Ontogenesis of main organs in *Litopenaeus vannamei*

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General Introduction

The aquaculture has an important role to overcome the growing demand of food all around the world.

In this regard, number of commercially important marine and fresh water species are being cultured all around the world including China mainland.

Production

145 million tonnes global fisheries, (2009)

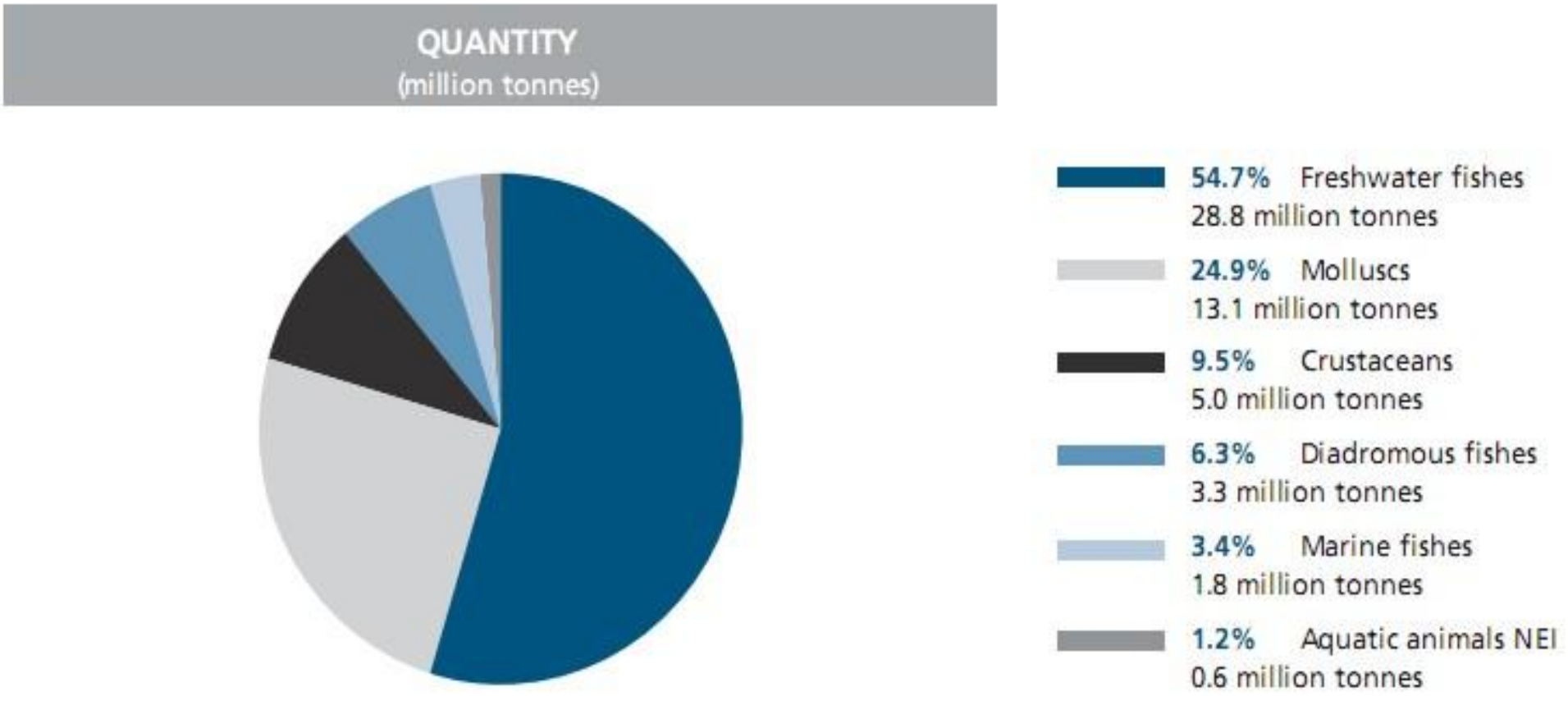
45.1% is shared by in land

Rest of it exploited from Marine waters.

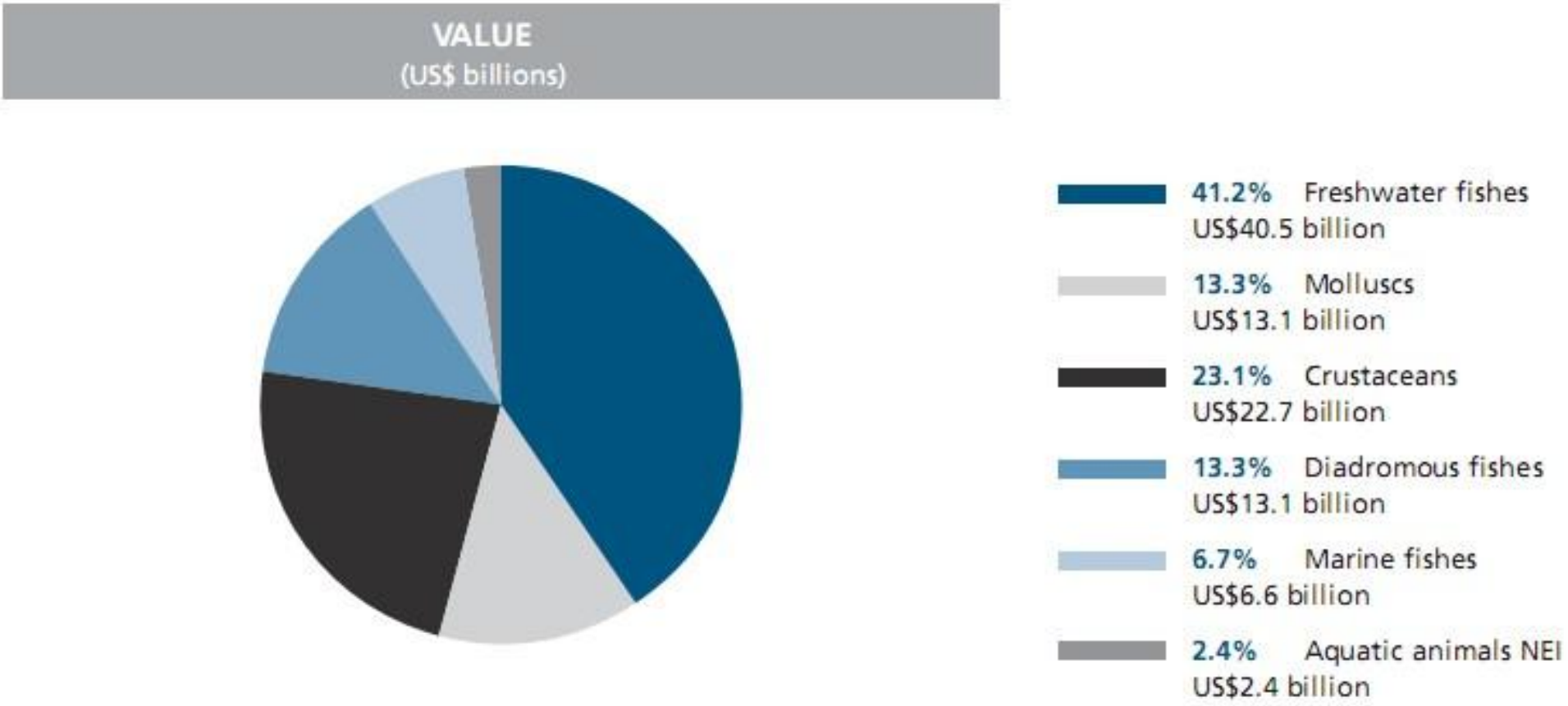
120 million tonnes utilized for human consumption and 27.3 million tonnes for non food uses.

FAO, 2010

Categorical Production



Categorical Value



Comparatively the production of crustaceans is low than freshwater fishes and molluscs but it is worth noted that value of crustaceans followed the value of freshwater fishes.

Culture of Major Groups in China

40 major marine species are being cultured in China.

Fish

- Sea bream, milkfish, sea perch,
- Japanese flounder, mullet, yellow croaker

Shrimp

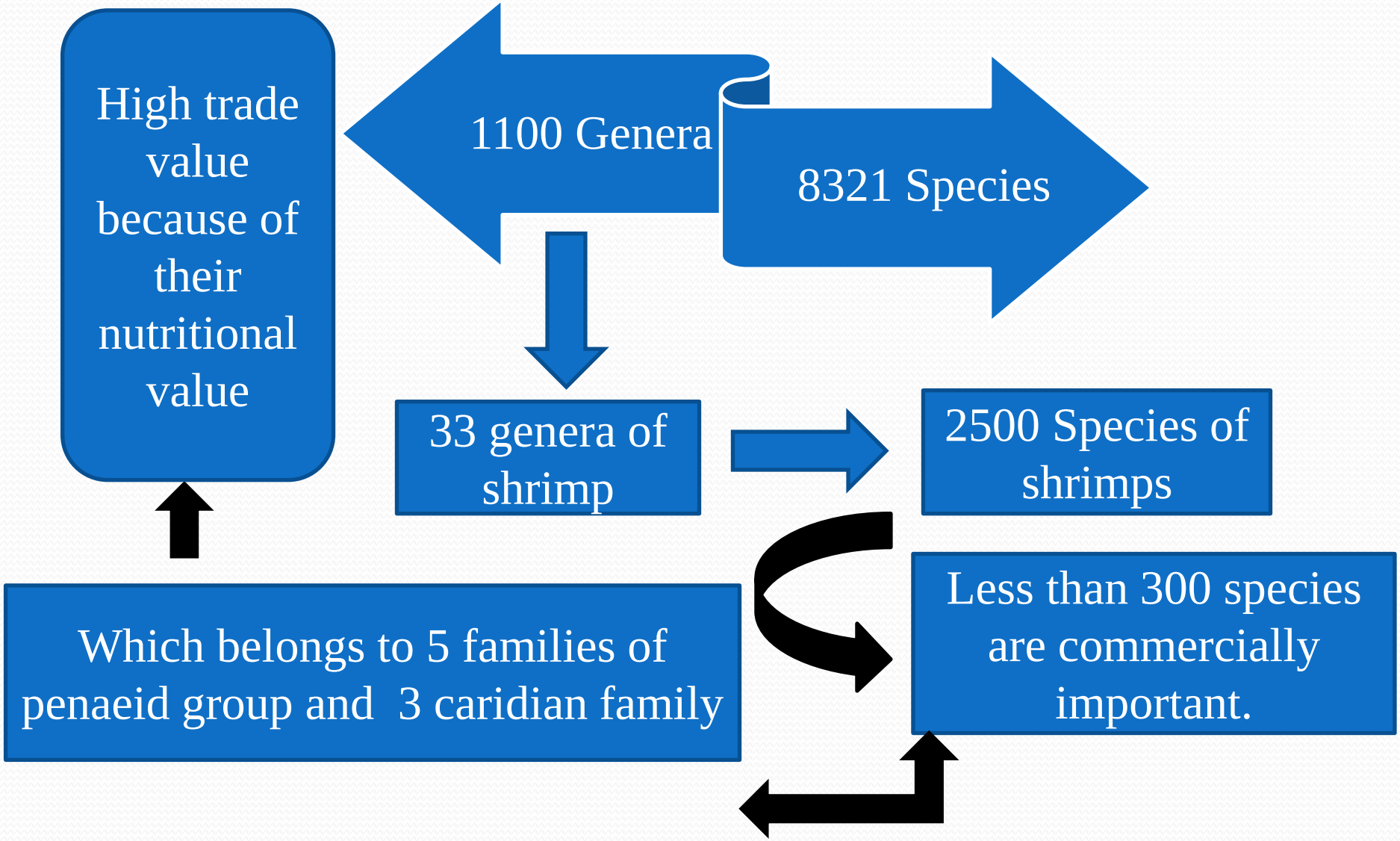
- *Fenneropenaeus chinesis, Penaeus japonicas,*
- *P. monodon, P. penicillatus, and Litopenaeus vannamei*.

Mollusks

- Oyster, Clams, Scallop, Manila clams
- Abalone etc.)

