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STUDIES ON THE TIME COURSE OF METABOLIC RESPONSE TO SALINITY STRESS IN THE FRESHWATER CRAB **OZIOTELPHUSA** (PARATELPHUSA) **SENEX SENEX** (FABRICIUS)

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By

K. APPASWAMY PILLAI, M.A.,
Department of Zoology
S. V. UNIVERSITY, TIRUPATI.

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D E D I C A T E D

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PREFACE

Osmoregulation in animals involves two important processes. The first one is the control of intracellular fluid osmolarity in relation to changes in extracellular fluid. The second one is the control of extracellular fluid osmolarity in relation to changes in external medium. The first one has been called as the "isosmotic regulation of the intracellular fluid" and the second one as the "anisosmotic regulation of the extracellular fluid"(Florkin, 1962). Whereas the first mechanism can be traced down to Protozoa, the second one seems to be a characteristic feature of highly evolved animal groups such as arthropods and vertebrates.

Life in fresh water seems to be more difficult than in sea water. Most of the fresh water animals have to maintain their body fluids hyperosmotic to the ambient medium whereas marine forms are mostly osmoconformers. As a consequence the energy requirements of fresh water forms should naturally be greater compared to marine forms. But studies on oxygen consumption in animals in relation to different external salinities have revealed confusing and contradictory trends (Reviews by Gilles, 1973b, 1974a, 1975). For instance, Schwabe (1933) showed no change in oxygen

consumption in Eriocheir sinensis when transferred to diluted media but Lee and Pyung (1970) measured higher oxygen consumption in diluted media in the same species. Kuenen (1939) measured higher oxygen consumption in Artemia salina acclimated to higher salinities whereas Eliassen (1953) showed depression of oxygen consumption in the same species in higher salinities. Gilles comments (1979) that "in many cases investigators have measured respiratory rates soon after the salinity change and without sufficient knowledge about the time course of adaptation process". It has been shown that cell volume regulation to hyper-osmotic shocks is a relatively slow process whereas it is accomplished rather quickly under hypo-osmotic conditions (Gilles, 1973a, 1974a). Such meaningful interpretations of data are not possible in experiments that are not carried out on a time scale. The present work involves time course of metabolic response to salinity stress in the crab Oziotelphusa (Paratelphusa) senex senex (Fabricius) and thus gains relevance.

Some aspects of metabolic response to salinity stress in the fresh water crab Oziotelphusa (Paratelphusa) senex senex (Fabricius) have been studied earlier (Ramamurthi, 1965, 1966, 1967a,b; Venkata Reddy, 1976). In these studies either the immediate response to salinity stress or response at the

end of acclimation period have been reported. In the context of reports that the blood amino nitrogen showed an elevation in two crustacean species after they were exposed to sea water (Madanmohan Rao and coworkers, 1969; Venkatachari and Ramamani Keshavan, 1973) which appears to be contrary to the trends reported by several others, Gilles (1979) laments "Unfortunately, no time scale experiments have been performed .... It is therefore difficult to discuss these results .....". Present studies on the time course of metabolic response to salinity stress in the crab Oziotelphusa (Paratelphusa) are aimed at understanding the temporal sequence of changes in 1. oxygen consumption, 2. water and major inorganic osmotic effectors, 3. major organic osmotic effectors and 4. some key enzymes of amino acid metabolism. Literature on the reverse acclimation of this crab from sea water to fresh water is completely wanting. It is with a view to bridge this gap to the extent possible, the present studies on time course of metabolic response to salinity stress both in the forward and reverse directions were programmed.

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GENERAL

INTRODUCTION

The ability of organisms to adjust to osmotic stresses is an important factor in the establishment of populations in different ecological niches. The colonization of aquatic populations in such diverse habitats as estuaries, intertidal zones, mangroves, lakes and ponds is proof of such osmoregulatory capacities conferred on animals in the course of their evolutionary history. Salinity stress is known to have a direct influence on the organismic and molecular abilities of organisms to ensure proper water and salt balance in their intracellular and extracellular fluids. Salinity stress may also become a limiting factor in the survival of organisms when acting together with one or many other stress conditions such as oxygen tension of the medium, temperature, pollution, parasitism, starvation and so on. The combined effects of all these factors are of the utmost importance for the survival of a population in a given habitat. It would be rather interesting and fruitful if studies on such combined effects on organisms are undertaken. Some attempts to understand the combined effects of temperature and salinity on animal metabolism were made under laboratory conditions (Kinne, 1964, 1971; Dehnel, 1960, 1962, 1965; Parvatheswara Rao, 1965; Ramamurthi, 1967a; Venkataramaiah *et al*, 1974; Lakshmi *et al*, 1978; Huni, 1979). Vivares *et al* (1980) made some studies on the combined effects of season, salinity, temperature and parasitism on

amino acid metabolism in carcinus mediterraneus. However, it is rather difficult to comprehend and quantify such combined effects in field or laboratory studies owing to a large number of parameters involved. As such most of the present day laboratory studies on the effects of salinity stress are directed towards the understanding of such direct effects as energy metabolism water balance and cell volume regulation, regulation of organic and inorganic osmotic effectors of the extracellular and intracellular fluids. The present work is limited to such direct effects of salinity stress on the fresh water field crab Oziotelphusa (Paratelphusa) senex senex (Fabricius).

The osmoregulatory problems of animals have been extensively studied and reviewed by several investigators (Krogh, 1939; Robertson, 1957, 1960; Beadle, 1957; Prosser and Brown, 1961; Prosser, 1973; Lockwood, 1962; Florkin, 1962; Gross, 1964; Potts and Parry, 1964b; Kleinzeller, 1972; Maetz, 1973, 1974; House, 1974; Schoffeniels and Gilles, 1970, 1972; Gilles, 1972b, 1975, 1979; Dick, 1966, 1970, 1979; Potts, 1955, 1976; Kirschner, 1977, 1979; Kotyk and Janáček, 1975, 1976; Schmidt-Nielsen, 1972, 1975; Hoffmann, 1977; Evans, 1977; Rorive and Gilles, 1979; Koefoed-Johnsen, 1979).

Crustaceans and fishes, with representatives from diverse media like the sea, brackish water and fresh water,

have received copious attention. Mechanisms involved in the regulation of extracellular osmoconcentration of crustaceans and fishes in relation to ambient medium have been investigated by numerous workers (Schwabe, 1933; Krogh, 1939; Beadle and Cragg, 1940; Ramsay, 1954; Lockwood, 1959, 1961, 1964; Lockwood and Inman, 1973; Potts, 1955; Potts and Parry, 1964a; Parry, 1957; Gross, 1955, 1957a,b, 1958, 1959; Croghan, 1958a, b, c; Shaw, 1959a, c; 1960a,b; 1961a, b; Bryan, 1960a, b,c; Shaw and Sutcliffe, 1961; Sutcliffe, 1968; Potts and Eddy, 1973; Potts et al, 1973; Baldwin and Kirschner, 1976; Evans, 1967, 1969, 1973; Evans et al, 1976; Cornell, 1979, 1980; Cameron, 1978; Romero and Zanders, 1980; Sabourin and Stickle, 1980; Sharp et al, 1980; Tan and Choong, 1981; Castille and Lawrence 1981a, b; Greenaway, 1981; Gupta and Hanke, 1982). Beadle (1957) postulated that in the marine crabs "there are atleast two types of active processes at work in the gills and in the excretory organs which are responsible for the ionic imbalance between blood and sea water". Even the marine animals "which maintain an essentially isosmotic blood concentration with reference to ambient medium do possess good levels of ionic regulatory capabilities". Mechanisms of active ion transport across cell membranes with particular reference to hyperosmotic regulators have been studied and reviewed extensively by several investigators (Krogh, 1937, 1938, 1939; Ussing, 1947, 1949, 1952, 1960; Kirschner, 1955, 1970; Zerahn, 1956; Engback and Hoshiko, 1957; Koefoed-Johnsen and Ussing, 1958; Koch, Evans and Schicks, 1954; Shaw, 1960a, 1961a; Maetz and Garcia

Romeu, 1964; Potts and Parry, 1964b; Biber, Chez and Curran, 1965; Skou, 1965; Sutcliffe, 1968; Bentley, 1968; Kamia and Utida, 1969; Garcia Romeu et al, 1969; Kerstetter et al, 1970, 1972, 1974; Biber and Curran, 1970; Jampol and Epstein, 1970; Greenwald, 1971; Biber, 1971; Kristensen, 1972, 1973; Schwartz et al, 1972; Kirschner et al, 1973; Maetz, 1956, 1973; Payan et al, 1975; Glynn and Karlish, 1975; Dharmamba et al, 1975; Towle et al, 1976; Helman and Fisher, 1977; Cala et al, 1978; [illegible] et al, 1978a; 1978b; Cameron, 1978; Busev and Semenkov, 1979; Neufeld et al, 1980; Greenaway, 1981).

The role of kidneys and antennal glands in hyperosmotic regulation has been investigated and reviewed. These organs are primarily responsible for maintaining ECF volume constant against an osmotic influx of water (Smith, 1951; Potts, 1955; Bunger, 1957; Schmidt-Nielsen and Laws, 1963; Kamemoto et al, 1962; Kirschner, 1967; Schmidt-Nielsen et al, 1968; Binns, 1969; Hickman and Trump, 1969; Reigel, 1972; Peterson and Loizzi, 1974).

Literature on the problems encountered by hypo-regulators and the mechanisms involved is very extensive (Smith, 1936; Croghan, 1958a, b, c; Potts and Parry, 1964b; Motais et al, 1966; Potts, Fletcher and Eddy, 1973; Maetz, 1974; Prosser, 1973; Geddes, 1975a, b; Baldwin and Kirchner, 1976).

Several investigators have made extensive studies on the role of salt excreting cells or 'chloride cells' in gills and salt glands and the energetics of sodium and chloride transport in hyporegulators (Philpott and Copeland, 1963; Philpott, 1966; Motais, Garcia Romeu and Maetz, 1966; Copeland, 1967; Epstein et al, 1967, 1973; Kamiya and Utida, 1969; Maetz, 1969, 1973, 1974; Jampol and Epstein, 1970; Kamiya, 1972; Motais and Garcia Romeu, 1972; Potts and Eddy, 1973; House and Maetz, 1974; Kirschner, Greenwald and Sanders, 1974; Kerstetter and Kirschner, 1974; De Renzis, 1975; Maetz and Bornancin, 1975; Potts, 1976; Karnaky et al, 1976; Evans, 1977; Kirschner, 1977).

The field of excretory physiology of hypo-regulators and osmoconformers has received copious attention and was reviewed by Kirschner (1967, 1979), Hickman and Trump (1969), Riegel (1972) and Schmidt-Nielsen (1972). Significant contributions were made in this respect by several investigators (Prosser, Green and Chow, 1955; Parry, 1955; Green and coworkers, 1959; Riegel and Lockwood, 1961; Munz and Mc Farland, 1964; Beyenbach and Kirschner, 1975, 1976; Burger, 1967; Chan, Phillips and Chester-Jones, 1967; Lahlou, 1967; Hickman, 1968a, 1968b; Chester-Jones, Chan and Rankin, 1969; Forster and coworkers, 1972; Payan and Maetz, 1973).

Mechanisms of osmoregulation by 'water intake' through the gut or 'drinking' in hypo- and isosmotic regulators have been investigated by several workers (Croghan, 1958c; House and Green, 1965; Skadhauge, 1969, [illegible]; Shehadeh and Gordon, 1969; Motais and coworkers, 1969; Evans, 1969; Oide, 1970, 1973; Huang and Chen, 1971; Skadhauge and Lotan, 1974; Geddes, 1975b; Ando, Utida and Nagahama, 1975).

All species living in media of changing salinities are necessarily euryhaline at least to some extent. They may have efficient or poor osmoregulatory capacities depending on the salinity range they can withstand. Osmoregulation in aquatic animals has been achieved in two different ways. One way is to keep the osmotic concentration of the body fluids constant irrespective of the salinity changes in the external medium. A second method is to maintain the intracellular fluid concentration in line with changes in extracellular fluid concentration. These mechanisms involve the "anisosmotic regulation of the extracellular fluids" and the "isosmotic regulation of the intracellular fluid" (Florkin, 1962).

Only a few euryhaline forms are homoeosmotic like Palaemonetes varians (Panikkar, 1941) and they are capable of maintaining a fairly constant level of osmotic concentration of their body fluids irrespective of the level of osmoconcentration of the environmental medium. They belong to the category

of "hyper- hypo-osmoregulators" which are able to hyper-regulate their blood osmolarity in lower salinities and hypo-regulate it in higher salinities. This type of regulation is "the most elaborate adaptation to salinity stress and it is the rule in teleost fishes and in higher aquatic euryhaline vertebrates" (cf: Gilles and Jeuniaux, 1979). Some crustaceans also exhibit this mechanism (Panikkar, 1941; Jones, 1941; Croghan, 1958a; Lockwood, 1962; Barnes, 1967; Romero and Zanders, 1980).

A second category of osmoregulators consists of the hyper-osmoregulators which maintain a more or less hyper-osmotic condition of their body fluid in lower salinities but in higher salinities the blood becomes more or less iso-smotic with external medium. Most euryhaline crustaceans are hyper-regulators. Some well known examples are the crabs Carcinus maenas (Schwabe, 1933; Shaw, 1961a), Pachygrapsus crassipes (Rudy, 1966), Cancer magister (Hunter and Rudy, 1975), Potamon niloticus (Shaw, 1959c), Eriocheir sinensis (Shaw, 1961b), Astacus astacus (Shaw, 1959a, 1960; Duchateau and Florkin, 1961). Most of the euryhaline freshwater crabs belong to this type. The freshwater field crab Oziotelphusa (Paratelphusa) senex senex (Fabricius) also comes under this category (Venkata Reddy, 1976). Some of these hyper-regulators extend from sea water, in which they are isosmotic, to fresh-

water wherein they are hyper-regulators. Callinectes sapidus and Eriocheir sinensis are some good examples of this type (Cf: Kirschner, 1979). Some are confined to freshwater having lost the ability to live in sea water. Pseudothelphusa jouyi (Thompson, 1970 cited by Kirschner, 1979) and Oziotelphusa (Paratelphusa) senex senex belong to this type.

Hyper-osmotic regulators show an orderly sequence in their adaptation to freshwater from the sea water and from such a sequence one can visualise the stages by which invasion of fresh waters was achieved by the marine forms (Beadle and Cragg, 1940; Shaw, 1959a, 1959c, 1960, 1961a, 1961b; Shaw and Sutcliffe, 1961; Bryan, 1960a, b, c; Potts and Parry, 1964a, b). A third group of animals includes the osmoconformers which are found in media of fluctuating salinities and exemplified by several marine invertebrate groups and the myxines. They have no power to regulate their blood osmoconcentration but can tolerate fluctuations of blood osmolarity. As a consequence some of these species have evolved populations in estuaries coastal lagoons and other habitats where they encounter large short-term salinity fluctuations. Some typical examples are molluscs like the oysters and mussels and some sediment dwelling polychaetes and star-fishes and some crabs like Maja varrucosa. Osmo-

conformers rely "entirely on their mechanisms of intracellular fluid isosmotic regulation to cope with changes in environmental salinity" (Schwabe, 1933; Robertson, 1957; Staaland, 1970; Pierce, 1971; Bedford, 1971; Gilles, 1972a,b).

From the foregoing account it can be inferred that habitats with fluctuating salinities have been occupied by animals with strong or weak or no powers of osmoregulation of their extracellular fluids. Therefore, it is obvious that the extracellular fluid regulation alone is not a major factor limiting the distribution of animals. Osmoregulation of the intracellular fluid thus appears to be an inseparable part of osmoregulatory mechanisms that enable organisms to invade ecotones of changing salinities (Cf: Gilles and Jeuniaux, 1979).

Mechanisms relating to water movement and cell volume regulation in animals have been investigated and reviewed extensively (Dick, 1979; House, 1974; Hoffmann, 1977; Janáček, 1979; Gilles, 1974a, 1979; Rorive and Gilles, 1979).

Problems concerning cell volume regulation, water and ionic movements in tissues and cells under hypo- and hyperosmotic stress conditions have been studied by several workers (Shaw, 1958a, b; 1959b; Leaf, 1959; Skou, 1965; Whittam, 1969; Fugelli, 1967; Kregenow, 1971a, 1971b; Kleinzeller, 1972;

Hendil and Hoffmann, 1974; Schmidt-Nielsen, 1975, 1977; Sphorer and Civan, 1977; Kevers et al, 1979; Schröck and Hanke, 1979a, b; Ago Hegab and Hanke, 1982).

The role of free amino acids in cell volume regulation has been implicated by Lang and Gainer (1969), Gerard and Gilles (1972a, b), Gerard (1975), Hoffmann and Hendil (1976); Gilles (1973a, 1974a), Schrock and Hanke (1979a,b) and several others.

The invasion of marine forms into brackish and freshwater habitats seems to have become possible because of the ability of cells to tolerate and adapt to a great reduction in the total solute concentration of blood. In Eriocheir sinensis, acclimation from sea water to fresh water brings about a fall in its blood osmoconcentration from 1100 mosmol/l to 550 mosmol/l. If the blood osmolar concentration were to remain at sea water level, the cells would have to withstand an enormous swelling pressure of about 12 atmospheres as per the Vant' Hoff-Arrhenius equation (Gilles, 1974a). Therefore, quick and effective regulation mechanisms must be operating to prevent bursting out of cells. In Eriocheir sinensis the muscle tissue swells initially but this is regulated very rapidly in about 24 hours as understood by changes in water content of the tissue (Gilles, 1974a). Regulation of volume control of cells thus assumes importance in the adaptation

of organisms to hypo- and hyper-osmotic media. In Carcinus maenas regulation of cellular volume in hypo-osmotic medium is achieved after about 70 hours of transfer (Harris, 1976). In the isolated axons of Eriocheir sinensis, this volume regulation is noticed after four hours of hypo-osmotic shock whereas in hyper-osmotic conditions volume regulation does not seem to occur even after 360 hours (Gilles, 1973a). Such observations indicate that volume regulation in animals adapting to hypo-osmotic media seems to be relatively quicker while this is a slow process in hyper-osmotic media (Lang and Gainer, 1969; Gerard and Gilles, 1972b; Gilles, 1973a, 1977, 1978, 1979; Schmidt-Nielsen, 1977). The ability for quicker volume regulation in hypo-osmotic media should have conferred a great advantage on the marine organisms in their invasion of brackish and fresh waters. In this dissertation an attempt is made to understand the time course of volume regulation process on the basis of changes in water content in two tissues of the crab in response to hyper- and hypo-osmotic shocks.

Problems of water influx by osmosis and loss of salts by diffusion faced by freshwater forms must have been partly solved by evasive mechanisms such as a general reduction of permeability of body surface (Schoffeniels and Gilles, 1970) and by a reduction of osmotic gradients between the blood and ambient medium. Beadle and Cragg (1940) postulated that the

blood chloride level is an important factor in the adaptation of animals to freshwater. They pointed out that reduction of blood chloride level confers on animals greater ability to survive in freshwater medium. They also postulated that lower the chloride gradient between blood and tissue, greater would be the adaptability of an organism to freshwater. Several studies suggest that the inorganic ion concentration of the ECF, particularly chloride, seems to trigger metabolic responses which bring about modulations in enzymatic activity related to amino-acid catabolism (Snoke, 1956; Chaplin, Huggins and Munday, 1965; Schoffeniels and Gilles, 1963; Schoffeniels, 1964, 1966; Gilles, 1969, 1974a, c; Gilles, Hogue and Kearney, 1971). The present dissertation also makes a reasonable attempt to correlate the metabolic responses of the crab with sodium and chloride gradients between the blood and medium and to understand the relationship between these inorganic ions and some enzymes of amino acid metabolism.

That increasing salinities bring about elevated levels of intracellular free amino acids and decreasing salinities result in depressed levels is well understood. The intracellular amino-acid content is higher in marine animals than in fresh water forms. In the crabs like Eriocheir sinensis, Callinectes sapidus acclimated to sea water, the intracellular amino acid content accounts for nearly 70% of the total osmolarity of the tissues. The remaining could be accounted for

mostly by the inorganic ions Na^+, K^+ and Cl^- (Gilles, 1974a, 1979). When the marine animals are transferred to fresh water, the intracellular concentration of inorganic ions, except chloride, falls only slightly but there is a greater decrease in amino acid content which accounts for most of the decrease in total osmolarity of the intracellular fluid. Literature on the role of amino acids as intracellular organic osmotic effectors in invertebrates and fishes is very extensive (Duchateu and Florkin, 1961; Schoffeniels, 1960, 1976; Florkin, 1962; Bricteux-Gregoire and coworkers, 1962; Florkin and coworkers, 1964; Huggins and Munday, 1968; Gilles and Schoffeniels, 1969a; Florkin and Schoffeniels, 1969; Schoffeniels and Gilles, 1970, 1972; Pierce and Greenberg, 1970; Cholette, Cagnon and Germain, 1970; Vincent-Marique and Gilles, 1970a, b; Chaplin and coworkers, 1970; Huggins and Codley, 1971; Lasserre and Gilles, 1971; Siebers, 1972; Gerard and Gilles, 1972a,b; Gilles, 1972c, 1973a, 1974a, 1975, 1979; Venkatachari, 1974; Gilles and Gerard, 1974; Hendil and Hoffmann, 1974; Hoffmann and Hendil, 1976; Tan and Choong, 1981).

