5. SUMMARY

- The present study documents the environmental relevant concentration of nonylphenol in surface water and sediment along the Chennai coast which includes Ennore, Cooum, Adyar and Muttukadu estuaries. Bioassay studies were performed by subjecting the marine shrimp *Fenneropenaeus indicus* at varying concentrations of nonylphenol, detected in the environment, to augment its impact on the reproductive physiology of shrimp.

- Nonylphenol levels were found to be maximum in sediment samples compared to surface water. The level of nonylphenol in the surface water was maximum at Muttukadu creek estuary (5.24 µg/l) followed by Ennore creek (4.06 µg/l), Cooum (3.22 µg/l) and Adyar (1.22 µg/l). Sediments from the creeks registered the maximum (19.86 µg/Kg) concentration of nonylphenol compared to mouth (6.22 µg/Kg) and offshore (1.30 µg/Kg). Similar to surface water, in sediments maximum concentration of Np was detected in Muttukadu (31.08 µg/Kg).

- Bioassay studies were performed in *F.indicus* using the average concentration of nonylphenol detected from the environment. Five concentrations of nonylphenol (control, control-acetone, 13 µg/l, 64 µg/l, 103 µg/l, 180 µg/l and 259 µg/l) were selected for short (7, 14 days) and long term (42 days) exposure studies. Effect of nonylphenol was not much pronounced during short term exposure. However, a slight decrease was observed in growth related traits at higher concentration of 180 µg/l and 259 µg/l nonylphenol treated groups. Whereas, shrimp exposed for long term showed significant changes in the body weight and reproductive activity such
as GSI, HSI, oocyte maturation in female and sperm count in male compared to control.

- Histological studies clearly revealed prominent changes in the architecture of hepatopancreas, ovary and testis in treated shrimp compared to control. In control shrimp, hepatopancreas showed normal hepatopancreatic tubules, lumen and basement membrane. In contrast shrimp treated with nonylphenol at higher concentrations of 103 µg/l, 180 µg/l and 259 µg/l of nonylphenol showed swelling of hepatopancreatic tubule, abnormal lumen and decrease in basement membrane thickness.

- Gross morphology of ovary in control shrimp showed normal architecture of ovary with proliferating germinal zone and early vitellogenic oocytes. Shrimp treated with varying concentrations of nonylphenol (13µg/l & 64µg/l) did not show much variations in their morphology compared to control. However shrimp treated with higher concentrations (103 µg/l & 180µg/l) of nonylphenol exhibited the presence of both early and late vitellogenic oocytes surrounded by prominent follicle cells indicating precocious maturation. In contrast shrimp treated with 259 µg/l of nonylphenol showed degeneration of oocytes.

- Testis in control shrimp showed mature spermatozoa packed in seminiferous tubules. Long term exposure to nonylphenol at 103µg/l showed marginal decrease in spermatozoa concentration. At higher concentration treated groups (180µg/l & 259µg/l) the testis showed remarkable decrease in concentration of spermatozoa along with disrupted seminiferous tubules. Overall the effect of nonylphenol in male
shrimp was found to be dose dependent as the increase in concentration of nonylphenol (103µg/l, 180µg/l & 259µg/l) remarkably decreased the spermatozoa concentration.

- Biochemical constituents such as protein, lipid, glycogen and glucose content varied in hepatopancreas, hemolymph and ovary at different concentrations of nonylphenol treated shrimp. Compared to control biochemical constituents were found to be reduced in hepatopancreas, hemolymph and ovary in shrimp treated with higher concentration of nonylphenol. Remarkably, the major energy source like glucose levels was found to increase in hemolymph while glycogen content decreased in hepatopancreas in treated groups.

- Quantification of vitellogenin content was carried out in the hemolymph and hepatopancreas of both male and female shrimp. During short term exposure, vitellogenin levels remained consistent at lower concentration of 13µg/l and 64µg/l in hemolymph and hepatopancreas. Whereas, at higher concentration of nonylphenol (180 µg/l & 259 µg/l), vitellogenin content increased after 14 days of exposure. Similarly long term exposure showed gradual increase in vitellogenin content in both hepatopancreas and hemolymph. Captivatingly vitellogenin content in hepatopancreas and hemolymph of male shrimp increased gradually in treated groups from 103µg/l to 259µg/l. However, a remarkable increase in vitellogenin content was observed in the shrimp subjected to 259 µg/l nonylphenol exposure.

- Immunohistochemical studies with specific antivitellin (primary antibody) clearly indicated the presence of vitellin content in the ovary which was evident through
immunostaining. Control shrimp showed moderate vitellin content in the ovary. Nonylphenol treated shrimp, however, showed increased vitellin content compared to control. Interestingly at 103 µg/l and 180 µg/l of nonylphenol, shrimp showed increased vitellin content as evident through intense staining with antibody.

- Comet assay clearly indicated that there was no DNA damage observed in the shrimp during short term exposure with Np (14 days). However shrimp subjected to long term exposure clearly showed slight increase in the tail DNA (0.94%) and OTM (Olive tail moment) (0.32). Thus least concentration of NP (13µg/l) showed the least DNA damage among all the treated groups. Maximum DNA damage was found in group treated with highest concentration of NP (259µg/l) with of tail DNA (33.43%) and OTM (15.92). Overall, the DNA damage was pronounced with an increase of nonylphenol concentration.

- Thus the present study, for the first time, provide a baseline data on the occurrence of nonylphenol along the Chennai coast, South east coast of India. Prevalence of nonylphenol was recorded in the order of creek>mouth>shoreline with respect to its varying concentrations. Further bioassay studies clearly showed the possible impact of nonylphenol on the reproductive physiology of both male and female shrimp with the level of increase in biomarkers of vitellogenesis (vitellogenin and vitellin). Experimental study clearly emphasis that low level of Np, could alter the population structure of ecologically sensitive organisms.