Inland aquaculture, 2400 years/ Marine shell fish is dated back to 1700-2000 years.

Importance of Order Decapoda among Crustaceans



Why white leg shrimp *Litopenaeus vannamei* for present investigation?

- Exotic species in this region
- Prime aquaculture species
- Wide range of fluctuated salt tolerance

0.5 to 45.‰

The single healthy female gives off about 100000-250000 eggs and hatching takes place after 16 h of spawning.

Culture history of White leg shrimp *Litopenaeus vannamei*

1st time

- The first captive breeding of white leg shrimp occurred at Florida in 1973.

Immigration .

- Thailand, Indonesia, India, Brazil, Ecuador, Mexico, Venezuela, Honduras, Guatemala, Nicaragua, Vietnam, Malaysia, Cambodia, Philippines, Peru.

Intro China

- In China, exotic species (*L. vannamei*) had introduced in 1988 for experimental purposes and by 1998 is being successfully cultured in commercial scale.

Status of white leg shrimp world and in China

- Over 1000 hatcheries are in operation.
- 1386000 tonnes world production (2004) while in China 300000 tonnes 210000 tonnes harvested during 2003.
- In marine and freshwater farming respectively.

Qing and Hai, 2005

Advantages of exotic white leg shrimp

- Faster growth
- Tolerance of wide range of salinities (0.5- 45‰)
- Tolerance of low temperature,
- Higher survival rate (50-60%) in hatchery.

Smith and Brigg, 2003

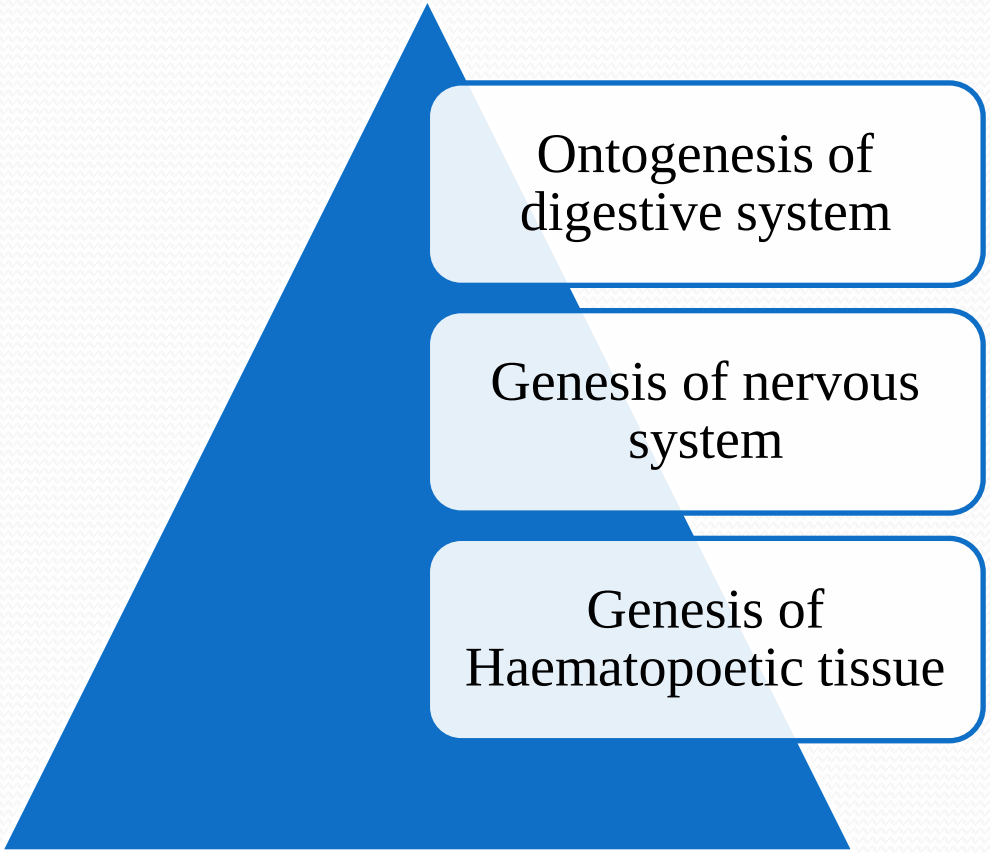
Objectives of present investigation

To determine the ontogenesis of main systems using classical histological techniques.

Such as digestive system and Nervous system

Out line

Genesis of Systems



Part 1 Genesis of system

Penaeid Shrimp

Shrimps are very important for wellbeing of mankind, for their sustainable culture and management, its necessary to study their basic systems.

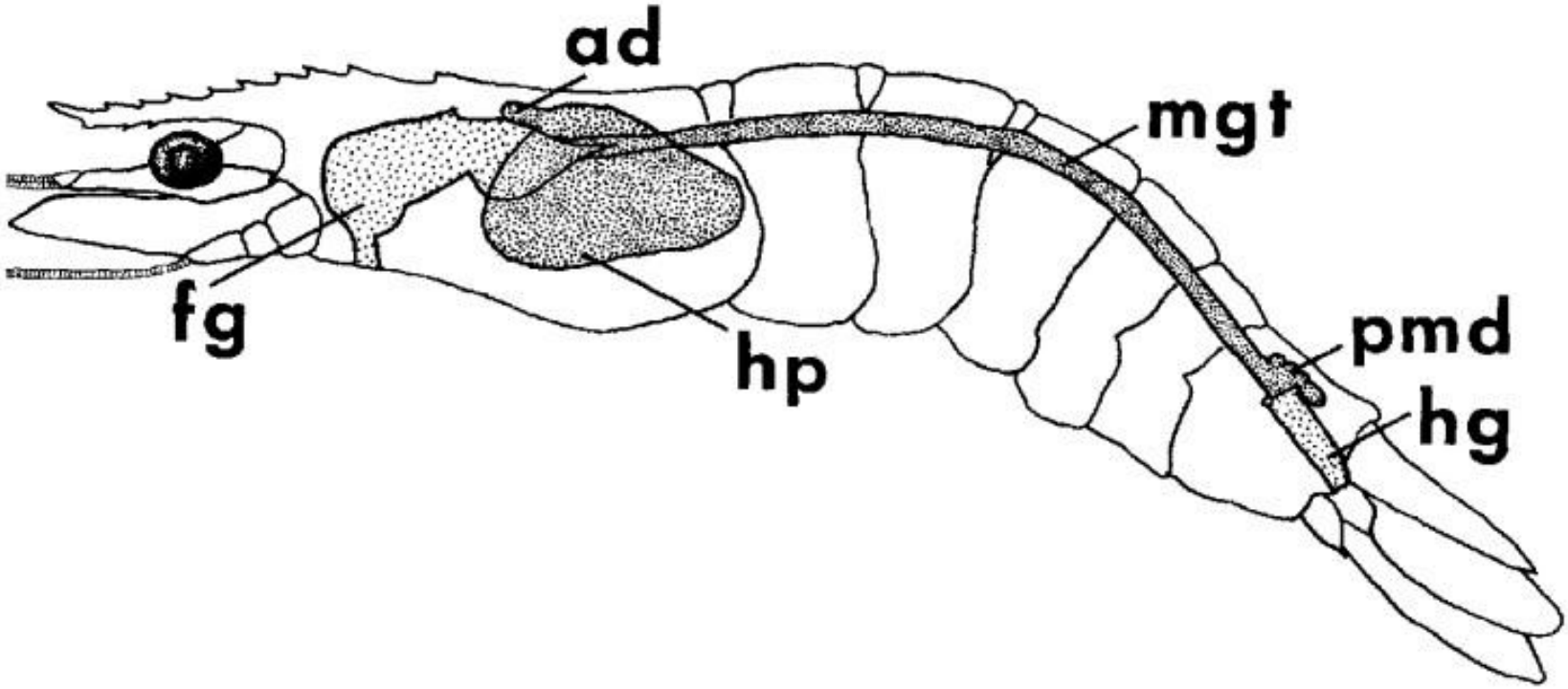
- Digestive system
- Nervous system
- Reproductive system
- Circulation system, Respiration system
- Defense mechanism system

Each of these systems play vital roles for survival of early and adult life of these commercially important animals.

