CHAPTER I

INTRODUCTION
1.1 OSMO- AND IONOREGULATORY MECHANISMS OF EURYHALINE CRUSTACEANS

Colonisations of diverse osmotic habitats by euryhaline organisms should understandably involve feasible levels of extracellular regulation with reference to the ambient medium, and regulation of intracellular concentration with reference to the osmotic pressure of the blood. The osmoregulatory mechanisms of aquatic organisms have been extensively investigated and reviewed (Krogh, 1939; Robertson, 1960; Prosser and Brown, 1961; Lockwood, 1962; Potts and Parry, 1964; Huggins and Munday, 1968; Schoffeniels and Gilles, 1970b; Kinne, 1971). These studies have evidenced the varying degrees of responses to osmotic stress. The responses to salinity stress range from osmoconformity through hyposmotic to hyperosmotic regulation.

The physiological mechanisms for hyposmotic regulation are comparable to those described by Hoar (1972) with reference to marine teleosts. In *Artemia salina* (Croghan, 1958a,b) it is accomplished by drinking water. Specialized cells in the gills like those of chloride cells (Copeland, 1964) or neck organ located at the top of the cephalothorax of *Artemia* (Conte et al., 1972) would help absorb the NaCl depending on the osmolarity of the medium.
Osmoregulatory capacities of marine and brackish water crustaceans have been extensively studied and reviewed (Lockwood, 1962; Schmidt-Nielsen and Laws, 1963; Potts and Parry, 1964; Kinne, 1971). The freshwater crustaceans, especially crabs have received only limited attention (Schwabe, 1933; Beadle and Cragg, 1940; Shaw, 1959a, b, c; Lockwood, 1959; Ramamurthi, 1966, 1967a, b; Venkatachari and Vasantha, 1973; Krishnamoorthy and Srihari, 1973; Krishnamoorthy et al., 1976).

Broadly, the osmoregulatory responses are classified into two types viz., i) those taking place on an evolutionary scale and ii) those taking place on short-term basis. The former category helps organisms to colonize new aquatic habitats of varying osmotic profiles, whereas the latter gains importance in such organisms that are subjected to short time fluctuations in the osmoticity of heterosmotic media. These can be occurring in intertidal, estuarine and brackish habitats.

Invasions to freshwater and land by crustaceans involved several physiological and biochemical changes during the evolutionary process which had taken place in geological ages. Reduction in the total solute concentration of the blood is one of the mechanisms that was undertaken to resist freshwater environment. Influx of water
by osmosis is one of the terrible problems that is encountered by the organism in freshwater. This problem is partly solved by a general reduction of permeability of body surfaces in the freshwater forms (Schoffeniels and Gilles, 1970). Beadle and Cragg (1940) have stated that blood chloride level is an important determinant in adaptation to freshwater. Also, they have pointed out (loc. cit.) that the blood-tissue chloride gradient is an important factor in freshwater adaptation; the lower the gradient, the greater the adaptability of the organism to freshwater. These postulations, however, do not emphasize intracellular osmotic problems accompanying the changes in blood concentration due to heterosmotic stress. It should be conceivable, in the physiologist's point of view that the importance of machinery operative at the cellular level must be charged with an establishment of osmotic parity between blood and intracellular fluid.

It is well known that several aquatic poikilotherms show response to osmotic stress in their metabolism (Remane and Schlieper, 1958; Florkin, 1960; Potts and Parry, 1964; Ramamurthi, 1965, 1966, 1967a, b). Salinity stress brings forth necessary adjustments in the metabolism of the animal to meet the expenditure incurred on regulatory works (Hoar, 1966). The oxygen consumption of the organism changes as a function of salinity. The relati
between the oxygen consumption and osmoregulation and ionic balance in crustaceans has been studied in greater detail (Beadle, 1957; Kinne, 1964; Potts and Parry, 1964; Krishnamoorthy et al., 1976; Subrahmanyan, 1979). The variations in oxygen consumption of euryhaline animals due to salinity stress, are classified into four types of responses (Vernberg and Vernberg, 1972): 1) lower oxygen consumption in higher salinities, 2) high oxygen consumption in both low and high salinities, 3) low oxygen consumption in both low and high salinities and 4) oxygen consumption unaffected by salinity. These patterns reflect different types of osmotic adjustments to salinity. These generalizations were made impossible when tissue oxygen consumption was compared.

