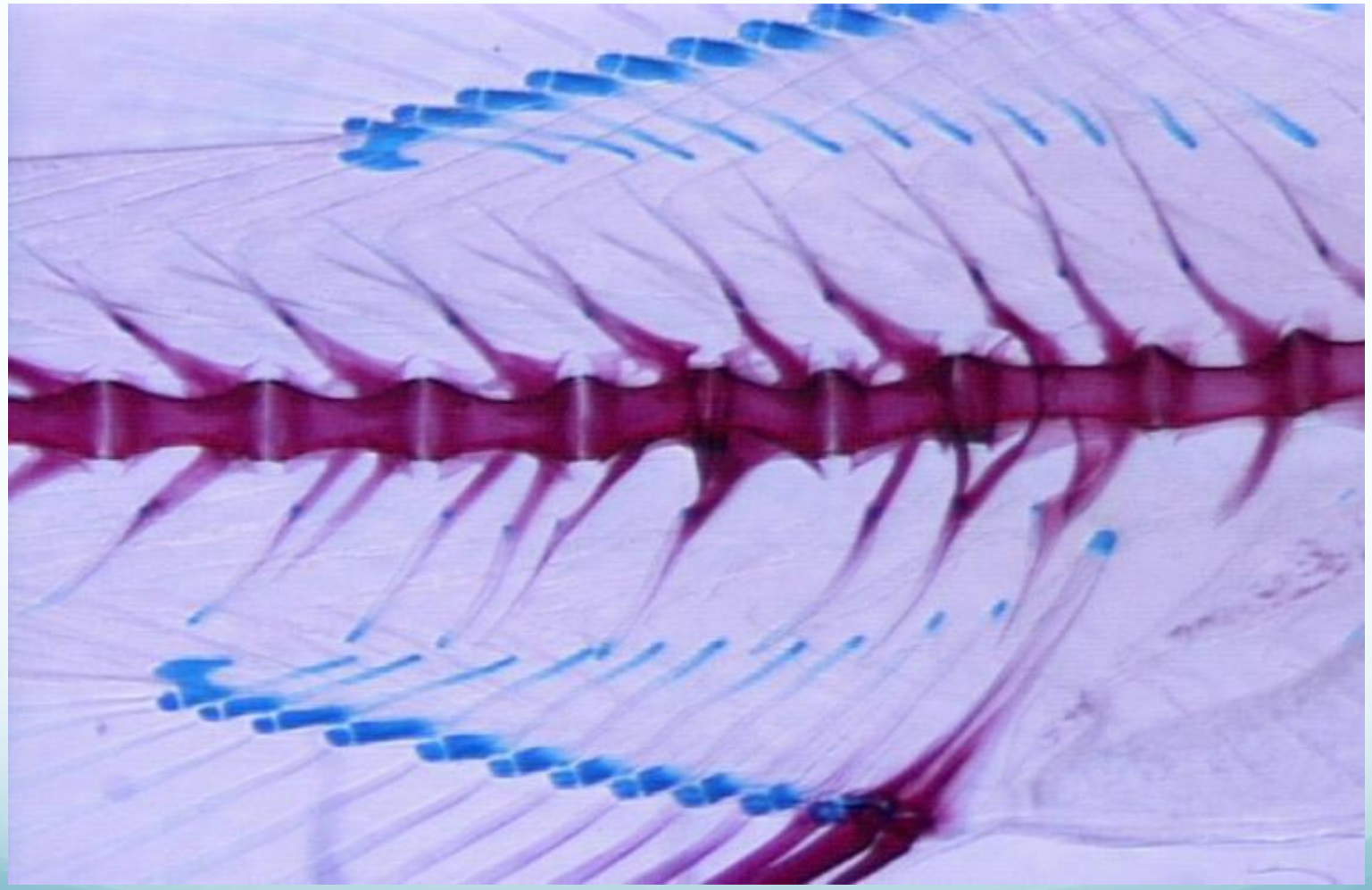
Sea bass (*Dicentrarchus labrax*) larvae at the age of 55 days dph, bones of the head and skeleton, 1x.



Sea bass (*Dicentrarchus labrax*) larvae at the age of 68 dph, almost all bones of the head and the skeleton except for some parts of the head and fins are red colored, 1x.



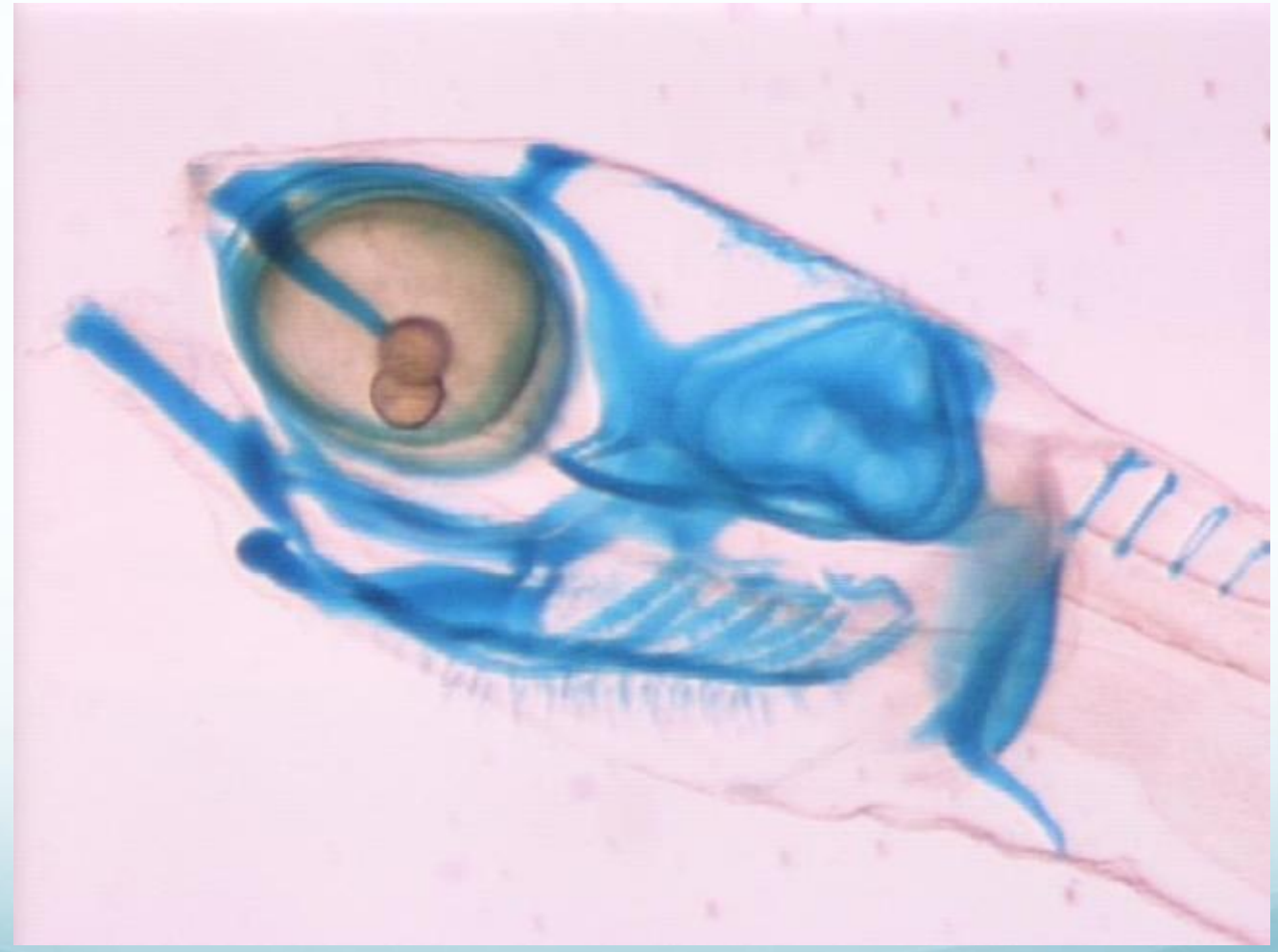
Sea bass (*Dicentrarchus labrax*) larva at the age of 68 dph, with visible mild deformity on the spine, 3x ,



Sea bass (*Dicentrarchus labrax*) larva at the age of 71 dph, almost all bones of the head and the skeleton except for some parts of the head and fins are red colored, 1x.



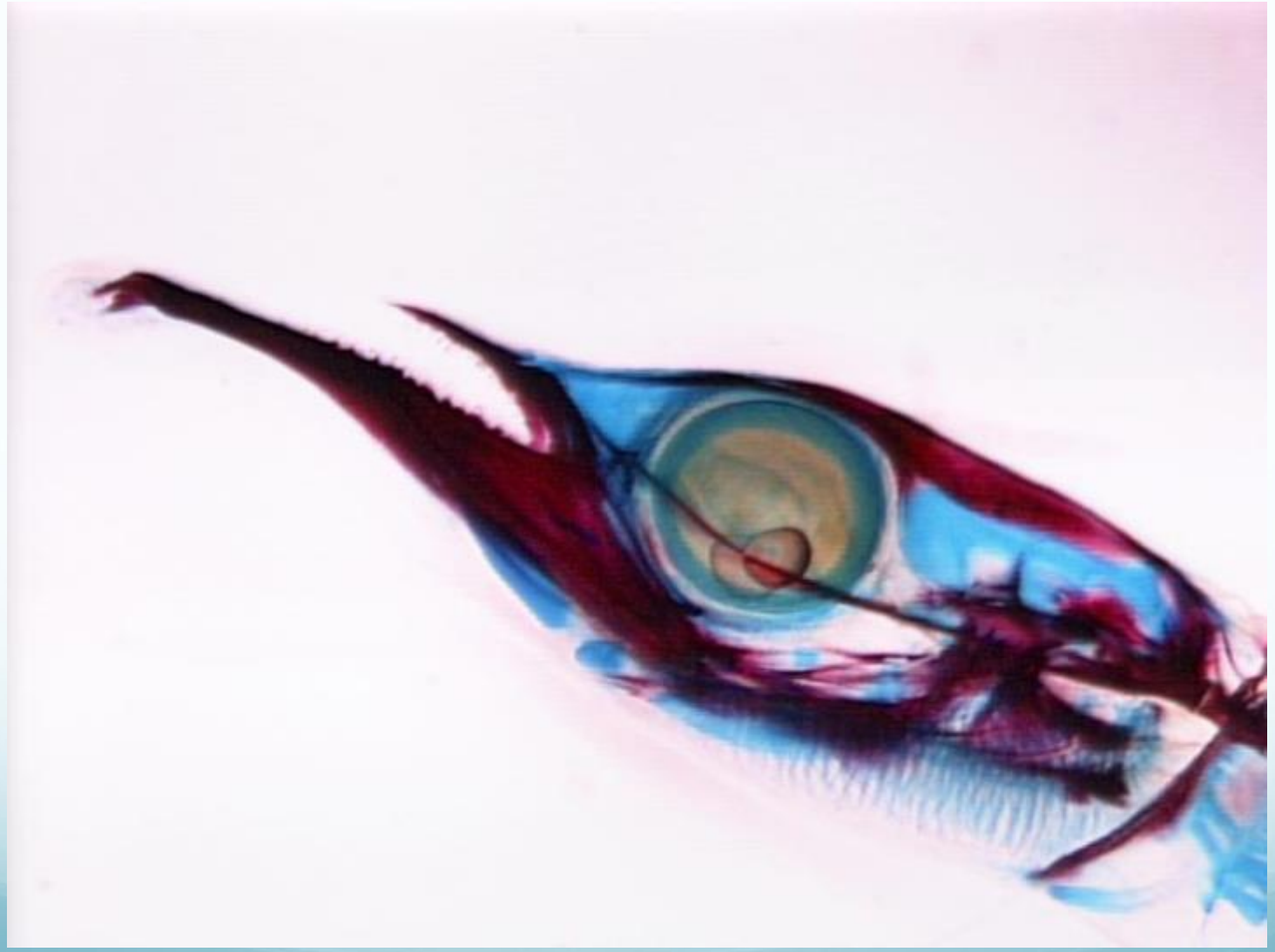
Chondrocranium of garfish (*Belone belone*) larvae at the age of 3 dph, 1x



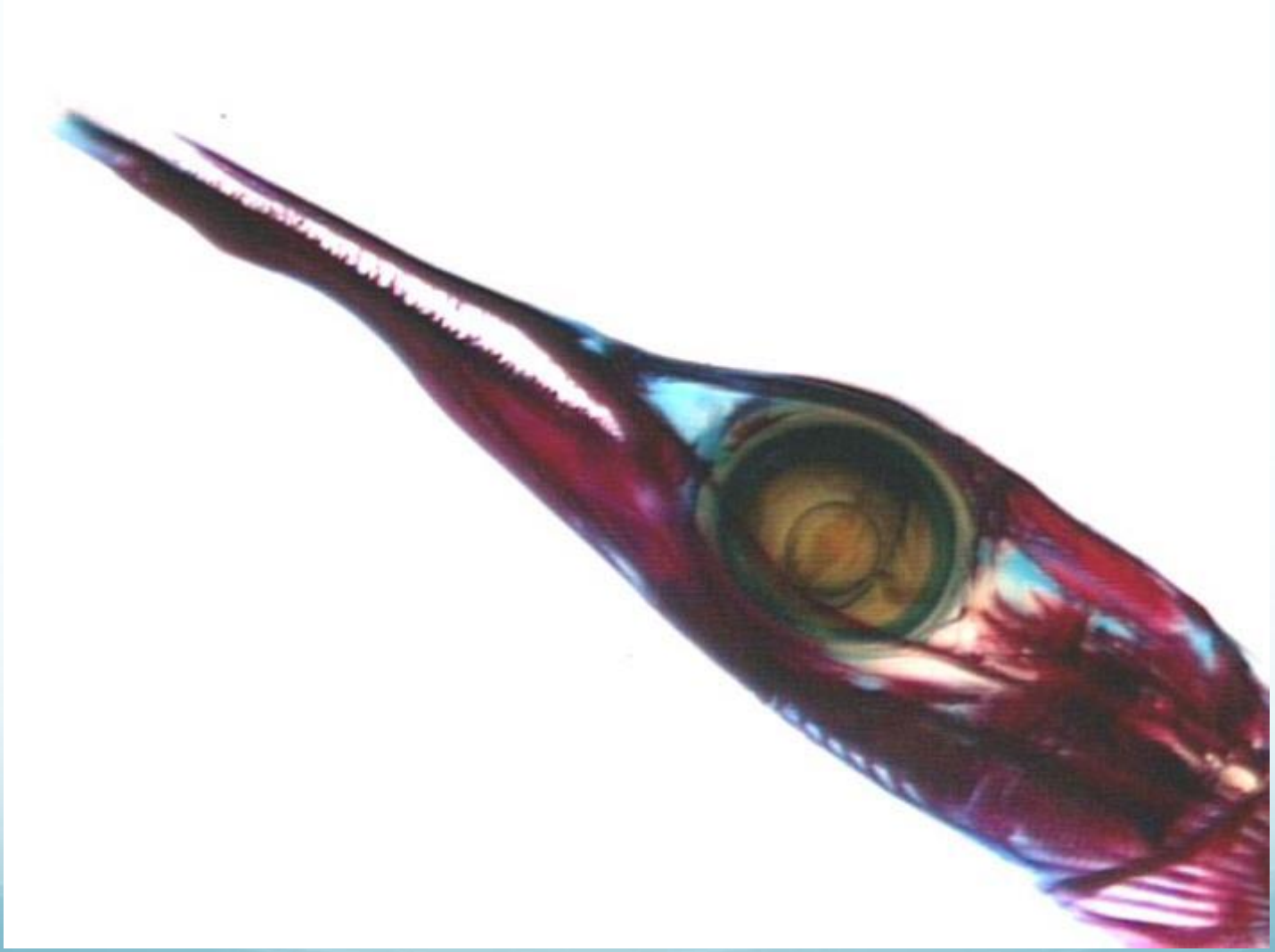
Chondrocranium of garfish (*Belone belone*) larvae at the age of 8 dph, x1.