Introduction of Digestive System

Young, 1959, divided digestive system in three functional parts

- Forgut or Stomodaeum
- Midgut or Mesentron
- Hindgut or Proctodaeum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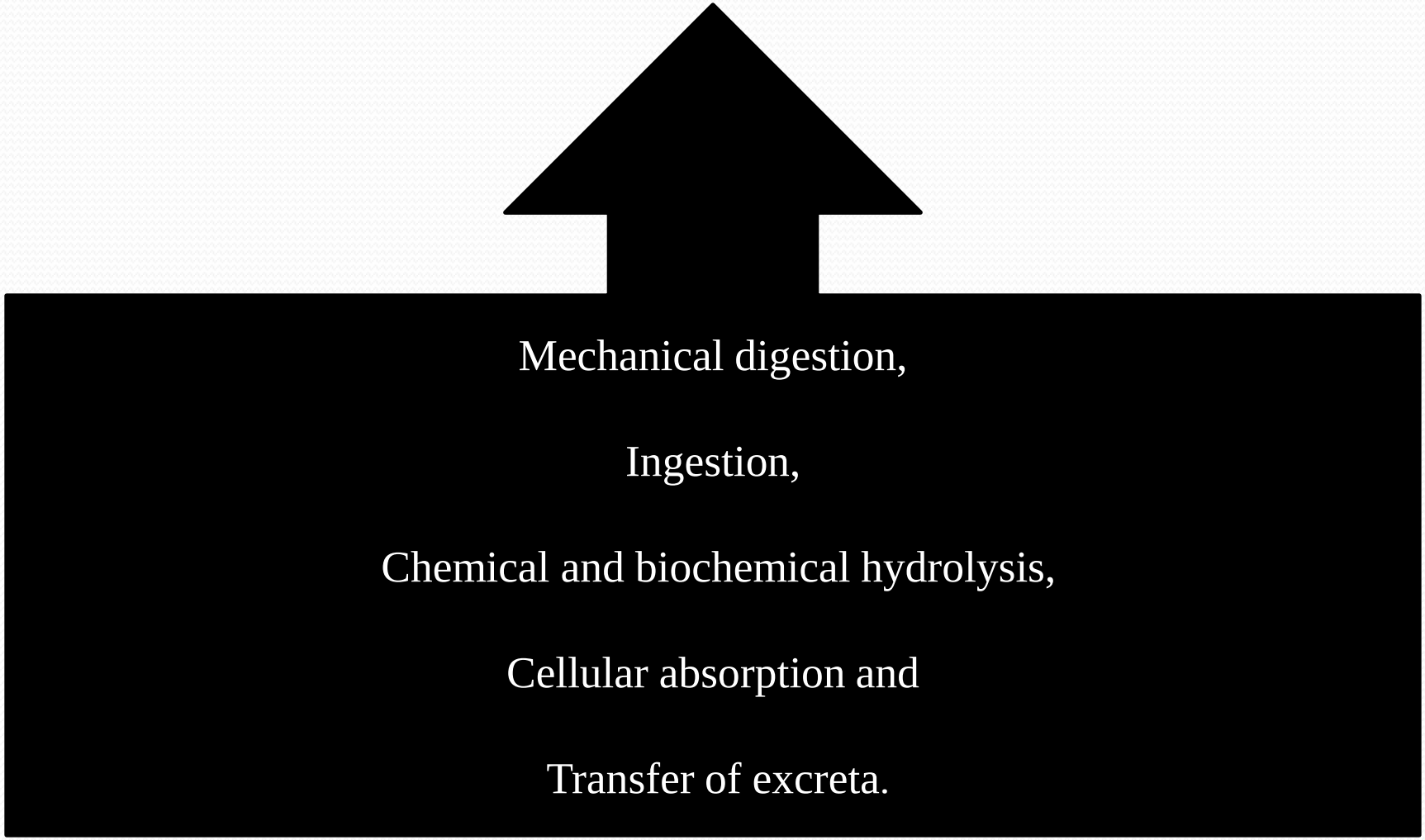
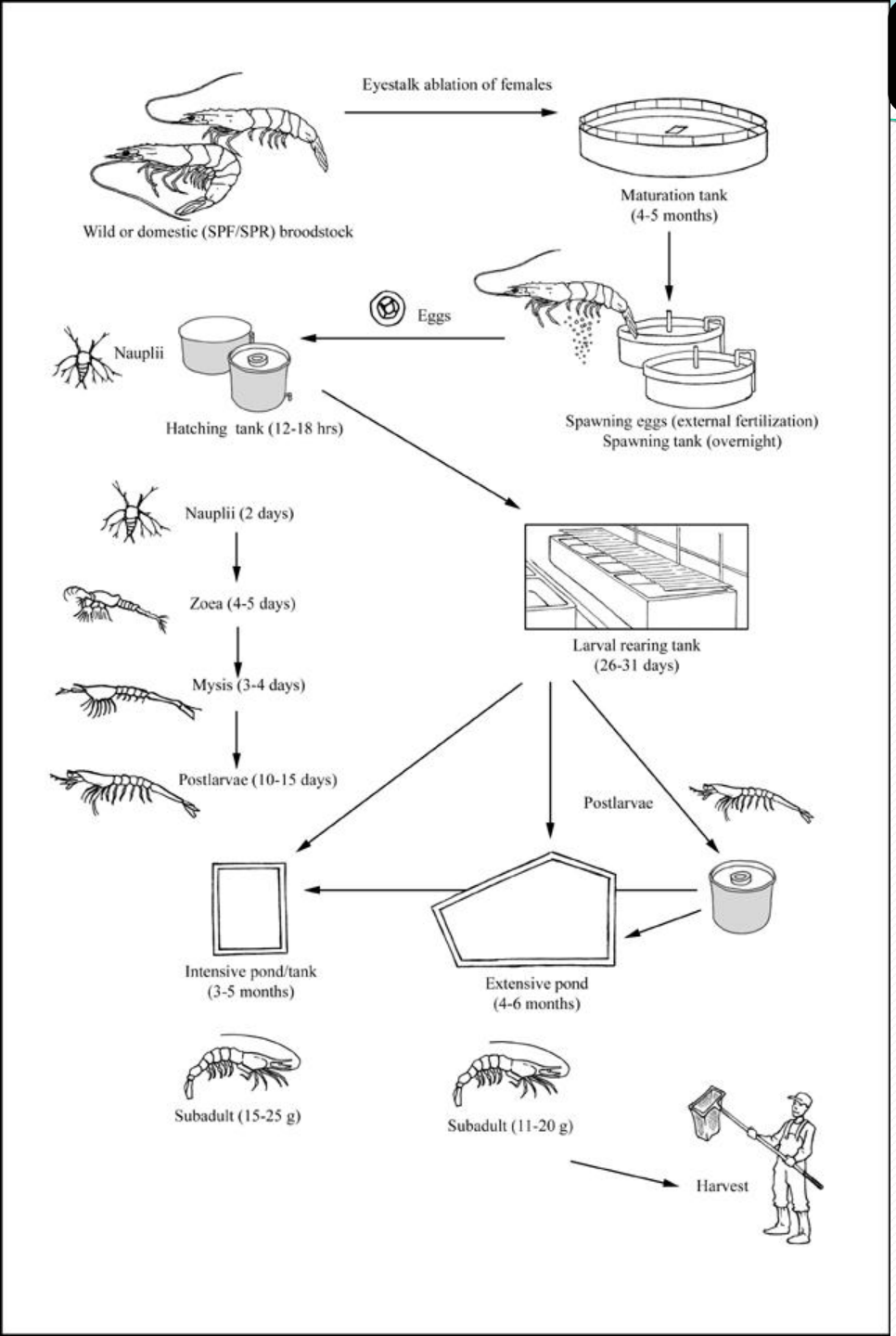
Roles of Digestive System

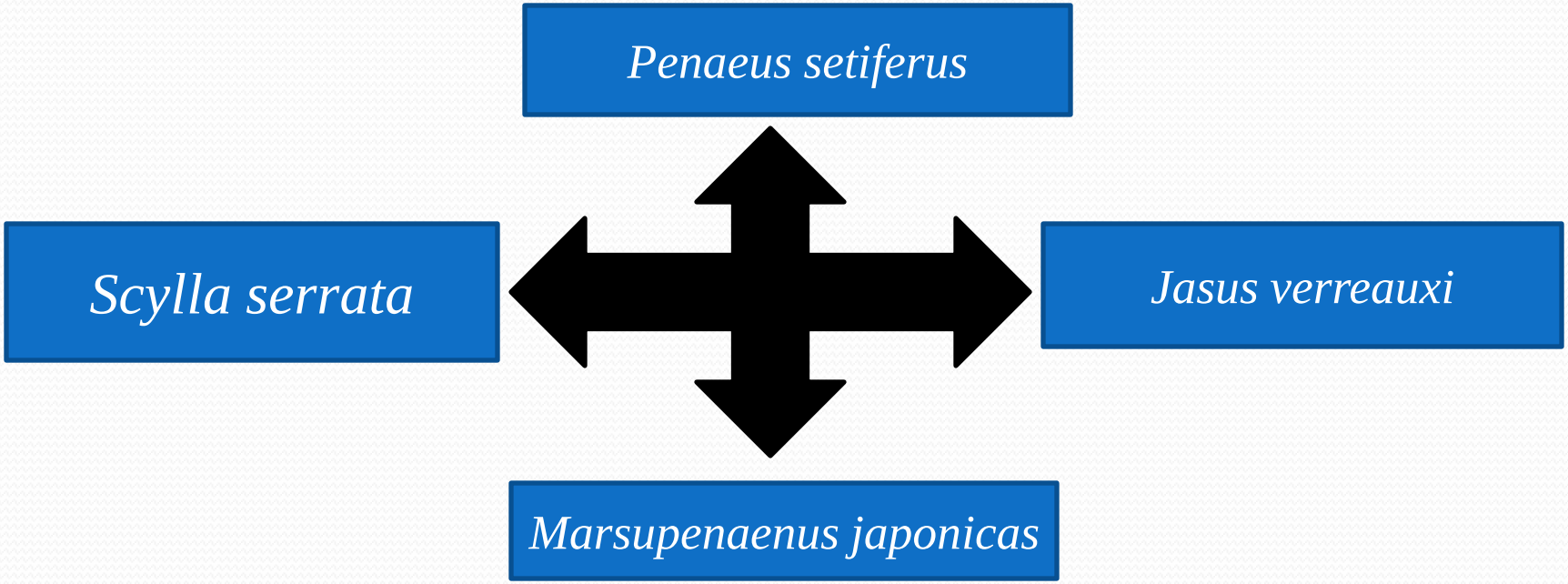




Production cycle of white leg shrimp

- Six stages of nauplius
- Three protozoeal stages
- Three mysis stages
- Post larval stages
- Diatoms, Flatellates, Artemia and Rotifers.
- The dietry shift appeals to investigate the genesis of digestive system.

Ontogenetic investigations in Decapoda

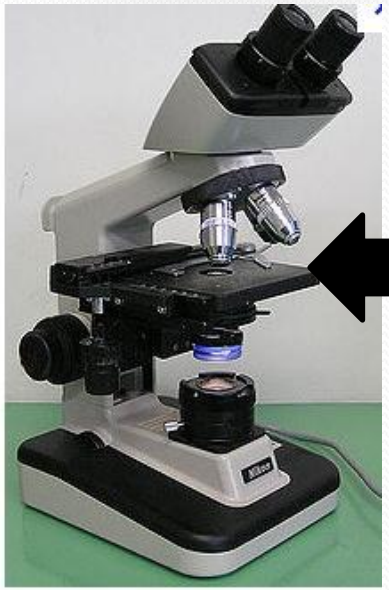


Materials and Methods

Classical Histological analysis for investigation of genesis.

The desired samples (Nauplius (N_1-N_6) Zoea (Z_1-Z_3) mysis. (M_1-M_3)and Post larval (P_1-P_{10})) obtained from shrimp hatchery Zhanjiang China, in 2009.

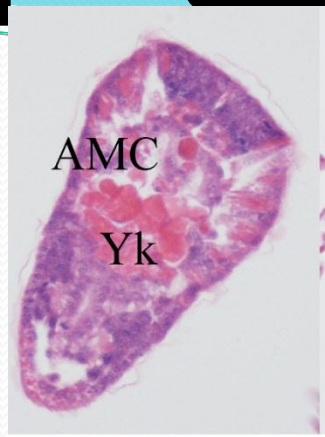
Standard histological procedure were followed.

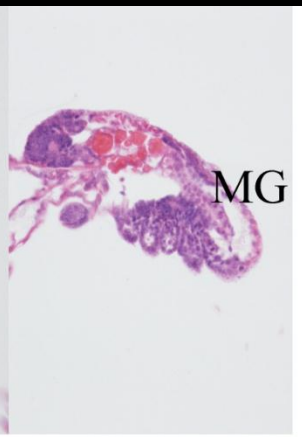


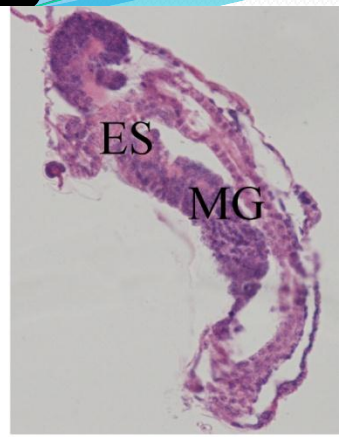
Genesis of digestive system

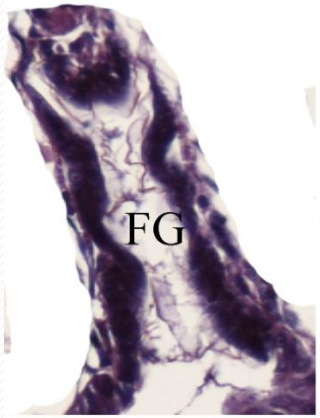
- N_5 yolk mass is the source of food
- Mouth open at N_6 stage
- The **foregut** was a simple lumen till M1 stage
- Anterior mid gut ceacum (AMC) first appears at N_3 stage
- The lateral mid gut caecum appeared from N_6 stage
- The gland filter first appeared in M_3 stage
- Hind gut appears in slight projected form at P_2 stage