Salinity may affect the structure and function of an organism differently at different ontogenic or physiologic stages (Huntsman and Hoar, 1939; Fontaine, 1954; Madan Mohan Rao, 1968; Ramamurthi, 1968). Efficiency of the metabolism is another aspect of physiological change occurring in salinity adaptions. There is voluminous literature on this aspect (Kinne, 1971; Pandian, 1975). Metabolic efficiencies are studied by comparing the rates of food conversion and conversion efficiencies. These are demonstrated in euryhaline fish Cyprinodon macularius (Kinne, 1960), Artemia salina (Reeve, 1963a,b; Hentig, 1970; Pandian, 1970).
Extreme salinities always exert over-reaching physiological burden on the organism, the net result of which would be a heavy expenditure of energy. In many organisms it is demonstrated that extreme salinities affect the oxygen consumption of the animal. In situations involving heavy expenditure of energy, the organism increases its calorific intake (Pandian, 1975). Generally, this is met with by increased food consumption or by utilizing reserve sources. Narasimhan (1973) working in this laboratory, suggested that the field crab Paratelphusa hydrodromous increases its fat metabolism on adaptation to higher salinities.

Salinity change influences the work performance of the organism (Kinne, 1971; Vernberg and Vernberg, 1972). Work performance like burrowing, boring and tube building activities, propulsion of feeding and respiratory currents in the media etc., are organismal responses sensitive to the salinity. Recently, Subrahmanyam and Krishnamoorthy (1979) reported that the freshwater field crab Osiotelphusa senex senex, on adaptation to higher salinities, changes its angle of orientation while moving on an inclined plane.

The free amino acid pool in the body fluids and tissues is related with the environmental salinity. In the muscles of crustaceans like, Eriocheir sinensis (Bricteux-Gregoire et al., 1962), Leander squilla and
Leander serratus (Jeumiaux et al., 1961), Astacus astacus (Duchateau-Bosson and Florkin, 1961), Carcinus maenas (Shaw, 1958a,b; Duchateau-Bosson et al., 1959), the amino acid pool is directly proportional to the environmental salinity. The number of free amino acids (qualitatively) has also been affected by the environmental salinity as in the case of Sesarma plicatum (Madan Mohan Rao et al., 1969). Variations in individual amino acid concentrations in many invertebrates were noted in salinity adaptations (Virkar and Webb, 1970; Baginski and Pierce, 1977). The extent of qualitative and quantitative variation of the amino acid pool is dependent on time course adaptations. For instance, Baginski and Pierce (1978) related the accumulation of free amino acids during initial stages of high salinity adaptation of Modiolus demissus. As switterions, the free amino acids contribute to the non-electrolyte osmotic pressure of the body fluids in the organism.

How regulation of electrolyte and non-electrolyte balances in the body fluids of euryhaline species is brought about, is not clearly understood. Probably, the mechanism involves both neural and hormonal pathways.

1.2. INTERACTION OF SALINITY AND AMMONOTELISM IN AQUATIC ORGANISMS

Needham (1938) first recognized a relationship between
the principal end-product of protein catabolism and osmo-
regulation. He suggested that the predominant nitrogenous
waste product is related to the water economy of the
organism. If ample water is available in the body, the
toxic ammonia will be rapidly excreted. If water avail-
ability is restricted, the organism excretes less toxic
urea or uric acid, in order to permit water conservation.
Needham (1938) followed later by Baldwin (1967) suggested
that ammonotelism is the most primitive condition while,
ureo- and urecotelism would have appeared during the course
of evolution. Smith (1956) has vigorously challenged
these statements. He proposes, at least in the verte-
brates, urea as the most primitive nitrogenous waste, and
the production of ammonia arising secondarily for the
regulation of acid-base balance and cation conservation
in aquatic animals. Recent work added new weight to the
point of view given by Smith (1956). Primitive crossoptery-
gian, Latemaria is ureotelic (Pickford and Grant, 1967).
The ammonotelic teleosts retain active ornithine-urea
cycle enzymes in their liver (Huggins et al., 1969).
According to Smith (1956) the ammonia formation is secondary
and peripheral. Ammonotelism has evolved in Crustacea and
Teleosts (Parry, 1960; Pequin and Serfaty, 1962; Goldstein
and Forster, 1965; Fromm and Gillette, 1968), where it
functioned through gill membranes. Krogh (1939) demonst
by his classical experiments that the gills of Crustacea and Teleosts, and the skin of Amphibia play a major role in the maintenance of the mineral balance. He was the first to emphasize that cutaneous excretion of ammonia may be related to the absorption of cations. Active transport of Na\(^+\) and Cl\(^-\) has been established in Crustacea (Shaw, 1959a,b,c, 1960a,b,c) and Teleosts (Romeu and Maetz, 1964; Maetz and Romeu, 1964; Moiais, 1967) and Amphibia (Maetz, 1972). These two ions are exchanged with the excretion of endogenous cation NH\(_4^+\) and anion HCO\(_3^-\) (Maetz and Romeu, 1964).