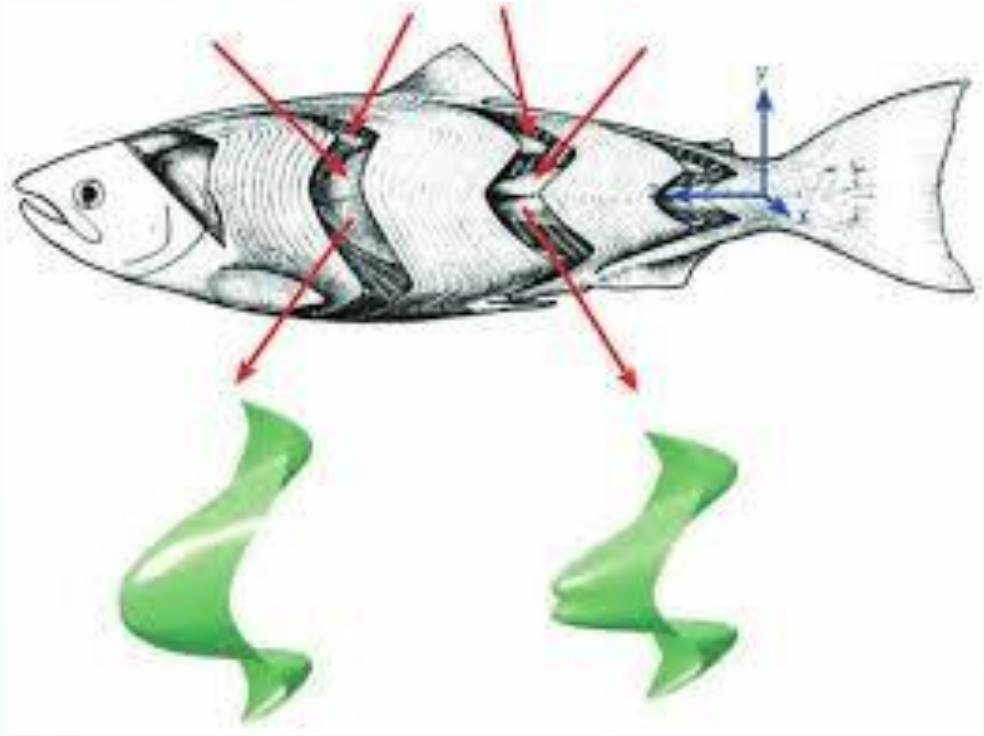
Chondrocranium of garfish (*Belone belone*) larvae at the age of 18 dph, x1.



Chondrocranium of garfish (*Belone belone*) larvae at the age of 21 dph, x1.



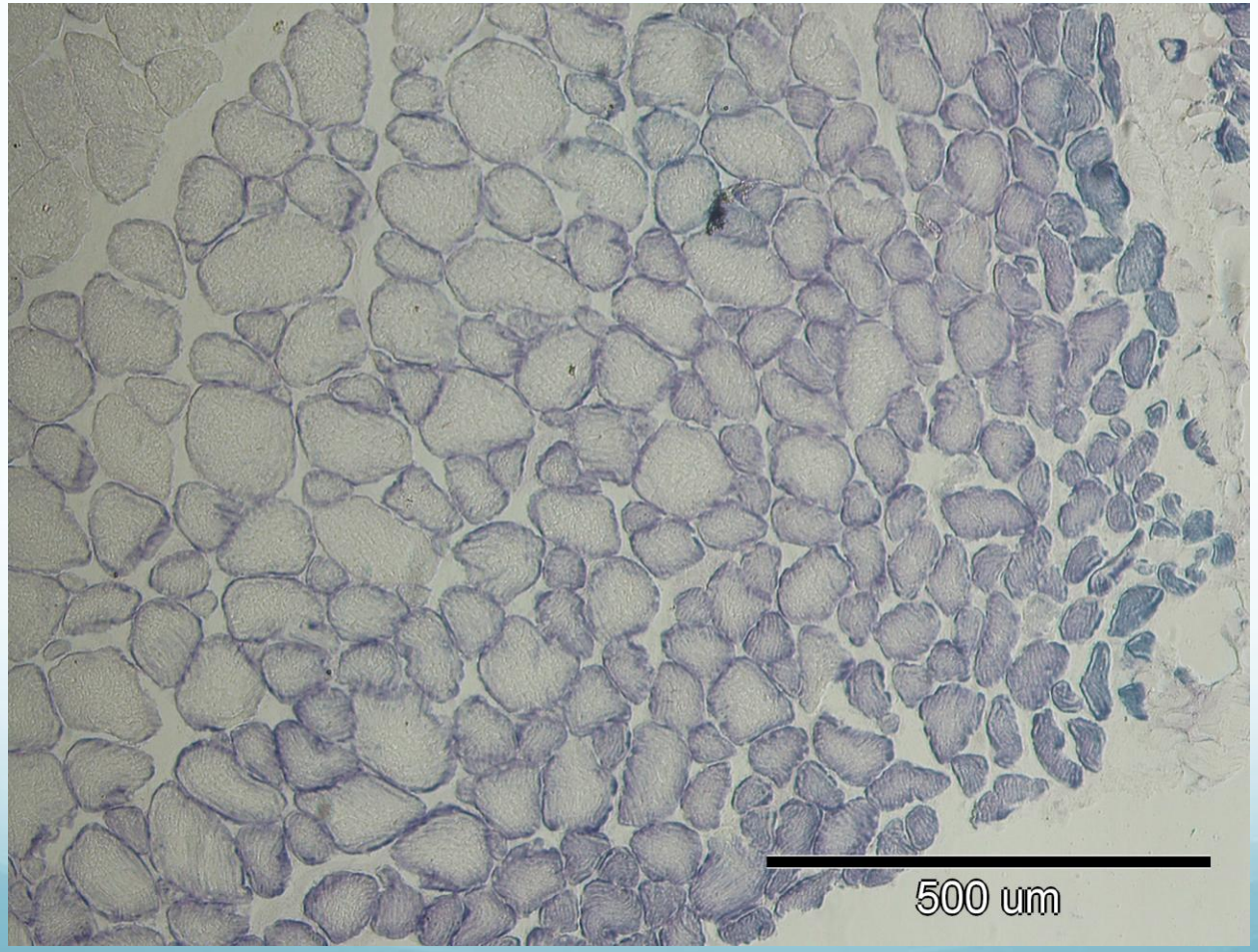
Myomeres shape in adult fish



J. L. Van Leeuwen. 1999

Fish muscles are markedly segmented. They are structured of muscle fibers and some connective tissue. The body and tail muscles support the backbone from the left and the right side and beneath and above the vertebrae, epibrachially and hypobrachially. There are separate muscles along the fins and head bones.

- **The major part of the trunk and tail muscles is structured of white muscle fibers. Red fibers are placed superficially at the lateral side of the body as dark muscles are clearly distinguished from the white ones. In some fish, between the superficial red and deep white muscle fibers, there is a layer of pink, or intermediary, fibers.**



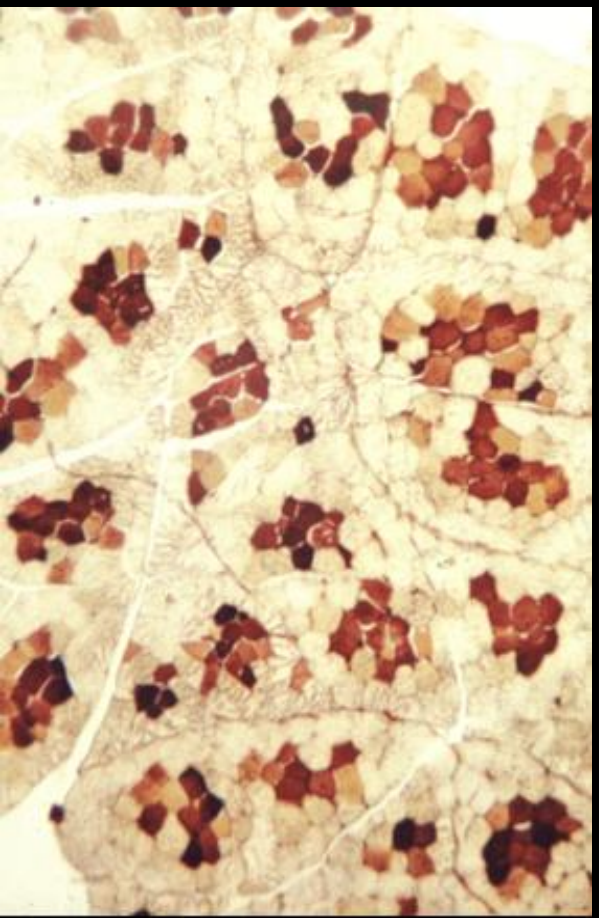
Histomorphological and histochemical characteristics of types of the muscle fibers

Muscle fiber type	red	white	Intermediary
Diameter	small	big	medium
Mitochondria	more	less	middle
Capillary network	rich	less rich	moderate
Myoglobin	increasing amount	small amount	moderate
Glycogen	small amount	increasing amount	moderate amount
Lipids	increasing amount	small amount	moderate
Activity of oxidative enzymes (LDH i SDH)	stronger	weaker	moderate
Activity of glicolitic enzymes (phosphorylase)	weaker	stronger	moderate
Activity of myosin ATP-ase	weaker	stronger	moderate
Metabolism	**aerobic degradation processes fatty acids**	anaerobic glycolysis	aerobic and anaerobic type of metabolism
Contraction	**slow contractive oxidative fibers**	fast contractive glycolytic	oxidative-glycolytic, fast or slow contractive

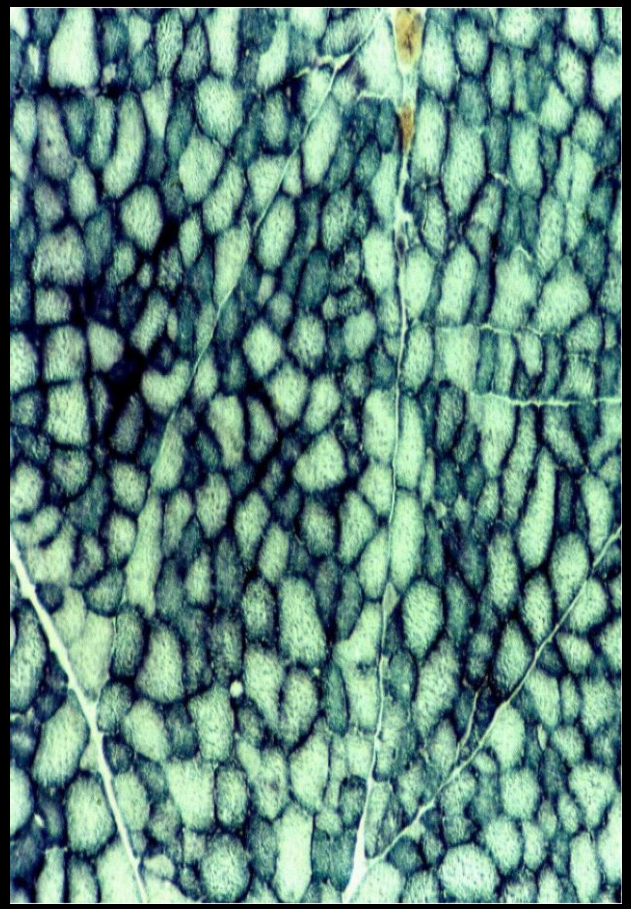
- **White muscle fibers are, by their metabolic characteristics, fast twitch glycolytic fibers used for forceful and rapid contractions during hunting for prey or escaping from predators and they tire quickly.**
- **According to their metabolic characteristics, red fibers are slow twitch oxidative fibers, designed for slow repetitive contractions i.e. for slow swimming. Fin muscles are structured mostly of red fibers. Proportions of red fibers increase caudally.**
- **The fiber diameter of intermediary oxidative glycolytic muscle fibers is larger than red and smaller than white.**
- **Skeletal muscles in fish develop by hyperplasia (genesis of new fibers) and hypertrophy (increase in fiber size-measurement of muscle fiber diameters).**

Types of muscle fibers with respect to:

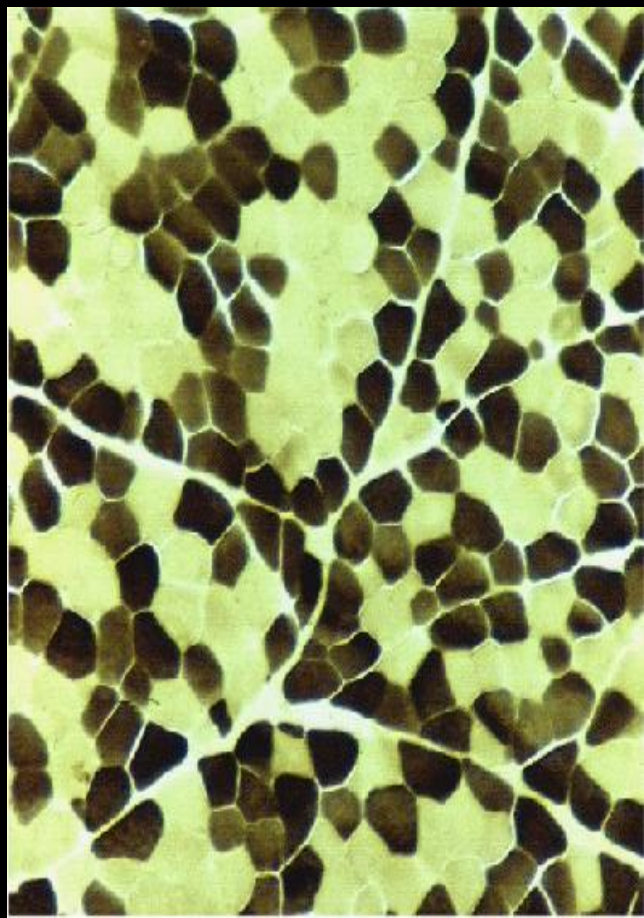
Amount of myoglobin



Activity of lactic acid dehydrogenase (LDH)

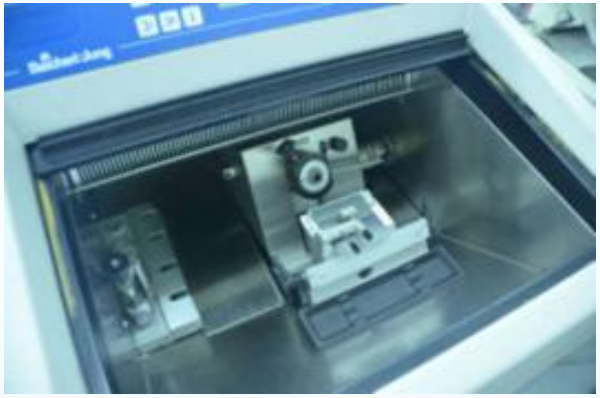


Activity of adenosin triphosphatase (ATP-ase)



Materials and methods

- **Samples of muscles of common pandora *(Pagellus erythrinus Lineatus, 1758)*, bogue, european hake (*Merluccius merluccius L. 1758)*, john dory (Zeus faber, Linnaeus, 1758), red mullet (*Mullus surmuletus*, Linnaeus, 1758), whiting *(Merlangius merlangus* L), saddled seabream (*Oblada melanura*) stargazer (*Uranoscopus scaber*, Linnaeus 1758), prussian carp (*Carassius auratus gibelio)*, european perch (*Perca fluviatilis)* were frozen in a liquid nitrogen and cut to 10 µm thin slices, which were then stained by hematoxyline and eosin (HE), (ROMEIS, 1968) for the analysis of muscular tissue.**



- **For the determination of metabolic activities of fibers, slices were subdued to procedures for showing: activity of succinic acid dehydrogenase (SDH) (PEARSE, 1972), lactic acid dehydrogenase (LDH) (PEARSE, 1972) and alkaline and acid stable adenosintriphosphatase (ATP) (Brooke and Kaiser, 1970).**

- **Van Gieson method (ROMEIS, 1968) slices were stained to show connective tissue, PAS method (ROMEIS, 1968) to show amount of glycogen in muscles (staind red) and Sudan Black B method treatment according to Lison (ROMEIS, 1968). for lipides inside muscles (stained black).**