The role of free amino acids seems to be mainly to exclude the unlimited entry of inorganic ions into the cells under hyper-osmotic conditions and to regulate cell volume. Higher concentration of inorganic ions seem to be injurious to cell metabolism. Therefore, the amino acids play the role

of osmotic effectors inside cells and check the excessive entry of inorganic ions. While this aspect is well understood, the mechanisms involved in the modulations of free amino acid content of cells are not properly understood. There appear to be three possibilities. One possibility is the influx and efflux of amino acids into and outside cells. Under hyper-osmotic conditions active uptake into cells of amino acids from the extracellular fluid has been postulated. Under hypo-osmotic stress conditions, the free efflux of amino acids from cells into the body fluid has been implicated (Fugelli, 1967; Gilles and Schoffeniels, 1969a; Lang and Gainer, 1969; Pierce and Greenberg, 1970; Gerard and Gilles, 1972b; Hendil and Hoffmann, 1974; Gerard, 1975; Pequeux et al, 1979). Such fluxes are supposed to be related to changes in the permeability properties of cell membranes and consequent changes in cell volume regulation process (Jonsson, 1971; Gerard and Gilles, 1972b). A second possible mechanism seems to involve alterations in the equilibrium between proteins and amino-acids. Evidence in support of this view is very scanty (Bedford, 1971; Venkatachari and Vasantha, 1973; Venkatachari, 1974). A third plausible mechanism to account for modulations in intracellular amino acid content under salinity stress seems to involve changes in the equilibrium between amino acid synthesis and degradation

(Schoffeniels and Gilles, 1970). Synthesis of amino acids under hyper-osmotic conditions is not likely as experiments involving measurement of specific activity of the amino-acids labelled from radio-active glucose and pyruvate do not strengthen such a view (Gilles, 1974a, 1979). Further, the increase in free amino acid content of cells under hyper-osmotic stress involves not only the non-essential amino acids but also some of the essential ones (Gilles, 1974a, 1977).

Therefore, an explanation for the changes in intra-cellular amino acid content observed under salinity stress should be sought for in modulations in the catabolism of amino acids. Increased catabolism of intracellular amino acids in animals adapting to hypo-osmotic media and decreased catabolism of amino acids in hyper-osmotic media have been implicated as the possible mechanisms for decreased amino acid levels in diluted media and increased amino acid levels in concentrated media (Chaplin, Huggins and Munday, 1970; Gilles and Jobsis, 1972; Siebers et al, 1972; Gilles, 1974a, 1975, 1979; Wickes and Morgan, 1976; Schoffeniels, 1976). The biochemical mechanisms implicated in the modulations of the intracellular amino acid concentration and the enzymatic patterns in relation to amino acid catabolism are far from clear. Florkin and his coworkers have suggested that the

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differences between various types of osmoregulators "must be searched for in the way the reducing equivalents are geared towards amino acid or energy metabolism" (Schoffeniels and Gilles, 1970). Some of the enzyme patterns studied in relation to amino acid metabolism will be considered separately (Chapter IV). These patterns have been reviewed by Schoffeniels and Gilles (1970), Gilles (1974a, 1975) and Schoffeniels (1976). The activity of all the oxidative enzymes studied so far, including GDH seems to decrease under increasing concentrations of NaCl in the incubation medium, suggesting a decreased activity of the Krebs cycle. In hypo-osmotic media, the activity of the Krebs cycle enzymes appears to increase. Such observations are in agreement with lowered oxygen consumption in animals adapting to higher salinities and higher oxygen consumption measured in animals acclimating to lower salinities (Schlieper, 1929; Beadle, 1931; Fox and Simmonds, 1933; Lowenstein, 1935; Gopalakrishnan, 1953; Lee and Pyung, 1970; Venkata Reddy, 1976). However, the variations observed in oxygen consumption of animals exposed to osmotic stress seem to be a subject of controversy (Ramamurthi, 1962, 1965, 1967b; Gilles, 1973b, 1974a; Shumway and Jones, 1981; Linton, Davies and Wrona, 1982). Therefore, this aspect of energy metabolism has been investigated in the present work, using a new technique for the continuous measurement of oxygen consumption in the crab keeping in view of the time course of metabolic response.

MATERIALS AND METHODS

Biology of Oziotelphusa (Paratelphusa) senex senex (FABRICIUS)

Oziotelphusa is a freshwater field crab commonly available in the paddy fields and canals around Tirupati and many districts in Andhra Pradesh. It makes burrows in soft mud along the edges of paddy fields and canals and lives in them partially filled with water. In a sense it can be described as semi-terrestrial as it lives partially immersed in water and can live for longer periods on land. It is carnivorous, feeding on worms, insects etc. It is also a cannibal feeding on younger crabs. It is not found either in brackish water or sea water and hence is a strict inhabitant of freshwater and yet it has the unique capacity to tolerate even direct transfer to 100% sea water. Interestingly Oziotelphusa (Paratelphusa) is eurythermal (Pampapathi Rao and Venkata Reddy, 1962) as it is euryhaline (Ramamurthi, 1962, 1965; Venkata Reddy, 1976).

Maintenance of crabs

When the crabs were brought to the laboratory, they were kept in large glass or polythene troughs partially immersed in freshwater (tap water). They were exposed to the natural

photoperiod. The water temperature was 26 ± 1°C in winter and 31 ± 1°C in summer. They were fed with earthworms, their natural food, ad libitum. Water in the troughs was replaced daily with freshwater. The crabs were maintained thus for atleast one week to obviate the effects of environmental changes. During this period nearly 50% of crabs do not survive. Once they survive the effects of change from the field conditions to laboratory conditions, generally there will be no further casualities. Crabs selected for experiments were generally of the size range from 20 - 40 grams. Both the sexes were used in equal numbers to avoid the influence of sex. Only animals in the intermolt stage were used. Gravid or egg-carrying females were not used. Injured crabs were avoided.

Method of acclimation

Natural sea water (S.W.) brought from the Madras coast was used as the acclimation medium. The salinity of the sea water was 33 - 34.5%.. Further dilution of sea water was made with distilled water. Only three different concentrations were used for the experiments viz., 100% S.W., 50% S.W. and freshwater (F.W.) (Natural medium). The crabs kept in freshwater served as controls. During the course of acclimation, the animals were transferred directly to 50% and

100% SW contained in glass troughs (size 4" x 6"). Only one crab was kept in each trough. Before transfer to the acclimation medium each crab was weighed and numbered. The crabs withstand direct transfer to 50% and 100% SW. Survival in 50% SW was assured for any number of days with a low mortality rate (about 10%) but survival in 100% SW. for longer periods was difficult. The mortality rate was around 50% by about the 3rd day of acclimation and thereupon the mortality rate decreased. The locomotor ability decreased and the crabs were paralysed in 100% SW by about the 3rd day, and there upon they were reluctant to feed and invariably stopped feeding. A period of 15 days was felt sufficient for acclimation to SW (Venkata Reddy, 1976). Similar durations for attainment of steady state have been reported for other crabs (Madan Mohan Rao et al, 1969; Venkatachari and Vasantha, 1973). Change in the pattern of excretion in this crab (Switch over to ureotelism from ammonotelism) occurred on the eleventh day (Krishnamoorthy and Srihari, 1973). So it is felt that 15 days of sojourn in a changed acclimation medium is sufficient for this crab to attain steady state conditions. During the acclimation period also, the animals were fed with earthworms once in 24 hours. They were starved for one day before measurement of oxygen consumption or any biochemical analysis was carried out to obviate any possible

variations due to differential feeding. The water in each glass trough was replaced daily.

Set up to measure the time course of oxygen consumption

Oxygen consumption was measured by the "continuous flow method" using the set up shown in Fig. 1 (improvised by the present investigator). The set up consists of a respiratory chamber described by Saroja (1959), an aspirator bottle of 10 litre capacity and a huge carboy of about 100 litre capacity that functionsas a reservoir. The set up is so arranged that water flows at a constant rate from the reservoir to the aspirator bottle and from the aspirator bottle to the respiratory chamber and then out side through polythene delivery tubes. The respiratory chamber is a wide mouth bottle fitted with a four-holed rubber cork. Through one hole of the rubber cork passes a glass tube that is connected to a delivery tube (No.D1) for the incoming water from the aspirator bottle. The lower end of the glass tube is just below the cork. Through the other hole passes a glass tube connected to a delivery tube (No.D2) for the out-going water. The lower end of this glass tube extends upto the bottom of the respiration chamber so that the incoming water does not pass out directly without being used by the animal kept in the chamber. A short glass tube passes through a third hole and its lower end is flush with the

FIG. 1: Assembly for the measurement of oxygen consumption by the "continuous flow method".

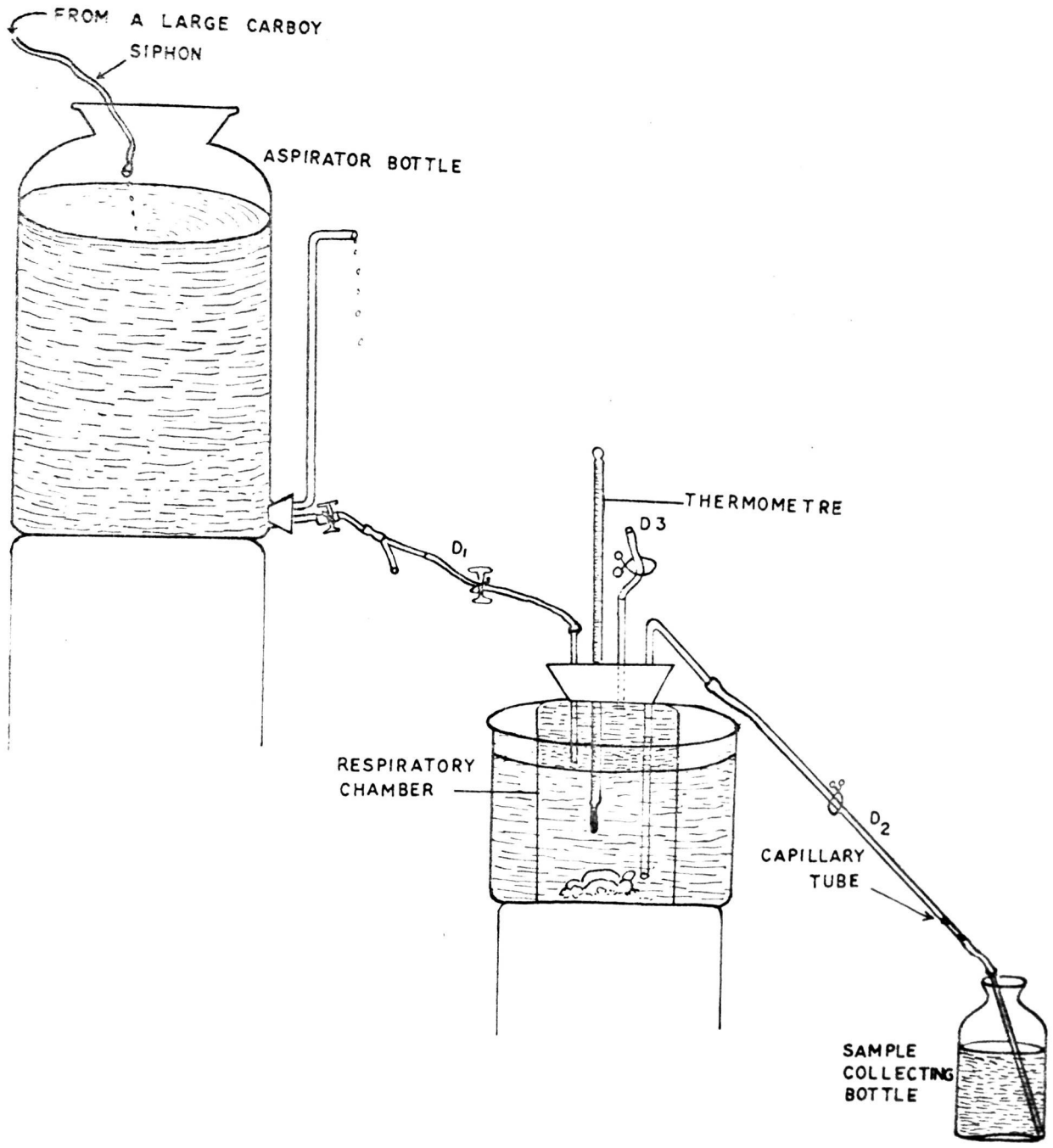


FIG. 1. RESPIRATION SET UP

lower surface of the rubber cork whereas the upper end is connected to a short rubber tube (No.3) to which a pinch cock is applied. This serves to check up the airtightness of the respiratory chamber and also to remove any air bubbles that may get into the chamber in the course of experimentation. Through the fourth hole of the rubber cork passes a thermometer. 4 respiratory chambers of the type described were used simultaneously to measure the oxygen consumption of 4 different crabs. All the chambers were coated with black paint in order to avoid changes in activity of the animal due to illumination. The respiratory chambers are placed in a glass trough containing water to reduce fluctuations in temperature.

Delivery tube No. 1 is connected to the outlet of the aspirator bottle placed at a higher level than that of the respiration chambers. This outlet is fitted with a two holed rubber cork and through one hole passes a short glass tube and through the other hole passes a twice bent glass tube as shown in Fig. 1. The short glass tube is connected to a short polythene tube to which a screw cock is applied. This polythene tube is inturn connected to 4 delivery tubes(D_1) of 4 different respiration chambers by using three 'Y' tubes as shown in the figure. (Fig. 1)

All the delivery tubes are polythene tubes. Rubber tubing is avoided to prevent diffusion of air. The twice bent glass tube is upturned and stands at a certain height below the mouth of the aspirator bottle. This is meant to maintain a constant level of water in the aspirator bottle by adjusting the flow between the reservoir and aspirator bottle in such a way that some excess water drips out at the upturned end. Constant level of water in the aspirator bottle ensures constant rate of flow. The reservoir is placed at a level higher than that of the aspirator bottle, and is connected to the aspirator bottle by a siphon. The required rate of flow of water is achieved by inserting one short glass capillary tube of appropriate bore into the delivery tube (No. 2) meant for the out-flowing water. Application of screw cocks to control the flow between the aspirator bottle and respiration chamber was found to be inconvenient as occasionally the flow was retarded or even stopped by the accumulation of fine air bubbles in the delivery tubes below the screw-cocks. Hence insertion of a capillary tube into the delivery tube carrying the out-flowing water was found to be very convenient to maintain a constant flow of water. After the insertion of capillary tube completely into delivery tube No. 2 the tip of the delivery tube is provided with a glass tube having a well drawn out nozzle.

Procedure

The reservoir is filled with the experimental medium. The same medium is taken into the aspirator bottle after closing the outlet with the screw cock. The water level in the aspirator bottle is so adjusted that some excess water drips out of the upturned delivery tube. The respiration chambers are also filled with the same experimental medium. Previously weighed and numbered crabs are introduced each into a respiration chamber and the mouths are tightly closed with the rubber corks. The time is noted. Now the screw cock on the outlet of the aspirator bottle is opened to allow a free flow of water from the aspirator bottle to the respiration chambers. This sets up a steady flow of water from the respiration chamber to the outside. Now the pinch cock applied on the short delivery tube (Tube No. 3) is opened and water from the respiration chamber is allowed to overflow through it, after stopping for a while the flow through the delivery tube No. 2. This removes air bubbles, if any, inside the respiration chamber. Then the pinch cock is applied again on delivery tube No. 3. The tips of all the delivery tubes (No. 2) carrying the out-flowing samples are placed in a large glass trough or any other container kept at appropriate height and the outflowing water samples are collected at planned intervals in 4 narrow mouth reagent bottles and the samples so collected

are used for the estimation of oxygen content.

Collection of samples and estimation of oxygen consumption

Sample collecting bottles of 65 ml were used. The rate of water flow was maintained around 1000 ml/hour. However, this rate was measured every time when the outflowing sample was collected. Slight fluctuations in the rate of water flow would still be there and these variations were measured each time and incorporated into the calculations. Dissolved oxygen content of the water samples was estimated by the standard Winkler's iodometric method as described by Welsh and Smith (1960). A sample of water from the aspirator bottle (2nd reservoir) was collected using a siphon, sometime before the collection of each outflowing sample. The difference in the oxygen content of the reservoir sample and the oxygen content of the outflowing sample gives the amount of oxygen consumed by the animal per hour when the rate of flow is taken into account.

Supposing the rate of flow is 1100 ml/hour, the standard equation is modified thus:

$$O_2 \text{ consumption (in ml/hour)} = \frac{K \times 200 \times 0.698}{\text{Vol. of sample}} \times \text{Difference between the volume of hypo } (0.025N.\ Na_2S_2O_3) \text{ run down for the reservoir sample and for the outflowing sample} \times \frac{1100}{1000}$$

As it is known that metabolic responses to salinity stress are influenced by other environmental factors especially temperature, sufficient care was taken to carry out these experiments at constant temperature (26 ± 1°C). In order to ensure a constant oxygen pressure, an aerator was introduced into the aspirator bottle and water was aerated whenever the pressure of oxygen was too low.

Dissolution of atmospheric oxygen into the samples of water during collection was prevented by introducing a few drops of paraffin oil into the sample bottles which floats as a thin layer on water.

This method has the following advantages over the one described by Saroja (1959):

1. This enables us to collect outflowing samples at short intervals over long periods of time, without dismantling the set up and distrubing the animals inside the respiration chambers.

2. This ensures a continuous supply of oxygen to the animal preventing hypoxia.

3. It also ensures continuous removal of metabolic wastes like CO_2 and NH_3 from the respiration chambers, thereby, avoiding their toxic effects on the animal.

4. These metabolic wastes in the outflowing samples can also be estimated quantitatively on the time scale of the experiment.

Procedure for the estimation of ions

Ions in blood

Sodium

Blood was drawn from crabs by inserting a hypodermic syringe through an arthrodial membrane of chelate leg. It was immediately treated with 10% trichloroacetic acid (10% W/V) to precipitate the proteins. 0.2 ml of blood was treated with 5 ml of trichloroacetic acid. The samples were filtered after 10 minutes through Whatman No. 1 filter paper. After making appropriate dilutions with glass distilled water sodium was determined by Flame Photometer (Elico Flame Photometer).

Chloride

Chloride was determined according to the method of Schales and Schales (1941). The mixture contained 0.2 ml of blood, 3.5 ml of distilled water, 0.5 ml of 0.36 molar H_2SO_4 and 0.5 ml of sodium tungstate reagent. After mixing well, this was allowed to stand for 5 min and centrifuged at 3000 rpm. The supernatant was used for titration. The

tungstic acid protein free filtrate was titrated with mercuric nitrate solution in the presence of diphenyl carbozone as the indicator.

Ions in tissues

Sodium

The crabs were dissected open and the hepatopancreas was collected. The muscle tissue was obtained by cutting the chelate legs and removing the muscle with the help of a forceps. The tissues were blotted and weighed in an electrical balance to the nearest milligram. The weighed tissue was wet ashed in 1:1 (V/V) concentrated perchloric acid: concentrated nitric acid (Dall, 1967). For the estimation of sodium ion, 100 mg of each tissue was dissolved in 10 ml of the said acid mixture. After keeping the tissue in the 'wet-ashing' solution for one hour or until the tissue was completely dissolved, the wet-ash solution was evaporated on a sand bath at 60-70°C. The residue was dissolved in a few ml of glass distilled water to obtain 10% W/V solution and filtered through Whatman No. 1 filter paper. Further appropriate dilutions were made prior to estimation of sodium by Flame photometry.

Chloride

Chloride was determined according to the method of Schales and Schales (1941). In this method a tungstic acid protein free filtrate of 4% (W/V) tissue homogenate was

titrated with mercuric nitrate solution in the presence of diphenyl carbozone as the indicator.

Water content in tissues

The water content of the tissue was determined from the difference in weight before and after drying the tissue at 110°C for 24 hours.