In recent years, evidence for NH\(_4^+\)/Na\(^+\) and HCO\(_3^-\)/Cl\(^-\) exchange underlying salt absorption has been accumulating. These have been demonstrated in the crayfish Astacans pallipes (Shaw, 1959a, 1964), the goldfish, Carassius auratus (Romeu and Maetz, 1964; Maetz and Romeu, 1964) and in larval amphibian, Ambystoma (Alvarado and Dietz, 1970; Dietz et al., 1967). Maetz (1972) showed that Na\(^+\) and Cl\(^-\) are absorbed independently from the external milieu. In other words, there will be differences in the absorption of ions of different charges. This can be explained with the laws of electroneutrality of solutions.

The (Na\(^+\) + K\(^+\))-ATPase, considered to be a protein dimer imbedded in the phospholipid bilayer of the plasma membrane, uses energy derived from the ATP hydrolysis to
move Na$^+$ outwards across the membrane. While doing so, an inward flux of counterion (K$^+$ or NH$_4^+$) exists across the membrane (for a recent review see Albers, 1976). Gill epithelium, possessing this enzyme, is thus able to move monovalent cations across the layer of the tissue. When euryhaline teleosts and crustaceans are subjected to various environmental salinities, the activity of the gill (Na$^+$ + K$^+$)-ATPase is consistent with the degree of Na$^+$ pumping expected. In the blue crab, Callinectes sapidus, (Na$^+$ + K$^+$)-ATPase activity increases as the adaptation salinity decreases (Towle et al., 1976). Euryhaline teleosts adapted to sea water showed higher gill (Na$^+$ + K$^+$)-ATPases than those in freshwater (Kamiya and Utida, 1969). In such cases, it is expected that large quantities of Na$^+$ is extruded into the sea water, thereby maintaining blood sodium concentration at the physiological level. Mangum and Towle (1977) feel that Na$^+$ regulation by the (Na$^+$ + K$^+$)-ATPase activity seems to be closely linked in an enantiostatic fashion with respiratory adaptations and nitrogen excretion during salinity adaptation. Correlations between gill (Na$^+$ + K$^+$)-ATPase activity and Na$^+$ gradient have been reported in several fish (Butler and Carmichael, 1972; Towle et al., 1977) and in Artemia salina (Conte, 1977). Na$^+$ uptake is done by the chloride cells present in the gills. These ion-transporting cells were demonstrated
in many crustaceans (Copeland, 1968; Copeland and Fitzjarrell, 1968; Milne and Ellis, 1973). Thomson and Sargent (1977) made a correlation between the number of chloride cells and the specific activity of (Na\(^+\) + K\(^+\))-ATPase in common eel *Anguilla anguilla*. Increased number of cells occurred with an increased amount of (Na\(^+\) + K\(^+\))-ATPase activity in the silver eel.

The sites of (Na\(^+\) + K\(^+\))-ATPase localizations differ variedly in aquatic invertebrates. They may be localized in one anatomically distinct area on the mantle (Mangum and Towle, 1977; Mangum et al., 1978; Saintsing and Towle, 1978). Ouabain inhibits ammonia excretion in Rangia cuneata (Mangum et al., 1978). Hedgepeth (1974) reported the occurrence of (Na\(^+\) + K\(^+\))-ATPase in the gills of the oyster *Crassostrea virginia* and the hard clam *Mercenaria mercenaria*. Saintsing and Towle (1978) maintain the view that the enzyme is localized in the mantle and regulates the Na\(^+\) ion and eliminates the ammonia.

1.3. RELATIVE ROLES OF CARBONIC ANHYDRASE AND ION STIMULATED ATPases IN THE MAINTENANCE OF ELECTROLYTE BALANCE IN FRESHWATER POIKILOOTHERMS

The carbonic anhydrase may play, in the freshwater organisms, the central ionoregulatory role which is commonly attributed to the (Na\(^+\) + K\(^+\))-ATPase system under marine
conditions. This speculation was well supported by Kerstetter and Keeler (1976) and Houston and McCarty (1978) in the case of fishes. They found that acetazolamide reduces the uptake of Na\(^+\) more than that of Ouabain. These findings did not corroborate the views of Payan et al. (1975). Observations of Murphy and Houston (1974) and McCarty and Houston (1977) showed that branchial and renal carbonic anhydrase activity, through their effects on HCO\(_3^-\)/Cl\(^-\); H\(^+\)/Na\(^+\) exchanges and possibly conversion of NH\(_3\) to NH\(_4^+\) prior to NH\(_4^+\)/Na\(^+\) exchange, are consistent and may provide relatively thermostable basal rates of sodium and chloride uptake. Temperature promotes increased electrolyte depletion which is largely associated with increased (Na\(^+\)/K\(^+\))-ATPase and erythrocyte carbonic anhydrase. Salinity also brings forth similar changes.