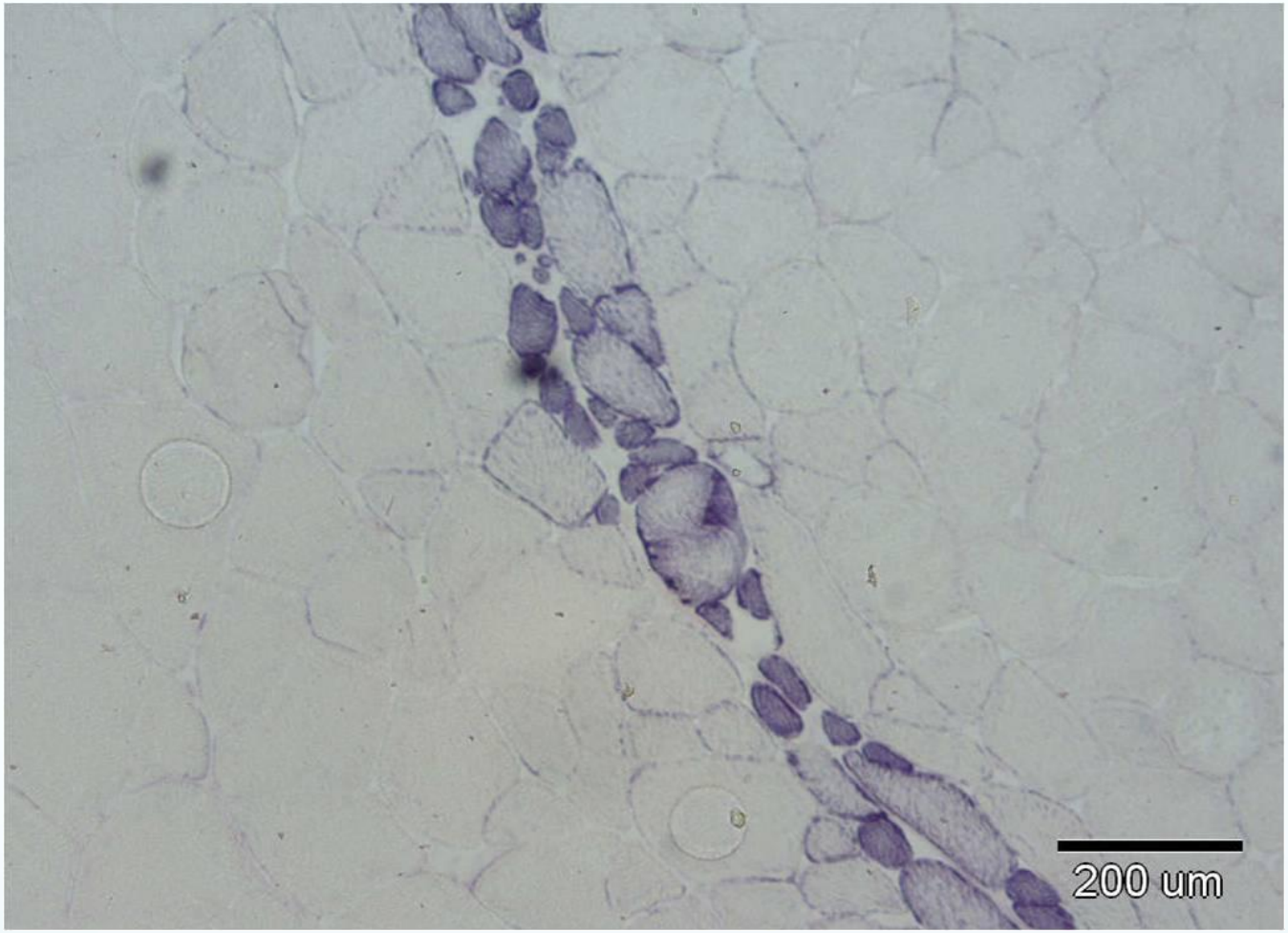


Stargazer, muscle fibers in the trunk muscle dorsal of the horizontal septum, hematoxyline and eosin, magnification 10x (further magnification).

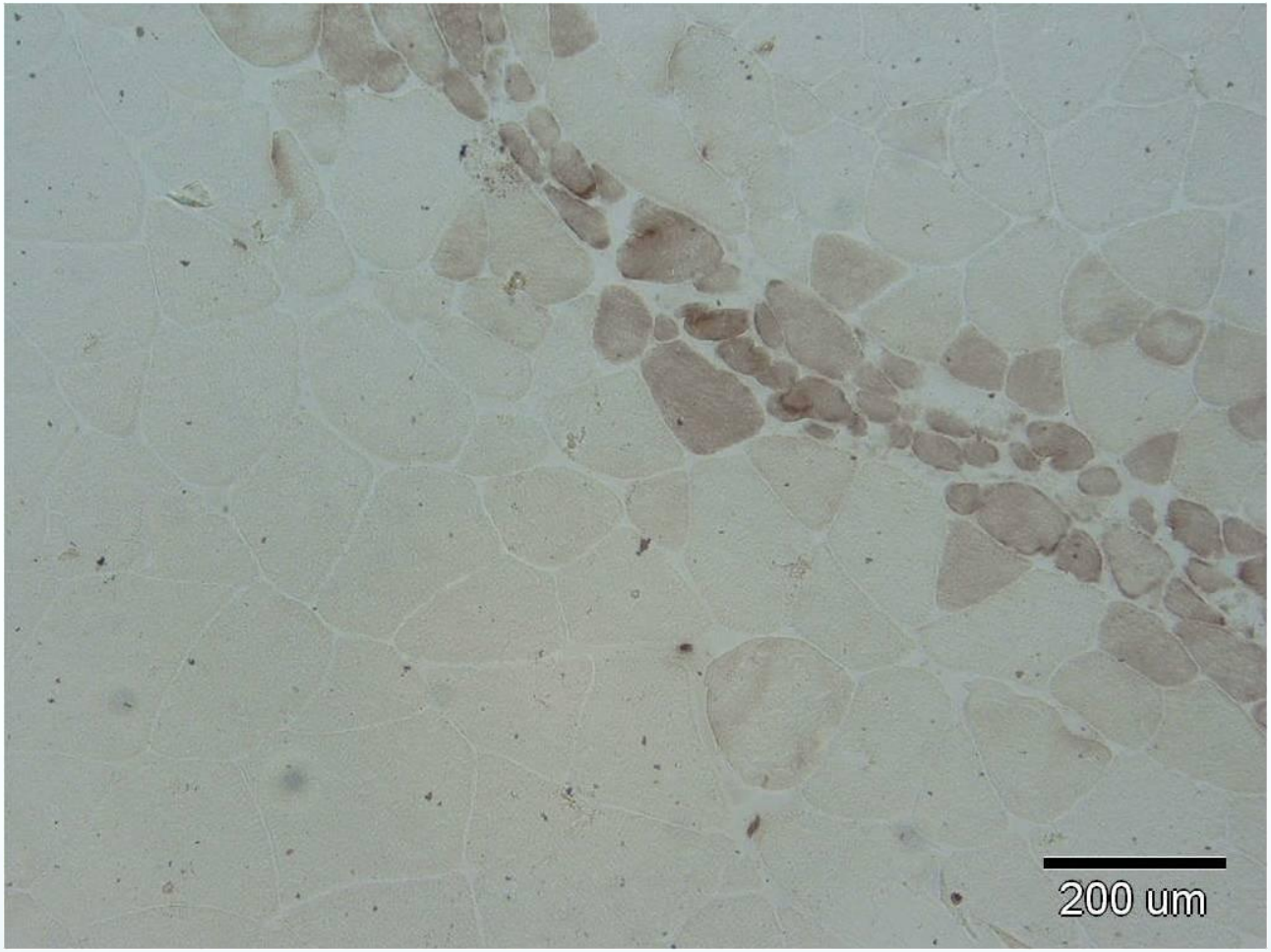
The activity of LDH and SDH as well of the alcaline stable ATP was weak but the activity of acid stable ATP was strong in white fibers. The intermediary oxidative glycolytic muscle fibers have got moderate to strong activity of SDH, LDH, acid and alcaline stable ATP. The activity of LDH and SDH, as well of the alcaline stable ATP was strong, but the activity of acid stable ATP was weak in red fibers. The red fibers were placed superficially at the lateral side of the body, as dark muscles clearly distinguished from the white ones, but in stargazer, small red and intermediary (pink) muscle fibers run as a band into the white dorsal muscle.



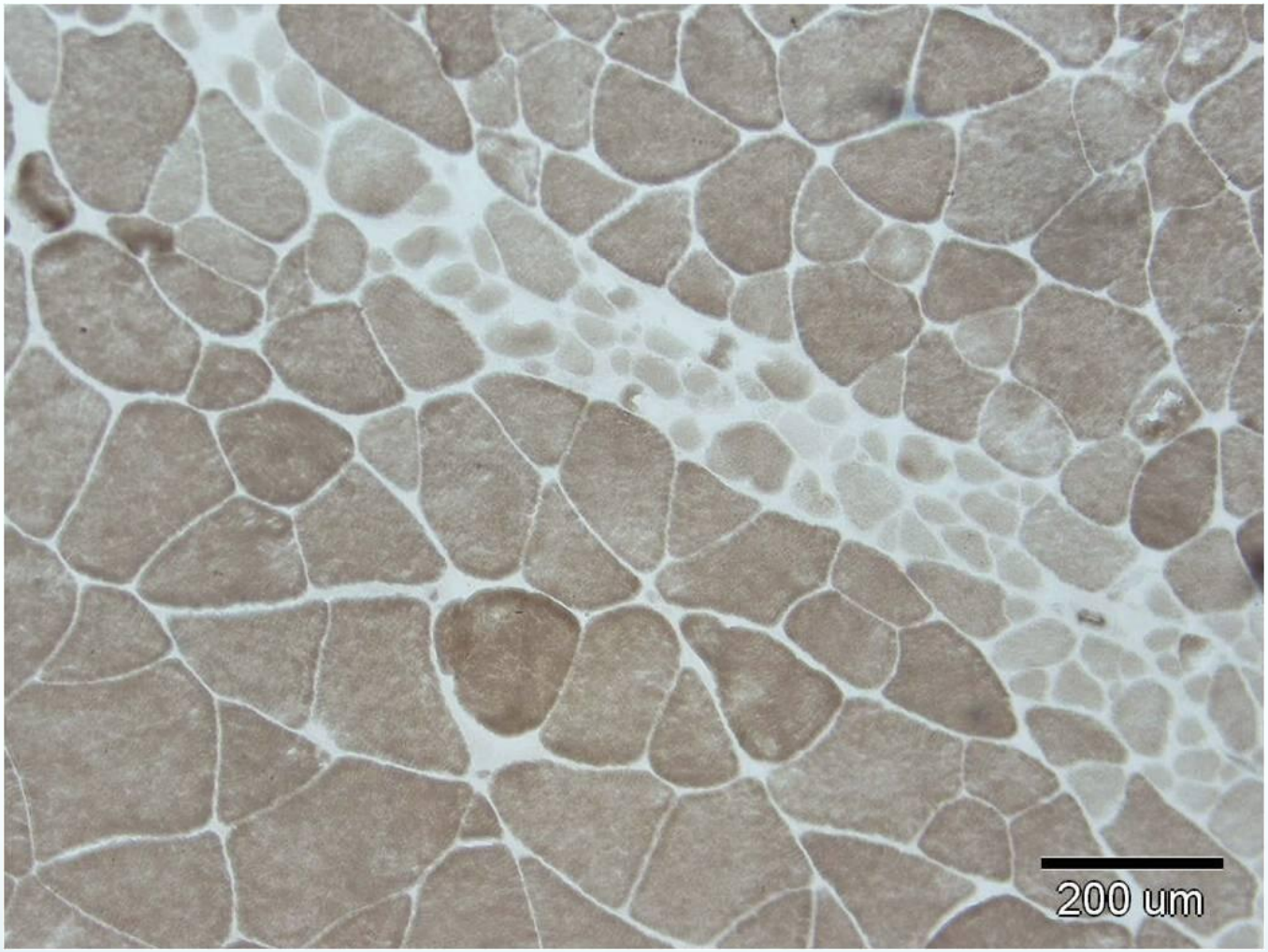
stargaser (*Uranoscopus scaberr, Linnaeus 1758)*



Stargazer, muscle fibers in the trunk muscle dorsal of the horizontal septum, the activity of lactic acid dehydrogenase (LDH), magnification 10x (further magnification).



Stargazer, muscle fibers in the trunk muscle dorsal of the horizontal septum, the activity of alcaline stable adenosinetriphosphatase (ATP) 9.4, magnification 10x (further magnification).



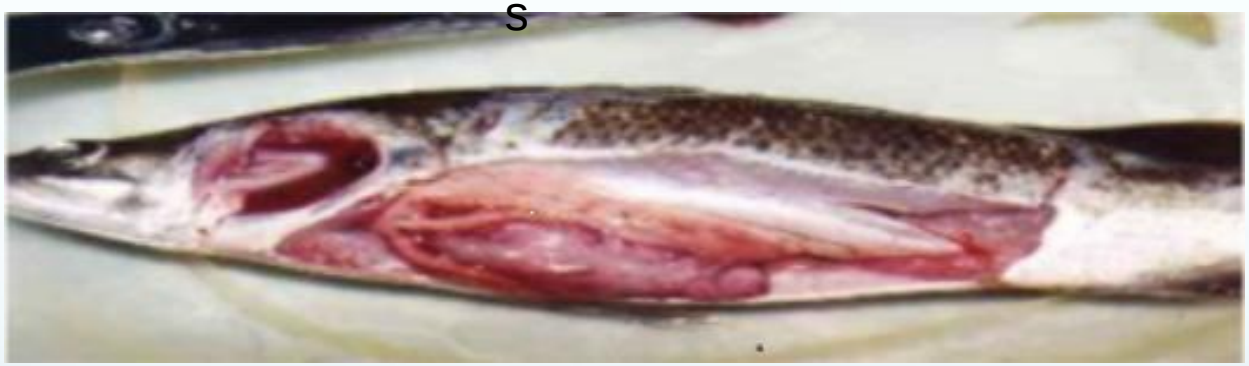
Stargazer, muscle fibers in the trunk muscle dorsal of the horizontal septum, the activity of acid stable adenosinetriphosphatase (ATP) 4.3, magnification 10x (further magnification).