Calculation of osmotic effectors for tissue water

The osmotic effectors in the case of tissues were first determined for unit weight of wet tissue. These values were recalculated per kg tissue water taking into account the levels of hydration of tissue thus:

$$\frac{\text{Concentration of ions in m moles/Kg wet tissue}}{\text{Water content in grams per Kg wet tissue}} \times 1000$$

= mosmols/Kg. tissue water.

Correction of osmotic effector concentration of tissues for the ECF volume of the tissue

Since the tissue contains blood in the extracellular spaces, the ion concentration/Kg tissue water includes ions of the blood. Hence, the ion concentration of blood in the tissue space should be subtracted from the ion concentration

of tissue water so as to arrive at the levels of ions in intracellular water. The volume of extracellular spaces (intercellular spaces) within the tissue is not known. No work has been done in this direction by the previous investigators. Venkata Reddy (1976) has assumed an arbitrary value of 20% as the volume of extracellular spaces within the hepatopancreas and muscle. This volume, although not very precise, serves the purpose to some extent. It is rather difficult to measure accurately the exact volume of the ECF of the tissues in invertebrates (Martin et al, 1958). Such techniques as have been reported by Schmidt-Nielsen (1975) and Schrock and Hanke (1979a) for the determination of ECF volume have not been perfected in our laboratories. Therefore, the values reported by other workers are being adopted. Lang and Gainer (1969) have reported an extracellular space of 22% for the muscle of Callinectes Sapidus. Keynes and Lewis (1956) have reported an ECF volume of 25% for the nervous tissue of the same crab. Hence the assumption of Venkata Reddy (1976) that the crab Oziotelphusa (Paratelphusa) sp. has 20% ECF volume in its hepatopancreas and muscle may be nearer to an approximation and the same has been adopted in this work for calculation of the intracellular concentration of ions and TNPS. The method used for this

this calculation is to deduct 20% ion or TNPS concentration per litre of blood from the ion concentration per Kg tissue water thus:

Intracellular ion concentration in mosmols/Kg cell water	=	Ion concentration per Kg tissue water	—	Ion conc. in 200 ml of blood

This method is a further simplification of the method followed by Venkata Reddy (1976) and both the methods yield almost the same values. It is worthwhile to know the precise volume of ECF inside the tissue but suitable experimental techniques adaptable in our laboratory are not forthcoming. Hence the data presented on intracellular ion composition (tables 12, 15) are only nearer to approximation. The same method was followed for the calculation of the intracellular amino acid content (table 19).

Statistical treatment

Data collected were treated according to standard statistical methods (Pillai and Sinha, 1968) as followed by Raghavaiah (1977). The individual values were subjected to analysis of variance ratios as several samples are involved. The calculated 'F' values were compared with table values at 5% significance level. Significant differences between

the values were determined by the 'critical difference' method or the 'least significant difference method' at 5%, 1% and 0.1% significance levels and the values were tabulated. For statistical comparison, the mean values were arranged only in the ascending order for uniformity and those mean values that were not significantly different at 5% level were grouped together by a common underline as was followed by Raghavaiah (1977) and Purushotham (1979).

Procedure for the estimation of total ninhydrin positive substances (TNPS)

Total ninhydrin positive substances or total free amino-acid (FAA) levels were estimated by the method of Moore and Stein (1954) as given by Colowick and Kaplan (1957) 5% tissue homogenates (W/V) were prepared in 10% trichloroacetic acid and centrifuged at 2000 rpm for 10 minutes. 0.5 ml of the above supernatant was taken into another clean test tube and 2 ml of ninhydrin reagent was added to it and was kept in boiling water bath for exactly 6.5 minutes and was cooled immediately. The solution was then made upto 10 ml with distilled water and the bluish pink colour was read at 570 m μ in spectrophotometer (Elico-Hyd., India). Standard graph was prepared with tyrosine.

The free amino-acid content of tissue was expressed as m. moles of tyrosine per kg tissue wet weight. This value was corrected for ECF volume of the tissue. 0.2 ml of blood was treated with 5 ml of 10% trichloroacetic acid and the mixture was centrifuged for 10 minutes at 2000 rpm. 0.5 ml of the supernatant was used for the estimation of TNPS as described above. The values were expressed as m. moles of tyrosine per litre of blood.

Enzyme Assays

Aspartate aminotransferase (AAT) (L-aspartate : 2 - Oxoglutarate aminotransferase : E.C. 2.6.1.1)

AAT activity was assayed by the method of Reitman and Fraenkel (1957) as described by Bergmeyer (1965). The incubation mixture in a final volume of 1.5 ml contained 100 μ M of potassium phosphate buffer (pH 7.4), 50 μ M of L-aspartic acid (pH 7.4) and 2 μ M of α - ketoglutaric acid (pH 7.4) and 0.5 ml of freshly prepared 3% (W/V) tissue homogenate in ice cold 0.25 M sucrose solution. After incubation for 1 hour at 37°C the reaction was stopped by the addition of 1 ml of 2,4- dinitrophenyl-hydrazine solution (0.001 M in 1 N HCl) and allowed to stand at room temperature for 30 minutes. Zero time controls were maintained for all

the samples by the addition of 2,4-dinitrophenyl-hydrazine solution prior to the addition of the homogenate. Colour was developed by the addition of 10 ml of sodium hydroxide (0.4 N) to all the test tubes, and it was read at 545 m μ in spectrophotometer (Elico-Hyd., India). The enzyme activity was expressed as u M of pyruvate formed per gram protein per hour.

Alanine aminotransferase (AlAT) (DL - alanine: 2 - Oxoglutarate aminotransferase : E.C. 2.6.1.2)

AlAT activity was determined by the method of Reitman and Fraenkel (1957) as given by Bergmeyer (1965). The incubation mixture in a final volume of 1.5 ml contained 100 u M of phosphate buffer (pH 7.4), 40 μ M of DL-alaline (pH 7.4), 2 μ M of α - ketoglutarate (pH 7.4) and 0.5 ml of 3% (W/V) tissue homogenate freshly prepared in ice cold 0.25 M sucrose solution. After incubation for 30 minutes at 37°C, the enzyme reaction was stopped by the addition of 1 ml of 2,4-dinitrophenyl hydrazine solution (0.001 M in 1 N HCI) and allowed to stand for 30 minutes at room temperature. Zero time controls were prepared for all the samples by the addition of 2,4-dinitrophenyl-hydrazine solution prior to the addition of the homogenate. Then the colour was developed by the addition of 10 ml of sodium hydroxide (0.4 N) and the same

was read at 545 m μ in the Spectrophotometer (Elico). The enzyme activity was expressed as μ M of pyruvate per gram protein per hour.

Glutamate dehydrogenase (GDH: Glutamate: NAD: Oxidoreductase, E.C.1.4.1.3)

GDH activity levels in muscle and hepatopancreas were determined using INT (2-para-iodopheny 1; 3-para-nitrophenyl; 5 phenyl tetrazolium chloride) as the terminal electron acceptor (Lee and Lardy, 1965). The incubation mixture in a final volume of 2.0 ml consisted of 40 μ M of sodium glutamate, 100 u M of phosphate buffer (pH 7.4), 0.1 μ M of NAD, 4.0 μ M of INT and 0.6 ml of 10% homogenate. After incubation for 30 minutes at 37°C the reaction was stopped by the addition of 6 ml of glacial acetic acid and the formazan formed due to reduction of the dye was extracted overnight into 6 ml of toluene at 5°C. The colour was read at 495 m μ in a Spectrophotometer (Elico). Zero time controls were maintained for all the samples by the addition of glacial acetic acid to the reaction mixture before incubation. The concentration of formazan equivalent to the optical densities were determined from standard graph. The enzyme activity was expressed in μ M formazan/gram protein/hour.

CHAPTER I

OXYGEN CONSUMPTION

Introduction

There have been several reviews on the metabolic response of aquatic animals to osmotic stress with particular reference to Crustacea (Remane and Schlieper, 1958, Shaw, 1960b; Florkin, 1960; Potts and Parry, 1964b; Potts, 1955, 1976; Kinne, 1964, 1971; Schoffeniels and Gilles, 1970, 1972; Gilles, 1973b, 1974a, 1975). Salinity stress brings about altered osmotic and ionic balance in animals coupled with metabolic adjustments (Beadle and Cragg, 1940; Beadle, 1957; Prosser and Brown, 1961; Prosser, 1973; Gross, 1964; Shaw, 1960b; Lockwood, 1962; Florkin, 1963; Potts and Parry, 1964b; Schoffeniels, 1976; Gilles, 1974a, 1979; Kirschner, 1979).

Eversince Schlieper (1929) showed that changes in the salinity of the medium influenced the oxygen consumption in Carcinus, there has been a vast accumulation of literature on the energy metabolism of animals in response to salinity stress. Several workers have reported that the oxygen consumption is minimal in isosmotic media and increases with increasing hyper- and hypo-osmotic media (Schwabe, 1933; Krogh, 1939; Flemister and Flemister, 1951; Lofts, 1956; Gross, 1957a; Pampapathi Rao, 1958; Dehnel, 1960; Madanamohan

Rao and Pampapathi Rao, 1962; Ramamurthi, 1965, 1967b; Shumway and Jones, 1981; Venkatachari and Vasantha, 1981). However, Fox and Simmonds (1933) showed in several species of gammarids and isopods "that the freshwater animals have higher rates of oxygen consumption than the marine ones". Since then several investigators have demonstrated that the oxygen consumption of aquatic animals is higher in lower salinities and lower in higher salinities(Lowenstein, 1935; Bock and Schlieper, 1953; Eliassen, 1953; Gopalakrishnan, 1953; Lee and Pyung, 1970; Siebers and coworkers, 1972; Gilles, 1973b, 1974a, 1975, 1979; Venkata Reddy, 1976). Some investigators measured no change in oxygen consumption of certain species when exposed to higher or lower salinities (Schwabe, 1933; Gilchrist, 1956; Bielawski, 1960; Mc Farland and Pickens, 1965; Linton, Davies and Wrona, 1982). For a very long time it was argued that metabolic energy might not be utilised for osmotic work. The increased oxygen consumption under salinity stress was attributed to "escape reactions" (Krogh, 1939) and the consequent increase in locomotor activity (Gross, 1957a,b). There is no change in oxygen consumption of the curyhaline crab, Eriocheir (Schwabe, 1933) and the freshwater gastropod Theodoxus (Bielawski, 1960) when exposed to heterosmotic media. Potts (1954) concluded that Eriocheir uses a negligible part of its

metabolic energy for the osmotic work. However, it has been shown in the cray fish (Schwabe, 1933), Palaemonetes varians (Lofts, 1956), Metapenaeus monoceros (Pampapathi Rao, 1958), Sesarma Plicatum (Pampapathi Rao and Madanamohan Rao, 1963) Paratelphusa, Hirudinaria, Pila and Lamallidens sp. (Ramamurthi, 1965) and Barytelphusa guerini (Venkatachari and Vasantha, 1981) that the oxygen consumption in various salinities is a function of the osmotic gradient between the blood and the medium. Greater the osmotic gradient, higher is the oxygen consumption, lesser the gradient, lower is the oxygen consumption.

Metabolic responses of the crab Oziotelphusa (Paratelphusa) senex senex when exposed to sudden changes in salinities (15 minutes to 1 hour after exposure) have been studied (Ramamurthi, 1967b). But a complete picture of the time course of metabolic response to salinity stress, when this fresh-water field crab is directly exposed to 50% sea water and 100% sea water and then reversed to freshwater, is not available. The present work makes an attempt at filling up this lacuna. Stress is laid on the first stages of acclimation and reverse acclimation processes but without losing sight of the middle and end stages of adaptation. This provides an entire sequence of the mechanisms involved in the acclimation of this crab to hyper-osmotic (100% SW) isosmotic (50% SW) and hypo-osmotic media (FW).

Oxygen consumption in relation to sex and size

Using the 'continuous flow' method of estimation of oxygen consumption described earlier, some preliminary data on oxygen consumption of the crabs in relation to sex and size were collected. Oxygen consumption of each crab was measured 6 times in a day and the mean value for each crab was taken into account.

Results and Discussion

The weight specific metabolic rate (O_2 µl/g/h) for different sized crabs and for both sexes are shown in table 1. The weight regression curves fitted by the method of least squares are given in fig. 2.

The regression coefficients ('b' values) for the two variables, viz., weight and metabolic rate are reported along with the curves. It is clear that the rate of oxygen consumption was higher in males than in females. This agrees with the findings reported earlier (Ramamurthi and Sainath Janak, 1973). This difference was kept up throughout the size range. The greater oxygen consumption in males has been attributed to greater activity of vital processes in males (Prosser et al, 1950). In several

FIG. 2: Weight-metabolism regression lines in the crabs under normal laboratory conditions.

Males:

'b' value = -0.6898

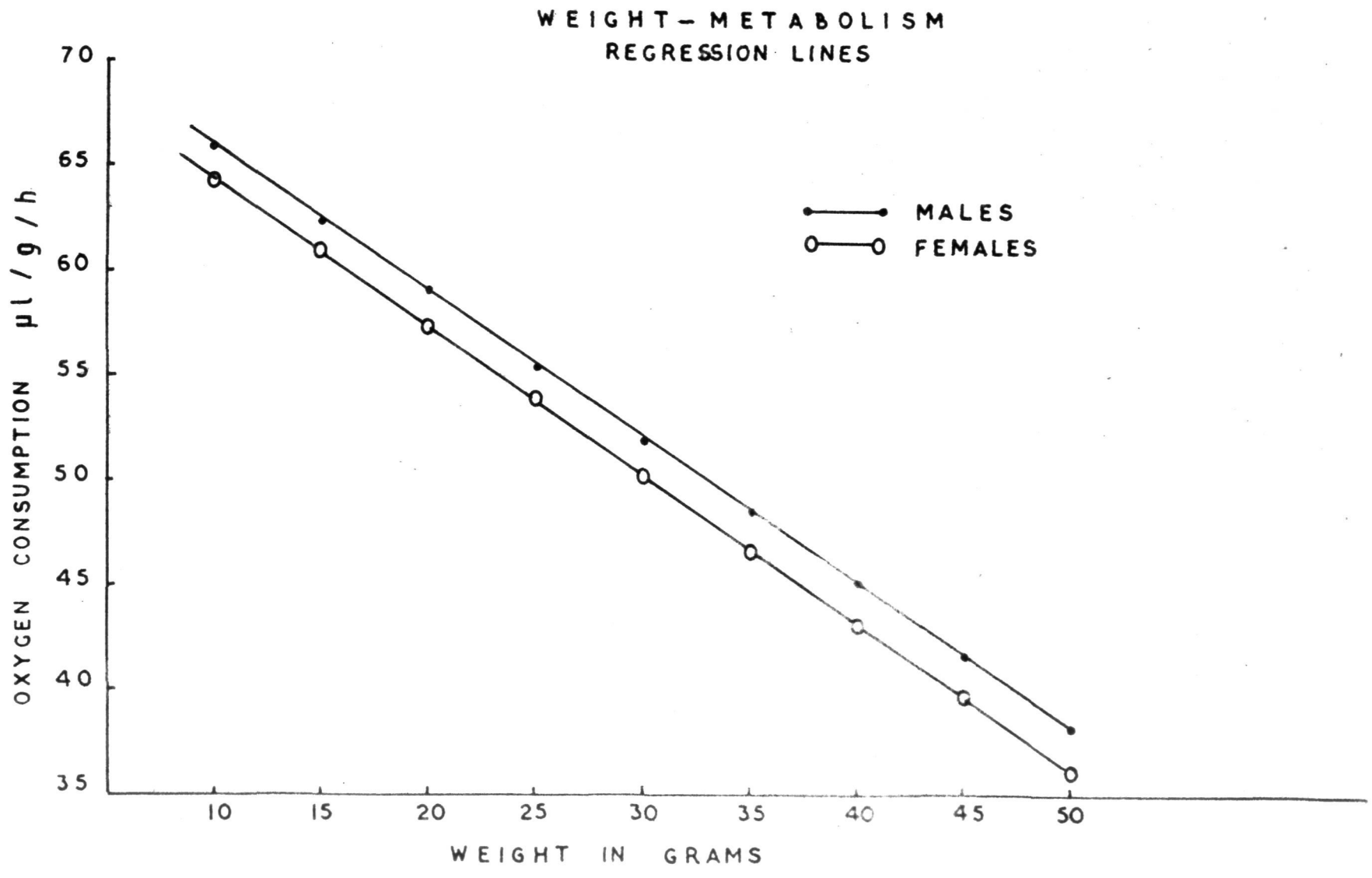
Regression equation = y = 72.7691-0.6898x

Females:

'b' value = -0.7036

Regression equation = y = 71.4147-0.7036x

FIG. 2


WEIGHT – METABOLISM
REGRESSION LINES
MALES
FEMALES
OXYGEN CONSUMPTION µl/g/h
70
65
60
55
50
45
40
35
10
15
20
25
30
35
40
45
50
WEIGHT IN GRAMS

decapod crustacea males tend to grow at a greater rate than the females (Orton, 1936). From this it is evident that at any given weight males would be younger than females. The relationship between body size and metabolism was reviewed exhaustively by Zeuthen (1953) and Bertalanffy (1951, 1953, 1957). The 'b' values were close to 2/3 rule indicating relationship between surface and metabolic rate. In the present work the males showed a 'b' value of -0.69 and the females showed a 'b' value of -0.70. These values are closer to 2/3 value, and as such agree with the surface rule. The unit oxygen consumption (O_2 ul/g/h) of smaller crabs was found to be greater than that of larger animals. The active growth processes may be responsible for the greater oxygen consumption of smaller animals (Loc. cit). Apparently, the growth process in larger crabs is not as active as it is in smaller crabs.

Diurnal rhythms

Method

Using the set up described earlier, the oxygen consumption of summer and winter crabs was studied at intervals of 2 hours in order to understand diurnal rhythms of oxygen consumption. Crabs were introduced in respiration chambers at 6.00 A.M. and samples of the outgoing water were collected once every 2 hours. This was continued till 6.00 A.M. the next day without disturbing the crabs in the chambers. A continuous and steady flow of water through the respiration chambers was ensured throughout the course of the experiment. The samples of water were fixed immediately after collection and titration work was done during day time.

Results and Discussion

The data on diurnal variations in oxygen consumption of crabs during summer months are shown in table 2, and the mean values are plotted in fig. 3. Data on diurnal variations for crabs during winter are shown in table 3, and the mean values are plotted in fig. 4. The data were subjected to statistical analysis of variance ratios (Pillai and Sinha, 1968). Variance ratios for data given in table 2 are shown in table 2-1 and the statistical

FIGS. 3 & 4: Diurnal rhythms of oxygen consumption in the crabs under normal laboratory conditions.