Acclimation to higher temperatures was associated with significant increases in the carbonic anhydrase activities of blood and kidney, whereas, that of gill preparations actually declined (Lindskog et al., 1971; Girard and Istin, 1975).

Maetz and Romeu (1964) and Maetz (1972) proposed a model for Na\(^+\) and Cl\(^-\) absorption by the teleostean gill considering tight coupling between Na\(^+\) uptake and NH\(_4^+\) excretion and between Cl\(^-\) uptake and HCO\(_3^-\) release mediated
by carriers located on the external face of the epithelial cells. Carbon dioxide and ammonia may cross the sarcosomal membrane in unionized form. Carbonic anhydrase plays a pivotal role in catalyzing production of $\text{HCO}_3^-$ and $\text{H}^+$. $\text{H}^+$ is presumably captured by $\text{NH}_3$ to form $\text{NH}_4^+$. A major portion of $\text{NH}_3$ excreted results from enzymatic deamination of amino acids. The mobile carriers are supposed to be situated on the external membrane of the epithelial cell. If they were located on the serosal side, $\text{Na}^+$ entering the cell would compete for the carrier with $\text{NH}_4^+$ and $\text{Cl}^-$ would compete for $\text{HCO}_3^-$. However, Maetz (1972) discusses that stoichiometric relationship does not exist between $\text{NH}_3$ excretion and $\text{Na}^+$ uptake in many cases. No correlation between $\text{Na}^+$ uptake and $\text{NH}_4^+$ production exists in the crayfish Astacus sp. (Romeu and Salibian, 1983).

Evans (1975a) proposed that marine teleost fishes extract $\text{Na}^+$ from sea water in order to drive a $\text{Na}^+$/NH$_4^+$ and $\text{Na}^+$/H$^+$ ionic exchange system which functions in nitrogenous waste excretion and acid-base regulation. Both the sea water-acclimated molly Poecilia latipinna and the marine pinfish Lagodon rhomboides displayed a saturable NH$_4^+$, H$^+$ and Amiloride inhibited Na$^+$ uptake when rapidly transferred to solutions of low NaCl concentration (Evans, 1973, 1975b; Carrier and Evans, 1976). Injection of 2 $\mu$M NH$_3$ = [(NH$_4$)$_2$SO$_4$]/g fish stimulated Na$^+$ uptake by Opsanus beta.
and also stimulated ammonia efflux, 50% of which was dependent upon external $Na^+$ (Evans, 1977).

It is reported that the carbonic anhydrase activity is involved in the above ion-exchanges (Maetz and Romeu, 1964; Maren, 1967). The inhibition of the enzyme produces significant plasma-chloride increase in the marine fish, *Serranus* sp. (Maetz, 1972) and a considerable decrease in the freshwater *Perca* sp. (Maetz, 1956). Krishnamoorthy and Virabhadrachari (1969) have demonstrated a reduced carbonic anhydrase activity in the gills of a euryhaline fish, *Etroplus maculatus* on acclimation to higher salinities. Their data showed that the enzyme activity increases in media where active chloride uptake is necessary. The same fish have the so called 'chloride cells' (Virabhadrachari, 1961), in the gills which absorb $Cl^-$ actively in hyposmotic media and excrete chloride in hyperosmotic media. Krishnamoorthy and Virabhadrachari (1969) also showed a close relationship existing between the chloride balance and carbonic anhydrase activity in *Etroplus maculatus* adapted to higher salinities.

Aldridge and Cameron (1979) described the pattern of $CO_2$ excretion in the gills of the blue crab, *Callinectes sapidus* and constructed a computer simulation model to explain the $CO_2$ exchange at the gill surface. This model explains the movement of $CO_2$ out of the gill directly as
HCO$_3^-$, a possibility first suggested by Krogh (1938), but not demonstrated directly so far. In both freshwater and salt water adapted crabs (Callinectes sapidus), the rates of Cl$^-$ influx are very high. The rate of CO$_2$ movement was two times higher in freshwater adapted crabs (Aldridge and Cameron, 1979), but the proportion of Cl$^-$ movement that is linked to Cl$^-$/HCO$_3^-$ exchange is not known but likely to be small (Cameron, 1978).