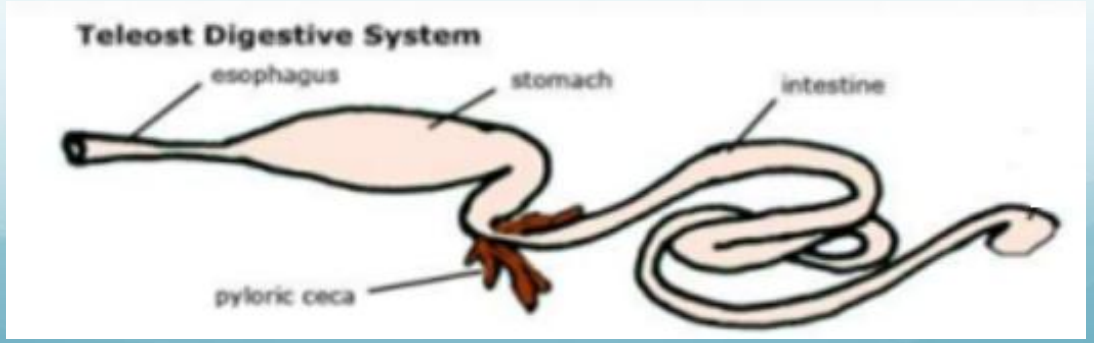
DIGESTIVE SYSTEM

- **The fish digestive system shows a marked diversity of both morphology and function and it is in a correlation with the different feeding habits as well as with the body shape.**

- **Carnivorous fish digestive processes differ from the same in herbivorous fish and their alimentary tract is shorter than in herbivores or omnivores.**

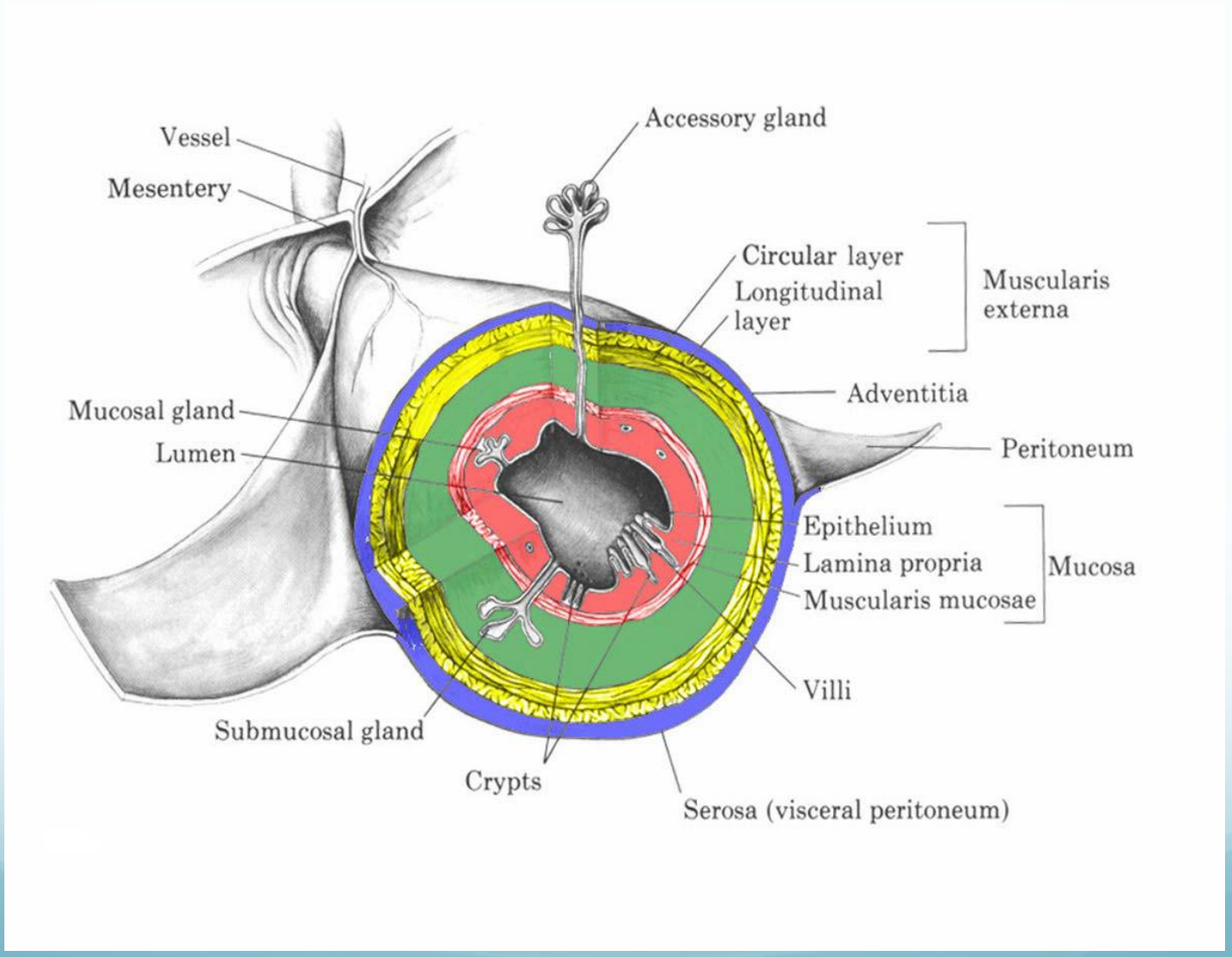
S





,

Gastrointestinal Tract



J. Morrison, 2007

Materials and methods

- **Parts of esophagus, stomach and intestines tissue of Atlantic bluefin tuna (*Thunnus thynnus*, L.) fixed in buffered 10% formalin and embedded in paraffin and sectioned into 10 µm thick slides .**





Materia nd methods

- **Dewaxed sections wer d with:**

- haematoxylin and E) (ROMEIS, 1968) for general histological purposes.

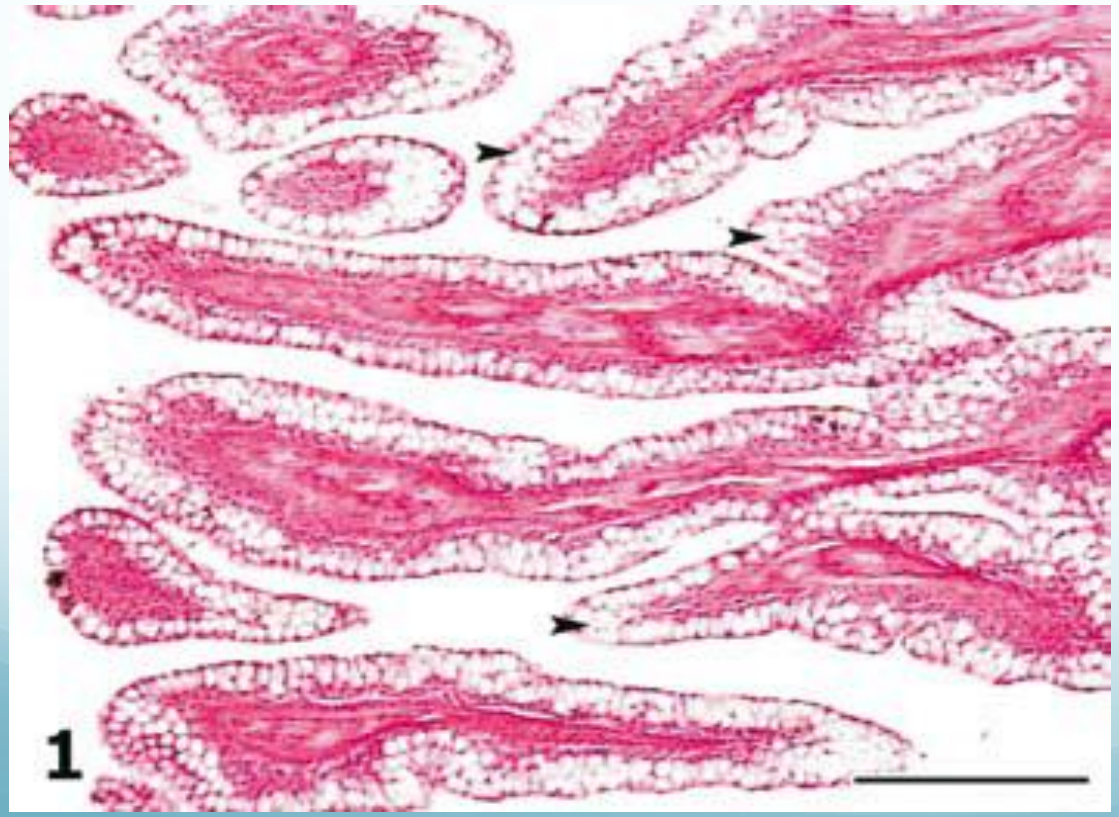
- periodic acid-Shiff (McMANUS, 1948) was used to demonstrate neutral gly gates.

- alcian blue (AB) at nd 1.0 (LEV and SPICER, 1964) and metachromasia w idine blue (TB) were used to demonstrate various ki acid glycoconjugates (PEARSE, 1968).

- **AB-PAS at pH 1,0 (*et al*.,1967)**

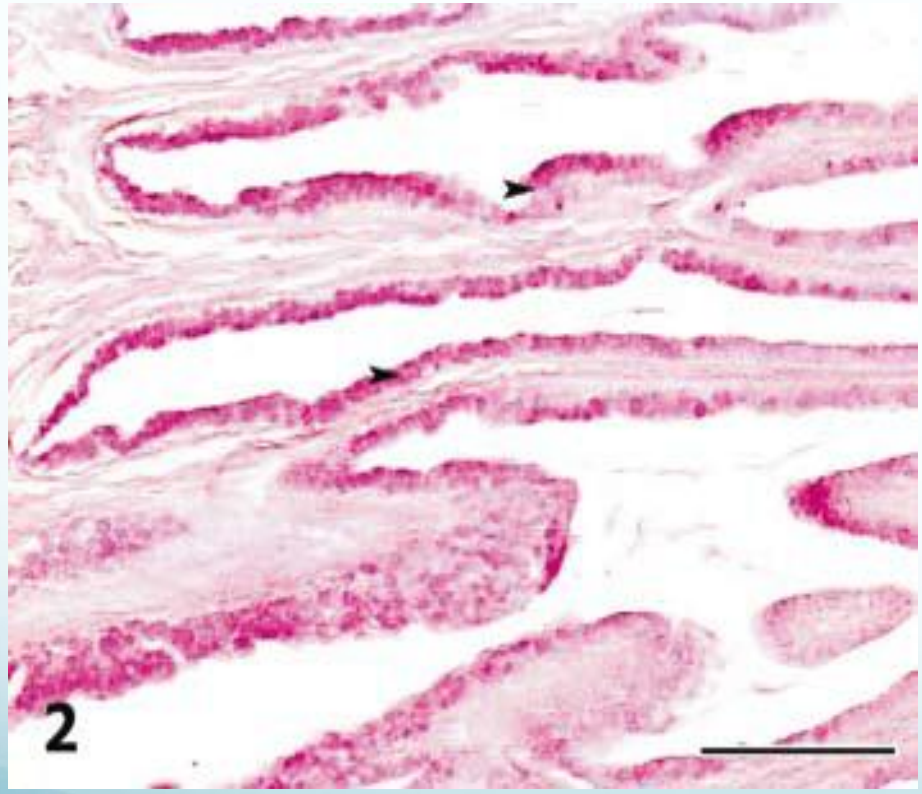
- **The alimentary tract of Atlantic bluefin tuna (*Thunnus thynnus*, L.) is short in accordance with their predatory nature and consists of the esophagus, stomach and intestine.**

- **The short esophagus has deep longitudinal folds and two basic cell types in the epithelial layer: basal and superficial epithelial cells, as well as saccular mucous cells.**

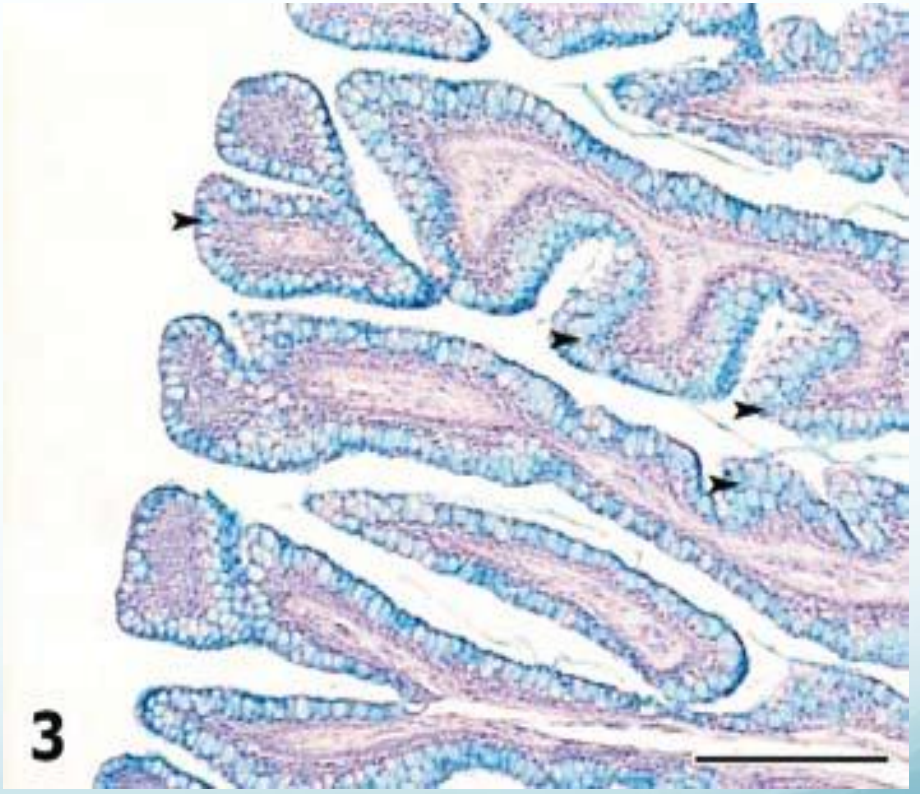


Atlantic bluefin tuna, the esophagus is lined with stratified epithelium with many mucous cells (arrow head). H&E; scale bar = 200 µm.

The most prominent and distinctive feature of the esophagus epithelium is the presence of numerous mucous cells that synthesize a large quantity of neutral as well as sulphated and silylated acid GPs. The histochemical methods employed showed high PAS-positive, AB at both pH values as well as TB metachromatic GPs in these cells.

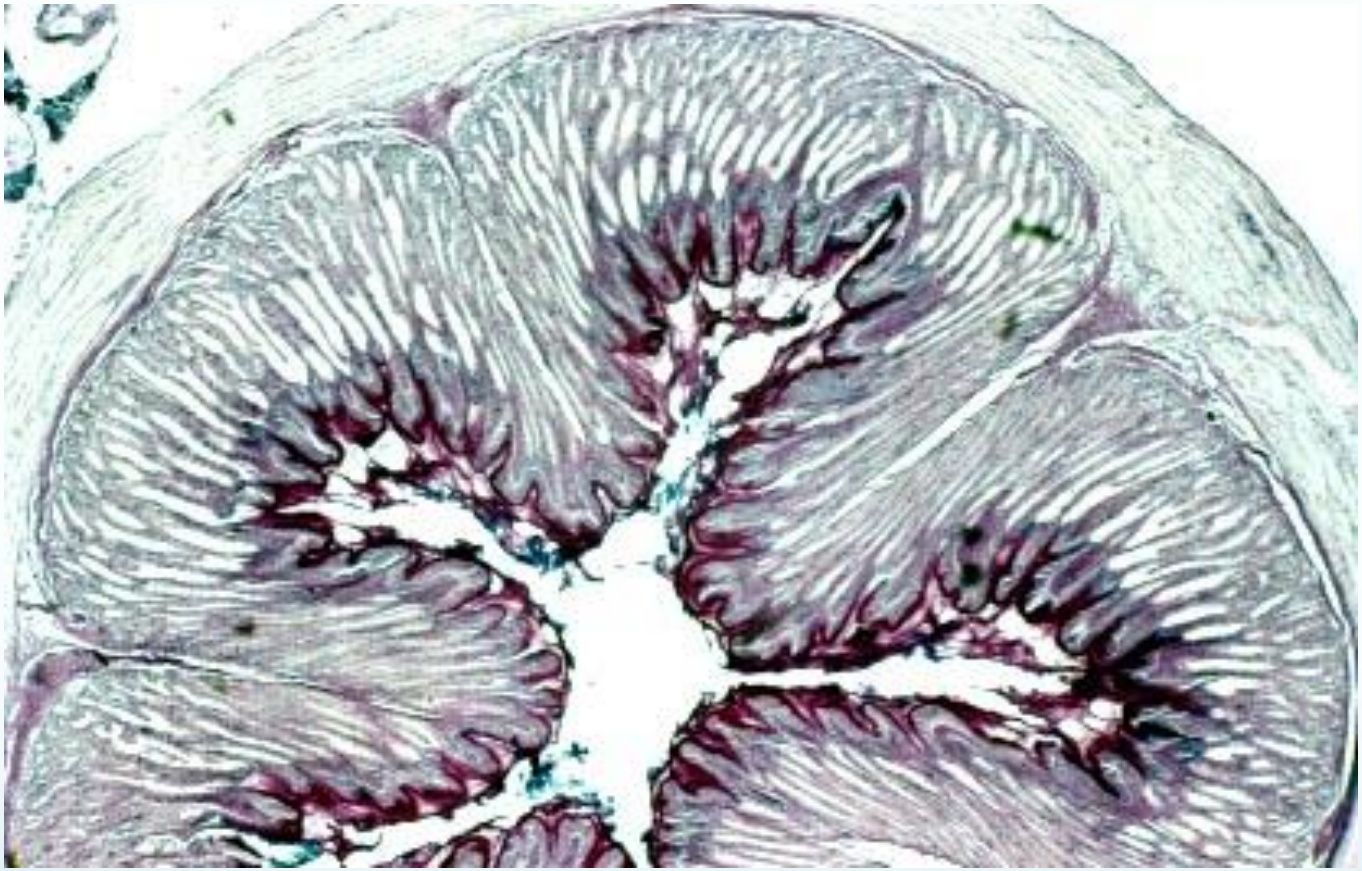


Atlantic bluefin tuna, esophagus, intensive stained mucous cells of mucosa (arrow head). PAS; scale bar = 200 µm.



