CHAPTER V

SUMMARY AND GENERAL CONCLUSIONS
1. The changes in the oxygen consumption, blood ionic composition, caloric intake, excretory metabolism, membrane-bound enzymic activities and lipid composition of the gill tissue, in the freshwater field crab, *Oziotelphusa senex senex* Fabricius 1798 on adaptation to higher salinities have been studied under laboratory conditions.

2. The oxygen consumption was high in low salinities. The lowest rate was found in 50% sea water medium. In 100% sea water, the rate was higher than that found in 50% sea water. When the rate of oxygen consumption was plotted against the salinity, it described a parabola. This type of response was similar to that found in *Ocypode* sp.

3. Low chloride gradients were obtained in crabs adapted to 50% sea water, whereas low sodium gradients were found in 70% sea water. The crab shows functionally both hyper- and hyposmotic regulation.

4. The $K^+$ and $Ca^{++}$ levels in the blood followed a gradual increase as the salinity of adaptation increased. The levels of the total ninhydrin positive substances (TNPS) showed a significant non-linear trend as the salinity of adaptation increased.
5. The salinity stress increased the lipase activity and decreased the amylase activity in the hepatopancreas. Protease activity was unaffected by the salinity stress.

6. Ingestion of food stuffs varied with reference to salinity. Protein and carbohydrate calorific intake reduced, whereas fat calorific intake increased on adaptation to salinity. Though the calorific intake of individual food stuff was altered with salinity, the percent assimilation was not altered for carbohydrate and proteins. The assimilation of fats increased.

7. Quantitative and qualitative variations in the excretion of nitrogenous wastes occurred as a result of salinity adaptation. In freshwater, the crab excreted ammonia, urea, uric acid and trimethylamine. Ammonia excretion gradually decreased as the salinity of adaptation increased. A contrary trend was observed with urea excretion. Uric acid and trimethylamine excretions also increased gradually as the salinity of adaptation increased.

8. A plot of urea:ammonia (U/A) excretion ratios over the salinity range described a typical S-shaped curve. The trend for a gradual increase in the U/A ratios in relation to increasing salinities persisted irrespective of the type of food consumed. The blood urea level during
the salinity adaptation was stable. The glutamic acid concentration increased in the blood of the crab in contrast to that of glutamine, due to salinity stress. Reduced glutamine levels in higher salinities are corroborated by the increased U/A ratios.

9. The synthetic rate of urea by slices of hepatopancreas in vitro increased non-linearly as the salinity of adaptation increased. The data on in vitro synthetic rates of urea authenticated the data on increased ureotelism on adaptation to higher salinities. The arginase activity in the hepatopancreatic tissue of the crab increased with salinity stress corresponding to urea synthetic rates. The glutaminase activity in the gills reduced gradually as the salinity of adaptation increased.

10. The (Na\(^+\) + K\(^+\))-ATPase activity in the mitochondrial and cytoplasmic fractions of the gill decreased steadily as the salinity of the medium increased. The decrease followed a non-linear trend, with steep falls noticed in the range of 0 to 60% sea water salinities. The per cent reduction of the activity of the mitochondrial enzyme described a non-linear increase with increasing U/A ratios. Ouabain, potentially inhibited the enzyme from all the crabs adapted to different saline media. The compounds, acetazolamide (Diamox \(\oplus\)), actinomycin D and amiloride
inhibited the enzyme from salinity adapted crabs to varying degrees. Greater inhibition was recorded with actinomycin D and acetazolamide on the enzyme from freshwater crabs. The inhibitory effect of these compounds on the enzymic activity reduced at higher salinities. Phospholipase digestion inhibited the ATPase activity. This inhibition was more in higher salinities.

11. The Ca$^{2+}$ ATPase activity in the gill tissue fractions followed the same trend as that of (Na$^+ + K^+$)-ATPase with reference to salinity of adaptation. Ca$^{2+}$ was sensitive to sodium azide.

12. The activity of carbonic anhydrase in the gill tissue reduced progressively as the salinity of adaptation and the U/A ratios increased.

13. The cholesterol and phospholipid concentrations of the gill tissue varied with reference to salinity stress. With reference to unit protein, cholesterol increased whereas phospholipid decreased on adaptation to salinity. The C/P ratio increased, following a S-shaped curve, with increasing salinities.

14. Six phospholipid fractions of gill lipids were isolated on TLC. Five of them were identified as phosphatidyl choline, phosphatidyl ethanolamine,
phosphatidyl serine, sphingomyelin and cardiolipin. The sixth fraction was not identified. The last four of the identified fractions did not vary quantitatively with reference to salinity of adaptation. Phosphatidyl choline and phosphatidyl ethanolamine concentrations varied; the former decreased whereas the latter increased as the salinity of adaptation increased.

15. All these results were discussed with reference to osmotic stress and nitrogen metabolism of the crab. The results offered evidence that phospholipid configuration of the gill brings forth membrane-bound enzyme modulations when the crab is subjected to higher salinity stress. These modulations are oriented for Na\(^+\) uptake and excretion of ammonia in the freshwater habitats. These are no more necessary when the crab is adapted to higher salinities (above 50% sea water) as the urea biosynthetic rates increase favouring ureotelism. Thus, ureotelism in higher salinities is a compensatory emergency devise for the crab to overcome the salinity stress.