The gill lamellae of freshwater-adapted Callinectes sapidus consist of epithelia of two distinct types (Copeland, 1964; Copeland and Fitzjarrel, 1968; Aldridge, 1977). One type, the thick epithelium, is presumably the site of active ion uptake or regulation (Copeland, 1964; King, 1965; Mantel, 1967; Copeland and Fitzjarrel, 1968; Smith and Linton, 1971; Engel and Eggert, 1974; Engel et al., 1975). It averages 10 µm in thickness and plays a minor role in gas exchange, because of its long diffusion pathway. The other type, thin gill epithelium, is probably the primary site of respiratory exchange as it has a thickness of 0.5 µm. Thus functionally significant areas are demarked on the gill for exchange of gases and ion uptake. Subrahmanyan (1979) in the laboratories of the Department of Zoology, University of Agricultural Sciences, G.K.V.K. Campus, Bangalore, observed varying thickness of the lamellar epithelium in the freshwater crab, Oziotelphusa
senex senex along its length. Aldridge (1977) demonstrated the presence of carbonic anhydrase in all the eight pairs of gills in Callinectes sapidus but not in blood. In the vertebrate systems the carbonic anhydrase is located both in lungs and in red blood cells (Effros et al., 1978).

1.4. TRANSITIONS IN NITROTELISM

Elasmobranchs, lungfishes and certain amphibians show metabolic adjustments through increased production of urea in hyperosmotic stresses (Balinsky et al., 1961; Goldstein and Forster, 1965; Pickford and Grant, 1967). Campbell (1970) believes that crustacea are incapable of responding to hyperosmotic stresses by synthesizing urea, since the enzymes of urea cycle with the exception of arginase have not been reported in them. Many reports appeared, however, on transition in nitrotelism. For example, crayfish can shift from freshwater to salt water (Sharma, 1966). Despite these shifts, a steady state level of ammonia is always excreted. It is suggested that certain amount of ammonia derived from deamination of amino acids is not routed through urea cycle. Sharma (1966, 1968) suggests that urea is released in crayfish as a preformed substance from some metabolite. Probably, purines and arginine may be implicated for this urea synthesis (Sharma, 1966, 1968).
Sharma (1969) also concludes that the trigger for increased urea production was an increase in salt concentration and not an increase in osmotic pressure in the individual. Horne (1968) reports that in the terrestrial crab *Cardisoma guanhumi*, the pattern of nitrotelism is not altered in the course of dehydration. He observes that the excretory urea in dehydrated crabs is derived from the food rather than through the urea cycle.

Salinity effects on non-protein and non-amino acid nitrogen levels of the blood were studied by Venkata Reddy (1976) in *Paratelphusa hydrodromous*. The non-amino acid nitrogen level declines steeply with increases in salinity. The data on non-protein nitrogen excretion in *Paratelphusa hydrodromous* (Krishnamoorthy and Srihari, 1973) reveals a de facto conservation of nitrogen.

Heterosmotic stress is associated with changes in intermediary metabolism of aquatic animals (Prosser, 1973; Kinne, 1971). A direct proportionality exists between the free amino acid pool and the environmental salinity. This has been demonstrated in the muscles of crustaceans like *Eriocheir sinensis* (Bricteux-Gregoire et al., 1962); *Leander aquilla* and *Leander serratus* (Jeuniaux et al., 1961); *Astacus astacus* (Duchateau-Bosson and Florkin, 1961) and *Carcinus maenas* (Shaw, 1958a,b; Duchateau-Bosson et al., 1959). The number of free amino acids increases with the
salinity of the medium in *Sesarma plicatum* (Madan Mohan Rao, et al., 1969). Generally the marine organisms retain more amino acids in the blood than freshwater organisms (Florkin, 1960; Florkin and Schoeffeniels, 1965). In many species of crustaceans, the nitrogenous waste products and their excretion vary as a function of environmental salinity. Adaptation to higher salinity increases the trimethylamine oxide excretion in *Eriocheir sinensis* (Bricteux-Gregoire et al., 1962), *Penaeus* and *Metapenaeus* (Velankar and Govindan, 1960) and *Paratelphusa hydrodromous* (Krishnamoorthy and Srihari, 1973). The total nitrogenous excretion was not altered in *Paratelphusa hydrodromous* on adaptation to higher salinities (Krishnamoorthy and Srihari, 1973). Fifty per cent sea water seems to be the threshold to cause a shift from ammonotelism to ureotelism in these crabs. Salinity adapted crabs excreted more urea, uric acid and trimethylamine than those in freshwater. Starvation did not affect the quantitative excretion.