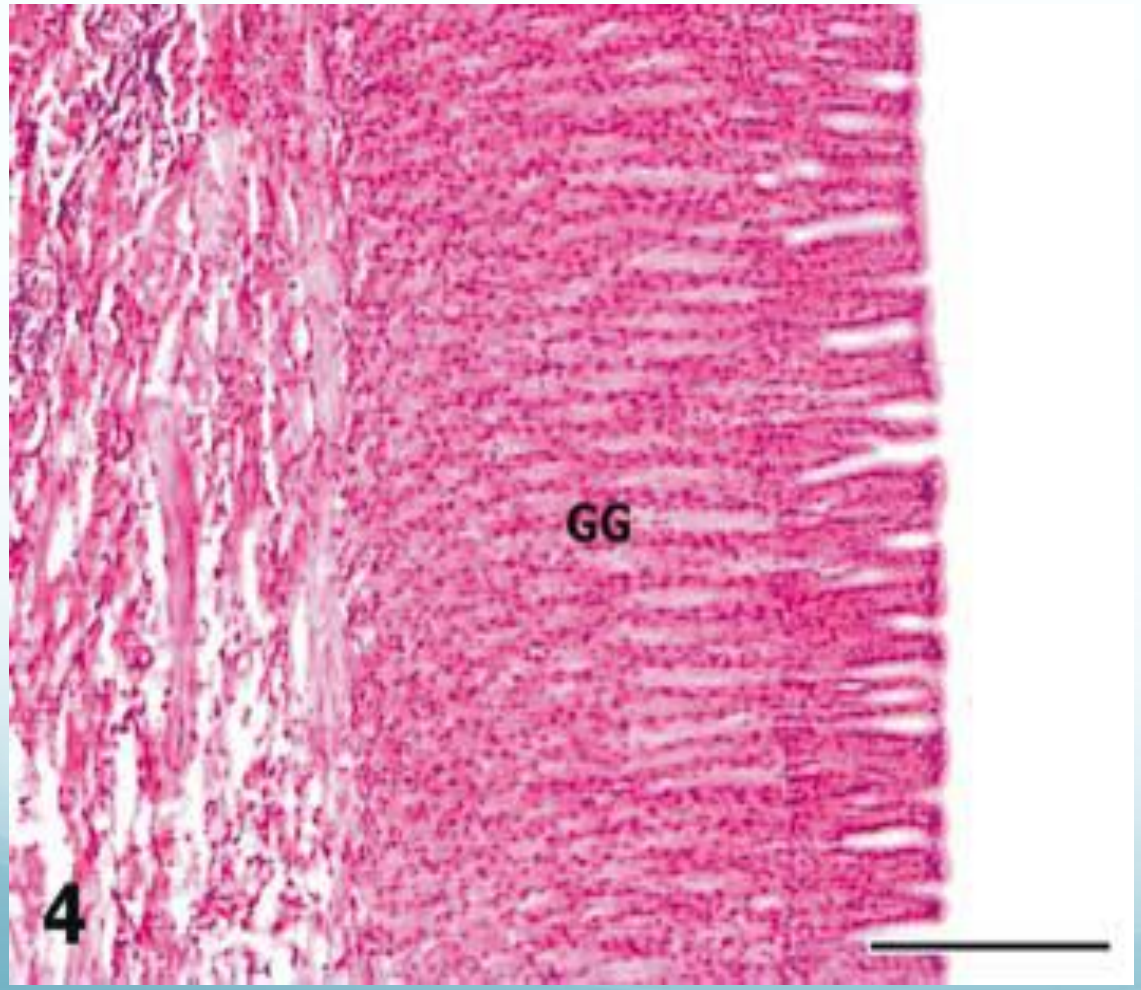
Atlantic bluefin tuna, esophagus, AB (2.5), mucous cells show strong AB reaction (arrow head). Scale bar = 200 µm.

- **A large quantity of PAS-positive substances in the mucus point to the presence of glycoproteins (GPs) with oxidizable vicinal diols and/or glycogen.**
- **Acid mucosubstances stained with AB (2.5) and TB-metachromatic suggest the presence of GPs with sialic acid and GPs with O-sulfate esters.**
- **The acidomucins present in the esophagus would confer high viscosity to the mucus, which aids in trapping small particles.**
- **Mucins in the posterior esophagus of some fish may play a role in pre-gastric digestion.**



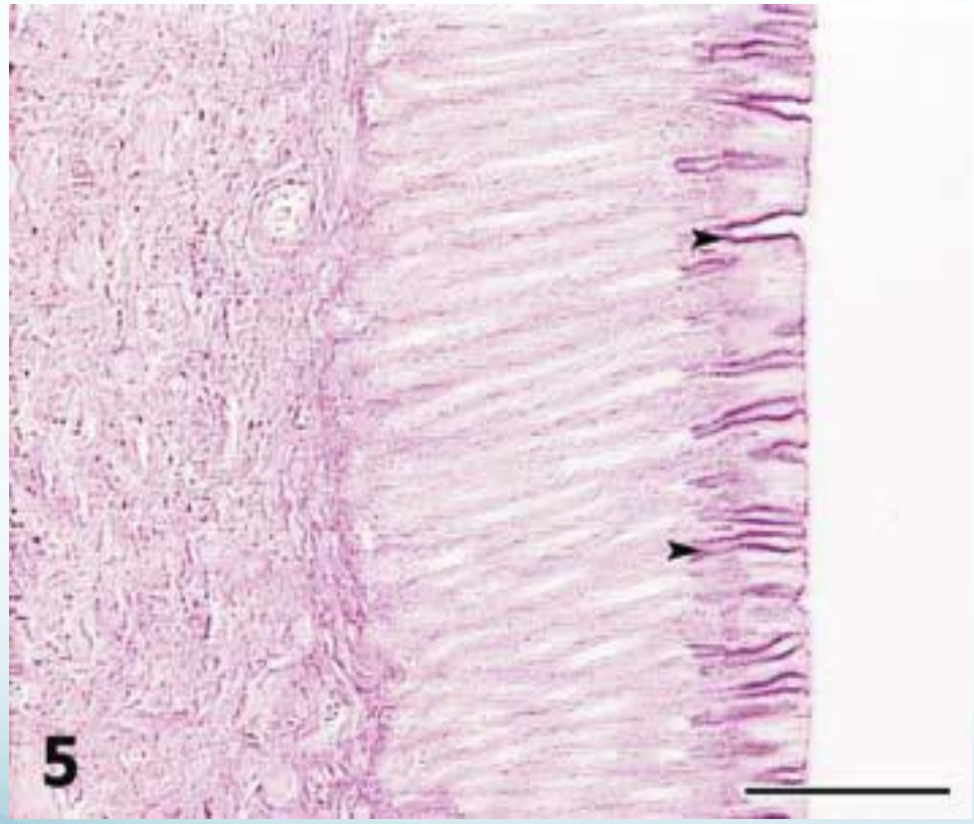
European pilchard (*Sardinia pilchardus* ,Walbaum), esophagus , AB- PAS, 63x

The stomach is an elongated sac-like structure. Superficial epithelial cells and simple tubular gastric glands in lamina propria form the mucosa.

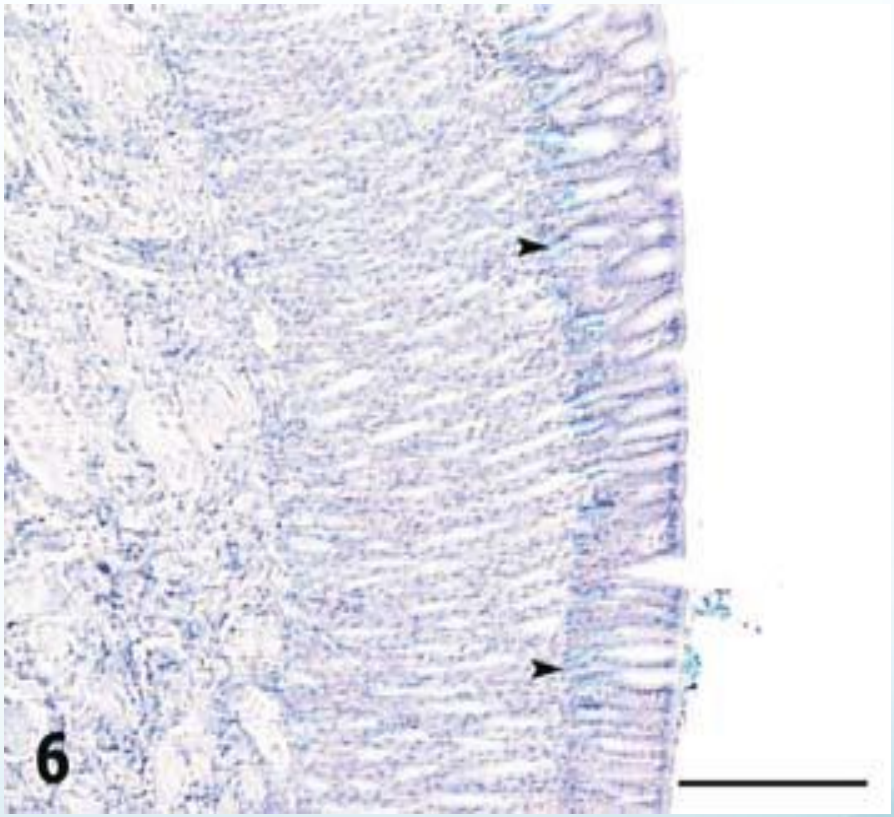


Atlantic bluefin tuna, stomach gastric glands (GG).
H&E; scale bar = 200 µm.

The epithelial cells, as well as the neck cells of gastric glands synthesize a mixture of neutral and acidic GPs. The mucosubstances facilitate the movement of large-sized food particles as well as protecting the gastric mucosa from a mechanical injury.

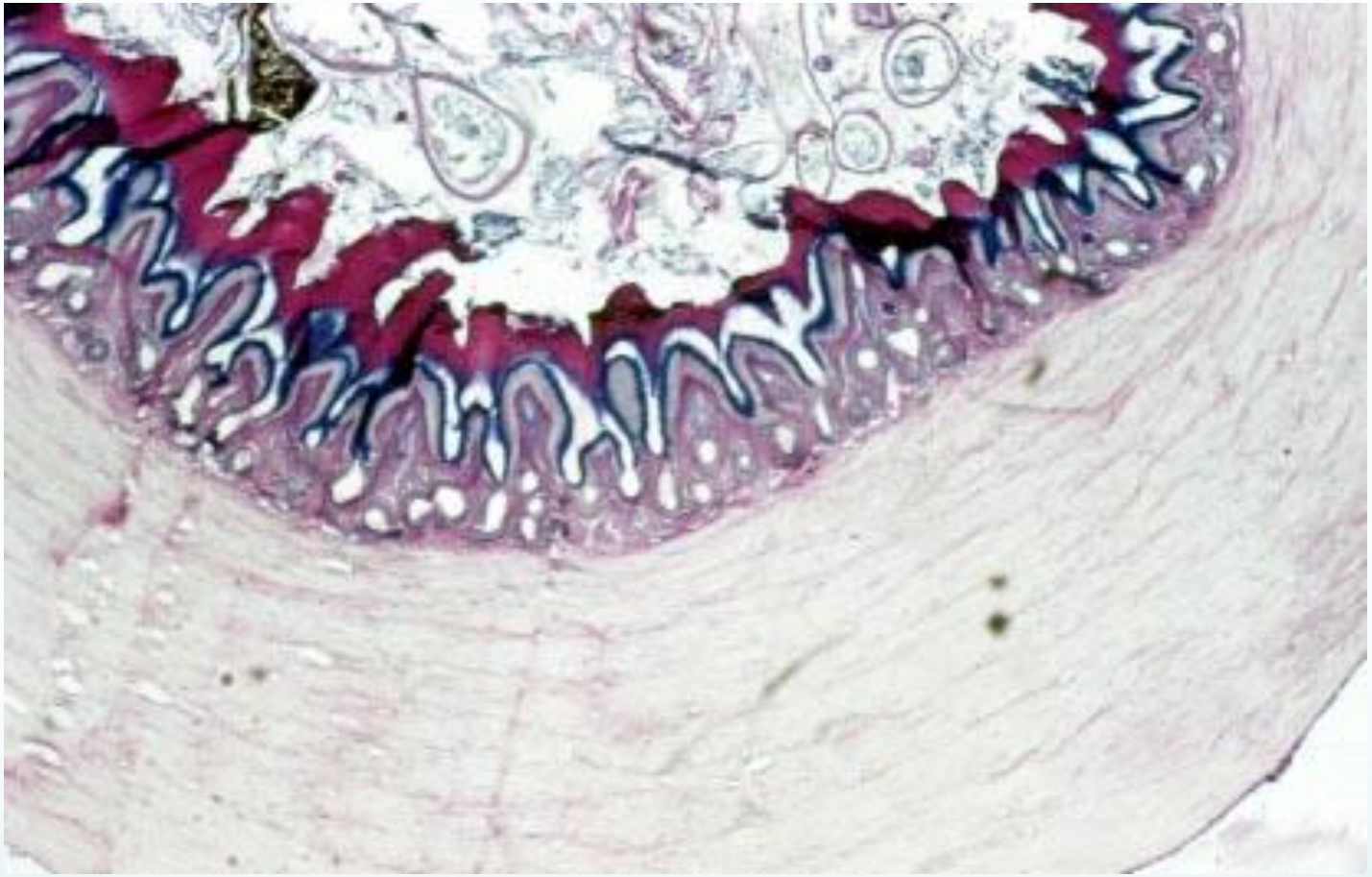


Atlantic bluefin tuna, stomach, PAS-positive GPs are present in apical cytoplasm of epithelial cells as well as in neck cells (arrow head). PAS; scale bar = 200 µm.