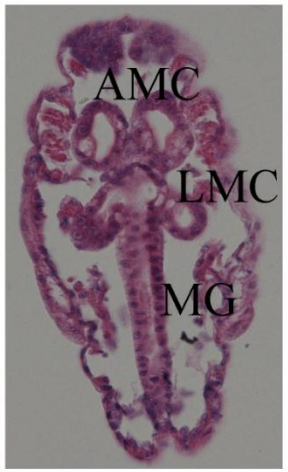
Results

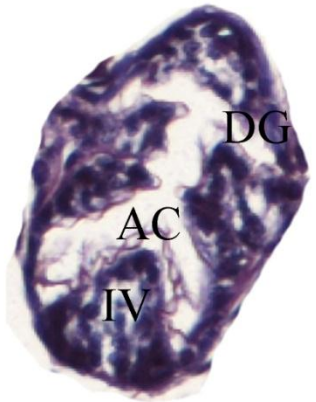


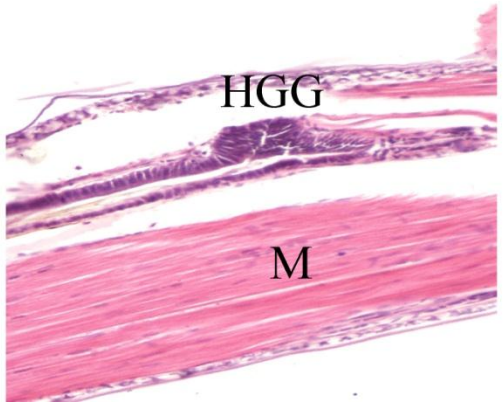




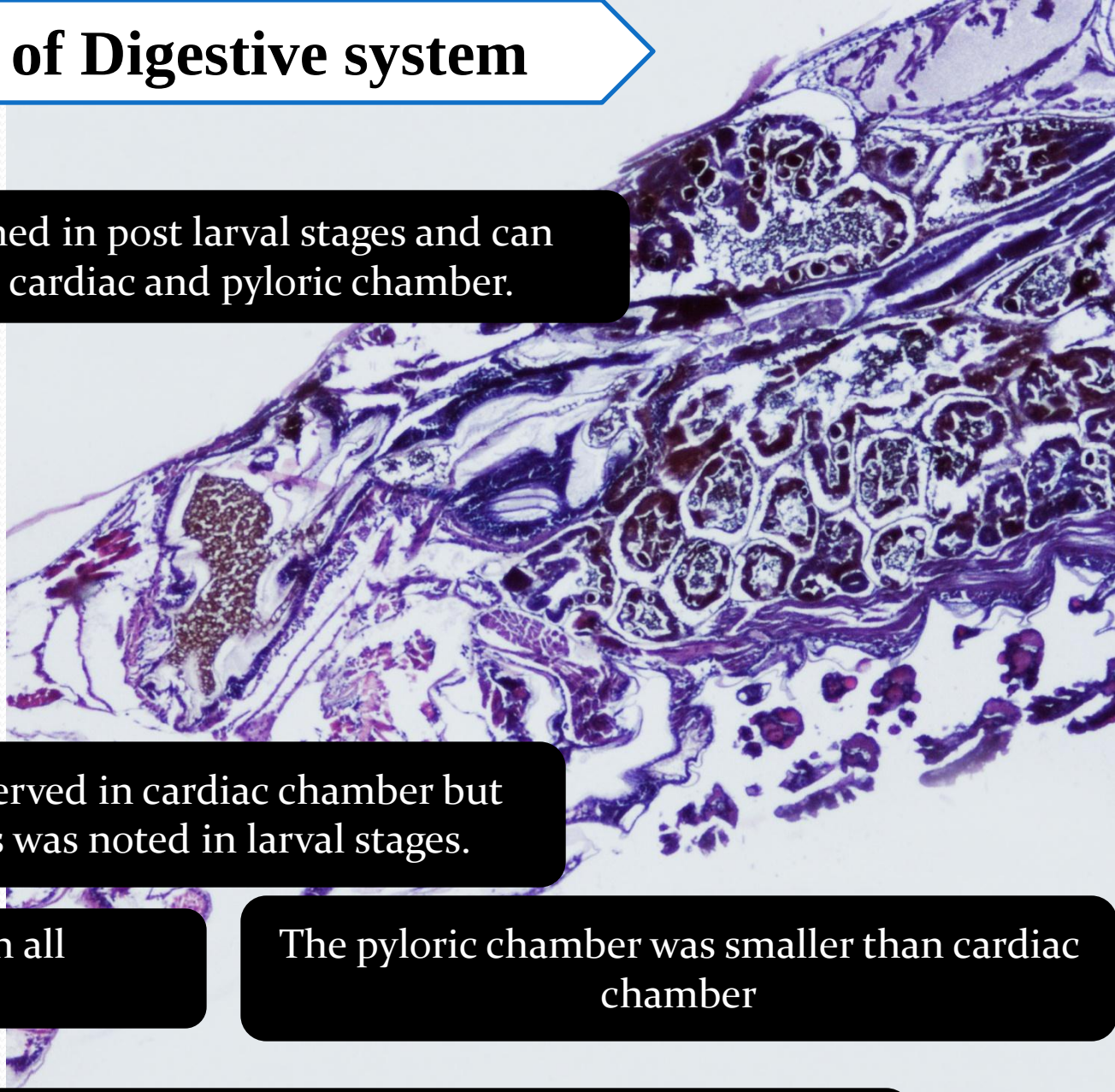








Development of Digestive system



The foregut was well defined in post larval stages and can easily be identified into cardiac and pyloric chamber.

No distinct setae were observed in cardiac chamber but gradual increase of folds was noted in larval stages.

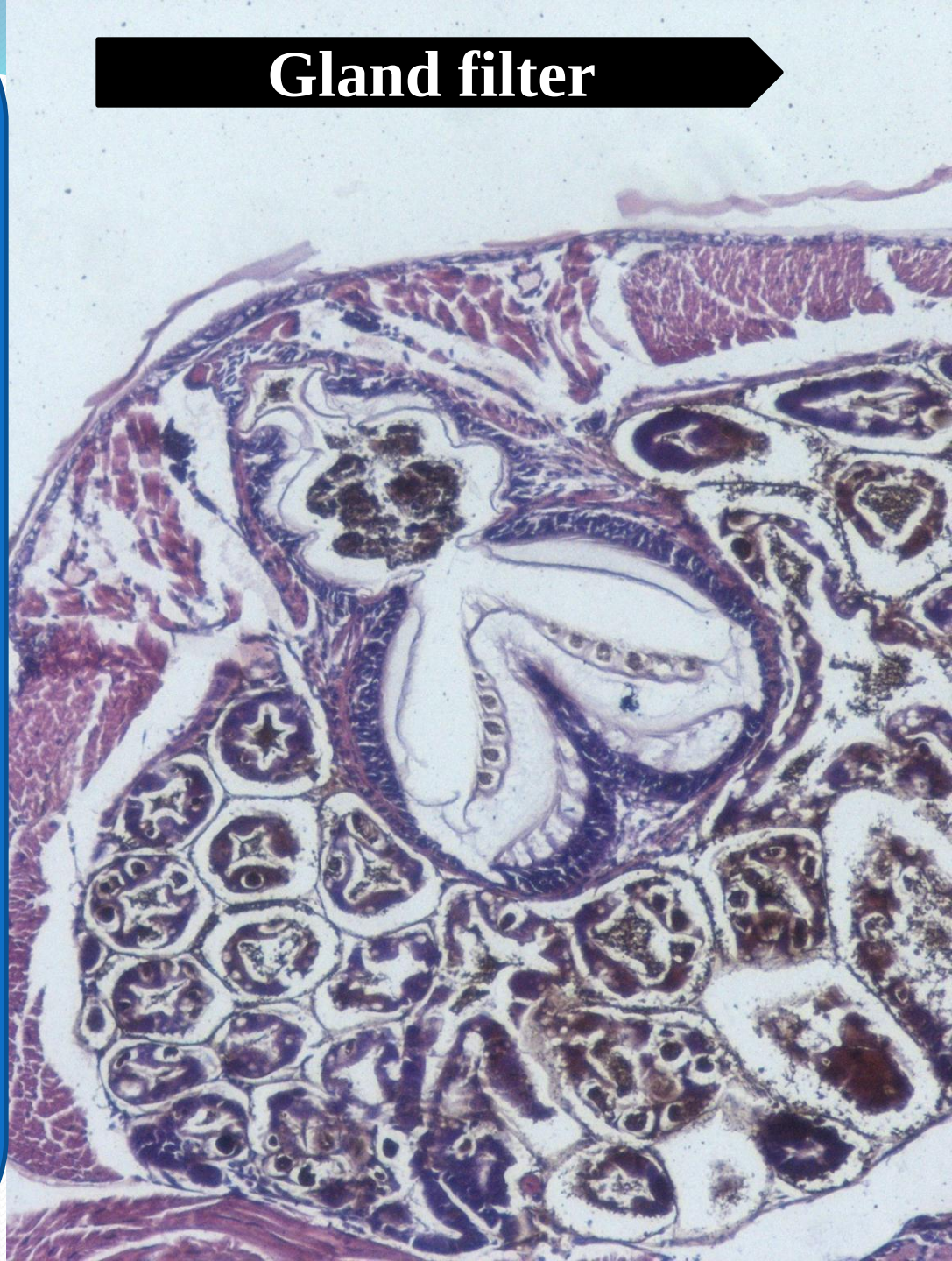
The gastric mill was absent in all investigated larval stages.

The pyloric chamber was smaller than cardiac chamber

The epithelial and cuticular layers were prominent in pyloric chamber.

Gland filter

The most important structure of pyloric chamber is gland filter and its setose ridges makes filter which preclude the food particles in collecting duct of hepatopancrease

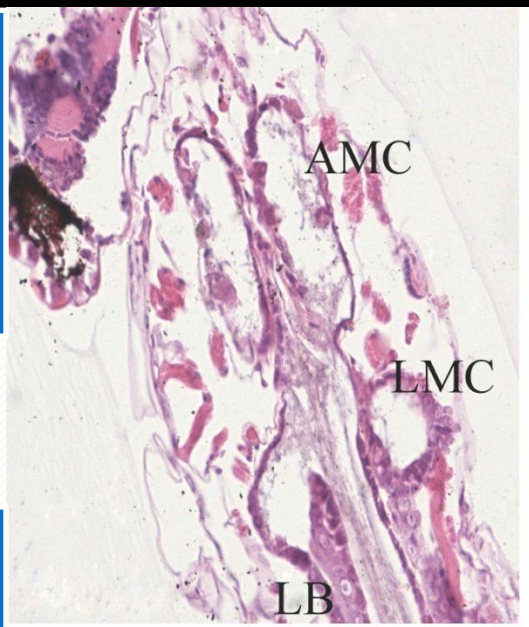


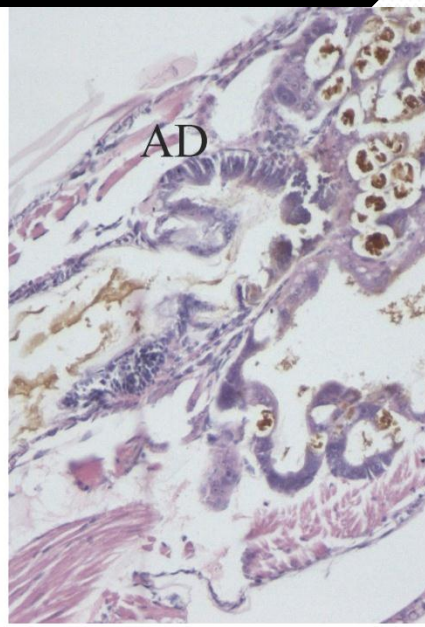
Anterior Mid gut ceacum/Anterior diverticum

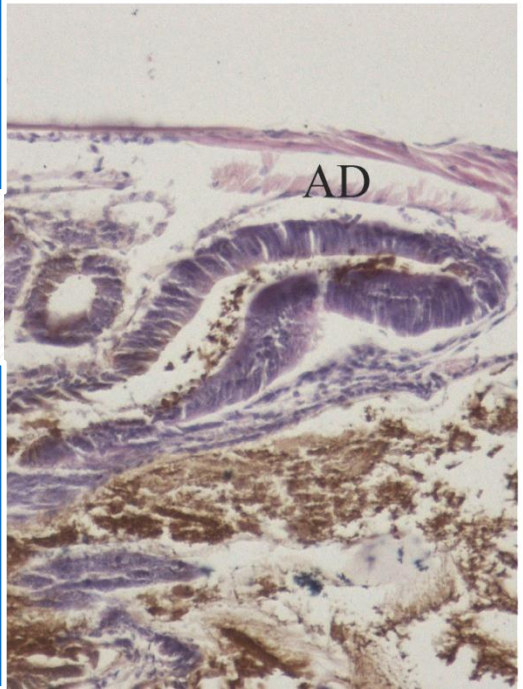
In zoea stages the AMC showed progressive continuation.

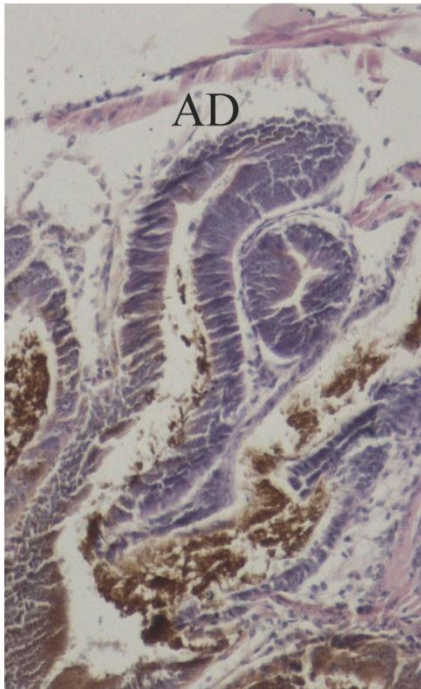
In mysis stages it showed reduction in size.