Mangum et al. (1976) showed that *Callinectes sapidus* increases its ammonia output as well as the Na\(^+\) uptake when the crab is in dilute sea water. A correlation thus is expected, coupling ammonotelism with Na\(^+\) uptake. Not only in crabs, but also in many other euryhaline invertebrate this is shown to be true. Emerson (1969) found that NH\(_3\) excretion rates increase invariably following transfers from 100\% sea water to 50\% sea water.
In the blue crab *Callinectes sapidus*, blood pH varies inversely with acclimation salinity (Mangum *et al.*, 1976). This is largely due to binding of $\mathrm{H}^+$ to the $\mathrm{NH}_3$ produced in catabolism of free amino acids, as intracellular fluids equilibrate with a dilute blood in low salinity. Also low salinity causes the formation of additional $\mathrm{NH}_4^+$ to provide a counter ion in order to balance the absorption of $\mathrm{Na}^+$ at the gill (Weiland and Mangum, 1975). Further, Mangum *et al.* (1976) demonstrated a balance between ammonia and urea output which shifts towards ammonotelism at low salinity in the blue crab. They (*loc.cit.*) hypothesized that excess ammonia in blood plays an influential role in osmoregulation as well as opposing the effect of salt reduction on the oxygen transport system.

The synthesis and urinary excretion of ammonium ions represent the acid-base homeostasis in the vertebrates (Fine *et al.*, 1972). The same should be true with the invertebrate system too. Metabolic acidosis may exist with excessive production of ammonium ion if it is not regulate properly (Owen and Robinson, 1963). The schema described in the earlier paragraph would probably check the production of ammonium ions. There is another regulation to neutralise the ammonium ions which occurs in the vertebrate. Under regulated acid-base homeostasis, the free ammonium ions in circulating body fluids are chelated by glutamic
acid and aspartic acid of the blood; as a result of which neutral and non-toxic glutamine and asparagine are formed (Shalhoub et al., 1963). At the excretory site, the -amidases viz., glutaminase and asparaginase present in the tissues, liberate ammonia from glutamine or asparagine and the ammonia is eliminated (Mahler and Cordes, 1966). Vertebrate kidney and the gill of aquatic organisms perform these functions. Gills in addition to involving in these reactions, also eliminate the respiratory waste CO₂. The mechanism of CO₂ excretion is explained in an earlier section. Occurrence and importance of ω-amidases have been reported in Paratelphusa hydrodromous (Krishnamoorthy and Srihari, 1973). These authors have reported a rapid decrease in the activities of asparaginase and glutaminase in the tissues of animals on adaptation to higher salinity.

1.5. MEMBRANE BOUND ATPases AND SALT ABSORPTION

(\(\text{Na}^+ + \text{K}^+\))-ATPases have been strongly implicated in the transport of cations into and out of the blood of euryhaline organisms. Their activity in the absorbing organs is often very high and this activity is enhanced when NaCl balance shifts from conformity to a gradient between blood and ambient medium (Towle et al., 1976; Conte, 1977; Subrahmanyan, 1979). The osmoregulatory role of the
\((\text{Na}^+ + \text{K}^+)\)-ATPases was questioned recently. Karnaky et al. (1976) proposed a hypothesis that they maintain a hyposaline blood, but it was not accepted (Mangum et al., 1979) as it is difficult to reconcile with the location of the enzyme on the inside of the epithelial barrier separating blood from the ambient medium. The injections of ouabain into crabs and eels may influence \(\text{NH}_4^+\) or \(\text{Na}^+\) movements and the animals develop conspicuous systemic effects resulting in death within several hours (Silva et al., 1977; Mangum et al., 1978). Exposure to exogenous ouabain, a specific inhibitor of this enzyme, is not lethal to simpler aquatic animals like annelids and molluscs though it depresses the \(\text{NH}_4^+\) excretion (Mangum et al., 1978). It is now shown that blood NaCl regulates the enzymatic activity (Mangum et al., 1979).

In recent years, ample evidence has been established to correlate the gill structure and ATPase activities. ATPase activities as related to salinity stress were studied in many euryhaline crustaceans. Hedgepeth (1967) and Mangum and Amendede (1972) described the survival of the blue crab, \textit{Callinectes sapidus} on transfers to heterosmotic media. This has become an excellent tool for biochemical experiments. It is a good osmoconformer in salinities of 27-35% sea water and maintains its hemolymph hyperosmotic nature in salinities below 27% sea water (Lynch et al., 1973;
This regulation involves, principally a regular uptake of \( \text{Na}^+ \) across the gill epithelium (Mantel, 1967; Schoffeniels and Gilles, 1970a,b; Smith and Linton, 1971; Gerald and Gilles, 1972; Colvocoresses et al., 1974; Engel et al., 1974).