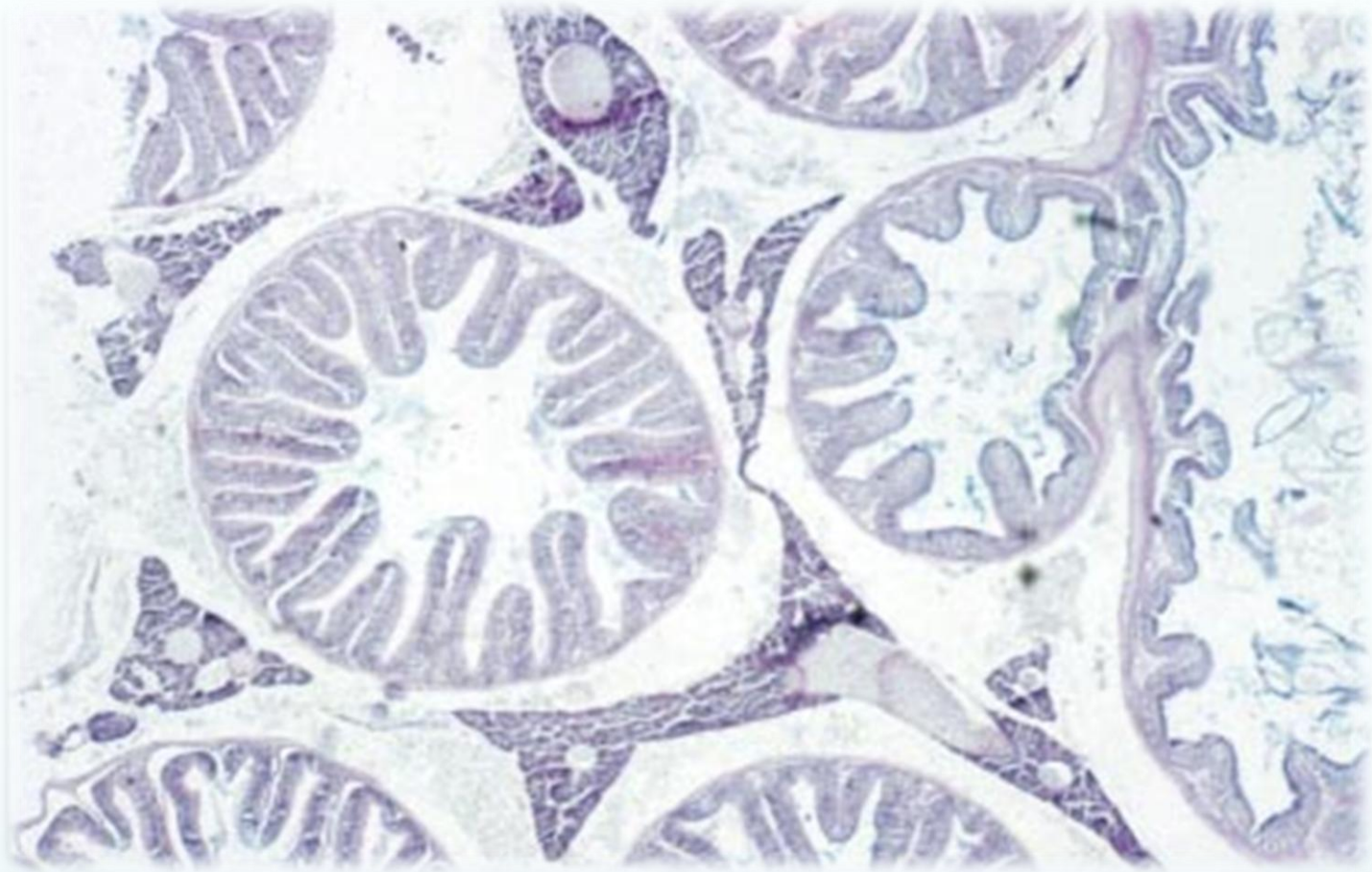


Atlantic bluefin tuna, stomach, AB (2.5), acid GPs are visible in apical cytoplasm of epithelial cells as well as in neck cells (arrow head). Scale bar = 200 µm.

- **Neutral GP-s identified in the apical portion of the epithelial lining cells and in the gastric pits of the stomach of the Atlantic bluefin tuna could serve to protect the underlying mucosa layer from the acid environment and proteolysis and may also be involved in digestive activity, by easily absorbing digested substances, such as disaccharides and short-chain fatty acids.**
- **The fish gastric glands synthesize both pepsinogen and acid and that neutral mucosubstances have a buffering effect on high acidity of stomach contents.**



European pilchard (*Sardinia pilchardus* Walbaum), stomach, AB-PAS, 75X



European pilchard (Sardinia pilchardus Walbaum) pyloric caeca AB-PAS, 75X

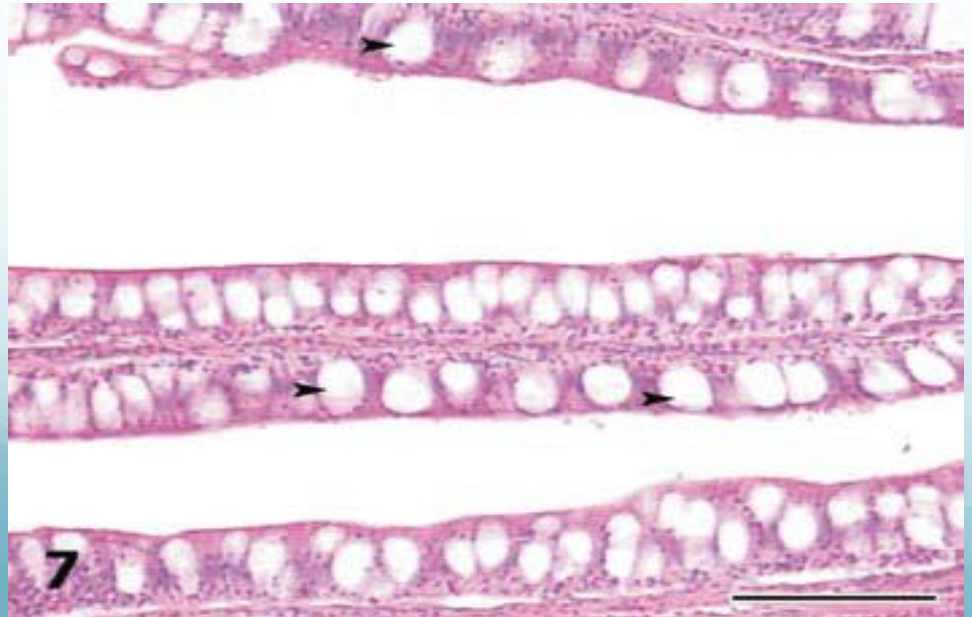
, ,

- **The intestine of bluefin tuna shows a uniform histological structure throughout its entire length. The intestinal mucosa consists of a single layer of columnar cells with many mucous goblet cells.**

- **The mucus secreted by goblet cells in the intestine may have many functions like lubricating and protecting the tunica mucosa against chemicals, parasites and acidity, and forming a diffusion barrier for various ions.**

- **The quality of gut mucosubstances is directly related to environmental conditions, which in turn may directly affect the function of the alimentary tract. The presence of mucosubstances, especially those sulphated in the intestine, possibly regulates the transfer of proteins, or a fragment of them, as well as of ions and fluids**

- **A possible role of mucosubstances in intestinal absorption processes**



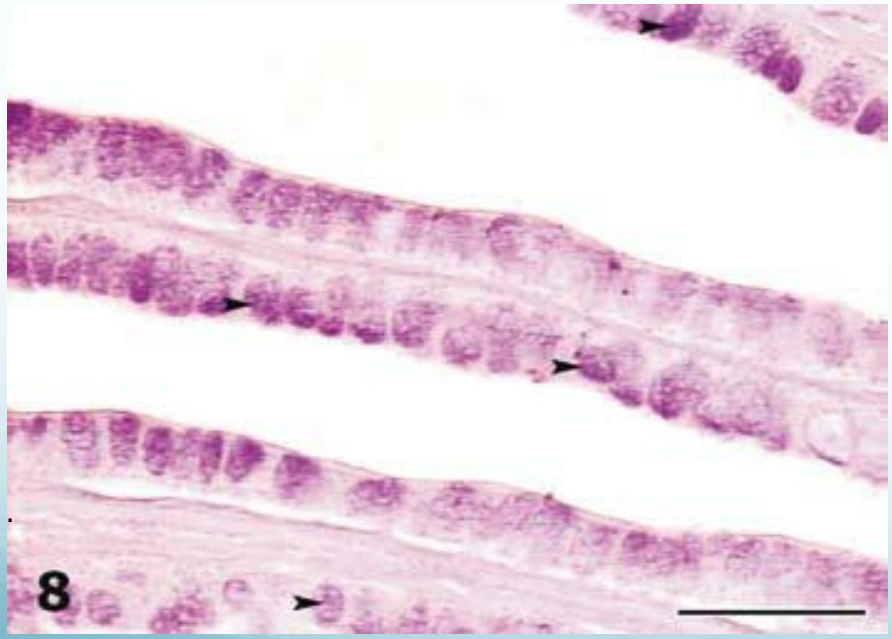
Atlantic bluefin tuna, intestine, goblet cells (arrow head). H&E; scale bar = 200 µm

The intestinal goblet cells synthesize neutral and acidic GPs. The acid GPs contain sialic acid and sulphated esters. A large amount of TB metachromatic substances in goblet cells points to the presence of sialic acid-rich nonsulfated GPs.

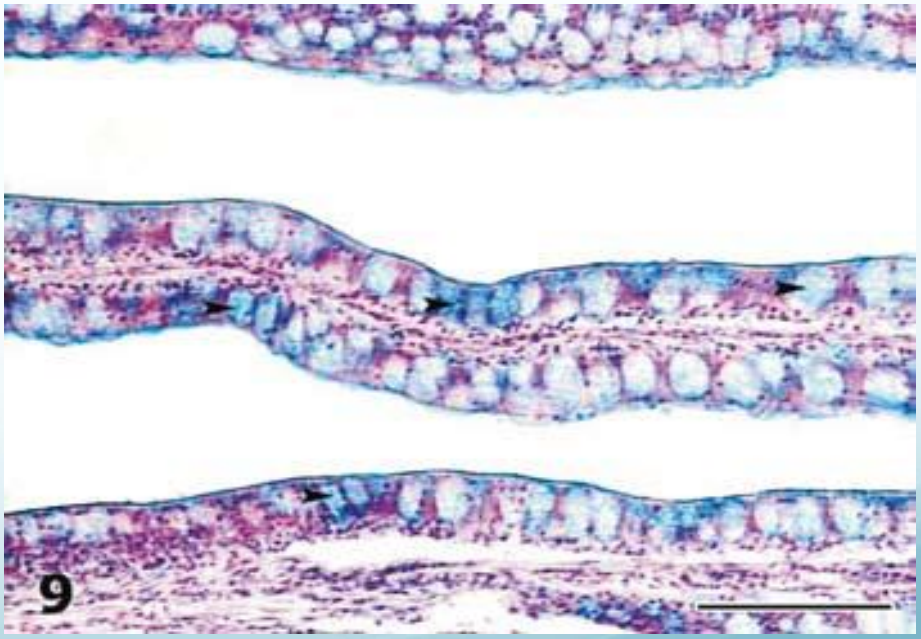
Acid GPs have been shown to coincide with increased viscosity of mucus in the fish alimentary tract and to lubricate the undigested materials for onward progression into the rectum.

The quality of gut mucosubstances is directly related to environmental conditions, which in turn may directly affect the function of the alimentary tract.

The quality of gut mucosubstances varies in different regions of the Atlantic bluefin tuna alimentary canal, and sustains functional harmony of the digestive tract.



Atlantic bluefin tuna, intestine, goblet cells contain neutral GPs. (arrow head). PAS; scale bar = 200 µm.



Atlantic bluefin tuna, intestine, AB (2.5), acid GPs in goblet cells (arrow head). Scale bar = 200 µm.

Intestine function

- **The anterior portion of the intestine functions:**

- **transport food material from the stomach to the posterior intestine;**
- **complete digestion by the secretion of enzymes from its walls and from accessory glands;**
- **to absorb the final products of digestion into blood and lymph vessels in its wall;**
- **secrete certain hormones (i.e. secretin, that stimulates pancreatic secretion).**

- **The posterior intestine functions include fluid absorption, mucous secretion (more goblet cells), some digestion which is accomplished by enzymes present in food material, and excretion.**

Digestive enzymes

- **The ability of fish to utilize ingested nutrients mainly depends on the presence of appropriate enzymes in appropriate locations of the wall and along the lumen of the gastrointestinal tract.**
- **The distribution intensity of gastrointestinal enzymes across the digestive tract varies with feeding habits and intestinal morphology, so better knowledge of digestive enzyme activities is essential for a deeper understanding of the physiology of fish nutrition.**
- **Carnivorous fish have a higher level of protein and lipids in diet. The protein and lipid content in diet is probably responsible for higher activity of esterase, alkaline phosphatase and aminopeptidase, enzymes involved in lipid absorption and protein digestion and absorption.**
- **The absorption of digested food particles and molecules generally takes place along brush border and cytoplasm of enterocytes where numerous digestive enzymes are localized. The enzymes of intestinal enterocytes carry out the final stage of digestion. The activity of intestinal enzymes demonstrated a small but clear difference in the regional distribution and localization across the intestinal mucosa.**

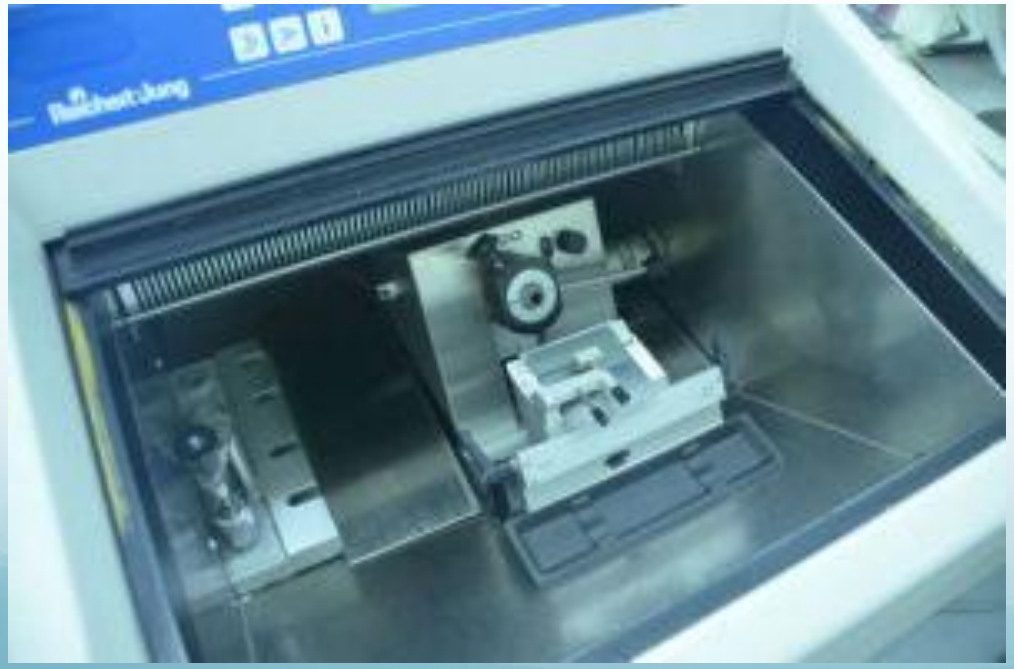
- **Two general types of digestion exist in the gut:**

-The first one is extracellular digestion, in which the enzymes are produced by cells into the gut lumen and digest food particles (luminal digestion).

-The second one is intracellular digestion. It is characterized by the decomposition of food by enzymes associated with cell structures and may be subdivided into cytoplasmatic and membrane digestion. Membrane digestion is associated with the brush border while intracellular digestion is connected to other cell structures, especially lysosomes.