It became a single structure called as Anterior diverticulum in post larval stages.



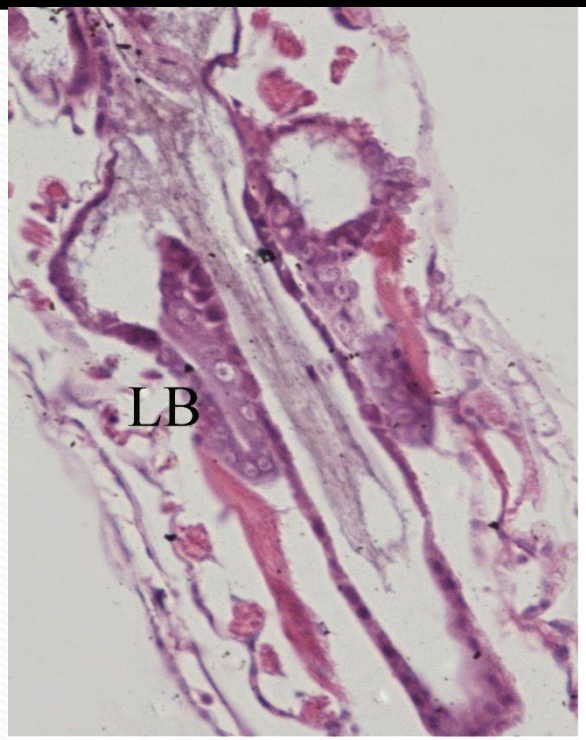


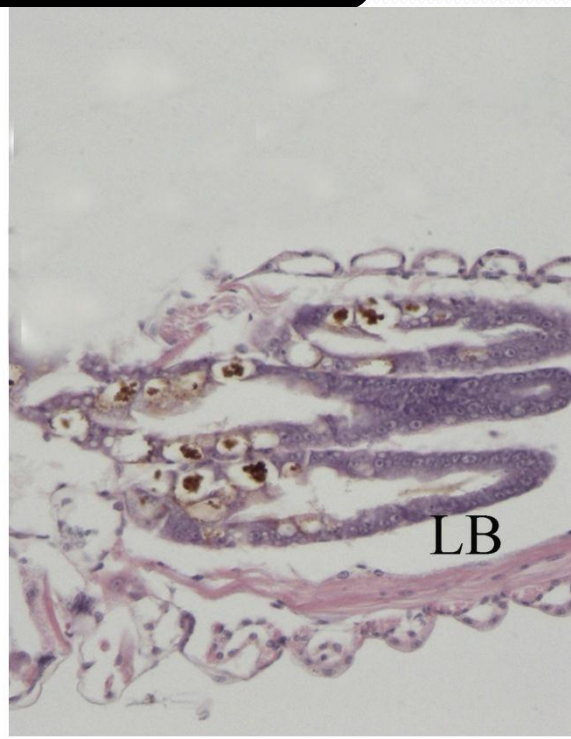


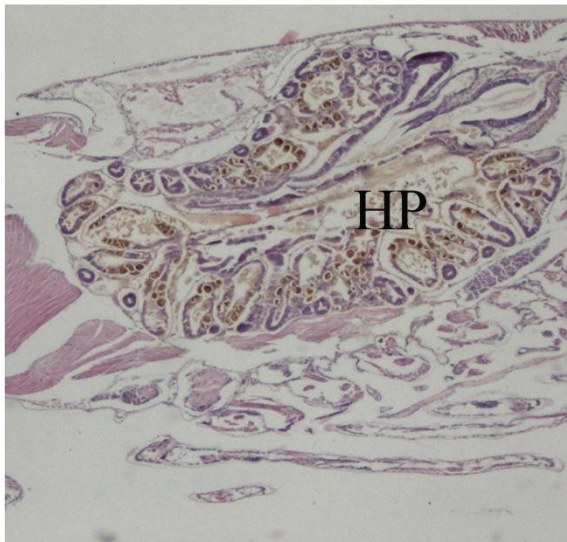


Lateral Mid gut caecum/Hepatopancrease

- These lobes increase in linear fashion .
- The number of lobes increases by ramifying themselves in myses stages and post larval stages.
- These lobes form compact structure which referred as **Hepatopancrease.**





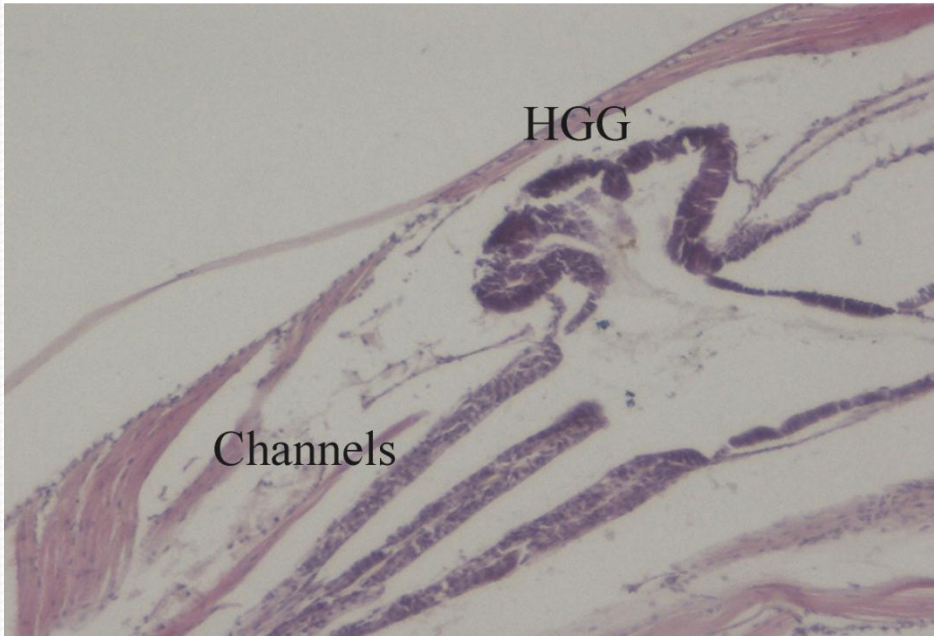


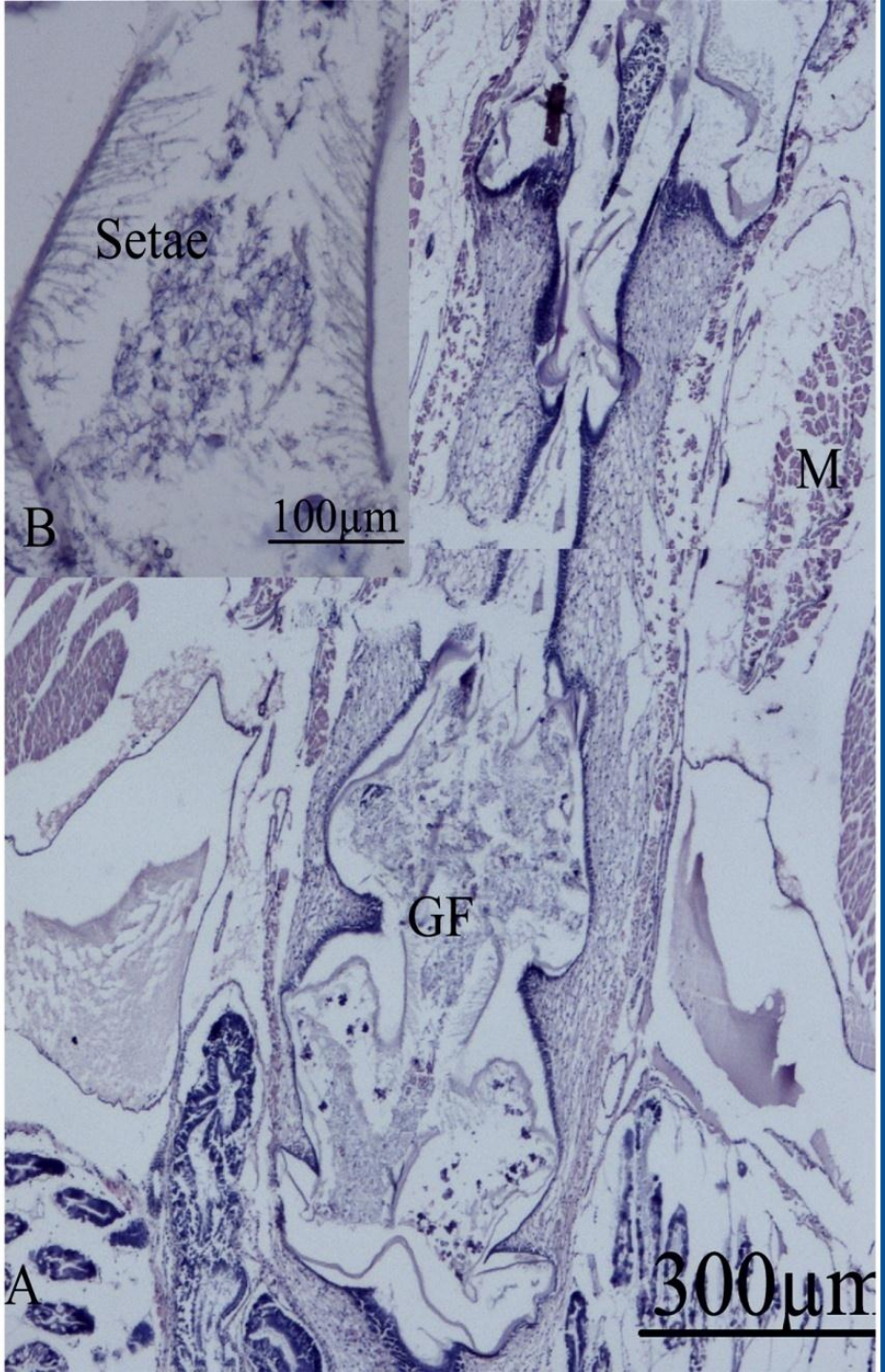
Mid gut/ Hindgut gland or posterior diverticulum

◆Mid gut is a simple tube – like structure through out the developmental stages.

◆ Hind gut gland in successive stages increases in size, changes in appearance and exhibits high protuberance.

◆ By P_7 stage it has two channels which join a single lumen at rectum.





◆Digestive system completely attained till P_{10}.

◆Elongation and hardness continue to grow such as the setae of gland filter.

◆ Size of hepatopancreas continue with respect of growth.

Discussion

Digestive system

- *L.vannamei*, in early stages the foregut is undifferentiated, from hatching up to N_5 stage, exclusively depends on endogenous source of nutrition.

- The foregut cannot be differentiated into cardiac and pyloric stomach in early stages; the gastric mill is absent in larval and post larval stages of *L.vannamei*, similar to *L. setiferus* and Caridean decapods.

- Unlike larvae of other decapods, (*H. americanus*, *P. argu*, *P. annulipe*, *C. anthonyii*, *P. pelagicus and M. mercenuria*) no gastric tooth was observed.

- The gland filter of *L. vannamei* is similar to other species of Penaeid.

- The ontogeny of digestive system of *L.vannamei* followed the same pattern to that of *L. setiferus*.

- However, differences in development of hindgut gland (posterior diverticulum) were noted whereas the hindgut gland appears at P_2 stage in *L. vannamei* while in *L. setiferus* it had noted in P_{21} (Lovett and Felder, 1989).

Conclusion

This study will be helpful for improvement of aquacultural development.

Genesis of Nervous system

Introduction

Investigation on nervous system remains the subject of interest, however a few studies are available on the genesis of nervous system.