Skou (1965) demonstrated that the active uptake of \( \text{Na}^+ \) is under the influence of \((\text{Na}^+ + \text{K}^+)\)-dependent ATPase. This enzyme exchanges \( \text{Na}^+ \) for \( \text{K}^+ \) across the membrane in many animal cells (Glynn and Karlish, 1975). \((\text{Na}^+ + \text{K}^+)\)-ATPase activities conferred hypersaline osmoregulatory ability in *Callinectes sapidus* (Towle et al., 1976). Therefore, \((\text{Na}^+ + \text{K}^+)\)-ATPase has been described to have some adaptive significance. Towle et al. (1976) showed increased specific activities of ATPase within two and a half hours of transfers of crabs from high salinity to low salinity. ATPase activities and fatty acid composition of gill tissue were correlated to the salinity stress (Towle et al., 1976, 1977; Thomson et al., 1977; Tondeur and Sargent, 1977).

\((\text{Na}^+ + \text{K}^+)\)-ATPase is a phosphoenzyme and is formed in the presence of MgATP and \( \text{Na}^+ \) is subsequently dephosphorylated in the presence of \( \text{K}^+ \) (Dahl and Hokin, 1974; Epstein, 1975). Enzymic properties of membrane bound enzymes have been worked out in many fishes. \((\text{Na}^+ + \text{K}^+)\)-ATPase from the gills of killfish *Fundulus*
heteroclitus acclimated to 16% sea water and to 30% sea water appear to be identical (Towle et al., 1976), indicating that the same enzyme may function to absorb Na\(^+\) in low salinities and excrete Na\(^+\) at gill level in higher salinities. Acclimation to short-term salinity changes may involve modifications in the catalytic rates rather than the number of (Na\(^+\) + K\(^+\))-ATPase molecules (Towle et al., 1976). In fishes these ATPases are influenced by the hydrostatic pressure of the medium (Pfeifer, 1978), increased pressures inhibiting the enzyme activity. Pequieux and Gill (1978) state that the membrane bound enzymes are so sensitive that any physical disturbance in the environment would culminate in the alteration of the specific activity of the enzymes.

The blue crab also possesses Ca\(^{++}\) activated ATPases (Fox and Ranga Rao, 1978). These ATPases function to transport Ca\(^{++}\) across the membrane. Salinity stress influencing gill membrane-bound ATPases were demonstrated in the crab, *Hemigrapsus sanguinensis* (Buser, 1977), in the Japanese eel (Motasis, 1970), in the salmon (Zaugg and McLain, 1970), in euryhaline teleosts (Kamiya and Utida, 1969), in the silver eels (Thomson and Sargent, 1977), in the eel *Anguilla anguilla* (Tondeur and Sargent, 1977) and in the crabs *Carcinus maenas* and *Libinia emarginata* (Mantel and Landsman, 1977).
1.6. METABOLIC COST, FOOD AND NITROGENOUS EXCRETION

General invertebrate nitrogen metabolism has been reviewed by Campbell (1970). Recently Nelson et al. (1977) focused particular attention on the effects of nutrient quality on nitrogen excretion of crustaceans. Both metabolic rate following ingestion and nitrogenous waste production have been used to evaluate the quality of diets for various organisms (Phillipson, 1962; Moshiri and Goldman, 1969; Kiriyama and Iwao, 1964; Brown and Cline, 1974; Nelson et al., 1977). An increase in metabolic rate following food intake (a meal) has been documented for some invertebrates (Heiman and Knight, 1975; Conover, 1956).

In Macrobrachium rosenbergii the ammonia production rate was not influenced by diet but was related to the weight of the individual (Nelson et al., 1977). A significant relationship exists between the protein level in the diet and the soluble nitrogenous excretory products in echinoderms (Diehl and Lawrence, 1979).

Kinne (1971), while discussing the terrestrial adaptations of crabs, pointed out that the active salt secretion and the reduction of the amount of nitrogen excreted per unit weight are some of the basic adaptations suited for terrestrial life. He maintains the view that these are considered genetic adaptations of crustacea to
land life. However, the persistence of these mechanisms in amphibians and euryhaline crabs is not totally overruled. When relative amount of nitrogen excretion is an indicator of the habitat, the food intake also should be influenced by the habitat conditions.

Intracellular concentration of nitrogenous metabolites contribute to bring forth adjustments of isosmotic regulation (Jeuniaux et al., 1961). How the total amino acids act as osmo-effectors has been pointed out in an earlier section of this dissertation. Changes in the qualitative and quantitative composition of amino acids are at the fulcrum of protein degradation (Mahler and Cordes, 1966). In Barytelphusa guerini the salinity stress alters the levels of insoluble and soluble proteins of the tissues (Venkatachari and Vasantha, 1973) indicating the possible degradation of proteins. Greater demand for utilization of amino acids were found in low salinity adaptations in many crustaceans (Chaplin et al., 1966; Huggins and Munday, 1968; Schoffeniels and Gilles, 1970b).