1. Females
2. Males
3. Males & Females

FIG. 3 RHYTHMS (SUMMER)

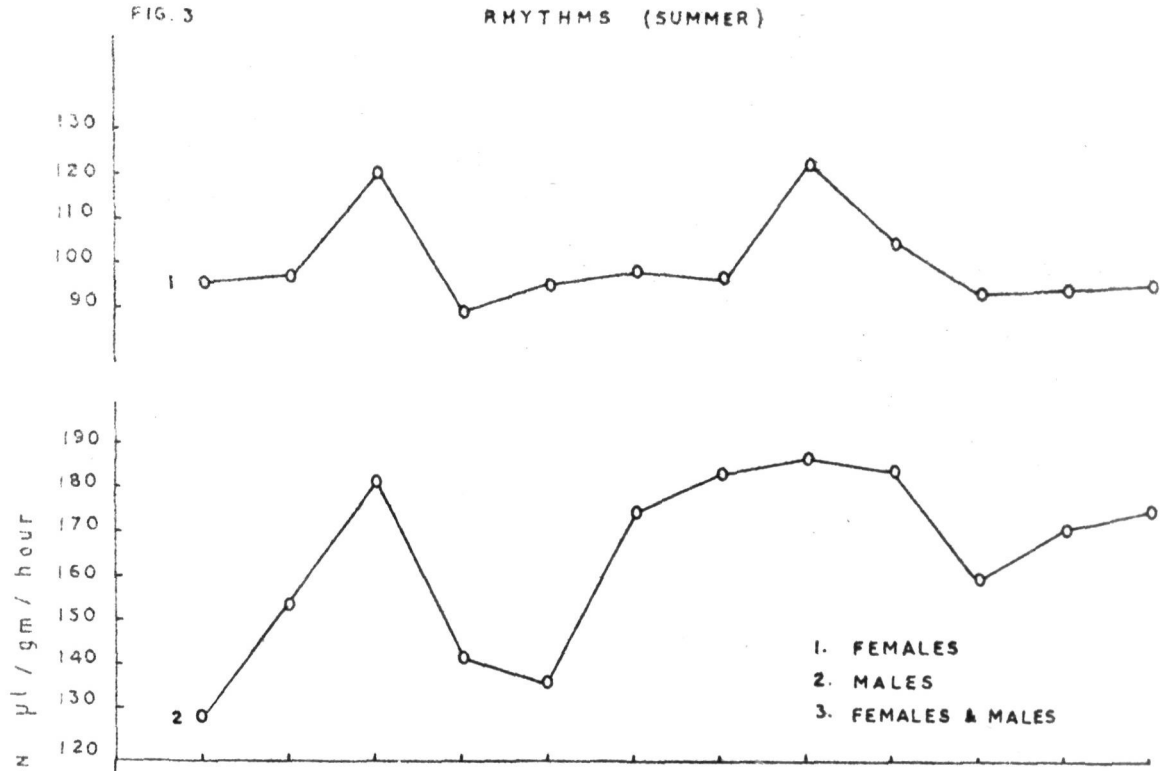
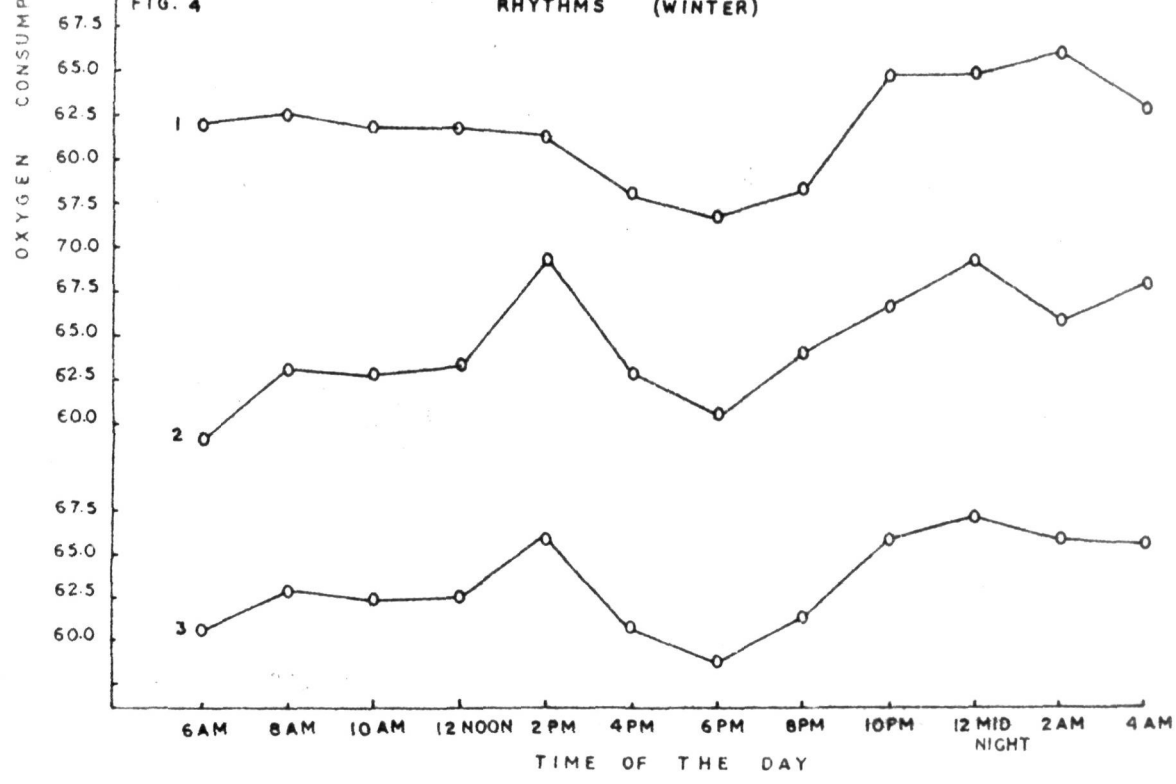


FIG. 4 RHYTHMS (WINTER)



comparison of the data is presented in table 2-2. Similarly, variance ratios for the data given in table 3 are shown in table 3-1 and statistical comparison of the corresponding data is presented in table 3-3. The values for summer and winter animals are plotted in figs. 3 & 4.

Diurnal rhythms of various physiological activities like colour change (Brown et al, 1953; Fingerman, 1955; Sheer, 1960) oxygen consumption (Brown et al, 1954; Ramamurthi and Sainath Janak, 1973) blood glucose level (Dean and Vernberg, 1965) and walking activity (Valente and Edwards, 1965) have been reported to occur incrustaceans. Ramamurthi and Sainath Janak (Loc. cit) have reported that oxygen consumption in Paratelphusa was maximal at 8.00 A.M. and at midnight. It is not clear whether these rhythms pertain to their summer activity or winter. Further the behaviour of each sex has not been studied separately. In the present studies, diurnal rhythms of oxygen consumption in both the sexes were examined in summer as well as in winter. In the summer, both sexes showed two peaks of oxygen consumption, one at 10 A.M. and the other at 8 P.M. Statistical comparison shows that these two peaks are not significantly different from each other but are significantly higher from most other values ($P < 0.05$). The

maximal activity in males was kept up from 6 P.M. to 10 P.M. and at 10 A.M. The maximal activity around 8 P.M. may be attributed to the feeding habits as the crabs are mostly nocturnal and come out of their burrows at dusk and go in search of prey. The same cannot be said for the peak metabolic activity at 10 A.M. which may be correlated to some other physiological activity influenced by temperature or some other environmental factor or factors.

The diurnal variation of oxygen consumption in winter showed a different picture. In the females there was only one peak at 2 A.M. but this value is not significantly different ($P > 0.05$) from most of other values (excepting the values at 4 P.M. & 6 P.M. which are significantly low). In the males there were two peaks one at 2 P.M. and the other at 12 mid night but these two values are not significantly different ($P > 0.05$) from most of the values (excepting the value at 6 A.M.). When both males and females were considered together, two maximal activities were observed, one around 12 at mid night and the other at 2 P.M. but these are not significantly different from most of the other values excepting those at 6 A.M., 4 P.M. and 6 P.M. which are significantly

low. The maximal activity from 10 P.M. to 2 A.M. may be interpreted as being due to nocturnal behaviour but the peak at 2 P.M. cannot be explained away thus. As a matter of fact, this second maximal activity is not significantly different from any other value except the value at 6 P.M. Thus, it can be concluded that in winter, crabs do not show significantly high peaks of metabolic activity. This is clear from the fact that the statistically calculated 'F' values are lower than table values for all the winter animals studied. Therefore the time of sampling during any part of the day during winter except at 6 P.M. and at 12 mid night may not have any significant influence on the physiological and biochemical studies in the field crab Oziotelphusa. However, the same is not true for summer animals as they show two maxima which are significantly high compared to other values. For this reason, most of the present work was executed in the months of December, January and February (Winter) and work in summer was avoided.

Time course of oxygen consumption upon transfer to 50% and 100% sea water and reversal to freshwater.

Method

Metabolic responses to stress conditions are normally divided into (1) first stages or short term responses and (2) long term responses or prolonged phase of adjustments and (3) the end stages when a steady state in metabolism is achieved. In the present work the entire sequence of responses during acclimation to salinity stress was proposed to be covered.

In addition, the sequence of responses during the reverse acclimation also formed part of the investigation. Reverse acclimation consisted of returning the animals to freshwater after they have been subjected to osmotic stress for atleast 15 days. During the forward acclimation and reverse acclimation processes the time scale followed for determination of oxygen consumption was 1, 2, 3, 5, 8, 12 and 24 hours after exposure of the crabs to the new medium and there upon once every day until a steady state was reached. The method described earlier (continuous flow method) is well suited for the collection of samples and estimation of oxygen consumption at various intervals planned. The intervals selected are arbitrary. However,

it was the intention to have greater insight into the first stages of metabolic response that prompted the author to follow several such closer intervals during the first day. Also the middle stages that are normally overlooked have been taken into account. Finally, the end stages (steady states) have not been lost sight of. As the work was planned in both the directions (forward and reverse acclimation) involving several days the parameters chosen were limited to oxygen consumption, water content in tissues, inorganic osmotic effectors, viz., Na^+ and Cl^-, organic osmotic effectors viz., free amino acids (TNPS) and enzymes viz., aminotransferases and GDH.

Oxygen consumption on the time scale indicated above was studied in 12 crabs after directly transferring them to 50% sea water. The oxygen consumption of each animal in its natural medium i.e. freshwater was determined 6 times, one day prior to transfer to sea water and the mean value was taken as the control value. Of the 12 animals 6 were males and 6 females. The size of the crabs ranged from 20.00 gms to 44.5 gms. The crabs used for forward and reverse acclimation were one and the same. Data on crabs that died during the course of acclimation were discarded. In the same way the time course

of oxygen consumption in 8 crabs exposed to 100% sea water was determined, both in the forward and reverse directions.

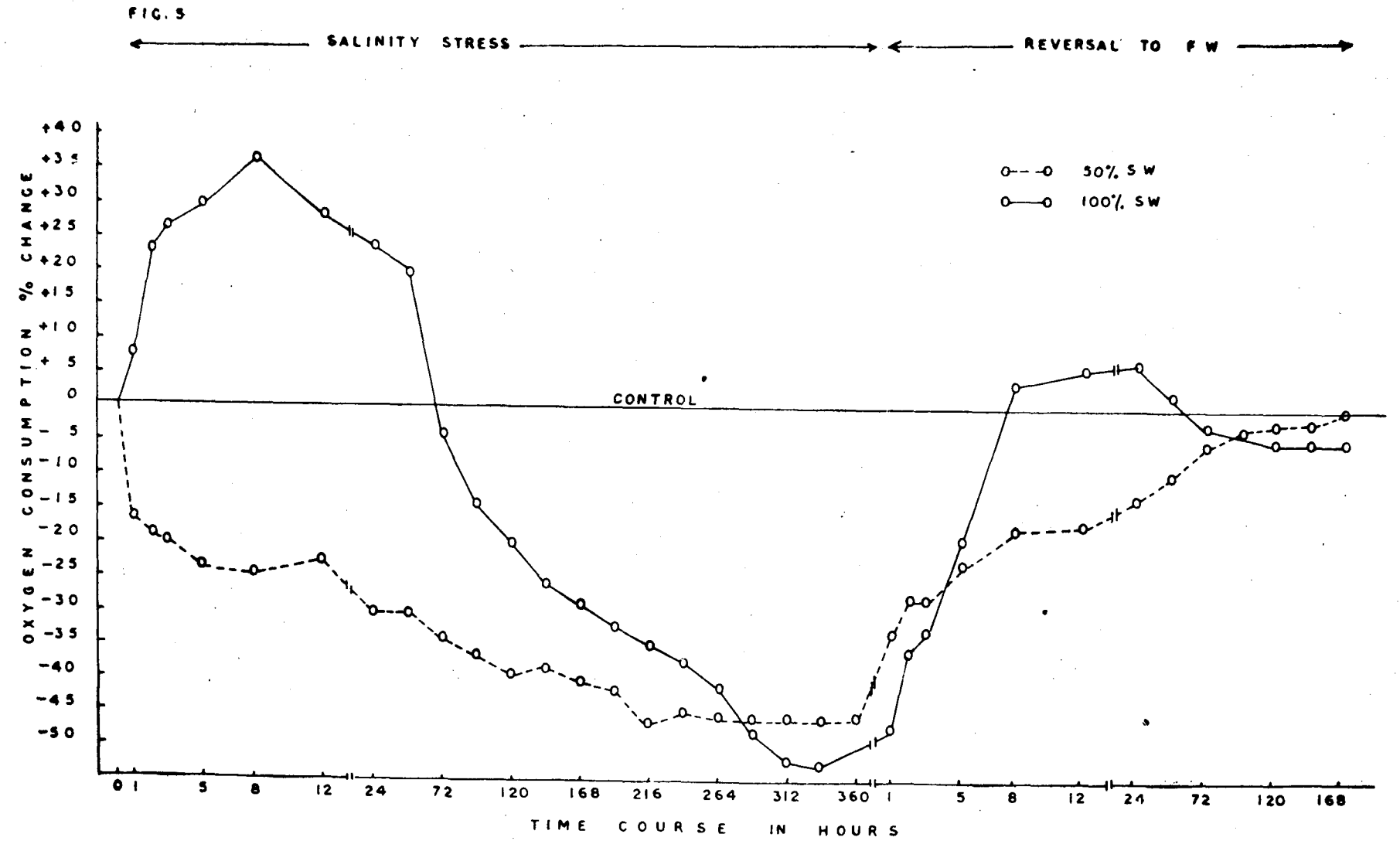
Results

Time course of oxygen consumption in 50% sea water

Mean values of time course of oxygen consumption in 12 crabs when they are directly transferred to 50% sea water from their natural medium (i.e. freshwater) are shown in table 4. The data were subjected to statistical analysis. Variance ratios and critical differences at 5%, 1% and 0.1% levels of significance are presented in table 4-1. Statistical comparison of the mean values is furnished in table 4-2. Percentage deviations of oxygen consumption from the normal values are plotted in figure 5. It is clear from these values that upon direct transfer of the freshwater crabs to 50% sea water, the oxygen consumption decreased to significantly low levels. This drop in oxygen consumption was evident even after the 1st hour which is highly significantly ($P < 0.001$) compared to control value. This decreasing trend continued upto 10 days and thereafter a steady state was reached. The steady state in oxygen

FIG. 5: Oxygen consumption of the crab, OZIOTELPHUSA during the period of its exposure to 50% & 100% sea water (SW) and during the reverse acclimation to fresh water (FW).

FIG. 5


SALINITY STRESS
REVERSAL TO F W
50% S W
100% S W
OXYGEN CONSUMPTION % CHANGE
+40
+35
+30
+25
+20
+15
+10
+5
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
CONTROL
0 1 5 8 12 24 72 120 168 216 264 312 360 1 5 8 12 24 72 120 168
TIME COURSE IN HOURS

consumption was maintained from the 10th day to the 15th day. It may be pointed out that the decrease in oxygen consumption occurred in several phases. From the 1st hour to 12th hour there was a gradual depression, but the 8th hour value is significantly low compared to the first and second hours. There was a significant drop in oxygen consumption between the 12th hour and 24th hour, between the 24th hour and 96th hour and between the 96th hour and 216th hour i.e., 10th day.

Reverse acclimation from 50% sea water to freshwater

* Mean values of time course of oxygen consumption of crabs during reverse acclimation (i.e., when they were transferred suddenly to freshwater after they had been acclimated to 50% sea water) are presented in table 5. Mean values for 12 animals are given. Variance ratios and critical differences at 5%, 1% and 0.1% levels of significance for the data in table 5 are given in table 5-1. Statistical comparison of the data is shown in table 5-2. Percent deviations from the control value of oxygen consumption are plotted in figure 5.

From the results obtained it is clear that reversal of 50% sea water acclimated animals to freshwater (or natural medium) resulted in a quick elevation of oxygen consumption

to normal levels. The immediate response i.e., after 1 hour, was a highly significant elevation ($P < 0.01$) from the level obtained in 50% sea water acclimated crabs and all the subsequent changes were significantly higher compared to acclimated values. Oxygen consumption after the 8th hour was a further significant increase compared to the 1st hour ($P < 0.01$). The increase after 72 hours was a further significant one compared to the 8th hour value ($P < 0.01$). The elevation in oxygen consumption after 3 days of transfer was significantly high and this was not significantly different from the value obtained in normal animals although there is a further gradual increase to normal level by the 7th day of return to freshwater. It is worth noting here that acclimation to freshwater in 50% SW acclimated crabs is quicker (3 days) than acclimation to 50% sea water which required a period of 10 days.

Acclimation to 100% sea water

Eight crabs were directly transferred to 100% sea water (hyperosmotic shock) and their oxygen consumption was estimated on the time scale indicated. As already reported, the behaviour of crabs in 100% sea water was not normal. By the 3rd day, they 'refused' to feed and

were found to be lethargic. On the first day, the crabs tried to avoid the medium by climbing one over the other or by climbing on to a stone when one was offered. But on the 2nd and 3rd days these escape reactions were not seen and on subsequent days they were almost immobilised. As many as 50% of them died during the course of the acclimation. 100% sea water is hyperosmotic to the body fluids of crab and perhaps the gradient is too large for the animals to withstand the hyperosmotic shock. However, some animals managed to survive the shock and were alive during the acclimation period of 15 days.

Results

Time course of mean values of oxygen consumption of crabs exposed directly to 100% sea water is presented in table 6. Mean values for 8 crabs are furnished. The data were subjected to statistical analysis of variance ratios and critical difference and are shown in table 6-1. Statistical comparison by the least critical difference method, of the mean values is given in table 6-2. The mean values of oxygen consumption are plotted against the time scale in fig. 5.

There was a sudden elevation of oxygen consumption immediately after direct transfer to 100% SW. In the first hour there was a non-significant increase but from the 2nd hour to 48th hour there was a highly significant increase ($P < 0.001$) in oxygen consumption. After reaching a peak at the 8th hour, there was a downward trend in the metabolic rate but upto the 48th hour, the rate was significantly higher than the level obtained in freshwater ($P < 0.001$). By the 4th day the rate of oxygen consumption decreased steeply and this significant depression ($P < 0.001$) continued throughout the experimental period, reaching very low level by the 15th day of exposure on (-53.25% d. from normal). The initial elevation of activity is worth remembering here. As will be discussed later, this may be attributed either to an escape reaction (Krogh, 1939) or to some homeostatic mechanism coming into operation (Pampapathi Rao, 1958). It is also worth noting here that the metabolic rate continued to decrease throughout the period of acclimation. However, after 12 days of exposure, a steady state in metabolic rate seemed to occur, as the values obtained from 288 hours onwards were not significantly different ($P > 0.05$) from each other. But, the steeply falling curve (fig. 5) points to the contrary.

Reverse acclimation to FW from 100% SW

Crabs exposed to 100% SW for a period 15 days were directly transferred to FW and their oxygen consumption was estimated on the time scale indicated already. The mean values of oxygen consumption during this reverse acclimation are shown in table 7 and the variance ratio for this data is given in table 7-1. Statistical comparison of the data is presented in table 7-2. Figure 5 shows the mean values plotted against time course.

While the crabs were in 100% SW they appeared to be highly lethargic and it looked as if some pathological condition had set in. The escape muscles were paralysed. Astonishingly, on the third day of their transfer to freshwater, they seemed to have recovered almost completely. After return to freshwater from 100% SW the oxygen consumption at the end of the first hour was not significantly different but it increased to a significantly higher level by the 3rd hour ($P < 0.001$) and thereafter there was an elevation of metabolic rate to normal levels by the 8th hour. Subsequent changes were not significantly different ($P > 0.05$) from the values obtained in freshwater crabs (normal values). As in the case of reversal from 50% SW it is worth noting here that acclimation to freshwater seems to be quicker than to 100% sea water.

Weight Regression Lines:

A) Acclimation to 50% sea water and back to freshwater

The regression coefficients of oxygen consumption in relation to body weight have been calculated for crabs acclimated to 50% SW and also for crabs in reverse acclimation process.

The weight regression lines are shown in figures 6 and 7. The 'b' values are reported along with the regression lines.

It is seen from these regression lines that in 50% SW acclimated crabs, the metabolic rate of smaller crabs was higher than that of larger crabs in all stages of acclimation. There was depression of metabolic rate in both smaller and bigger crabs throughout the acclimation period but the depression was greater in smaller animals than in larger animals at all stages. Smaller animals showed greater response to stress conditions than larger ones. Figure 10 shows the metabolic response after transfer to 50% SW and back to FW in 6 individual crabs. Greater response of smaller crabs to a change in the ambient medium is also evident from this figure. There is a sudden decrease in 'b' value on transfer from FW. to 50% SW. even at the

FIGS. 6 & 7: Changes in weight-metabolism regression curves during the course of acclimation of the crabs to 50% sea water and reversal to fresh water.