Fenneropenaeus chinensis

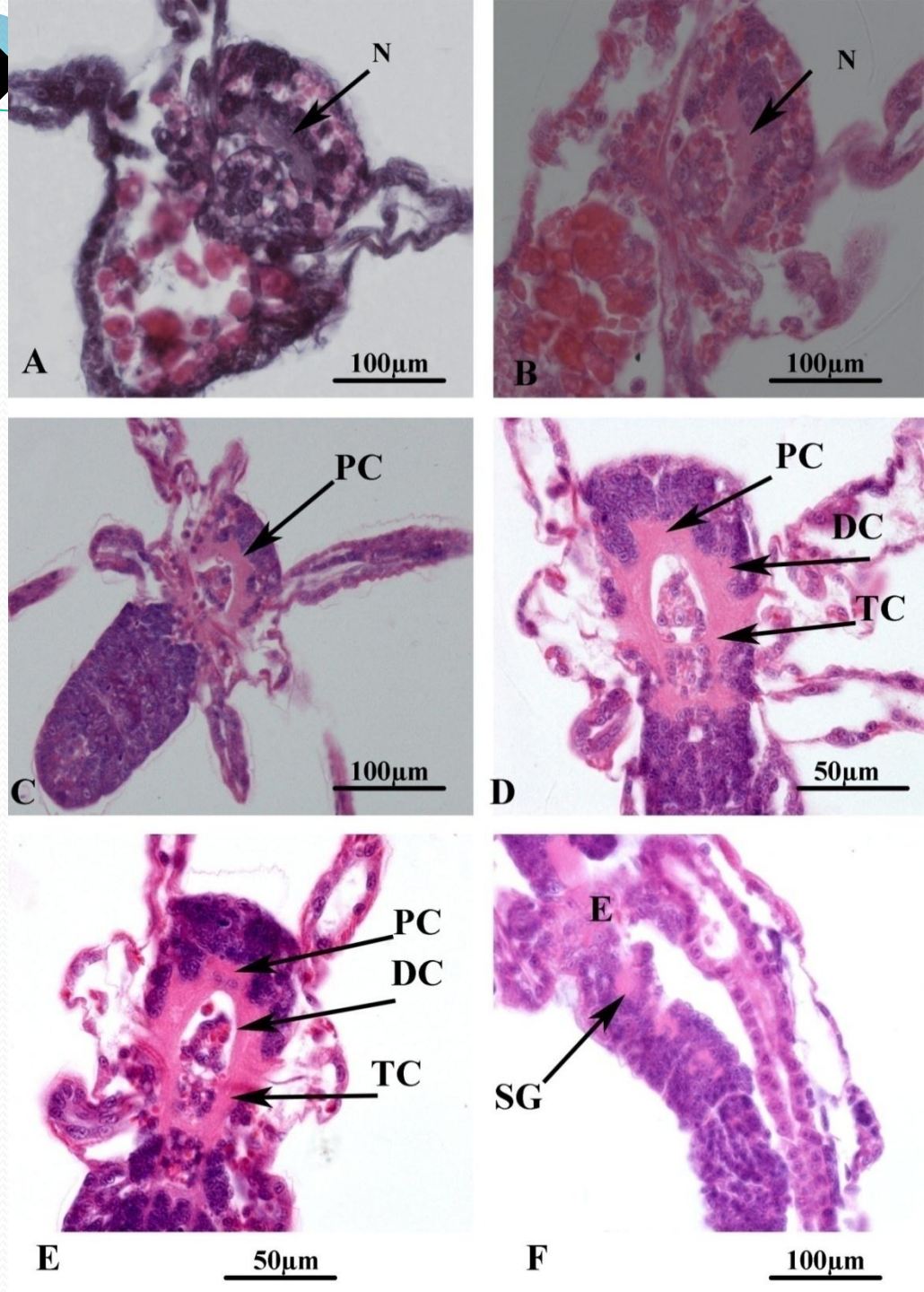
Marsupenaeus japonicus

Material and Methods

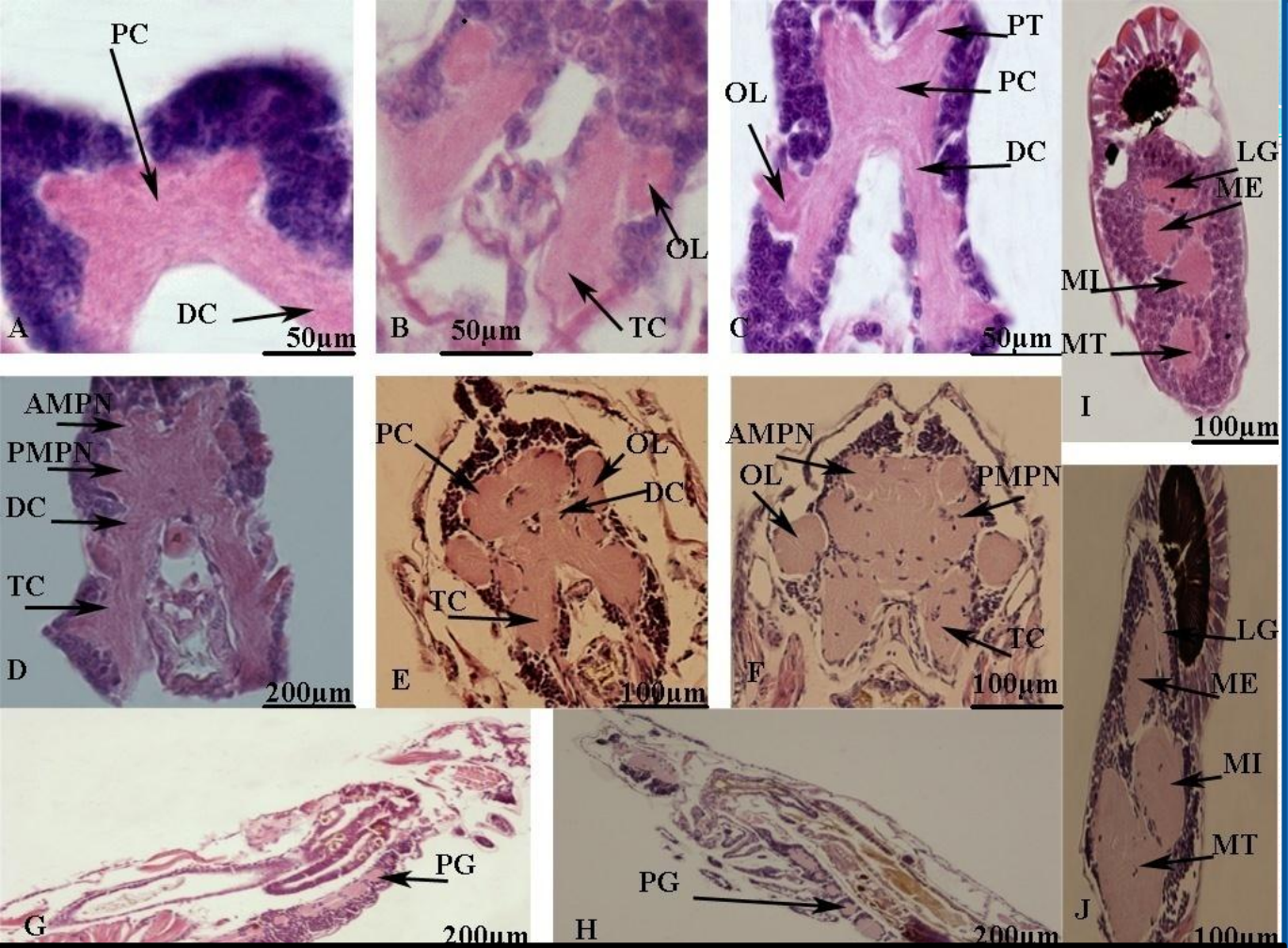
Classical histological sections were done as per procedure stated in earlier slide.

Genesis of nervous system

- Neuropil distinct at N_1 (A).
- The initiative appearance of Proto deuto- and tritocerebrum occurred in N_5 (D-E)



Development



◆ Olfactory lobes were demarcated at Z_1 stage, it is important chemoreceptor.

◆The ganglion of compound eye appeared in zoea stages The optic ganglions are connected with protocerebrum by protocerebral tract.

◆ Pleon ganglion are well organized in N_6 stage and onward.

◆During Zoea stages the brain becomes larger and more distinguishable, (Fig A-C).

◆Until Z_3 (Fig D) the protocerebrum differentiated into two parts.

◆ Brain showed expansion in mysis and post larval stages.

Discussion

Nervous system

- The result are accord with the *F. chinesis* (Zhang, et al., 2007), but disagreement with *P. japonicus* where the slight differentiation of nervous tissue was observed in N_2 (Nakamura and Seki, 1990).

- In *L. vannamei*, the complete willing movement and balancing begin from N_6 and zoea because olfactory lobes are well demarcated in zoea which play role in behavioral activities.

- Our results did not show any disagreement in basic structure of CNS with that of the family Penaeidae.

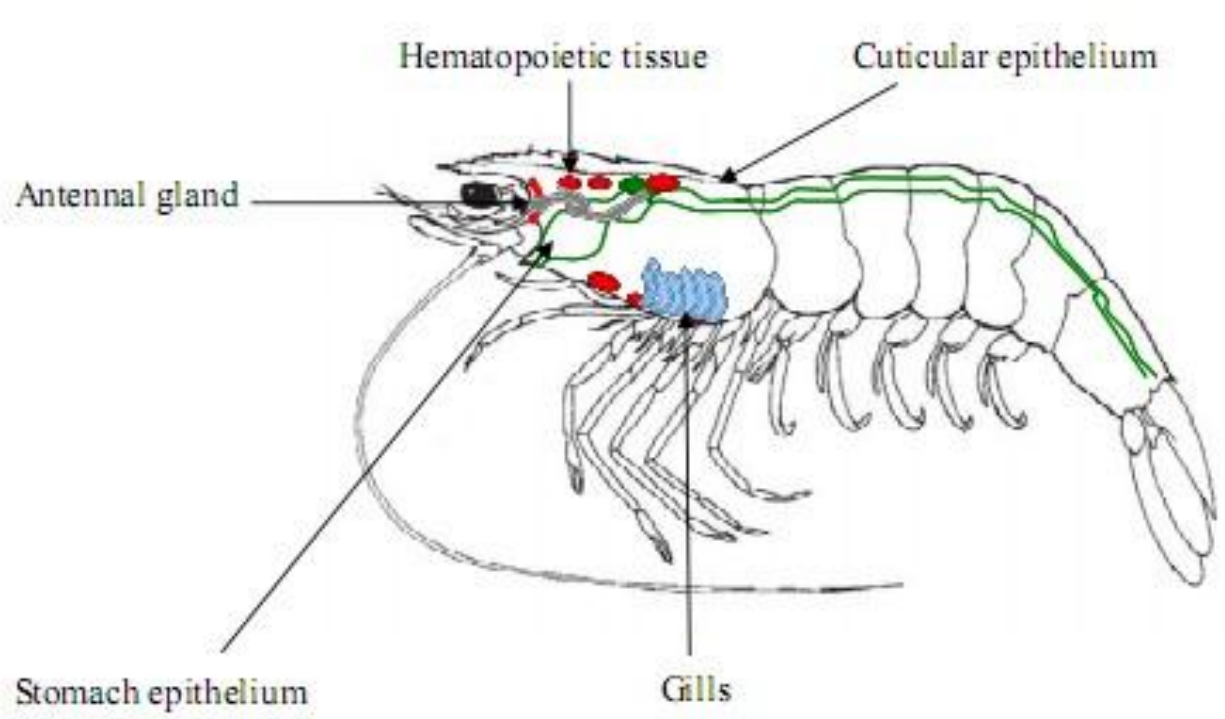
Conclusion

The present results are contribution to basic structure of nervous system, however in depth knowledge still needed to understand the behavioral function of shrimp.

Hematopoietic tissue (HPT)

Introduction

HPT described in Crab, Lobster, Cray Fish Penaeid shrimp.