1.7. OSMOTIC BUDGET

It is assumed that tissue cells are isosmotic with blood (Schoffeniels, 1967; Florkin and Schoffeniels, 1969). On adaptation to a heterosmotic medium, the blood of an euryhaline organism establishes a new level of osmo-
concentration, depending on its extracellular anisosmotic osmoregulatory capacity. When blood alters its osmotic properties, correspondingly the tissues establish their osmotic profile. The isosmotic regulatory potentialities are often assessed by examining the osmotic effector profile of different tissues of the animal under adaptation. Schoffeniels and Gilles (1970b) and Gerard and Gilles (1972a) investigated the tissue osmoregulatory mechanisms.

Decapod crustaceans regulate their ionic balance through gills, gut and antennary (maxillary) glands. Partly, the urinary bladder and other organs and tissues may also be used for this ionoregulation. The degree of activity of these regulatory sites varies in different species and is also dependent on the ionic gradients between cell fluids and body cavity fluids and those between the body fluids and the external environment. In low salinities, lamellar (gill) epithelium and branchial epithelium (gill chamber) actively absorb ions. Gut picks up ions from the food in low salinities and reabsorbs water in high salinities. Kidneys maintain hypertonic regulation in low salinities (K\(^+\)-reabsorption in tubules) and hypotonic regulation in high salinities (Na\(^+\) and Ca\(^{++}\) excretion). Antennary glands and the urinary bladder regulate Mg\(^{++}\) or other specific ions and reabsorb glucose as in *Pachygrapsus crassipes* (see Kinne, 1971 for references). Many authors
(Hukuda, 1932; Gross, 1958; Robertson, 1949, 1953; Lockwood, 1962; Potts and Parry, 1964) proposed extravascular 'salt pools' which act as reservoirs in crabs for Na\(^+\) and K\(^+\) balances under salinity stress. The respiratory lamella of the crab gill consists of a highly interdigitated epithelium (Copeland, 1968; Flemister, 1959; Subrahmanyan, 1979). The folds of this epithelium are rich in mitochondria and form mitochondrial pumps helping active ion uptake from the medium. The intercellular spaces observed in these folds conform to the morphological requirements of the model system proposed by Diamond (1962, 1965) and Diamond and Tormey (1966) for water transport outlining the theory of standing osmotic gradients. The organic composition of blood also helps congenially in maintaining and regulating ionic ratios. For example, the blood of crabs is rich in protein content which helps the formation of Ca-protein complexes (Robertson, 1953). Most of the internal K\(^+\) is held electrostatically by non-diffusible organic ions, organic phosphates and protein, and low internal Na\(^+\) levels are maintained by active expulsion of Na\(^+\). These effluxes are controlled by the membrane bound ATPase activities.
1.8. STATEMENT OF THE PRESENT PROBLEM

The brief review of literature cited in earlier pages gives a comprehensive account of the ecophysiology of the crabs with reference to environmental salinities. However, the nitrogen metabolism of these crabs, in relation to salinity stress is not very well understood. No attempts have been made to elucidate whether the changes in nitrogenous excretion with reference to salinity are dietary or real manifestations of salinity influences. The exhaustive literature available on membrane-bound enzymes and excretion of nitrogenous wastes prompts one to examine whether there is any correlation between osmotic gradient and excretory pattern. The central idea of this dissertation is to attempt drawing out such a correlation. As Bangalore is far away from the sea cost (250 miles from East or West Coast of India), the euryhaline crab chosen for this study was a semiterrestrial freshwater crab, *Oziotelphusa senex senex* Fabricus 1798. This crab tolerates wide ranges of experimental salinities. In India the semiterrestrial crabs by virtue of their euryhaline and eurythermal nature, are often used for laboratory investigations (Rao and Venkatareddy, 1962; Ramamurthi, 1965, 1966, 1967a,b; Krishnamoorthy and Venkatareddy, 1968; Venkatareddy and Aravinda Babu, 1969;
The results of this dissertation are aimed to answer the following questions which sum up the physiological and biochemical mechanisms of osmotic stress and nitrogen excretion in euryhaline crabs.

a) What are the salient features of the osmotic budget of the crab exposed to heterosmotic media?

b) What are the general aspects of oxidative and assimilatory mechanism of the crab in relation to osmotic stress?

c) What is the quantitative and qualitative profile of excretory products in relation to salinity adaptation?

d) What are the possible correlations between membrane bound enzyme activities, molecular markers and the excretory metabolism of the crab?