Fig. 6:

A :	Control (FW);	'b' value = -0.9341
B :	1 hour after transfer to 50% SW;	b = - 0.5760
C :	24 hours after transfer to 50% SW;	b = - 0.2167
D :	72 hours after transfer to 50% SW;	b = - 0.1155
E :	168 hours after transfer to 50% SW;	b = - 0.1355
F :	360 hours after transfer to 50% SW;	b = - 0.0239

Fig. 7:

A :	360 hours after transfer to 50% SW;	b = - 0.0239
B :	1 hour after reversal to FW;	b = - 0.6820
C :	24 hours after reversal to FW;	b = -0.5056
D :	72 hours after reversal to FW;	b = - 0.7598
E :	168 hours after reversal to FW;	b = - 0.9488

FIG. 6

WEIGHT — METABOLISM REGRESSION LINES

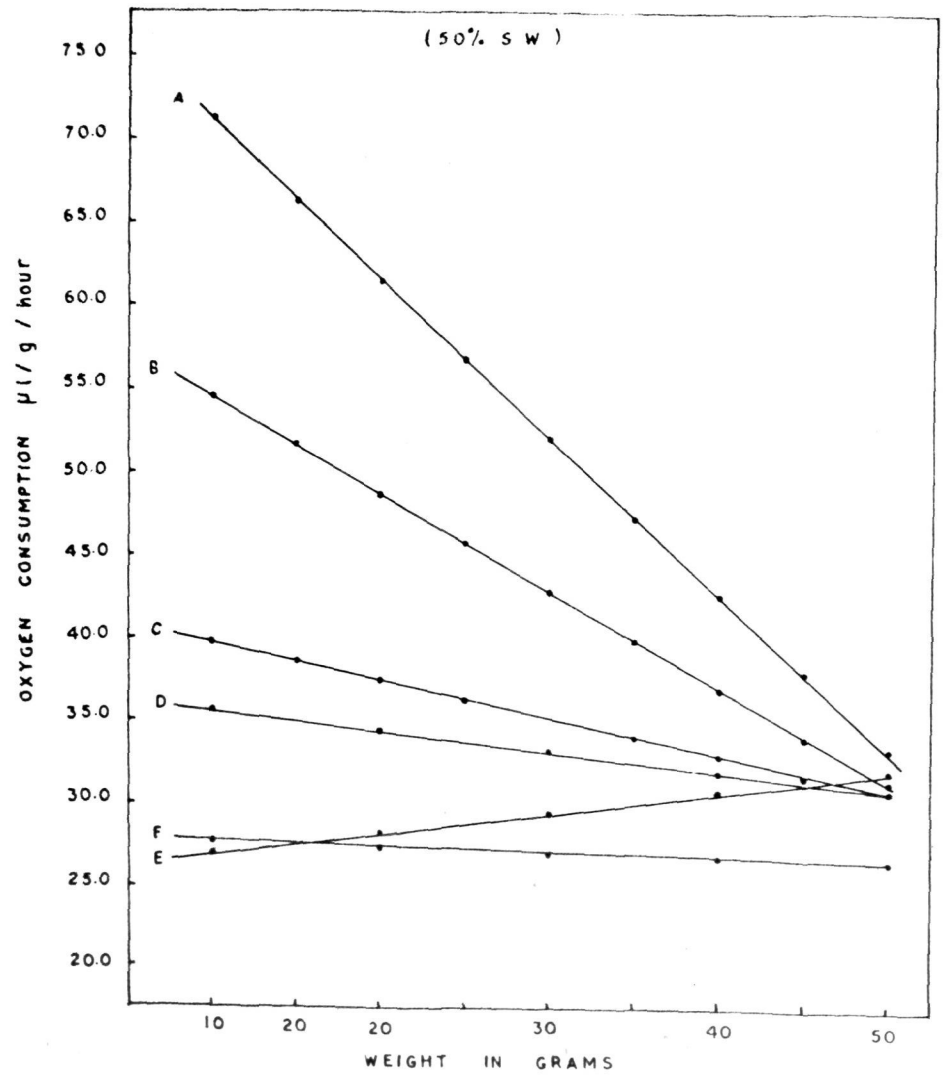
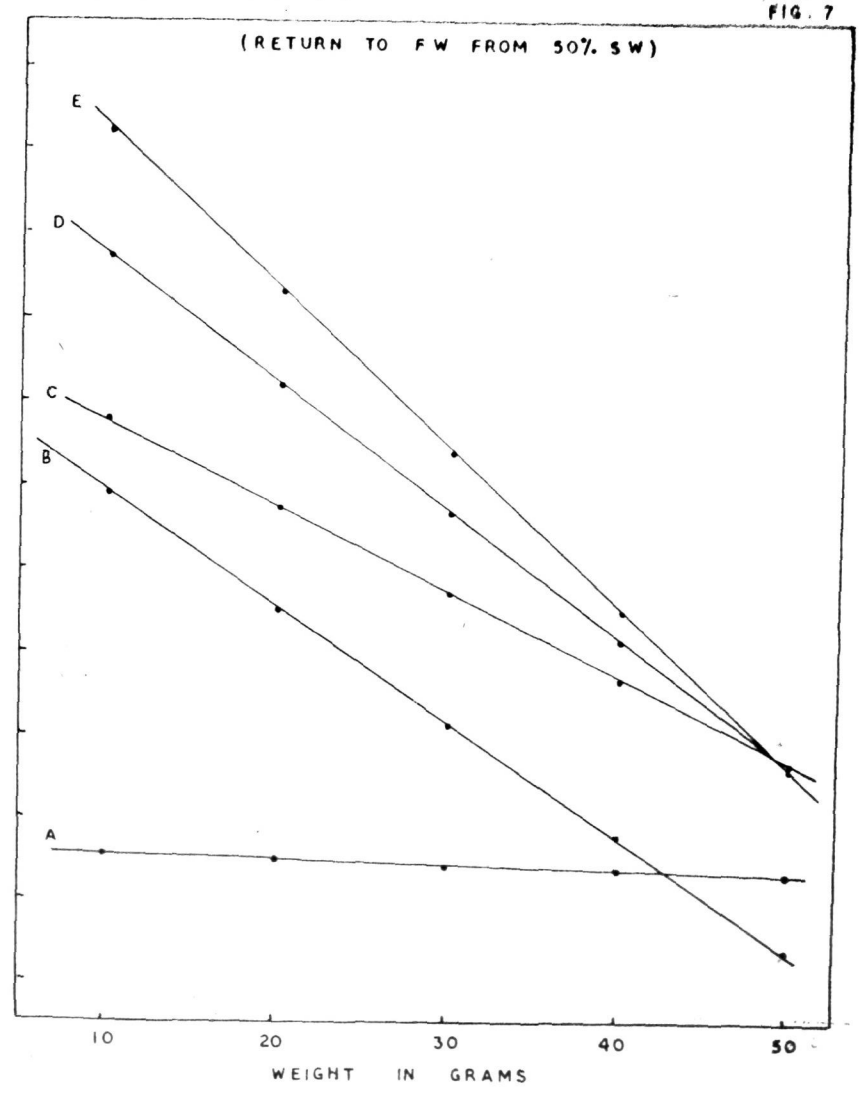


FIG. 7



end of 1st hour of transfer and this trend generally continued upto 360 hours. The 'b' value on the 7th day is at variance from the other values. Upon reversal to FW. from 50% SW. the regression lines also show a reversal of the trend. The 'b' values generally increase gradually along the time scale. There was elevation in metabolism in both smaller and larger crabs upon reversal to freshwater but the elevation is greater in smaller animals than in larger animals. The 'b' values reach the control value by the 7th day of reverse acclimation to freshwater.

B) Acclimation to 100% sea water and back to freshwater

The weight regression lines for crabs acclimated to 100% SW. and for those returned to FW.(reverse acclimation) are shown in figure 8 and 9. These lines do not show any pattern of change except that the regression coefficients are elevated upon transfer to 100% SW in the 1st and 24th hours with a later depression on the 3rd and subsequent days. On the 3rd and 14th days the depression in 'b' values is more pronounced and as expected the decrease in metabolic rate was greater in smaller crabs than in larger ones. In the reverse acclimation, the 'b' values gradually increase to normal levels by the 7th day.

FIGS. 8 & 9: Changes in weight-metabolism regression curves during the course of exposure of the crabs to 100% sea water and reversal to fresh water.

Fig. 8:

A : Control (Fresh water); b = - 0.9472
B : 1 hour after transfer to 100% SW; b = - 0.8032
C : 24 hours after transfer to 100% SW; b = - 0.7693
D : 72 hours after transfer to 100% SW; b = - 0.1070
E : 168 hours after transfer to 100% SW; b= -0.3527
F : 336 hours after transfer to 100% SW; b = -0.1776

Fig. 9:

A : 336 hours after transfer to 100% SW; b = -0.1776
B : 1 hour after reversal to FW; b = -0.4395
C : 24 hours after reversal to FW; b = -0.5108
D : 72 hours after reversal to FW; b = -0.5153
E : 168 hours after reversal to FW; b = -0.6725

FIG. 8

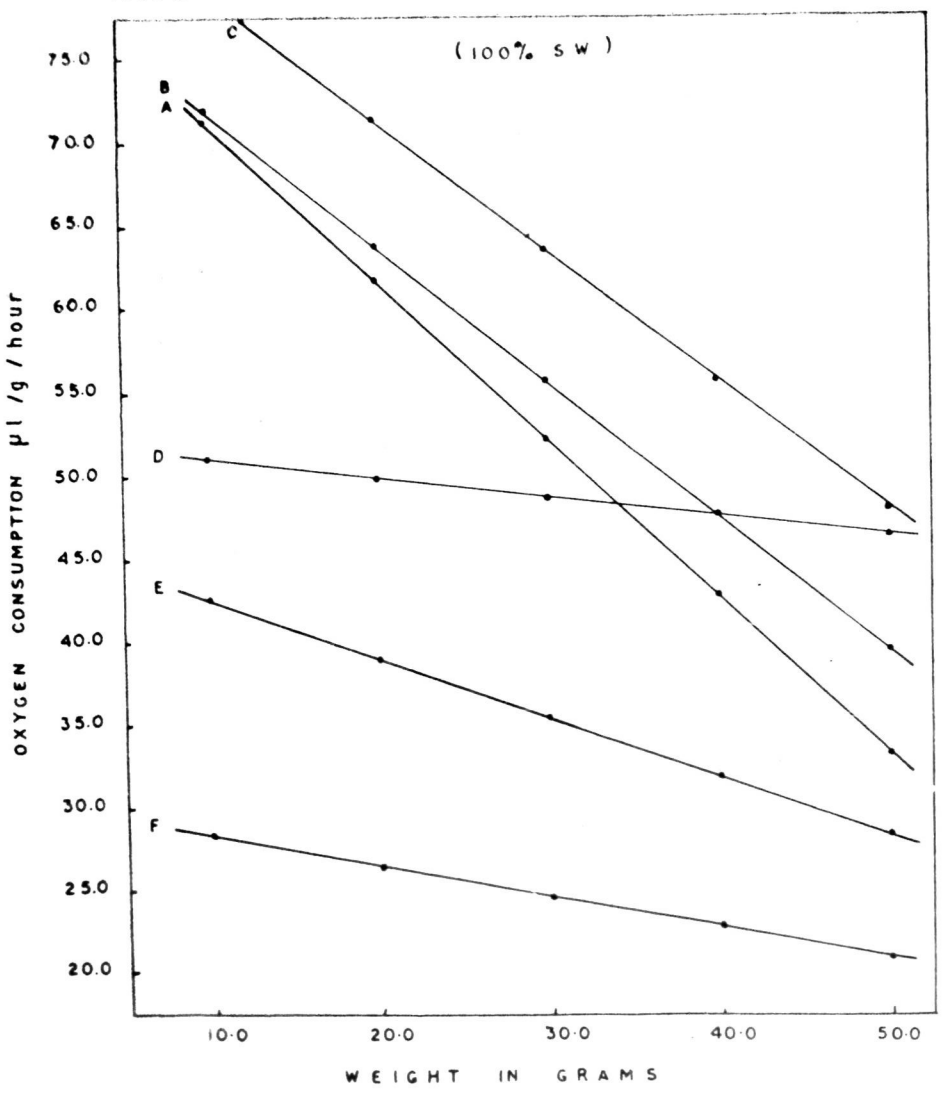

(100% SW)
OXYGEN CONSUMPTION µl /g / hour
75.0
70.0
65.0
60.0
55.0
50.0
45.0
40.0
35.0
30.0
25.0
20.0
C
B
A
D
E
F
10·0
20·0
30·0
40·0
50·0
WEIGHT IN GRAMS

FIG. 9

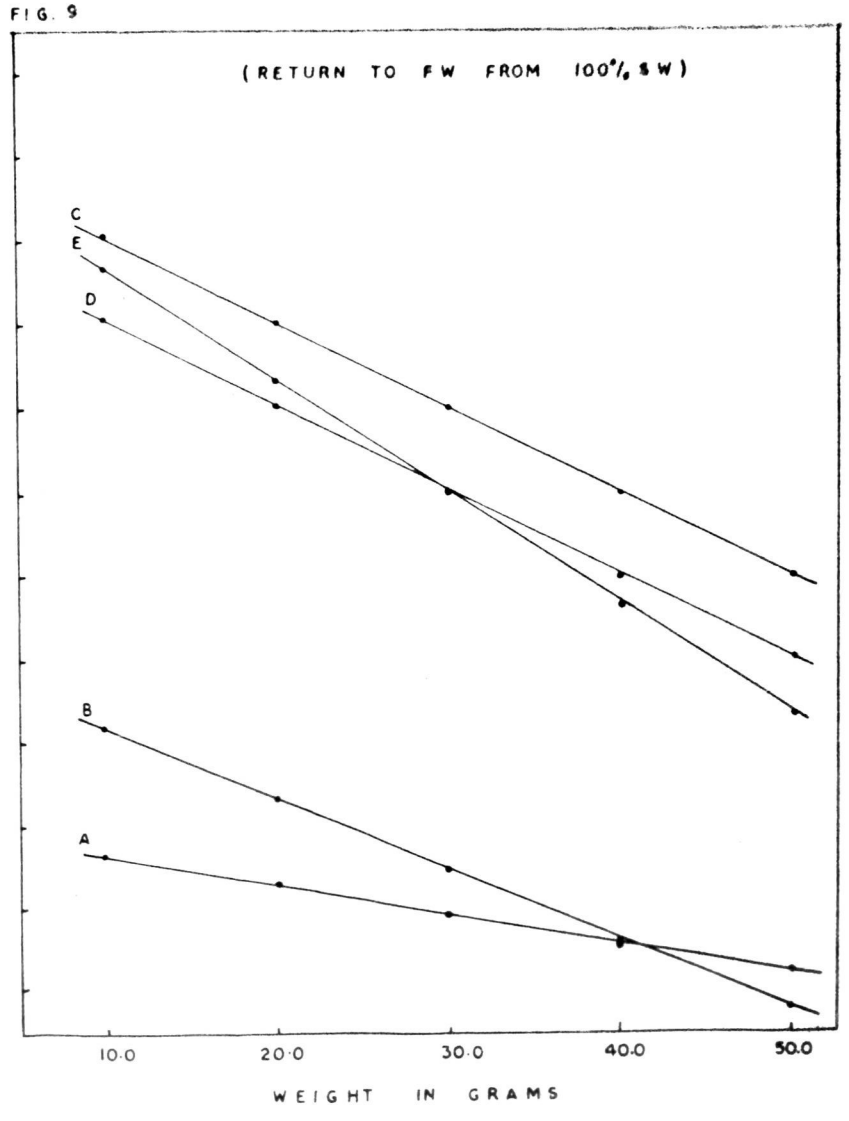

(RETURN TO FW FROM 100% SW)
C
E
D
B
A
10·0
20·0
30·0
40·0
50·0
WEIGHT IN GRAMS

FIG. 10: Unit metabolism of 6 individual crabs during one day prior to exposure to 50% SW and during acclimation to 50% SW and also in the reverse acclimation process.

1. Female : 44.5 gms; 2. Male : 40.2 gms.
3. Female : 35.1 gms; 4. Male : 31.3 gms.
5. Male : 25.0 gms; 6. Female : 20.3 gms.

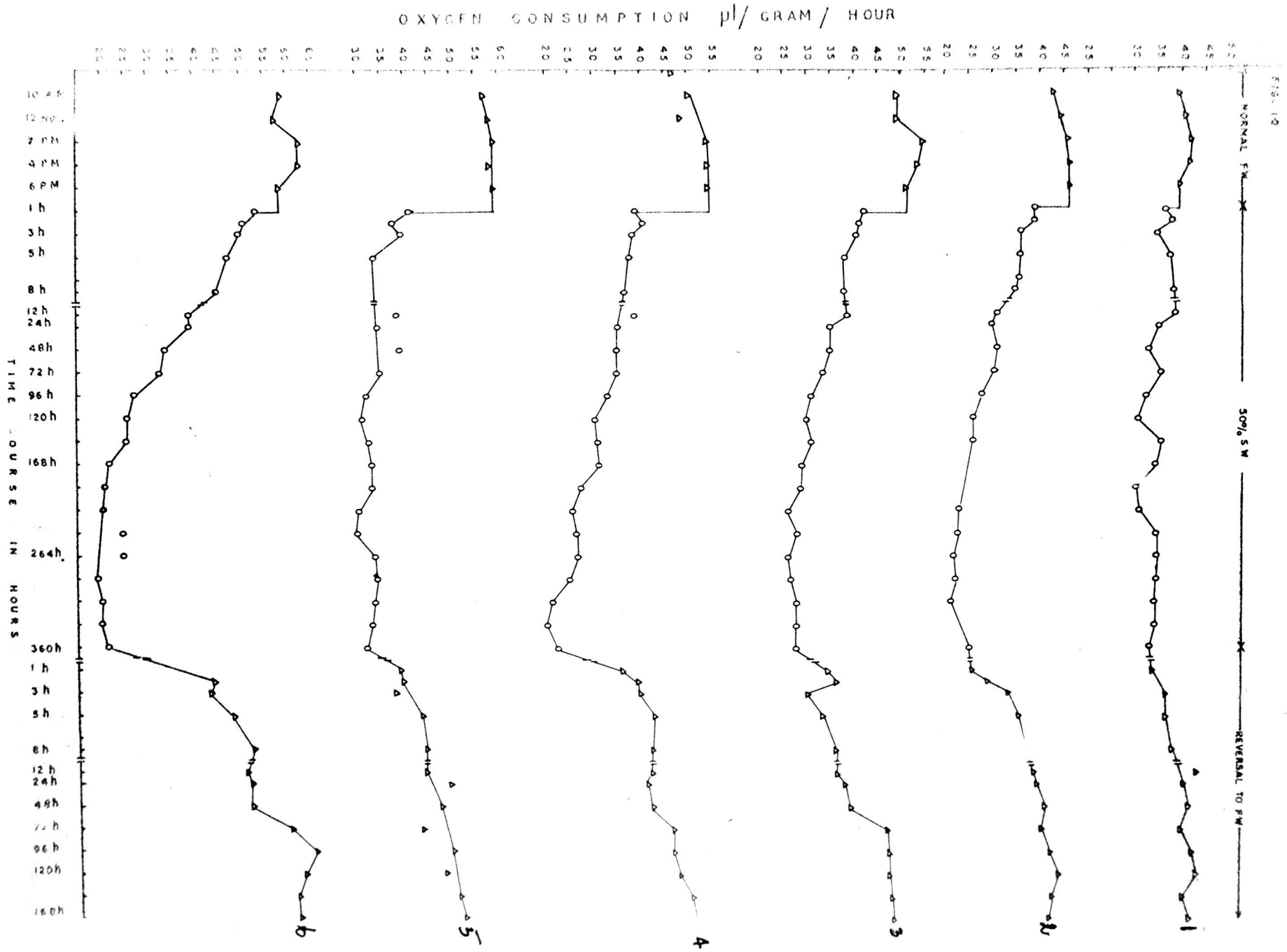

OXYGEN CONSUMPTION µl/ GRAM / HOUR
TIME COURSE IN HOURS
10 A.M.
12 NOON
2 P.M.
4 P.M.
6 P.M.
1 h
3 h
5 h
8 h
12 h
24 h
48 h
72 h
96 h
120 h
168 h
264 h.
360 h
1 h
3 h
5 h
8 h
12 h
24 h
48 h
72 h
96 h
120 h
168 h
NORMAL FW
50% SW
REVERSAL TO FW
1
2
3
4
5
6

FIG. 10

Discussion

Decreased levels of oxygen consumption in higher salinities and increased levels in lower salinities is the usual pattern of metabolism in most Euryhaline crustaceans. However, variations from this pattern remain the subject of much controversy (Gilles, 1973b, 1974a). In some species, the oxygen consumption remains unaffected. A few cases are reported so far wherein there is no change in oxygen consumption; these are the crustaceans Eriocheir sinensis (Schwabe, 1933); Artemia salina (Gilchrist, 1956) Palaemonetes varians (Mc Farland and Pickens, 1965), hirudinoid Erpobdella and Nephelopsis (Linton, Davies and Wrona, 1982) and a freshwater gastropod Theodoxus (Bielawski, 1960). However, Lee and Pyung (1970) showed higher oxygen consumption of Eriocheir sinensis in diluted media. While Kuenen (1939) measured higher oxygen consumption in A. Salina acclimated to concentrated media, Eliassen (1953) showed depression of oxygen consumption in the same species in concentrated waters. Whereas Lofts(1956) showed variations in oxygen consumption in Palaemonetes varians, Mc Farland and Pickems (1965) showed no such variations. In some species, the oxygen consumption was found to be lower in a diluted or concentrated medium than in the natural

medium. In the estuarine mud crab Helice crassa, Shumm-way and Jones (1981) reported lower oxygen consumption in 50% SW and higher rates in 10%, 100% and 150% SW. Para-telphusa hydrodromous and Macrophthalmus japonicus, are reported to show a relatively higher oxygen consumption in diluted media than in concentrated ones (Lee and Pyung, 1970; Venkata Reddy, 1976). While measuring the initial response (45 minutes after transfer to the medium) in paratelphusa hydrodromous, Ramamurthi (1965, 1967b) reported maximal oxygen consumption in its natural medium (FW), minimal oxygen consumption in 50% SW and an elevated level in 100%SW. In the same crab, Venkata Reddy (1976) showed a decreasing trend in oxygen consumption in increasing salinities (25%, 50%, 75% SW and 100% SW) after acclimation of these crabs to these four concentrated media for a period of 15 days. Thus the problem of metabolic response to salinity changes remains to be a subject of controversy . While referring to such confusing. Work done by the author assumes significance in this connection. Time course of oxygen consumption and related aspects in relation to salinity stress were not followed in the earlier studies. The present studies lay emphasis on time course in order to have a broader perspective of the problem.