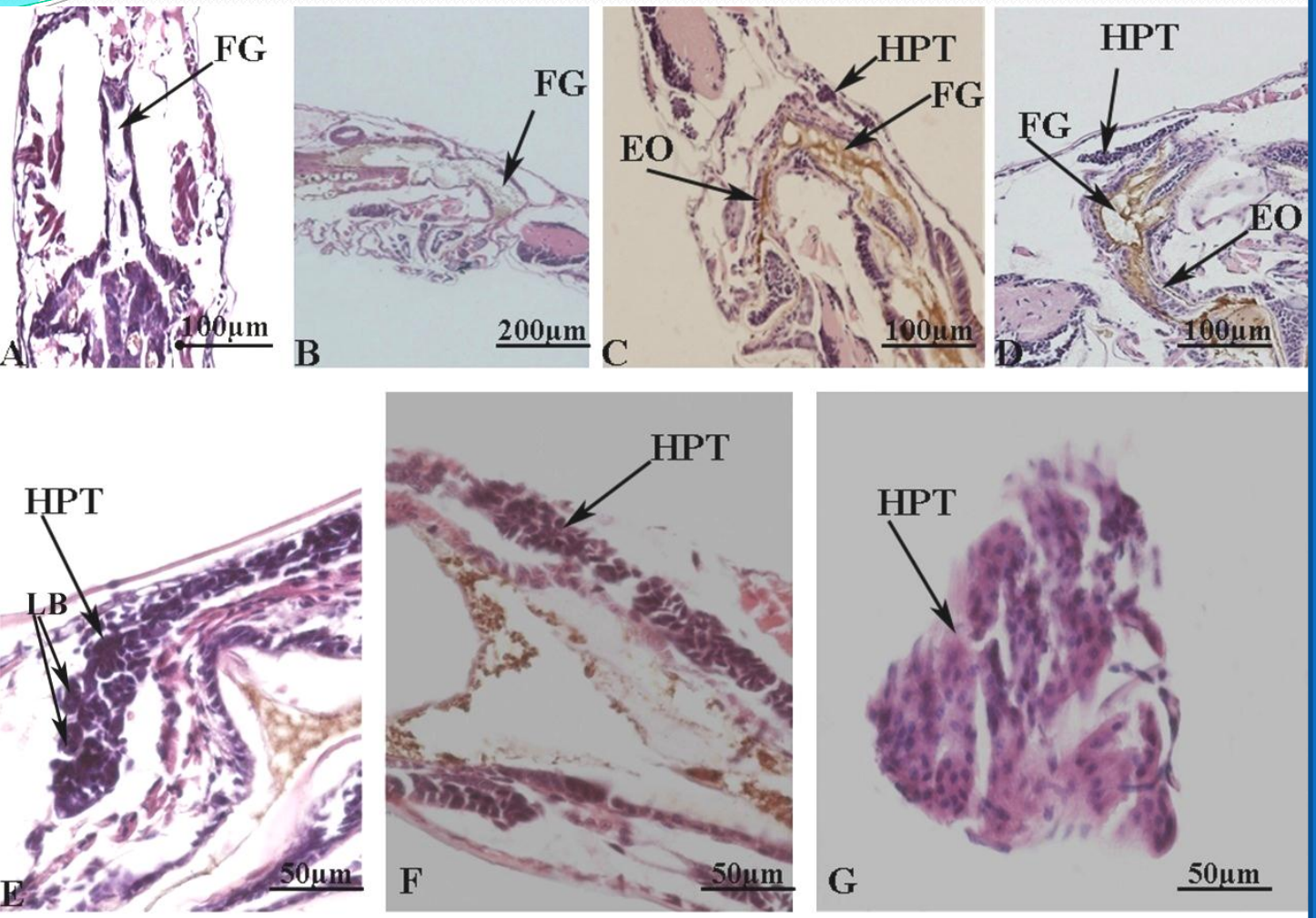
HPT lies above the front part of forgut, beneath the carapace.

HPT is responsible for haemocyte production and haemocytes has several immune functions, Such as phygocytosis, encapsulation, medication of cytotoxicity .

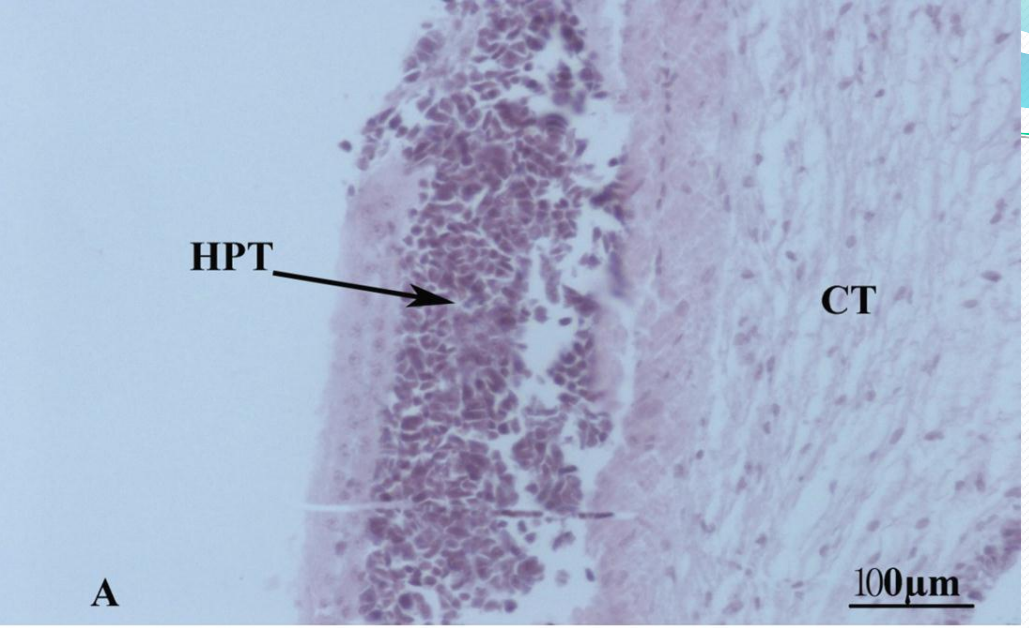
Material and Methods

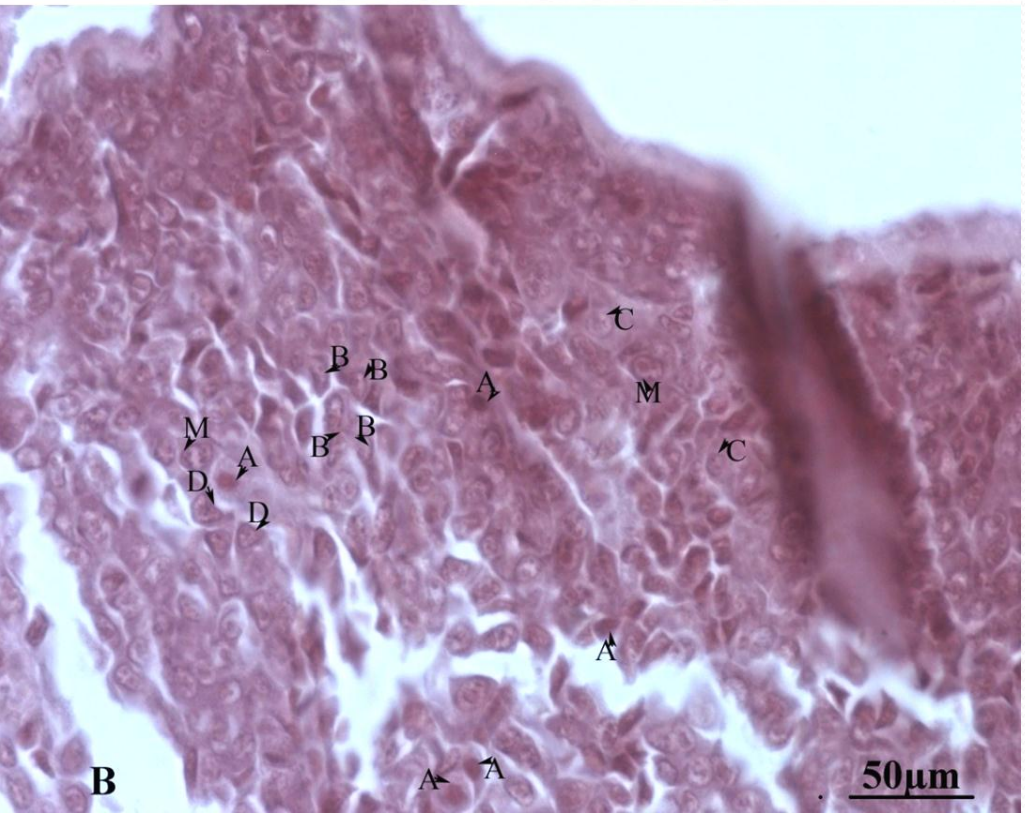
All histological procedures taken as per stated earlier.

Genesis of Hematopoietic tissue (HPT)



- Did not found HPT in mysis and first two post larval stages.
- The first occurrence of HPT was noted in P_3.
- Does not show any structural change during development.



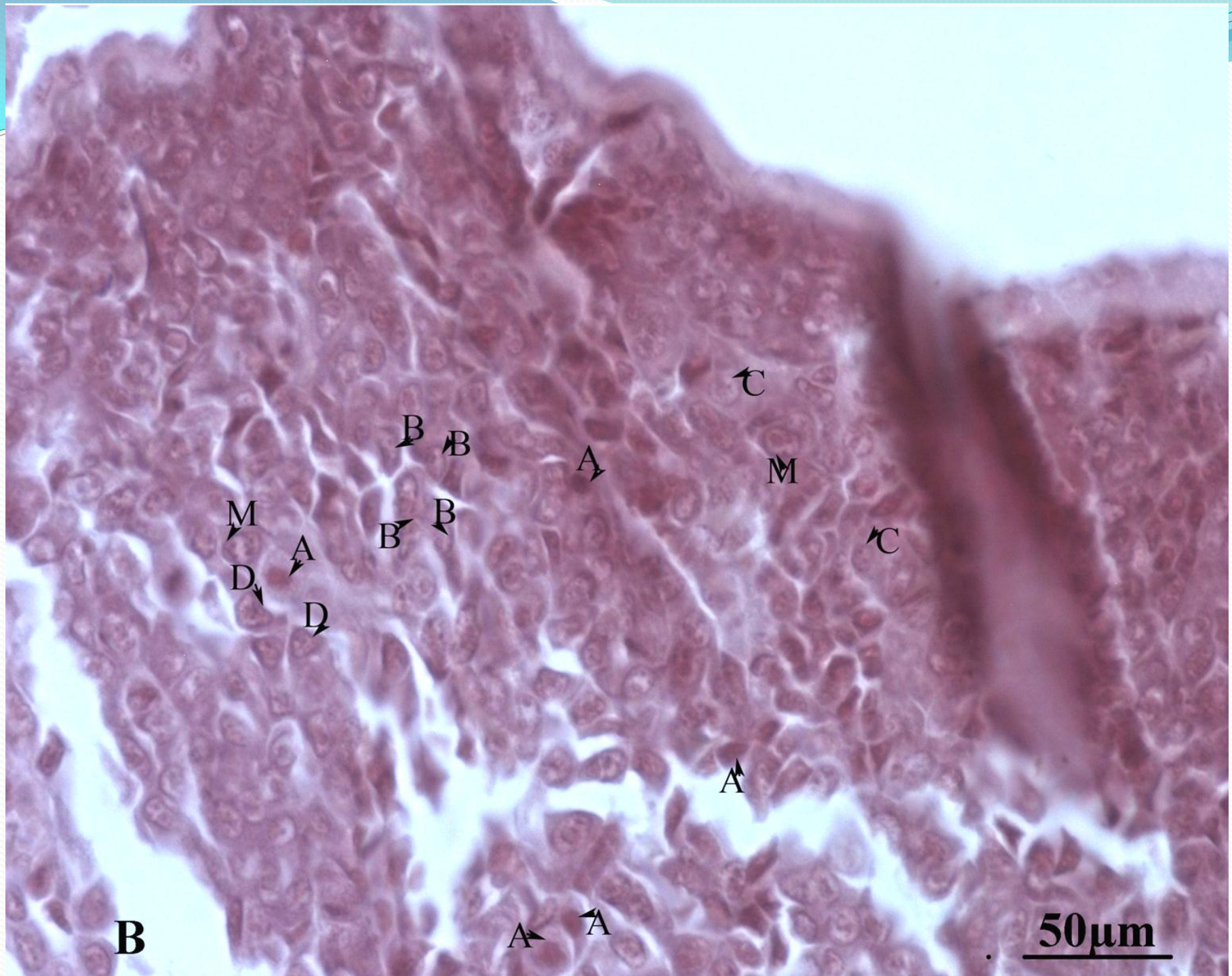


1- A type cells are deeply stained and their nuclei is bigger than others.