Materials and methods

- **Immediately after catching of free-living hake (*Merluccius merluccius* L. 1758) and common two-banded seabream (*Diplodus vulgaris*, Geoffroy St-Hilaire 1817) samples of esophagus, stomach, and two parts of intestine were fixed in cold (4 °C) formol-calcium for 24 hours and then transferred to ice-cold sucrose solution. The tissues were sectioned in a cryostat into 10 µm thick slides and used for histochemical detection of enzymatic activity.**





Materials and methods

- **Histochemical techniques for detecting enzymatic activities were taken from LOYDA et al. (1979) for the following enzymes:**

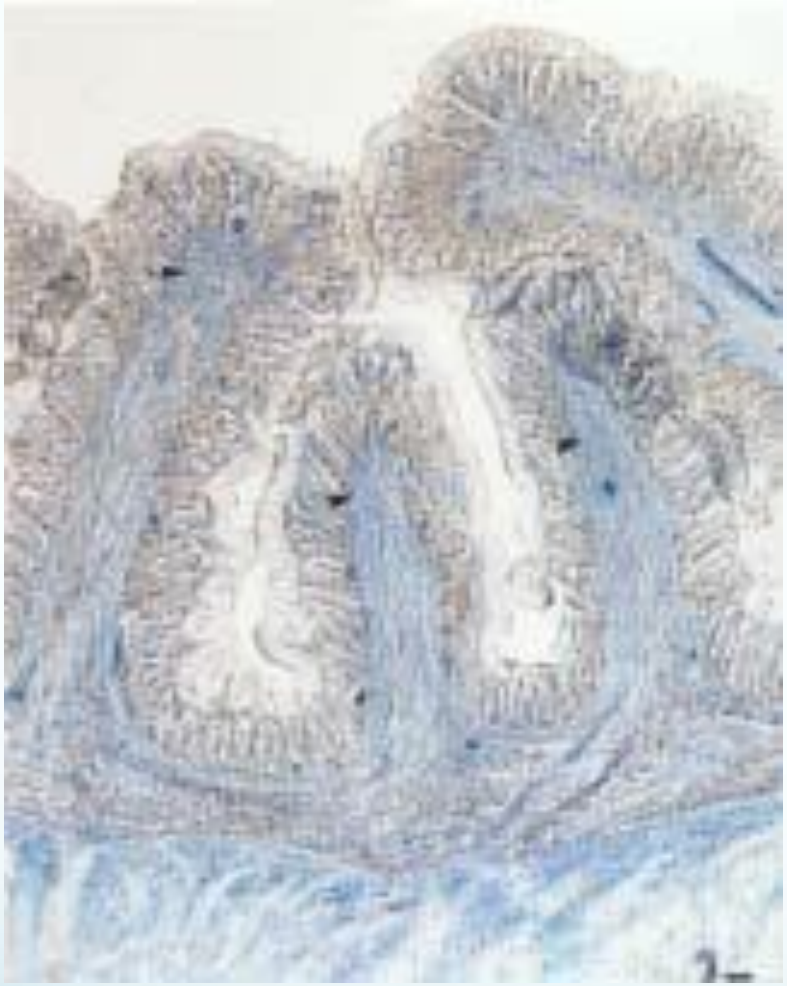
- non-specific esterase (E.C.3.1.1.)-substrate 1- naphthyl-acetate (pH 6.5);

- alkaline phosphatase (E.C.3.1.3.1)-substrate, sodium β-glycerolphosphate (pH 9.4);

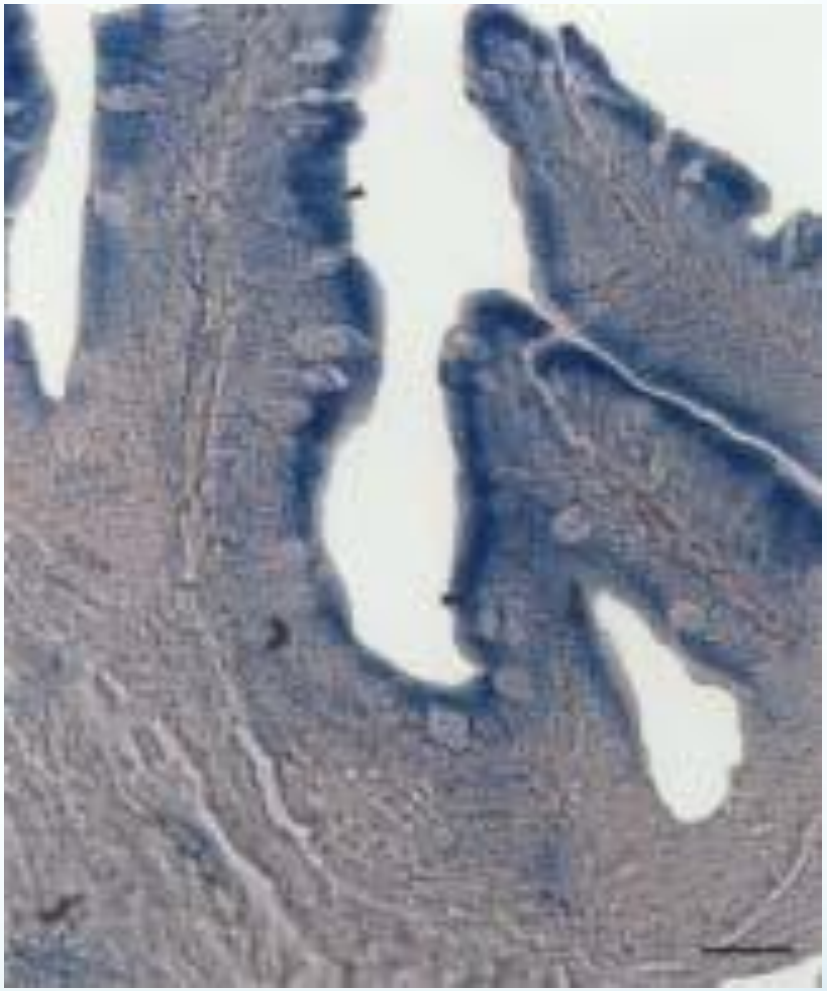
- acid phosphatase (E.C.3.1.3.2.)- substrate, sodium β-glycerolphosphate (pH 5.5).

Aminopeptidase, supstrate-L-Leu-4-MNA (pH 6.5), were taken from PEARSE (1968) .

Enzymatic activity was analyzed visually and described further according to the intensity of the color reaction observed: no enzymatic reaction, weak (barely detectable reaction), moderate reaction and strong reaction.



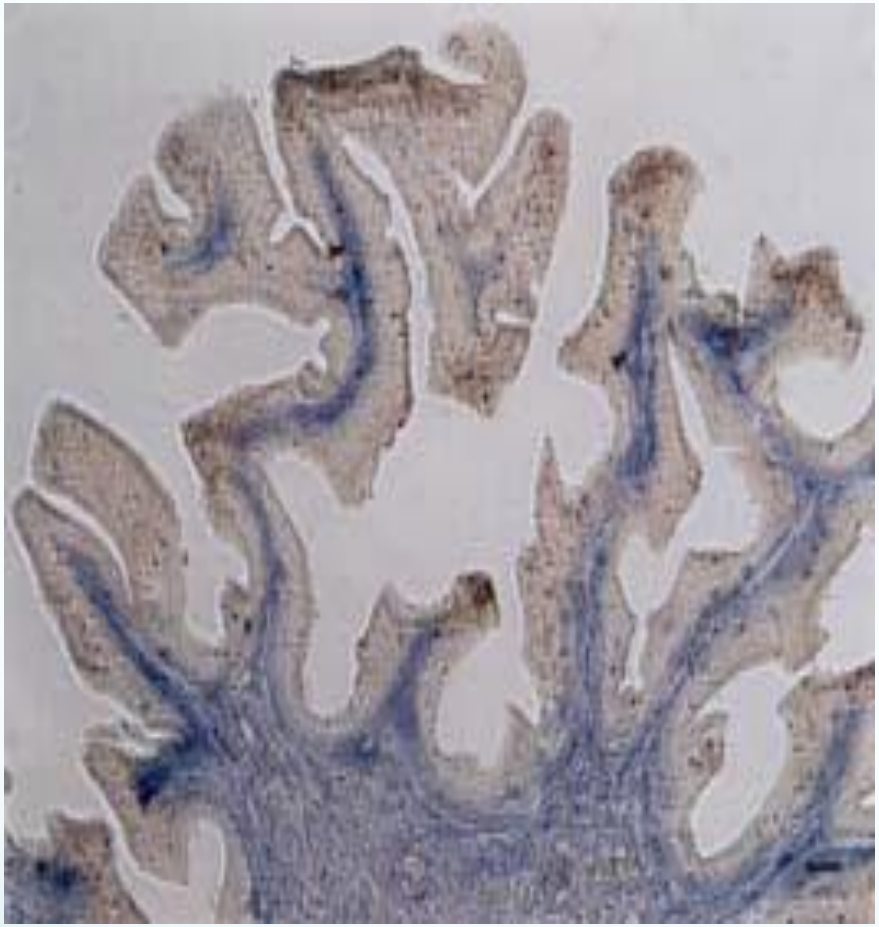
Hake stomach, non-specific esterase. Enzyme activity is observed in lamina propria (arrowheads).
Scale bar = 50 µm.



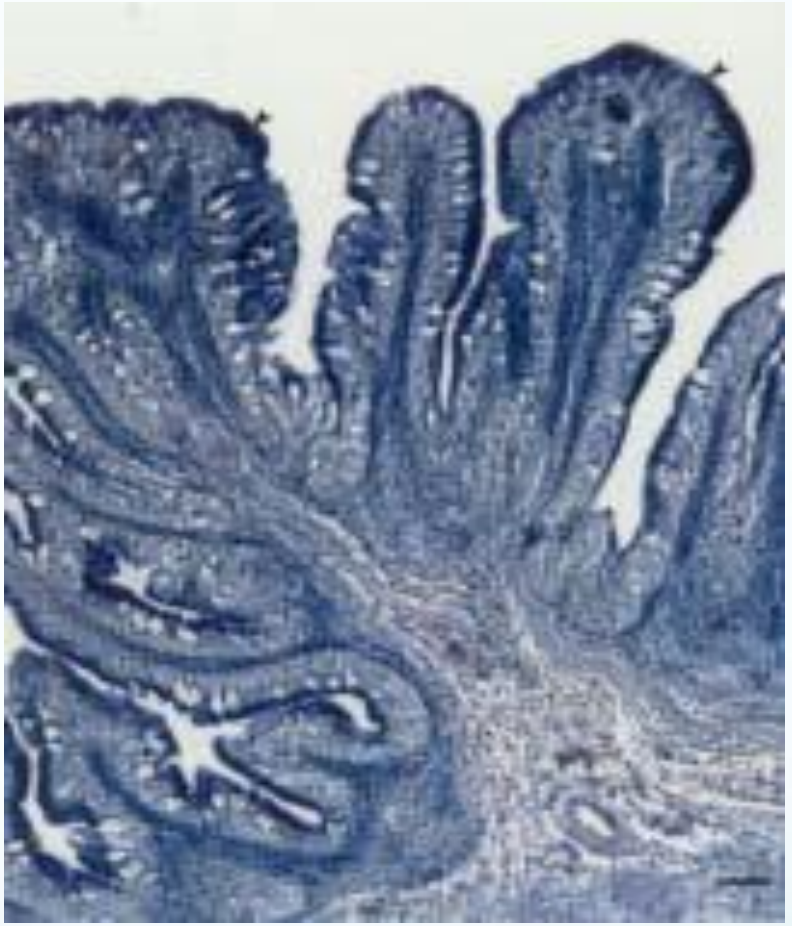
Hake intestine, non-specific esterase. Enzyme activity is localized in cytoplasm and brush border of epithelial cells (arrowheads). Scale bar = 50 µm.

Non-specific esterase

- **Non-specific esterase enzyme may be of particular importance because fish utilize lipids as their main nutritional source rather than carbohydrates and proteins.**
- **Differential distribution of esterase activity could reflect differences in function on particular parts of post-gastric regions.**
- **The intestine is generally associated with lipid absorption based on the appearance of lipid-containing vacuoles. Strong esterase activity had been noticed in intestinal epithelial cells than in esophageal and gastric ones.**
- **The low esterase activity in epithelial cells of esophagus and stomach leads us to conclude that hydrolysis of carboxylic esters (by non-specific esterase) occurs to a small degree in these regions of the gut of hake.**



Hake esophagus, alkaline phosphatase. A very strong activity of enzyme is observed in lamina propria (arrowheads).
Scale bar = 50µm



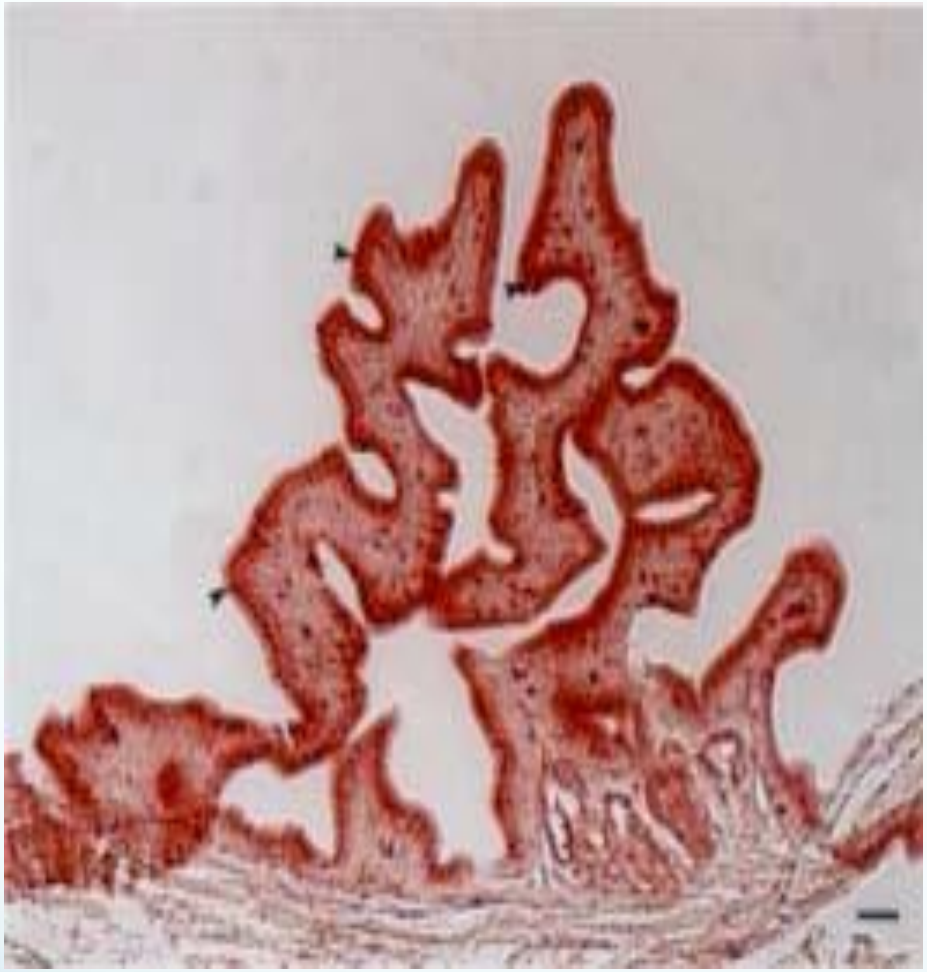
Intestine, alkaline phosphatase.Very strong activity in brush border, moderate activity in cytoplasm of epithelial cells is observed (arrowheads).
Scale bar =50 µm.

Alcaline phosphatase

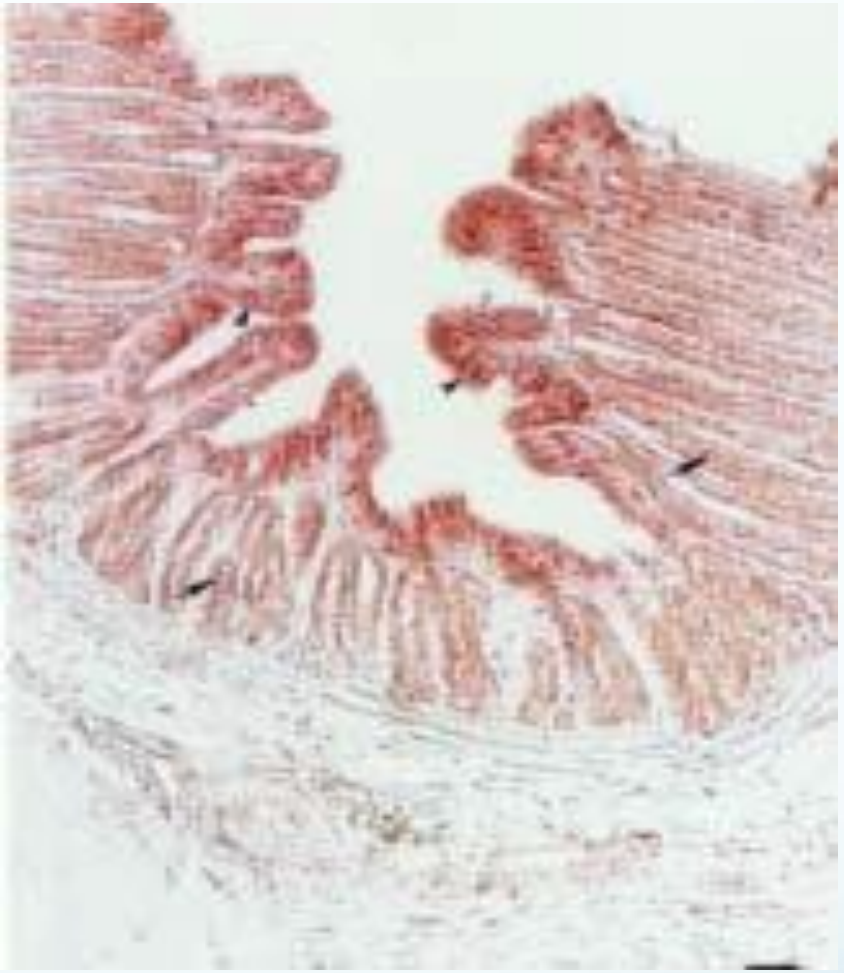
- **Alkaline phosphatase has a wide distribution and localization within gut segments. In hake, alkaline phosphatase is generally found in lamina propria. However, in both investigated intestine segments the highest enzyme activity was found in brush border of enterocytes and was stronger in the posterior region of the intestine which is some in common two-banded seabream (*Diplodus vulgaris*, Geoffroy St-Hilaire 1817).**