From the results obtained in the present work it is seen that oxygen consumption of Oziotelphusa (Paratelphusa) senex senex was significantly higher in its natural medium but significantly lower in 50% SW throughout the acclimation period ($P < 0.001$). This significant decrease commenced right from the 1st hour of exposure to 50% SW (table 4, fig. 5) and reached a steady state from the 10th day. This agrees with the results obtained by Ramamurthi (1965, 1967b) and Venkata Reddy (1976) for this crab. But the values obtained in 100% SW are interesting. Data presented in table 5 and the curve shown in fig. 5 indicate that there was a general decrease in oxygen consumption in 100% SW which is in line with results obtained by Venkata Reddy (1976) but this trend was seen only after the 3rd day of exposure to SW. The immediate response, i.e., oxygen consumption after 1 hour, was higher than the normal value but it was not significantly high compared to the normal ($P > 0.05$). This agrees with the findings of Ramamurthi (1965). But subsequently, the oxygen consumption increased to a significantly high peak by the 8th hour ($P < 0.001$) and later showed downward trend. However, a significant higher level was maintained upto 48 hours. Such a trend could not be recorded by Ramamurthi

(1965) and Venkata Reddy (1976) in the absence of time course studies. An explanation has to be provided for this higher metabolic rate in higher salinity for a period of 48 hours. Two probable interpretations are possible.

1. The increased metabolic rate in 100% SW may be attributed partly to an 'escape reaction' (Krog, 1939, 1964), and the consequent increase in the locomotor activity (Gross, 1957a,b). Immediately after transfer from FW to 100% SW some crabs were observed to avoid the changed medium. They were observed to mount upon each other. When a stone or other object was placed in the though, some crabs tried to climb on to such objects and avoid the stress condition. These observations lend support to the view suggested by Krogh (1964) and Gross (1957a,b) Here, the maguitude of the stress is so great that the animals find it rather difficult to withstand the stress and begin to avoid the stress. Nearly 50% of the animals perished in the first two days, indicating the deleterious effects of higher salinity stress on crabs which were adapted to freshwater over a long period in their evolutionary history. These crabs have not been reported either from estuaries or seas. They are strict freshwater forms. When such forms are directly transferred

to 100% SW many of them, if not all, are perhaps unable to withstand the stress and so they may show escape reactions accompanied by locomotor activity that increases oxygen consumption. Perhaps this explanation holds good for the elevation of oxygen consumption for some hours after the transfer, say upto the 8th hour. After the 8th hour, there was a decreasing trend in oxygen consumption but upto 48 hours it was significantly higher than the normal.

A second possible explanation for this elevated metabolism for 48 hours in 100% sea water may be sought in the existence of osmotic or ionic gradients between the milieu interieur and exterior. The osmotic and ionic concentration of the blood of crab is about the same as in 50% SW (Ramamurthi, 1965) at the beginning of the experiment. The osmotic and ionic concentration of 100% sea water is about double compared to that of blood. This large gradient imposes a great stress perhaps for several hours, probably upto 48 hours and the stress involves osmotic work that requires energy. It has been shown in the cray fish (Schwabe, 1933), brackish water prawn (Pampathi Rao, 1958), Palaemonetes varians (Lofts, 1956) that "the oxygen consumption in various salinities is governed by the osmotic gradient existing between the blood and the medium". The elevated oxygen consumption in the crabs in the first stages of sojourn in 100% SW may be particularly due to the

chloride gradient between the blood and the medium which is very high during the first stages of adaptation (Fig.20). The oxygen consumption is reported to follow the chloride ion gradient between the blood and medium in the marine crab, Ocypod albicans (Flemister and Flemister, 1951) in the brackish water crab, Sesarma plicatum (Pampapathi Rao and Madanamohana Rao, 1963) in the freshwater crab under investigation (Ramamurthi, 1965) and in a related crab Barytelphusa guerini (Venkatachari and Vasantha, 1981).

The depression of oxygen consumption in 50% SW and also in 100% SW (observed after 72 hours) fits into the general trend of energetics of osmoregulation indicated by numerous studies (Lee and Pyung, 1970; Siebers and coworkers, 1972; Gilles, 1973b, 1974a). In the crab under investigation, the explanation for lowered oxygen consumption in higher salinities may be sought in the more or less isosmotic conditions obtained in 50% SW between the blood and the external medium and also minimal chloride gradient (Ramamurthi, 1965). A similar situation is perhaps achieved in 100% SW after 72 hours (Chapter II). These decreased gradients minimise the energy requirements for osmotic work and thereby bring about a depression in oxygen consumption. As will be shown in a subsequent chapter, the

free amino acid levels also generally show an increase in the tissues of crabs acclimated to 50% and 100% SW which along with increased levels of inorganic ions probably bring about isosmoticity of the intracellular fluid with the extracellular fluids (Chapter III). This may further add to decreased osmotic work leading to depression of oxygen consumption in the whole animal. Yet another reason for lowered metabolism in higher salinities may be found in the interesting observation that the cell metabolism is geared toward anaerobic conditions which prevent some of the reducing equivalents from entering the krebs cycle(Schoffeniels and Gilles, 1970) under conditions of increased ionic concentration of cell ambient medium. The enzymic profile of tissues of Oziotelphusa (Paratelphusa) also reflects such a gearing towards anaerobic conditions (Chapter IV). It is also worth to remember here that transfer of a euryhaline animal from freshwater to sea water may result in dehydration of the tissues and consequent shrinkage especially of the muscle. Such a loss of water accompanied by shrinkage of muscle has been reported for Eriocheir sinensis exposed to hyper-osmotic shock. Resumption of the water content to the control value and thereby volume regulation occurs more slowly in hyperosmotic media than in hypo-osmotic conditions (Gilles, 1979). Dehydration of tissues may be one of the causes for the decreased metabolism (Pieh, 1936; Remane and Schlieper, 1958, 1971). Venkata Reddy (1976)

reported decrease in gravimetric status of hepatopancreas ("salogenous atrophy") of Paratelphusa hydrodromous acclimated to media of higher salinities (-34.66% in 50% SW and -61.3% in 100% SW). Such effects of salinity stress result-in anorexia may also be responsible for the decreased metabolism of animals under higher salinity stress especially in 100% SW leading to anaerobic conditions. Further, decreased catabolism of intracellular amino acids has been implicated for the augmentation of amino acids in the intracellular isosmotic regulation (Schoffeniels and Gilles, 1970; Gilles, 1974a). This aspect also explains the lowered oxygen consumption in this crab in 50% and 100% SW.

Reverse acclimation of 50% and 100% SW acclimated crabs to FW

Freshwater is the natural medium of these crabs. Transfer of 50% and 100% SW acclimated crabs to FW involves hyper-osmotic regulation. From the data presented in tables 5 and 7 and figure 5, it is observed that in both the cases oxygen consumption became elevated upon transfer to freshwater and reached a steady state after 72 hours in 50% SW acclimated crabs and after 8 hours in 100% SW acclimated ones. Literature on reverse acclimation of animals is almost wanting. However, literature on hyper-osmotic regulation is voluminous. Several studies on hyper-osmotic regulation indicate increased oxygen consumption in animals transferred to lower salinities (Remane

and Schlieper, 1958, 1971; Ramamurthi, 1965; Lee and Pyung, 1970). In Carcinus maenas acclimated to a diluted medium, $^{14}Co_2$ production from injected labelled glutamate increased by 50% (Chaplin, Huggins and Munday, 1970). Increase in Co_2 production indicates a corresponding increase in O_2 consumption. Increase of metabolic activity in freshwater of euryhaline forms could be explained on the basis of the osmotic and ionic gradients. The osmotic gradient between the ambient medium and 50% and 100% SW acclimated crabs must be very high and hence the high O_2 uptake. Further as already explained, the chloride gradient is also high between the freshwater medium and blood of the crab. This also explains the higher levels of oxygen CONSUMPTION in the crabs passing through reverse acclimation process. Oziotelphusa (Paratelphusa) is a good hyperosmotic regulator in freshwater and in lower salinities whereas it appears to be an osmo-conformer in higher salinities (Ramamurthi, 1965; Venkata Reddy, 1976). As a strong regulator in hypo-osmotic media including freshwater it obtains the inorganic osmotic effectors such as Na^+ and Cl^- by active transport mechanisms. An active salt absorption mechanism has been demonstrated in this crab (Pampapathi Rao and Venkata Reddy, 1962). The chloride uptake in this crab has been shown to be an energy consuming process (Venkata Reddy and Aravindababu, 1969). Studies on the isolated skins of amphibians on Na^+ transport have thrown more light on Na^+

transport and oxygen uptake. Active transport of sodium is mainly energized by oxidative metabolism. This has been well demonstrated in amphibian skin and urinary bladder. Stoichiometry between sodium transport and net oxygen consumed is found to be about 18 Na^+ ions per molecule of O_2 in frog skin (Zerahn, 1956; Leaf and Renshaw, 1957) and toad bladder (Leaf, Page and anderson, 1959). This relationship persists whether Na^+ transport takes place against a concentration gradient or potential gradient or whether the transport is mediated by a hormone (Zerhan, 1956). In recent studies Na / O_2 ratio in Rana pipiens is found to be 15 equivalents of sodium per molecule of oxygen (Viera, Caplan and Essig, 1972; Lang et al, 1977). A linear relationship between net sodium uptake and net oxygen consumption under varying circumstances has been obtained in the skin of frog and bladder of toad (Loc. cit). Such studies throw more light on the relationship between oxygen consumption and active Na^+ uptake in animals transferred from sea water to freshwater.

Studies on water regulation and consequent volume control in tissues subjected to hyper- and hypo-osmotic shocks add further dimension to the discussion. Transfer of higher salinity acclimated crabs to freshwater brings about hydration of tissues and consequent increase in cell volume to normal levels. In the present studies, water content in muscle and hepatopancreas was found to increase (tables 8 and 9, figures 11 & 12) to normal levels

relatively quickly in hypo-osmotic medium (FW) rather than in hyperosmotic media (SW). It is interesting to note here that the oxygen consumption of 50% SW and 100% SW acclimated crabs generally follows the same trend as water content during reverse acclimation to freshwater. This shows that hydration of tissues and consequent swelling, or cell volume regulation increases oxygen consumption. Pieh (1936), Remane and Schlieper (1958, 1971) have implicated hydration of tissues as the causative factor for the elevation of metabolism in animals transferred from a marine habitat to more diluted media. Isolated axons of the Chinese crab Eriocheir sinensis subjected to hypo-osmotic shock show an initial phase of swelling due to hydration but the tissue volume resumes almost completely its control values in the time course of the experiment i.e., 4 hours. On the contrary, after the application of an hyperosmotic stress, the tissue shrinks and no regulation is observed in the time course of the experiment i.e. 6 hours (Gilles, 1973a). The results obtained in the present investigation on whole animals are comparable with these observations. Thus, oxygen consumption and tissue hydration and consequent volume regulation appear to be closely linked phenomena. It is worth noting here that regulation of O_2 consumption was found to be a slower process in 100% SW than in 50% SW and regulation in freshwater was relatively quicker than in both higher salinities studied. The same appears to be the case in the regulation of water content and the organic and inorganic osmotic effectors of tissues (See the forthcoming Chapters).

Table 1

Oxygen consumption in relation to size and sex in Oziotelphusa senex senex (FABRICIUS).
Values expressed as μ l/gram/hour. Temp: 26±1°C

MALES			FEMALES		
S. No.	Weight in grams	Oxygen consumption Mean±S.D. for 6 estimations	S. No.	Weight in grams	Oxygen consumption Mean±S.D. for 6 estimations
1.	13.5	65.66±4.37	1.	13.0	61.13±3.61
2.	14.0	61.35±4.82	2.	14.5	60.96±7.15
3.	20.0	57.29±2.65	3.	20.0	57.41±3.51
4.	25.0	56.59±5.83	4.	27.5	54.37±6.01
5.	26.0	55.19±5.73	5.	31.0	49.41±6.17
6.	31.0	51.32±5.22	6.	33.5	47.55±4.56
7.	41.5	47.52±5.83	7.	45.0	41.06±3.27
8.	44.5	41.24±4.06	8.	50.5	34.16±6.65
9.	50.0	36.30±6.11			

Table 2

Diurnal variations in oxygen consumption of Oziotelphusa senex senex in summer (Temp: 31±1°C).

Values expressed as μ l/g/h.

Time	8.00 A.M.	10.00 A.M.	12.00 Noon	2.00 P.M.	4.00 P.M.	6.00 P.M.	8.00 P.M.	10.00 P.M.	12.00 Mid night	2.00 A.M.	4.00 A.M.	6.00 A.M.
Mean values for 8 females	98.28	121.42	91.01	96.15	100.84	97.85	123.64	106.43	94.68	96.11	97.30	97.61
S.D. ±	8.25	12.03	12.18	12.56	8.45	6.64	10.24	16.12	10.26	5.15	5.76	14.78
Mean values for 4 males	155.05	183.37	142.85	138.55	176.05	185.35	189.30	185.62	160.77	172.27	176.30	129.45
S.D. ±	10.54	10.28	7.95	7.59	11.75	8.79	6.10	7.15	7.69	8.23	9.62	4.71

Table 2-1

Variance ratios for the data given in Table 2.

Sex	Variance ratio (F)		Critical difference	
	Table value at 5% level of significance	Calculated value	at 5% level of significance	1% level of significance
Females	2.17	2.85	15.34	20.42
Males	2.09	24.82	10.66	14.49

Table 2-2

Statistical comparison of the data given in table 2.

Mean values arranged in ascending order.

Sex	12 Noon	12 Mid-night	2 A.M.	2 P.M.	4 A.M.	6 A.M.	6 P.M.	8 A.M.	4 P.M.	10 P.M.	10 A.M.	8 P.M.
Females	91.01	94.68	96.11	96.15	97.30	97.61	97.85	98.28	100.84	106.43	121.42	123.

	6 A.M.	2 P.M.	12 Noon	8 A.M.	12 Mid-night	2 A.M.	4 P.M.	4 A.M.	10 A.M.	6 P.M.	10 P.M.	8 P.M.
Males	129.45	138.55	142.85	155.05	160.77	172.2	176.05	176.3	183.37	185.35	185.62	189.3

N.B.: Values grouped together by a common underline do not differ significantly amongst themselves at 5% level of significance.

Table 3

Diurnal variations in oxygen consumption of Oziotelphusa senex senex in winter (Temp: 26 ± 1°C). Values are expressed as μ l/g/h.

	8.00 A.M.	10.00 A.M.	12 Noon	2.00 P.M.	4.00 P.M.	6.00 P.M.	8.00 P.M.	10.00 P.M.	12 Mid-night	2.00 A.M.	4.00 A.M.	6.00 A.M.
Mean values of oxygen consumption for 5 females	62.87	61.92	62.05	61.66	58.28	56.93	58.51	64.96	64.79	66.19	62.94	62.50
S.D. ±	8.39	8.27	6.27	6.19	5.50	6.26	6.82	4.07	6.34	8.85	4.71	7.31
Mean values of oxygen consumption for 6 males	63.28	62.96	63.56	69.45	63.09	60.78	64.34	66.96	69.5	65.92	68.24	59.31
S.D. ±	9.94	9.69	7.77	8.42	9.24	7.81	5.99	9.47	11.00	6.15	7.48	11.00
Mean values for both males and females	63.09	62.48	62.87	65.91	60.90	59.03	61.69	66.05	67.36	66.04	65.83	60.76
S.D. ±	8.81	8.63	6.82	8.21	7.82	7.09	6.76	7.25	9.09	7.09	6.61	9.21

Table 3-1

Variance ratios for the data given in table 3

Sex	Variance ratios (F)		Critical Difference	
	F tab (5% level)	F cal	5% level	1% level
Females	2.12	0.8803	7.69	9.07
Males	1.96	0.004	9.41	12.26
Males & Females	1.88	1.27	6.29	8.63

Table 3-2.

Statistical comparison of the data shown in table 3. Mean values arranged in ascending order.

	6 P.M.	4 P.M.	8 P.M.	2 P.M.	10 A.M.	12 Noon	6 A.M.	8 A.M.	4 A.M.	12 Mid night	10 P.M.	2 A.M.
Females	56.93	58.28	58.51	61.66	61.92	62.05	62.50	62.87	62.94	64.79	64.96	66.19

	6 A.M.	6 P.M.	10 A.M.	4 P.M.	8 A.M.	12 Noon	8 P.M.	2 A.M.	10 P.M.	4 A.M.	2 P.M.	12 Mid night
Males	59.31	60.78	62.96	63.09	63.28	63.56	64.34	65.92	66.96	68.24	69.45	69.50

	6 P.M.	6 A.M.	4 P.M.	8 P.M.	10 A.M.	12 Noon	8 A.M.	4 A.M.	2 P.M.	2 A.M.	10 P.M.	12 Mid night
Males & Females	59.03	60.76	60.90	61.69	62.48	62.87	63.09	65.83	65.91	66.04	66.05	67.36

N.B: Values grouped together by a common underline are not significantly different from each other at 5% level of significance.

Table 4

Time course of oxygen consumption in the fresh water field crab Oziotelphusa senex senex when transferred to 50% sea water. Values are expressed as $\mu l/O_2/g/h$. Temp: 26±1°C (Figures in paranthesis denote number of individuals)

Control	50% S.W.									
(F.W.)	1 h	2 h	3 h	5 h	8 h	12 h	24 h	48 h	72 h	96 h
Mean 50.38	41.79	40.91	40.31	38.25	37.92	38.62	34.99	35.01	33.15	31.60
S.D. ± 7.83	5.56	4.23	5.56	3.94	3.16	4.05	2.23	3.16	2.38	4.01
(12)	(12)	(12)	(12)	(12)	(12)	(12)	(12)	(12)	(12)	(12)
% d	-17.05	-18.79	-19.98	-24.07	-24.73	-23.34	-30.54	-30.50	-34.20	-37.27

120 h	144 h	168 h	192 h	216 h	240 h	264 h	288 h	312 h	336 h	360 h
30.15	31.01	29.90	28.99	26.66	27.54	27.28	27.25	27.16	26.94	27.09
3.80	4.68	4.05	4.11	3.48	4.01	4.62	6.38	5.84	5.54	4.44
(12)	(12)	(10)	(10)	(12)	(12)	(12)	(12)	(12)	(12)	(12)
-40.15	-38.44	-40.65	-42.45	-47.08	-45.33	-45.85	-45.91	-46.08	-46.52	-46.22

Table 4-1

Variance ratio for data given in table 4

Whole animal (or) Tissue	Variance ratio (F)		Critical difference		
	F Cal	F tab at 5% level	5% level	1% level	0.1% level
Whole animal	45.53	1.70	2.78	3.97	5.13

Table 4-Z

Statistical comparison of the data presented in table 4. Mean values arranged in ascending order.

216 h	336 h	360 h	312 h	288 h	264 h	240 h	192 h	168 h	120 h	144 h	96 h	72 h	24 h	48 h	8 h	5 h	12 h	3 h	2 h	1 h	F.W.
26.66	26.94	27.09	27.16	27.25	27.28	27.54	28.99	29.90	30.15	31.01	31.60	33.15	34.99	35.01	37.92	38.25	38.62	40.31	40.91	41.79	50.38

N.B: Values grouped together by a common underline do not differ significantly amongst themselves at 5% level of significance.

