7.0 SUMMARY AND CONCLUSION

- Optimization of cell culture medium for crustacean cells by modifying different commercially available media for mammalian and insect cells with different supplements formed the crux of this investigation.

- Various modified media such as 0.2xL-15, 2xL-15, 2xMEM, 2xDMEM, 2xRPMI, 2xTC100, 2xIPL-14, 2xGRACE and 2xM199 with different supplements namely AS, CME/SME and NSW were used to develop the cell culture system.

- Different media with various supplements as above were tested for developing primary cell culture system for tissues of heart, ovary, lymphoid tissue, hepatopancreas, gill, eye stalk, muscle and hemocytes of S. serrata, L. vannamei and P. monodon.

- 2x L-15 medium was found to be more suitable and it was tried with different supplements for the development of primary culture of cells of different tissues from S. serrata, L. vannamei and P. monodon.

- Among the three supplements AS with 2x L-15 medium predominantly supported the growth of cells of hemocyte, heart and lymphoid tissues. The cells were attached, attained complete monolayer formation and could be subcultured.

- In CME/SME supplementation cells were attached, proliferation was observed but complete monolayer was not achieved in any of the media used.

- NSW supplementation did not promote the cell growth in any of the media used in this study.
Moderate attachment of cells were observed but proliferation was not seen in TC100, M199 and Grace's media while only few cells were attached in DMEM, IPL-14, MEM and RPMI with any of the supplements.

Cells of various tissues such as hemocyte, heart and lymphoid organ formed a confluent monolayer, sustained for a longer time and the cells could be subcultured in 2x L-15 medium with AS supplement.

Ovary cells were maintained for 45 days in 2x L-15 medium with AS supplement but cells could not be subcultured due to failure of reattachment of detached cells to the flask.

Proliferation of hepatopancreas and gill cells were observed and cells could not form a complete monolayer. After subculture, these cells were not able to replicate, which could be attributed to the lack of specific growth factors.

Eyestalk and muscle cells did not show any unique cell morphology in any of the media with supplements.

New shrimp cell culture medium was formulated based on the amino acid and biochemical concentration present in the hemolymph of *L. vannamei*.

Five different concentrations of media such as 0.025, 0.25, 0.5, 1 and 2x L-15 medium were prepared based on the hemolymph concentration with 15% FBS and screened with various tissues like hemocytes, heart, lymphoid organ, hepatopancreas, gill, eyestalk and muscle of *L. vannamei*.

Cell attachment and viability was found to be very less in 0.025x and 0.25x medium when compared to 0.5x medium, prepared based on the hemolymph composition that showed moderate attachment and cell viability.
Comparison between 1x and 2x L-15 medium revealed that 2x L-15 medium was prominent medium for growth and attachment of cells and further standardization of 2x L-15 medium was carried out.

2x L-15 medium with different supplements such as FBS, SME, FGF, NSW, and different physical parameters pH, mOsm, and Temp were screened for the growth hemocyte cells of *L. vannamei*.

Various concentrations of FBS 1%, 5%, 10%, 15%, 20% and 25% were used, but more than 10 to 20% FBS supported the cells growth, attachment and viability and in 25% FBS, inhibition of cell growth was observed.

Various concentrations of SME 1%, 5%, 10%, 20% and 25% were used as enrichment during cell growth. In 1%, SME supplement low attachment of cells were observed whereas in 5, 10 and 20% of SME supplemented with 2x L-15 medium showed rapid cell attachment, well defined morphology and more than 20% SME inhibited the cell growth. SME without FBS caused cell lysis in all the concentration used after 24 hrs.

Different concentrations of FGF such as 0.5, 1, 3 and 5ng/ml FGF were screened. Since FGF concentrations above 1ng/ml showed same pattern FGF at a level of more than 1ng/ml is not required for cell growth and attachment.

pH in the range between 6.8 and 7.2 coinciding with that of the hemolymph was found to be optimal that showed healthy cells with good morphology at 24 hrs observation.
The optimum temperature was observed to be 28°C for growth of cells. Cells incubated between 32 and 35°C and between 20 and 24°C for 24 hrs were found to be lysed.

Various osmolality such as 540, 730, 870, 1075, 1270 and 1470±20 mOsmkg⁻¹ were tried. Good cell viability and healthy morphological features were observed at 730±20 mOsmkg⁻¹.

Different concentrations of NSW at 0, 01, 05, 10, 15 and 20% were used for development of the hemocyte cell culture. Initially media colour was changed at 05, 10, 15 and 20% of NSW. More than 02% NSW formed cluster of crystal cells and cell viability also decreased.

Cytopathic effects were observed in the cells of hemocytes developed from *L. vannamei* and *S. serrata* against the bacterial ECP isolated from *V. parahaemolyticus* and *V. harveyi*.

Heavy metal toxicity for primary hemocyte culture from *S. serrata* and *L. vannamei* was noted and susceptibility decreased as confirmed by MTT assay when the cells were treated with various heavy metals such as arsenic, lead, cobalt and nickel.

Hemocyte culture developed from *P. monodon*, *L. vannamei* and *S. serrata* using a modified 2x L-15 medium served as a convenient model for WSSV replication. The adapted cells showed CPE like cell shrinkage and lysis.

The CPE, apoptotic cells and WSSV replication in hemocyte were confirmed by MTT assay, Hoechst staining, Propidium iodide staining and conventional PCR.
WSSV were adapted in three different insect cell lines namely Sf9, C6/36 and S2. The virus adapted in the insect cell lines, showed CPE but the viral load remained the same in the successive passage. Though the virus got adapted to these cell lines, it was unable to replicate which may be due to the absence of single or several promoters necessary for its replication, which required further study.