- **This enzyme was found primarily in cell membranes where active transport takes place and where this enzyme contributes to absorptive processes because it is considered to be a general marker of nutrient absorption.**

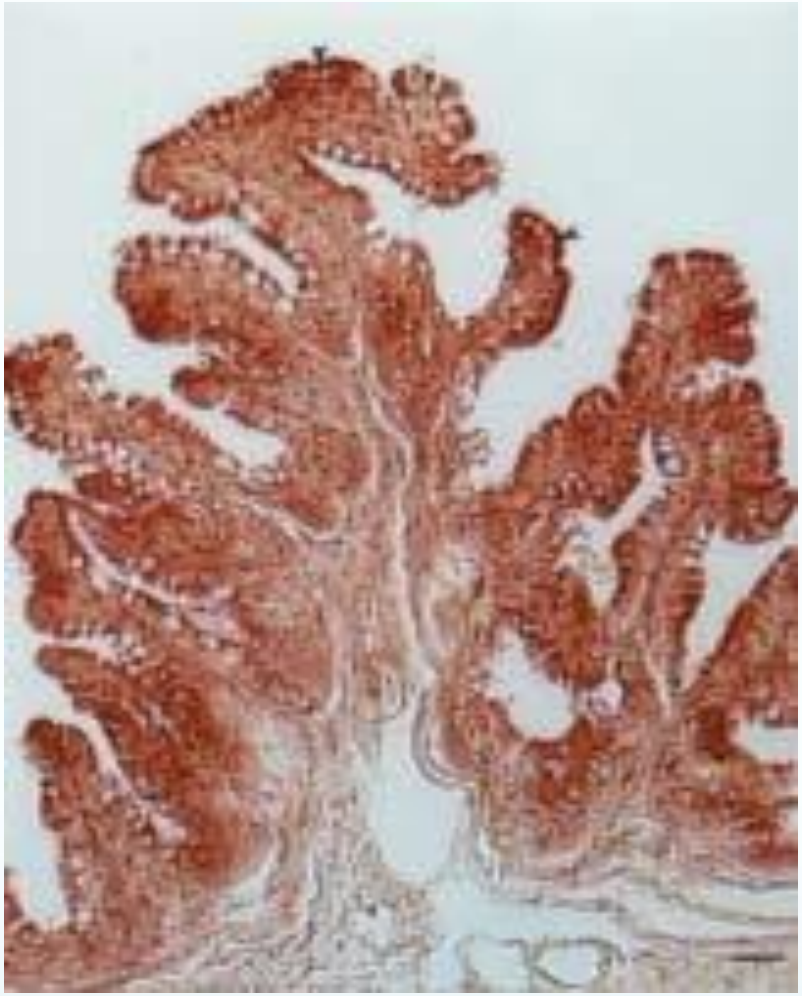
- **Small peptides and amino acids resulting from the action of digestive enzymes are transported with the collaboration of alkaline phosphatase through intestinal enterocytes membrane.**



Hake esophagus, acid phosphatase. The activity of enzyme is localized in supranuclear region of epithelial cells (arrowheads).
Scale bar = 50 μm.



Hake stomach, acid phosphatase. The activity of enzyme is observed in supranuclear region of epithelial cells (arrowheads) and in cytoplasm of glandular cells (arrows).
Scale bar = 50 μm.



Hake intestine, acid phosphatase. A very strong activity in epithelial cells (arrowheads), moderate activity in lamina propria is observed. Scale bar = 50 μm.

Acid phosphatase

- **Intestinal alkaline phosphatase is considered to be involved in the absorption of nutrients such as lipids, glucose, calcium and inorganic phosphate.**
- **The activity of acid phosphatase in investigate parts of hake gut is associated with epithelial cells. The enzyme is localized in supranuclear parts of enterocytes, but particular globular sites with enzyme activity are observed in lamina propria where they presented macrophages.**
- **Acid phosphatase is one of the marker enzymes for lysosomes, but its activity was also found outside lysosomes.**
- **From all investigated enzymes, acid phosphatase is the only one which is found in gastric glands of hake. Its activity is probably connected with processes of secretion and a strong activity of enzyme was found in the posterior region of the intestine.**
- **Alkaline and acid phosphatase, their activity appearing to be correlated with the presence of dense vesicle, containing proteins, in the posterior intestine enterocytes, indicating pinocytotic processes .**

Aminopeptidase

- **The activity of aminopeptidase was associated with brush border in the intestines of common two-banded seabream (*Diplodus vulgaris*, Geoffroy St-Hilaire 1817). Aminopeptidase activity was demonstrated in all investigated digestive tract segments (I-3 intestinal segments). These findings suggest that these enzymes participate in intracellular protein digestion and absorption, and take place in all intestinal parts.**

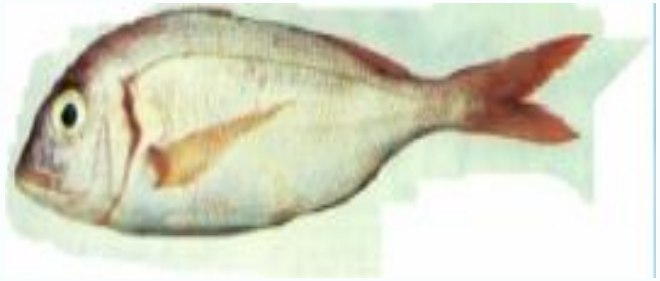


Common two-banded seabream (Diplodus vulgaris, Geoffroy St-Hilaire 1817), interstine. aminopeptidase, 10 x

- **The fine structure of the intestinal epithelium as well as enzyme activity in enterocytes depends on feeding habits of fish.**
- **The carnivorous fish exhibited a lower disaccharidase activity than the omnivorous and herbivorous fish, but lipase and protease are the highest in carnivorous fish.**
- **Common two-banded seabream is a carnivorous fish having a higher level of protein and lipids in diet. The results will contribute to knowledge on digestive morphology and physiology of common two-banded seabream as a candidate for aquaculture.**

LIVER STRUCTURE IN SOME SEA FISH FROM THE ADRIATIC SEA (CROATIA)

Sparidae family



common pandora (*Pagellus erythrinus* L.)



black sea bream (*Spondylosoma cantharus* L.)



saddled bream (*Oblada melanura* L.)



common two-banded sea bream
(*Diplodus vulgaris* E. Geoffroy Saint-Hilaire)

Merlucciidae family

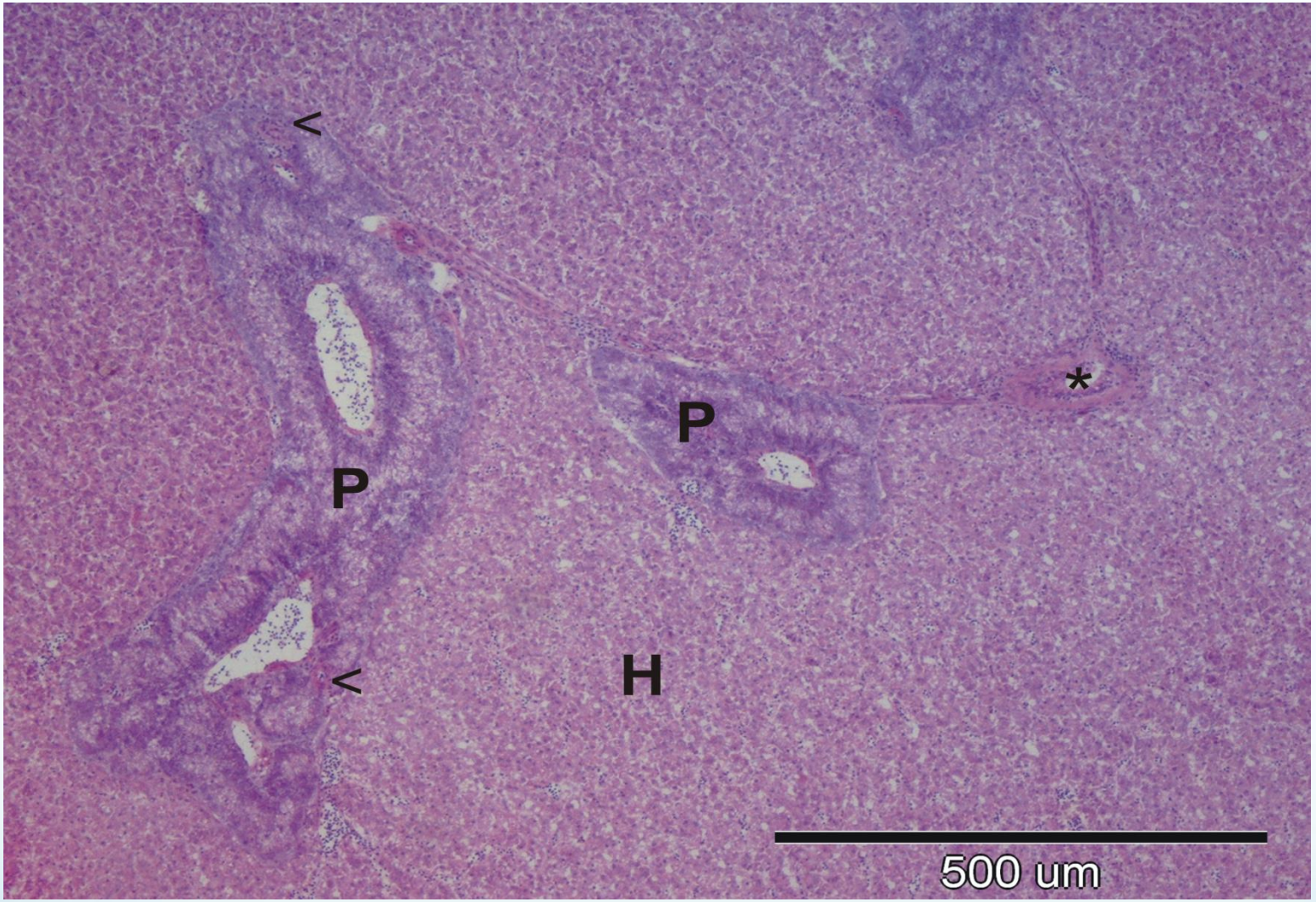


hake (*Merluccius merluccius* L.)

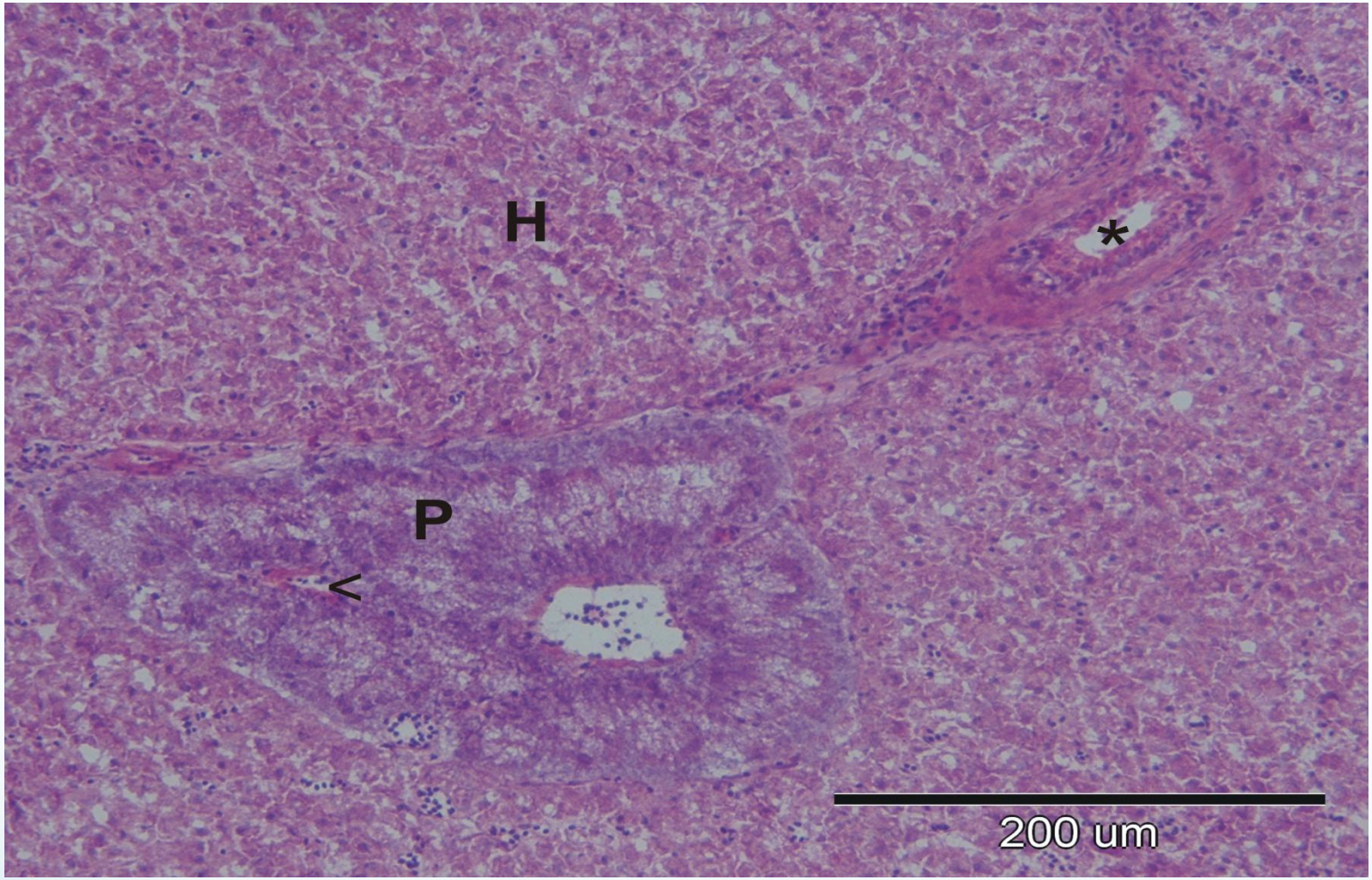
Labridae family



rainbow wrasse (*Coris julis* L.)



Hepatopancreas, common pandora (*Pagellus erythrinus* L.), hematoxyline and eosin, magnification 10 x (further magnification)
H- liver
P- pancreas
*- liver blood vessels
<- pancreas blood vessels



Hepatopancreas, common pandora (*Pagellus erythrinus* L.), hematoxyline and eosin, magnification 25 x (further magnification)
H- liver
P- pancreas
*- liver blood vessels
<- pancreas blood vessels

Conclusions

- In hepatopancreas of the hake (*Merluccius merluccius* L.) the biggest share of liver cells 88.96% and the smallest share of pancreatic tissue 4.30% was found, while in common pandora (*Pagellus erythrinus* L.) the smallest portion of liver cells 81.21% and the biggest part of the pancreatic tissue 14.21% was found.
- The share of blood vessels is the biggest in hake (*Merluccius merluccius* L.) 5.49%, while in common two-banded sea bream (*Diplodus vulgaris* E. Geoffroy Saint-Hilaire) it was the smallest 0.70%.
- Pancreatic blood vessels were at most in common pandora (*Pagellus erythrinus* L.), and at least in common two-banded sea bream (*Diplodus vulgaris* E. Geoffroy Saint-Hilaire) 0.04%.
- By analyzing histological preparations, division to liver lobuli and Glisson trias was not visible, which is generally the case in fishes.

Thank you very much





Common two bended seabream (*Diplodus vulgaris*)



Goldline (*Sarpa Salpa*)



European eel (*Anguilla anguilla*)



Large-scaled gurnard (*Lepidotrigla Cavillone*)



hake (*Merluccius merluccidus*)