Table 5

Time course of oxygen consumption in the fresh water crab Oziotelphusa (Paratelphusa) sp. during reverse acclimation i.e., from 50% S.W. to F.W. Values are expressed as μ l of oxygen/gram/hour. Temp: 26±1°C. (Figures in paranthesis denote number of individuals).

	Control Value (F.W.)	50% S.W. acclimated value (360 h)	Reversal to F.W. from 50% S.W.												
			1 h	2 h	3 h	5 h	8 h	12 h	24 h	48 h	72 h	96 h	120 h	144 h	168 h
Mean values	50.38	27.09	33.55	36.09	36.05	38.79	41.38	41.79	43.68	45.37	47.71	48.68	49.26	49.44	50.24
S.D. ±	7.83	4.44	5.51	5.85	4.47	4.57	5.53	5.01	4.83	5.32	7.60	8.01	7.79	7.78	7.66
	(12)	(12)	(11)	(12)	(11)	(12)	(12)	(12)	(12)	(12)	(12)	(12)	(12)	(10)	(10)
% d		-46.22	-33.41	-28.36	-28.44	-23.01	-17.86	-17.05	-13.29	-9.94	-5.29	-3.37	-2.22	-1.86	-0.27

Table 5-1

Variance ratio for data given in table 5

	Variance ratio (F)		Critical difference		
	F cal	F tab (5% level)	5% level	1% level	0.1% level
Whole animal	19.43	1.70	4.18	5.98	7.73

Table 5-2

Statistical comparison of the data presented in table 5. Mean values are arranged in ascending order.

360 h 50% SS	1 h	2 h	3 h	5 h	8 h	12 h	24 h	48 h	72 h	96 h	120 h	144 h	168 h	F.W.
27.09	33.55	36.05	36.09	38.79	41.38	41.79	43.68	45.37	47.71	48.68	49.26	49.44	50.24	50.38

N.B: Values grouped together by a common underline are not significantly different amongst themselves at 5% level of significance. S.S: Salinity Stress

Table 6

Time course of oxygen consumption in the fresh water field crab Oziotelphusa (Paratelphusa) senex senex (Fabricius) when transferred directly to 100% SW. Values are expressed as µl O_2/g/h. Temp: 26±1°C. Mean values are for 8 individuals.

Control (Normal) (F.W.)	100% S.W.											
	1 h	2 h	3 h	5 h	8 h	12 h	24 h	48 h	72 h	96 h	120 h	144 h
Mean 50.87	54.58	62.33	64.00	65.81	69.35	65.03	62.66	60.84	48.61	43.46	40.69	37.71
S.D. ± 8.58	8.69	7.85	6.83	7.01	7.02	5.05	7.63	4.12	6.24	5.30	3.62	2.54
% d	+7.29	+22.52	+25.81	+29.36	+36.32	+27.83	+23.17	+19.59	-4.44	-14.56	-20.01	-25.86

168 h	192 h	216 h	240 h	264 h	288 h	312 h	336 h
36.15	34.34	32.87	31.67	29.68	26.39	24.40	23.78
3.49	4.07	2.49	3.03	2.30	3.35	2.09	2.91
-28.93	-32.49	-35.38	-37.74	-41.65	-48.12	-52.03	-53.25

Table 6-1

Variance ratio for the data given in table 6

Tissue (or) whole animal	Variance ratio (F)		Critical difference		
	F cal	F tab (5% level)	5% level	1% level	0.1% level
Whole animal	249.30	2.02	4.30	5.66	7.46

Table 6-2

Statistical comparison of the data presented in table 6. Mean values arranged in ascending order.

336 h	312 h	288 h	264 h	240 h	216 h	192 h	168 h	144 h	120 h	96 h	72 h	Normal F.W.	1 h	48 h	2 h	24 h	3 h	12 h	5 h	8 h
23.78	24.40	26.39	29.68	31.67	32.87	34.34	36.15	37.71	40.69	43.46	48.61	50.87	54.58	60.84	62.33	62.66	64.00	65.03	65.81	69.35

N.B: Values grouped together by a common underline do not significantly differ amongst them at 5% level of significance.

Table 7

Time course of oxygen consumption in the fresh water crab Oziotelphusa (Paratelphusa) sp. during reverse acclimation i.e. from 100% SW to FW. Values are expressed as μl O_2 / g / h. Temp: 26±1°C. (Mean values are for 8 individuals). Size of crabs ranged from 20-40 grams.

	Control (Normal) (F.W.)	100% S.W. acclimated (336 h)	Reversal to F.W. from 100% S.W.											
			1 h	2 h	3 h	5 h	8 h	12 h	24 h	48 h	72 h	96 h	120 h	144 h	168 h
Mean values	50.87	23.78	26.75	32.52	34.07	40.71	52.66	53.95	54.25	51.96	49.53	49.24	48.25	48.34	48.07
S.D. ± (n)	8.58	2.91	4.04	5.62	8.08	8.57	5.61	4.07	5.05	6.94	4.99	4.84	4.49	3.80	3.18
% d		-53.25	-47.41	-36.07	-33.02	-19.97	+3.52	+6.05	+6.64	+2.14	-2.63	-3.20	-5.15	-4.97	-5.46

Table 7-1

Variance ratio for the data **given in table 7**

Tissue (or) whole animal	Variance ratio (F)		Crital difference		
	F cal	F tab (5% level)	5% level	1% level	0.1% level
Whole animal	52.42	2.10	4.98	6.67	9.01

Table 7-2

Statistical comparison of the data presented in table 7. Mean values arranged in an ascending order.

336 h 100% SS	1 h	2 h	3 h	5 h	168 h	120 h	144 h	96 h	72 h	(F.W.) Normal	48 h	8 h	12 h	24 h
23.78	26.75	32.52	34.07	40.71	48.07	48.25	48.34	49.24	49.53	50.87	51.96	52.66	53.93	54.25

N.B:- Values grouped together by a common underline are not significantly different from each other at 5% level of significance. S.S : Salinity Stress

CHAPTER II

INORGANIC OSMOTIC EFFECTORS

Introduction

When aquatic organisms encounter changing salinities, genetic mechanisms should enable them to tolerate a range of salinity variations. This range may be a limited one (stenohaline) or a wider one (euryhaline). In otherwards, the capacity of an organism to tolerate changed osmotic situations depends on its hereditary potentiality. It is this hereditary capacity for physiological adjustment that we often try to understand in our laboratories, quite often overlooking such aspects of heredity!

Several aquatic animals have evolved abilities to adapt to changed external osmotic conditions through toleration of concomitant changes of osmotic constituency of milieu interieur or through a regulation of this osmotic composition to reasonable levels of constancy.

Studies on aquatic crustaceans have thrown considerable light on osmoregulatory problems and their solutions. Crustaceans are mostly aquatic. Although most of them are marine, "crustaceans can be found in any body of natural waters from soft freshwater to crystallizing brine". As such, we find in this group members having remarkably different degrees of tolerance to a variety of osmotic conditions. Some are confined

to the oceans which are called stenohaline, marine animals. Others are confined to freshwater and they are called stenohaline, freshwater forms. Many species called euryhaline can cope with a broad range of salinities. Hence, their distribution ranges from oceans to estuaries and rivers. A few crustaceans like the brine shrimp can survive extreme environments from freshwater to saturated NaCl. The crab Oziotelphusa (Paratelphusa) senex senex selected for the present studies is confined to freshwaters and has not been reported from estuaries or seas. But, it is a euryhaline animal and can tolerate higher salinities including 100% SW (Ramamurthi, 1962, 1965; Venkata Reddy, 1976).

Early investigations of the mechanisms of ion transport were done in the laboratories of August Krogh in Copenhagen (1937, 1938, 1939). One of his students, Hans Ussing, continued these investigations, using isolated frog skin for studying active Na^+ transport (1947, 1949, 1952) and the model of Na^+ transport suggested by him (1960) has thrown much light on these mechanisms. A number of inverstigators have described the osmotic and ionic regulative capacities of aquatic animals with particular reference to crustaceans and fishes (Ramsay, 1954; Robertson, 1957, 1960; Beadle, 1957; Shaw, 1960b; Prosser and Brown, 1961; Lockwood, 1962; Krogh, 1964; Gross, 1964; Potts and Parry, 1964b; Scheer, 1965; Huggins and Munday, 1968;

Hickman and Trump, 1969; Schoffeniels and Gilles, 1970; Riegel, 1972; Schmidt-Nielsen, 1972; Prosser, 1973; Potts, 1976; Schoffeniels, 1976; Evans, 1977; Kirschner, 1977, 1979; Gilles and Jeuniaux, 1979). Beadle stated that in marine crabs "there are atleast two types of active processes at work, in the gills and in the extretory organs, which are responsible for the ionic imbalance between blood and sea water". He felt that "adjustments in the rates of these processes could have led to the evolution of both hypo- and hyper-osmotic regulation".

Mechanisms of osmotic regulation of extracellular fluid in relation to external salinity, and water and ion fluxes through the gills and excretory organs have been studied extensively by several investigators (Robertson, 1949, 1953; Gross, 1957a, 1958, 1959, 1964; Croghan, 1958a, 1958b; 1958c; Prosser, Green and Chow, 1955; Maetz, 1956, 1969, 1973, 1974; Dahnel, 1962, 1965; House and Green, 1965; Biber et al, 1965; Motais et al, 1966; Evans, 1967, 1969, 1973; Kerstetter et al, 1970, 1972, 1974; Geddes, 1975a, 1975b; Hunter and Rudy, 1975; Baldwin and Kirschner, 1976; Evans, Cooper and Bogan, 1976; Kristensen, 1972, 1973; Garcia Romeu et al, 1969; Kirschner, 1955, 1970; Hickman, 1968a, 1968b; Schmidt-Nielsen et al, 1968; Smith, 1969, 1976; Potts and Parry, 1976; Pequeux and Gilles, 1977; Romero and Zanders, 1980; Sharp et al, 1980; Castille and Lawrence, 1981a, b; Gupta and Hanke, 1981). Osmotic

regulation of marine, estuarine and brackish water crustaceans has been explored extensively (Schwabe, 1933; Robertson, 1957; Shaw, 1958a, 1958b, 1961a; Croghan, 1958a, 1958b, 1958c; Lockwood, 1962; Schmidt-Nielsen and Laws, 1963; Potts and Parry, 1964b; Davenport, 1972; Prosser, 1973; Cameron, 1978; Cornell, 1979, 1980; Sabourin and Stickle, 1980; Neufeld et al, 1980; Castille and Lawrence, 1981a, b). Osmotic and ionic regulation in freshwater crustaceans has been extensively studied (Schwabe, 1933; Beadle and Cragg, 1940; Shaw, 1959a, 1959b, 1959c, 1960a, 1961b; Lockwood, 1959; Parry, 1955, 1957, Bryan, 1960a, b, c; Ramamurthi, 1962, 1965, 1967; Horne, 1966, 1967, 1968; Kerly and Pritchard, 1967; Sutcliffe, 1967a, 1967b; 1968; Thomson, 1970; Venkata Reddy, 1976; Greenaway, 1981; Venkatachari and Vasantha, 1981).

From the studies of various investigators the following general conclusions can be drawn: Crustaceans exhibit a variety of responses to osmotic stress. These responses range from osmoconforming to hyper- and hypo-osmotic regulation. Most marine crustaceans are osmoconformers and their hemolymph concentrations of Na^+ and Cl^- and total osmotic pressure are the same as in sea water. There are no diffusive flows across the body surface. They are unable to maintain a relatively constant extracellular fluid whenever they face changes in the medium. Their cells also fail to function when the extracellular fluid concentration changes. Maja verrucose is a good example

(Schwabe, 1933; Potts and Parry, 1964b and Prosser, 1973). Pugettia producta, Libinia emarginate (Cornell, 1979, 1980) are some other examples of this type. The limiting factor in these forms is lack of an active ion transport system although the antennal glands handle an increased water load when these animals are transferred to diluted media (Cornell, 1980). Many species of crustaceans , however, have developed the ability to survive in more diluted media. They are able to maintain hemolymph concentrations above that of diluted salinities and at levels compatible with normal tissue functions. In diluted media these animals face diffusive loss of solute and osmotic influx of water. In order to maintain a constant composition of the body fluids, they must have homeostatic osmoregulatory mechanisms which require the expenditure of energy. Some of them are weak regulators such as many estuarine and shore crabs that include carcinus maenas (Schwabe, 1933; Shaw, 1961a) and cancer magister (Jones, 1941; Hunter and Rudy, 1975). In these two species there is a substantial reduction in osmotic permeability of body surface (Posm) but salt permeability (PNa) remains within the range of osmoconformers. They tolerate dilute media to some extent (60%-40% SW). Further dilution brings about reduction of body fluid concentration which is more or less parallel with the isosmotic line. This shows a

breakdown in osmoregulatory capacity but it also brings down the diffusion gradients, and prevents diffusive leaks. This is no doubt an evasive mechanism but is adaptive to the extent of ECF dilution being compatible with tissue function.

Another group has been called strong regulators. Their representatives are found over a complete range of environments from sea water to freshwater. In sea water they are isosmotic and isoionic and in dilute media they are hyper regulators, and maintain their ECF NaCl concentration far above that of ambient medium. Callinectes sapidus, (Cameron, 1978) Eriocheir sinensis (Shaw, 1961b), are two good examples of this type. The amphipod Gammarus duebeni also shows the same pattern (Shaw and Sutcliffe, 1961; Sutcliffe, 1967a). These forms have a relatively high NaCl concentration in the blood and the urine is isosmotic and with blood in lower salinities. A few strong regulators are confined to freshwater and have lost the ability to live in sea water. Oziotelphusa (Paratelphusa) and Pseudothelphusa jouyi belong to this category. Whereas the latter is known to survive only upto 80% SW (Thompson, 1970) the former which is under study survives direct transfer from FW to 100% SW for 15-20 days (Venkata Reddy, 1976) but not for longer periods. Potamon niloticus

(Shaw, 1959c) the east African freshwater crab is unable to live in media above 50% SW. The crayfish Astacus fluviatilis (Schwabe, 1933; Bryan, 1960a) some amphipods like Gammarus pulex (Lockwood, 1961) can only live in dilute media but cannot live in SW.

The mechanisms involved in hyper-osmoregulation may be summarised thus: Efficient cell functioning demands a relatively constant composition of the ECF. In most hyperosmotic regulators the ECF concentration is usually maintained more or less constant and above that of ambient. ECF of animals is separated from the external medium by epithelia that are usually permeable to both water and solutes which is a complicated situation. If a steady state is to be maintained the passive influx of water in dilute media has to be balanced by some active mechanism that should move water at equal rate but in the opposite direction. The same mechanism must hold good for ions. While osmoconformers and a few osmoregulators adopt evasive mechanisms to minimize diffusive flows by lowering or elevating the body fluid concentrations in line with that of the external medium, most hyper- and hypo-regulators adopt what are known as compensatory mechanisms to solve the problem. These mechanisms generate flows of

ions and water against concentration gradients making use of energy. These mechanisms have been studied intensively during the past 40 years. Adaptation to freshwater seems to have proceeded by two mainstages: (1) a slight reduction of salt level in the body fluid and reduction of permeable body surface (constituting evasive methods) and (2) active absorption of ions from the medium and evolution of renal salt reabsorption and excretion of dilute urine (compensatory mechanisms) (Beadle and Cragg, 1940). The cray fish maintains a constant composition of the body fluid by active sodium uptake via the gills (Krogh, 1939; Shaw, 1960a,b) production of copious hypotonic urine (Riegel and Kirschner, 1967; Riegel, 1972) and by some reduction in membrane permeability (Gross, 1957a). The role of antennary glands in hyperosmotic regulation in Pachygrapsus crassipes, has been suggested (Prosser, Green and Chow, 1955).

Isosmotic regulation of the intracellular fluids is an important aspect of osmoregulation in animals (Florkin, 1962). Cell volume regulation under conditions of changing salinities seems to be a closely related phenomenon. The capacity to maintain a relatively constant cell volume seems to be one of the most remarkable properties of living cells

(cf: Rorive and Gilles, 1979). Water and the major inorganic osmotic effectors of cells Viz: K^+ , Na^+ and Cl^- and the major intracellular organic osmotic effectors viz: the free amino acids are known to play an important role in cell volume control. Animal cell membranes are largely permeable to water, major inorganic ions and also larger molecules such as the free amino acids. When living cells are exposed to hypotonic media, a rapid increase in cell volume occurs due to the osmotic entry of water but the cell volume is regulated back to the normal level in a rather short time (Gilles, 1973a, 1974a; Schröck and Hanke, 1979a, 1979b). During this volume regulation phase ions like Na^+ , K^+ and Cl^- and even larger molecules, free amino acids, seem to leakout of the cells, depending on the cell types studied (Schmidt-Nielsen, 1975; Hoffmann, 1977). When living cells are exposed to hypertonic media, the cells respond by a rapid shrinkage due to osmotic loss of water and cell-volume regulation has not been observed in most cases involving in vitro studies. But, in vivo experiments suggest cell volume adjustment after prolonged exposure to hypertonic media (Gilles, 1973a, 1974a; Schröck and Hanke, 1979a, 1979b; Abo Hegab and Hanke, 1982). Animals seem to regulate cell volume in hypertonic media by an augmentation of the intracellular free amino acid content, while regulating the

entry of inorganic ions. Literature on cell volume regulation and associated mechanisms of water and ionic movements is very extensive and has been reviewed by several investigators (Kleinzeller, 1972; House, 1974; Schmidt - Nielsen, 1975, 1977; Hoffmann, 1977; Rorive and Gilles, 1979). The free amino acids have been implicated in cell volume regulation under conditions of salinity stress (Lang and Gainer, 1969; Gerard and Gilles, 1972a, 1972b; Gerard, 1975; Hoffmann and Hendil, 1976; Gilles, 1973a, 1974a, 1979; Schrock and Hanke, 1979a,b).

Some aspects of osmotic and ionic regulation of the euryhaline fresh water crab Oziotelphusa (Paratelphusa) senex senex have been studied (Ramamurthi, 1962, 1965; Venkata Reddy, 1976). However, these studies do not include osmotic and ionic changes during the period of acclimation. Either the immediate responses to osmotic stress (Ramamurthi, 1962, 1965) or changes after complete acclimation (15 days) (Venkata Reddy, 1976) were studied but not changes during acclimation. The patterns of change during reverse acclimation are not known. The purpose of the present study is to fill this gap atleast to some extent, if not fully. Time course of change in water content and ionic composition during the period of acclimation

to 50% and 100% SW and also in the reverse acclimation process has been studied and dealt with in this chapter. The problem of osmoregulation is closely linked with regulation of major ionic constituents of body fluids. Since NaCl is the main salt common to both the animal body fluids and all natural waters, attention has been paid only to these two major ions viz., Na^+ and Cl^- that constitute the two major inorganic osmotic effectors of body fluids.

Materials and Methods

Maintenance of crabs and method of their acclimation to SW and method of reverse acclimation are given in the section on "Materials and Methods". Procedures for the estimation of Na^+ and Cl^- in blood and tissues are also described therein. The method of statistical treatment of the data, the mode of calculation of ion concentration of the intracellular fluid are also described in the same section.

Time course of changes in water content

RESULTS

Tables 8 and 9 and figures 11 and 12 show the time course of changes in water content in tissues. Variance ratios and statistical comparison of the data on water content in tissues are presented in Table 8-1, 8-2. 9-1 and 9-2. The values indicate that on exposure of whole animals to SW the tissues were dehydrated. The degree of dehydration was different in the two tissues and in different salinities and at different periods of acclimation. On reversal to freshwater the tissues rapidly regained water.

Water loss was greater in both tissues in 100% SW than in 50% SW. In both the salinities the hepatopancreas lost more water than the muscle and it also lost water more rapidly than the muscle. In the reverse acclimation it gained water more slowly compared to the muscle. The loss of water in hepatopancreas was significantly high from 24 hours to 360 hours after transfer to both salinities and the peak value was recorded at 72 hours in 50% SW and at 168 hours in 100% SW ($P < 0.001$). In 50% SW there seemed to be some compensation for water loss at the end of acclimation period but it was not complete. The value at the end of the acclimation period was significantly lower compared to

FIGS. 11 & 12: Time course of changes in the water content of tissues in the crab, OZIOTELPHUSA.