2- Type B cells have comparatively light stain and nuclei is on either side of the cell.

3- The type C cells are almost round in shape.

4- Type D cells are pear shape in structure.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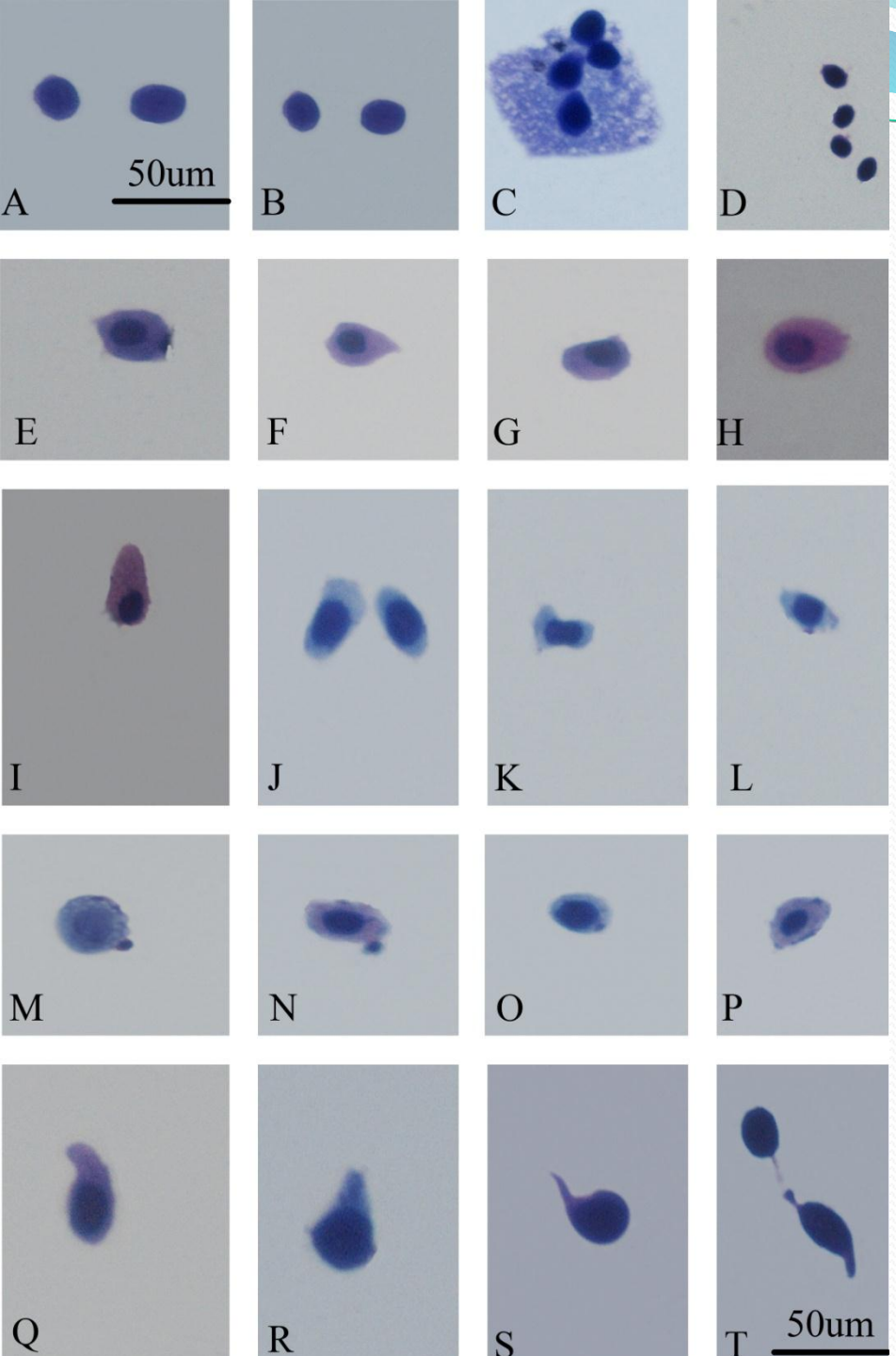


Wright Giemsa/ Giemsa staining

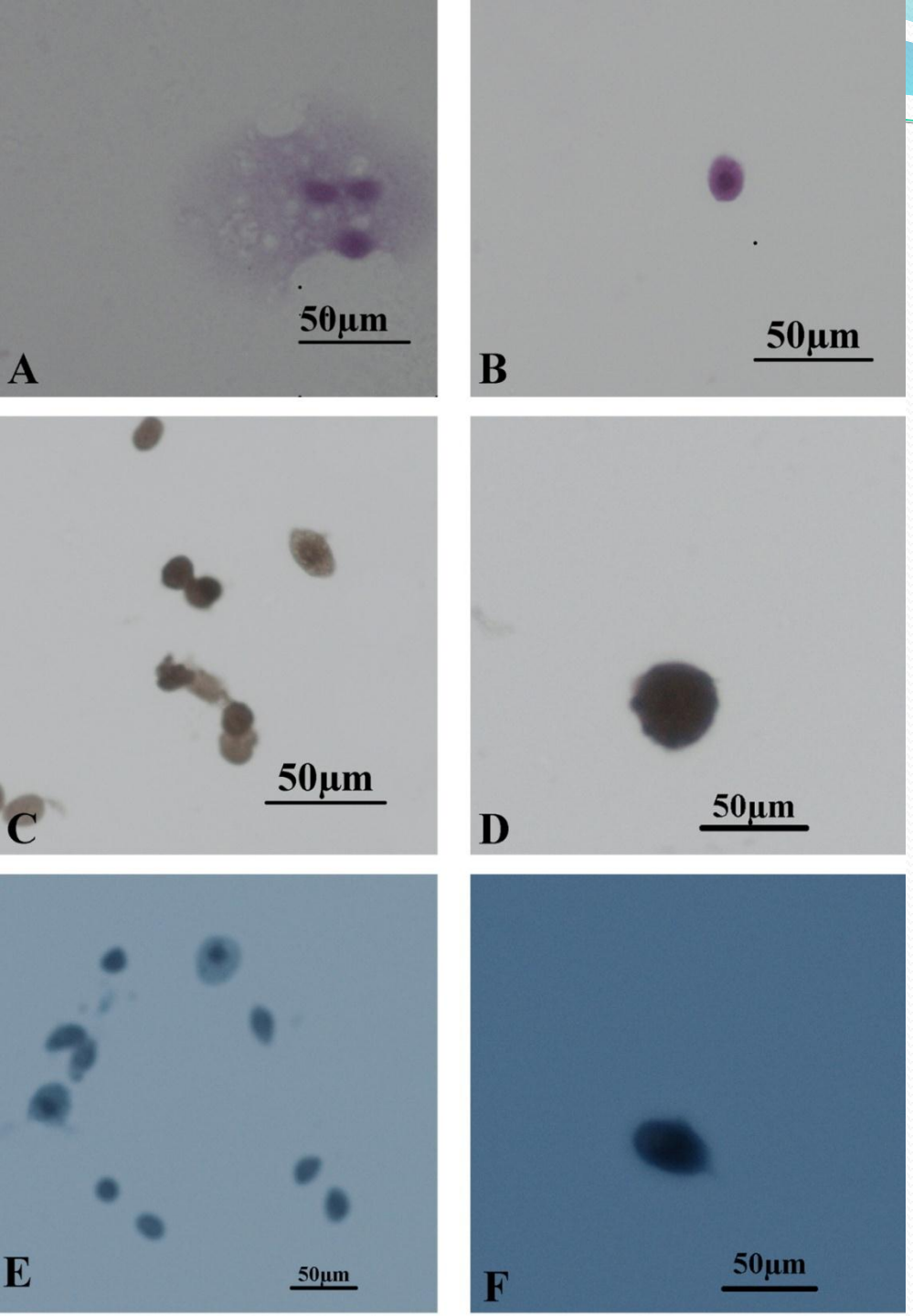
1- The nucleus occupies the largest part of cell and cytoplasm can hardly be demonstrated.

2- In type 11 cells the nucleus is more or less round in shape and cytoplasm is uneven.

3- The type III cells are elongated and flat in shape and cytoplasm is unequal to both of the ends of nucleus, however in few cells the nucleus is on edge of the cytoplasm.

4- Type IV cells have rough cytoplasm, containing small granule along the margins of the cells.

5- Type V cells have a protuberance and do not show any proper cytoplamic structure, however the shape of projection varies among cells while nucleus covers the whole cell excluding tiny projection.



Cytochemical analysis

- All the haemocytes are PAS positive .Type I and II showed more activity than others.
- The prophenoloxidase activity was mainly observed as scanty black pigment in the type I cells.
- All the haemocytes are weakly Sudan Black B positive.

Discussion

Hematopoietic tissue (HPT)

- The HPT producing haemocytes should begin at P_3 stage at least. However, Wang, et al., (2002) reported that haemocytes are visible firstly in nauplius of *Penaeus chinensis*, although type of cells is simple.

- The work is not enough on genesis of the HPT and early initiator of haemocytes in decapod, we could not predict how to generate haemoctyes at earlier larval stages in *L. vannamei*.

- The structure similarity between the HPT cells and the circulation haemocytes implied that haemocyte cells originate from haematopoetic tissue.

Conclusion

The co-relation of cell types in HPT and haemocytes revealed that HPT is the mother tissue for haemocyte production.

I am cordially Thankful to Professor Zhang Zhifeng and all my colleagues.

Acknowledgement

Thanks to China government cholarship council for providing PhD scholarship

List of Publications

•**Faiz Muhammad and** Sultana. R, **2007:** New record of edible jellyfish, *Rhizostoma pulmo* (Cnidaria: Scyphozoa: Rhizostomitidae) from Pakistani waters. *JMBA2* - Biodiversity Records. **Published on-line, pp 1-3**

•**Faiz Muhammad,** Zhang Zhi-feng, Shao Ming-yu, Dong Ying-ping and Muhammad Shafi, **2012**. Ontogenesis of digestive system in *Litopenaeus vannamei* (Boone, 1931) (Crustacea: Decapoda)**. Italian journal of zoology, 79:1, 77-85. (Impact facto 0.9)**

• **Faiz Muhammad,** Zhang Zhi-feng, Shao Ming-yu, Dong Ying-ping and Muhammad Shafi. **2012**. Development of nervous system in early stages of *Litopenaeus vannamei* (Boone, 1931) (Crustacea: Decapoda). **Sindh University Research journal (Science series) 44: (1) 29-34.**

•**Faiz Muhammad,** Zhang Zhi-feng, Shao Ming-yu, Shi Xiaoli and Muhammad Shafi. Cytochemical Study of Haemocytes and morphology of Haematopoietic tissue in *Litopenaeus vannamei* (Boone, 1931) (Crustacea: Decapoda) **(Pakistan veterinary Journal (Accepted)) (Impact facto 1.225)**

•**Faiz Muhammad,** Zhang Zhi-feng, Shao Ming-yu, Shi Xiaoli, Muhammad Shafi.and Xiao-Ling Liu . Molecular cloning and expression of cyclophilin A *Litopenaeus vannamei* (Boone, 1931) (Crustacea: Decapoda) **(Ciencias Marinas (Under review)) SCI**

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Thanks a lot for your kind attention.

谢谢！