FIG. 11

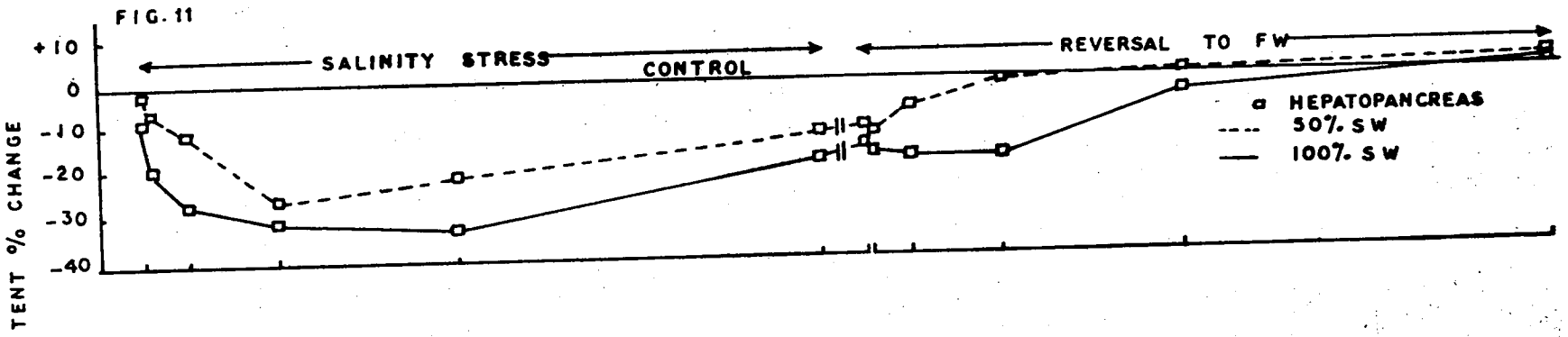
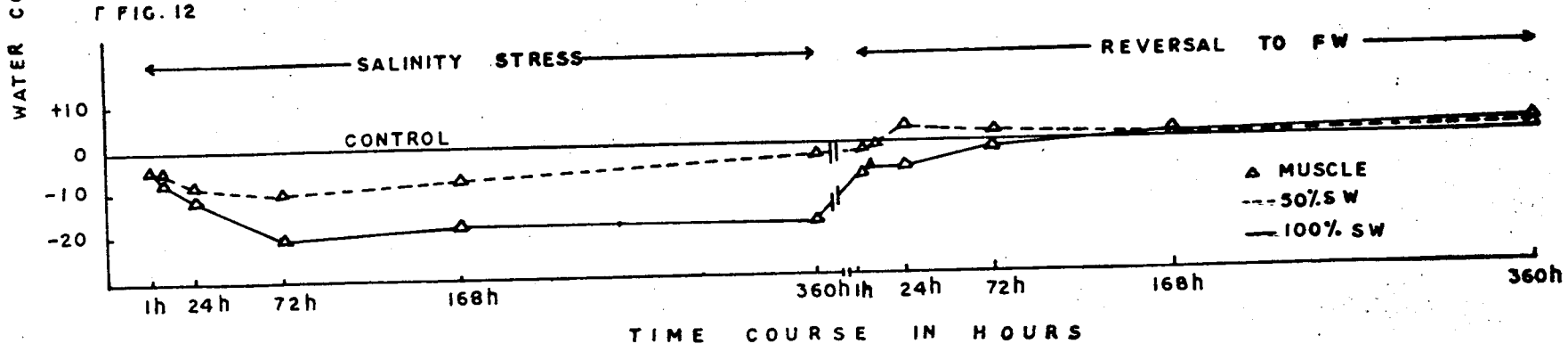

SALINITY STRESS
CONTROL
REVERSAL TO FW
+10
0
-10
-20
-30
-40
WATER CONTENT % CHANGE
HEPATOPANCREAS
50% SW
100% SW

FIG. 12


SALINITY STRESS
REVERSAL TO FW
CONTROL
+10
0
-10
-20
WATER CONTENT % CHANGE
MUSCLE
50% SW
100% SW
1h
24h
72h
168h
360h
1h
24h
72h
168h
360h
TIME COURSE IN HOURS

to normal ($P < 0.05$). In 100% SW there was no compensation whatsoever even after 360 hours and dehydration of hepatopancreas continued to be highly significant ($P < 0.001$). During the reverse acclimation, the water content in hepatopancreas rose to significantly higher levels by 72 hours in 50% SW acclimated crabs and by 168 hours in 100% SW exposed ones and thereafter the values were not significantly different from those of normal crabs. In other words the water content returned to normal levels more rapidly in 50% SW acclimated crabs than in 100% SW exposed animals during reverse acclimation.

The loss of water in muscle was significantly high between 24 and 168 hours in 50% SW acclimated animals and between 5 and 360 hours in 100% SW exposed ones ($P < 0.05$). The peak values were obtained at 72 hours in both salinities ($P < 0.01$ in 50%SW and $P < 0.001$ in 100%SW). Whereas there was full compensation for water loss at the end of acclimation period in 50%SW acclimated crabs, there was no compensation whatsoever in 100% SW. In the reverse acclimation process water content in the muscle did not increase significantly in 50%SW acclimated crabs but in 100%SW exposed animals there was a significant increase by 72 hours and thereupon the normal level was attained.

Discussion

Dehydration of tissues was observed in response to osmotic shocks (50% & 100% SW) to which the crabs were subjected. Such abrupt changes may not occur in nature in the lives of crabs. However, these crabs live in burrows partially immersed in water. The water in these burrows is subjected to extreme evaporative loss during summer with the result the ionic concentration may increase to greater levels. The crabs in nature must have evolved mechanisms to cope with this seasonal increase of ionic concentration of the ambient medium. Water loss observed in the present investigation may reflect the extent to which these animals have evolved adaptive mechanisms to tide over dehydrative conditions seasonally imposed on them. It is worth mentioning here that acclimation of the crabs from FW to SW leads to considerable decrease in water content of both tissues and return to normal levels is not achieved even after 15 days except in muscle in 50% SW. Whereas, during reverse acclimation to FW, tissue hydration resumes its normal value relatively quickly i.e., within 24 hours in 50% SW acclimated crabs and 72 or 168 hours in 100% SW exposed animals. This shows that on transfer to higher salinities the tissues lose water and probably shrink and there appears to be no cell volume control for a long period of time except in the

muscle of 50% SW acclimated crabs, whereas on transfer to freshwater i.e., hypo-osmotic medium, the tissues gain water quickly and consequently there is regulation of cell volume. This aspect gains greater significance in the understanding of osmoregulatory problems in the context of invasion of brackish and freshwaters by marine organisms. Acclimation of Eriocheir sinensis from SW to FW shows a transient increase in the muscle water content, but the tissue water level resumes its control value within the first day. On the contrary when the same crab is transferred from FW to SW the muscle tissue shrinks and resumes its control value more slowly (Gilles, 1973a, 1974a). Similar observations were made by several investigators for a variety of tissues and cell types (Fugelli, 1967; Kregenow, 1971a; Hendil and Hoffmann, 1974; Schrock & Hanke, 1979a,b). Lang and Gainer (1969) showed such a behaviour in the isolated muscle fibres of the crabs Callinectes sapidus. Isolated axons of C. sapidus and E. sinensis behave in the same way (Gerard and Gilles, 1972b; Gilles, 1973a). Water content and volume regulation of cells are closely related phenomena. In most of the tissues and cell types studied (Loc. cit) volume regulation in hypo-osmotic conditions has been reported to occur quickly while no volume control was observed in hyper-osmotic media. The results obtained in the present

work on water content in tissues agree with this general observation. The absence of regulation may be due to the amplitude of the osmotic shock. For an animal whose natural habitat is FW, 50% and 100% SW may be too large an osmotic shock that it fails to regulate its tissue water content in these salinities. Compensation of the water content in muscle in 50% SW seems to be an exception and this may have some significance. The crabs acclimated to 50% SW appear to be normal in their locomotory and feeding behaviour whereas crabs acclimated to 100% SW become lethargic without any escape reactions after 3 days of transfer, and they generally 'refuse' to feed. The dehydration of muscle in 100% SW without compensation may be one of several causes responsible for the inactivity of the animal in that medium. Muscle shows regulation of water content in 50% SW. This may be one of the reasons for the normal activity of the crab in 50% SW. However, it may be added that the hepato-pancreas fails to regulate its water content in 50% and 100% SW and the muscle in 100% SW. Hence, it may not be wrong to infer that Oziotelphusa fails to invoke compensatory method of osmoregulation in 100% SW but invokes such a method only partially in 50% SW. The crab seems to depend on evasive mechanisms such as reduction of osmotic gradients in higher salinities like the osmoconformers (See forthcoming account). Steep decrease in oxygen consumption of crabs seen in both the salinities

(Figure 5) strengthens this view. On the otherhand the oxygen consumption of crabs transferred to FW from SW (reverse acclimation) increases rather rather quickly to normal levels (Figure 5) reflecting the immediate operation of compensatory mechanisms. Sojourn of these animals in SW for 15 days does not seem to have caused any irreparable damage in those animals that survived 100% salinity stress. Another aspect worth remembering is the changes in ionic concentration. Loss of water in tissues of crabs acclimated to higher salinities should bring about increased ionic composition and hydration of tissues in FW (during reverse acclimation) should lead to decreased ionic concentration, although the changes observed in ionic concentration need not entirely be due to water fluxes. Changes in ionic composition may also be due to active or passive movement of ions into and/or outside the tissue and this aspect will be considered separately.

Ionic composition of blood

Results

Blood sodium

Data on time course of changes in Na^+ concentration are presented in tables 10 and 11. Variance ratios are given in tables 10-1 and 11-1. Statistical comparison of the data is presented in tables 10-2 and 11-2. The values are plotted in figure 13a as percentage deviations from the normal.

It is clear from the data obtained that Na^+ concentration in the blood of crabs exposed to 50% and 100% SW increased gradually and reached maximum levels at the end of acclimation period (360 hours) and on return to FW, the levels fell steeply but attained normal levels only after 168 hours. In 50% SW a significantly higher level of Na^+ was seen only after 72 hours of transfer ($P < 0.01$) but in 100% SW a significant ($P < 0.05$) increase was observed even after 1 hour of transfer. In 50% SW a further significant increase was evident only between 168 and 360 hours but in 100% SW highly significant ($P < 0.001$) increases were apparent at all other stages studied viz., 5, 24, 72, 168 and 360 hours. Whereas the maximum increase in 50% SW was only 27.37%, it was only 27.37%, it was 107.93% in 100% SW.

FIGS. 13a & 13b: Time course of changes in sodium ion content in the blood and tissues of the crab when exposed to 50% & 100% SW and then returned to FW.

FIG. 13 a

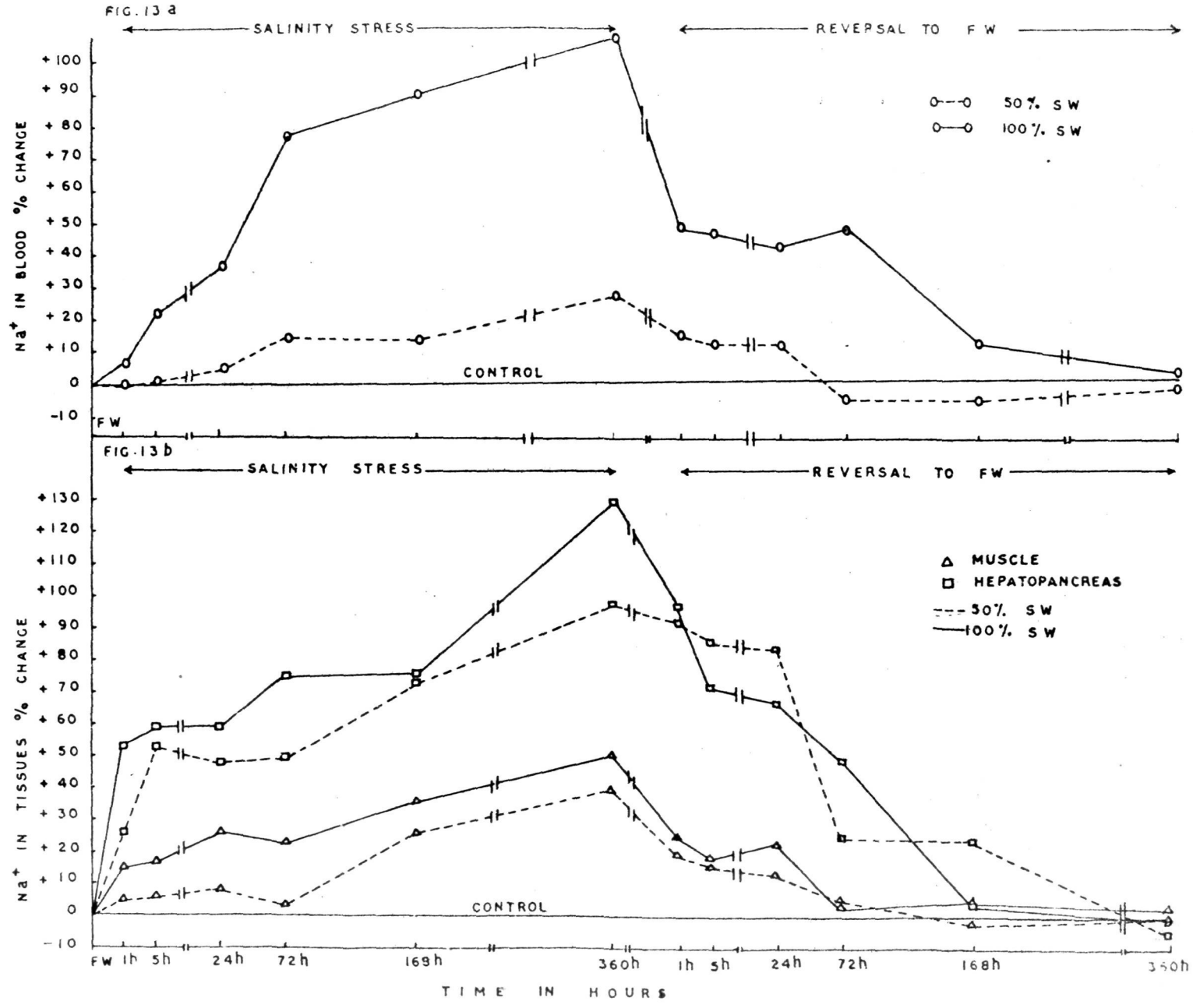


FIG. 13 b

During reverse acclimation to FW, Na^+ concentration of blood fell significantly after 1 hour in both 50% and 100% SW exposed crabs. This decreasing trend continued significantly upto 168 hours in 50% SW acclimated ones. The Na^+ level decreased significantly below the normal level between 72 and 168 hours as if there was a leakage and subsequently rose to normal level. In 100% SW exposed crabs Na^+ concentration of blood fell steeply during reverse acclimation. The decreasing values obtained after 1, 24, 72 and 168 hours were significantly different from each other and also from the normal values. However, the normal level was reached by about 360 hours.

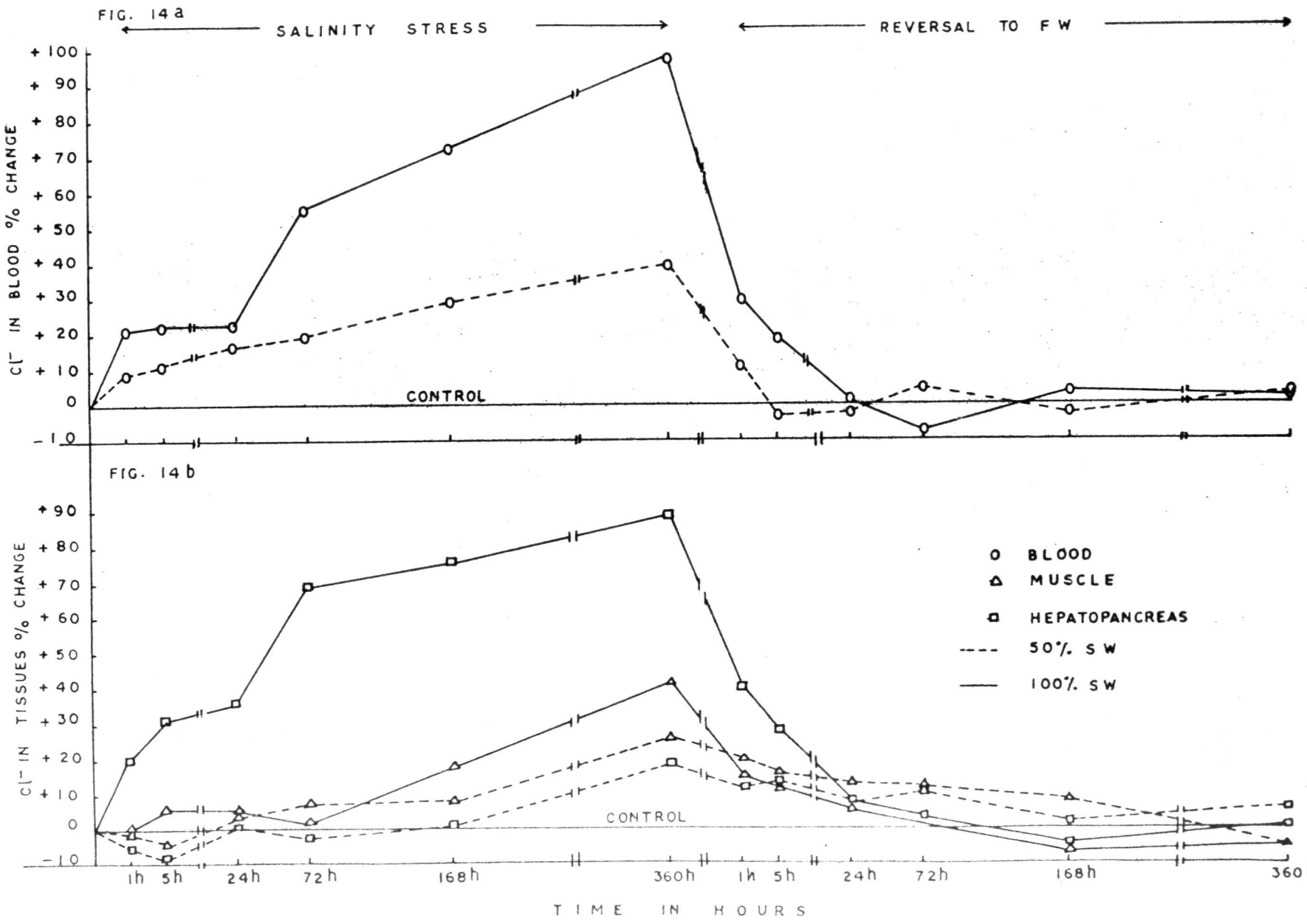
Blood chloride

Tables 13 and 14 show data on blood chloride levels in 50% and 100% SW acclimated crabs. Variance ratios are shown in tables 13-1 and 14-1. Statistical comparison of the data is given in tables 13-2 and 14-2. The values are plotted as percentage deviations from the normal (figure 14a).

There was an increasing trend in the Cl^- concentration of blood in both salinities studied and maximum levels were attained at the end of 360 hours (39.06% in 50% SW and 97.5% in 100% SW). In the reverse acclimation to FW, there was sudden and steep fall (10.8% and 29.37% in 50% and 100% SW exposed

FIGS. 14a & 14b: Time course of changes in chloride ion content in the blood and tissues of the crab when exposed to 50% & 100% SW and then returned to FW.

FIG. 14 a


SALINITY STRESS
REVERSAL TO F W
Cl⁻ IN BLOOD % CHANGE
+ 100
+ 90
+ 80
+ 70
+ 60
+ 50
+ 40
+ 30
+ 20
+ 10
0
- 10
CONTROL
FIG. 14 b
Cl⁻ IN TISSUES % CHANGE
+ 90
+ 80
+ 70
+ 60
+ 50
+ 40
+ 30
+ 20
+ 10
0
- 10
CONTROL
BLOOD
MUSCLE
HEPATOPANCREAS
50% SW
100% SW
1h 5h 24h 72h 168h 360h 1h 5h 24h 72h 168h 360h
TIME IN HOURS

crabs respectively). In both salinities significantly higher levels were obtained even after 1 hour of transfer to SW and further highly significant ($P < 0.001$) increases were recorded after 24, 168 and 360 hours in 50% SW, and after 24, 72, 168 and 360 hours in 100% SW. The amplitude of change was far greater in 100% SW than in 50% SW. In the reverse acclimation there was a steep fall by the first hour in the Cl^- of blood of 50% and 100% SW exposed crabs. Further significant decreases were recorded at the end of 5 hours in 50% SW acclimated crabs and at the end of 24 hours in 100% SW exposed ones. The levels of Cl^- attained by the 5th hour in 50% SW acclimated crabs and by the 24th hour in 100% SW exposed crabs were not significantly different from those of normal crabs. In otherwords Cl^- levels of blood returned rapidly to normal levels in the reverse acclimation from SW to FW.

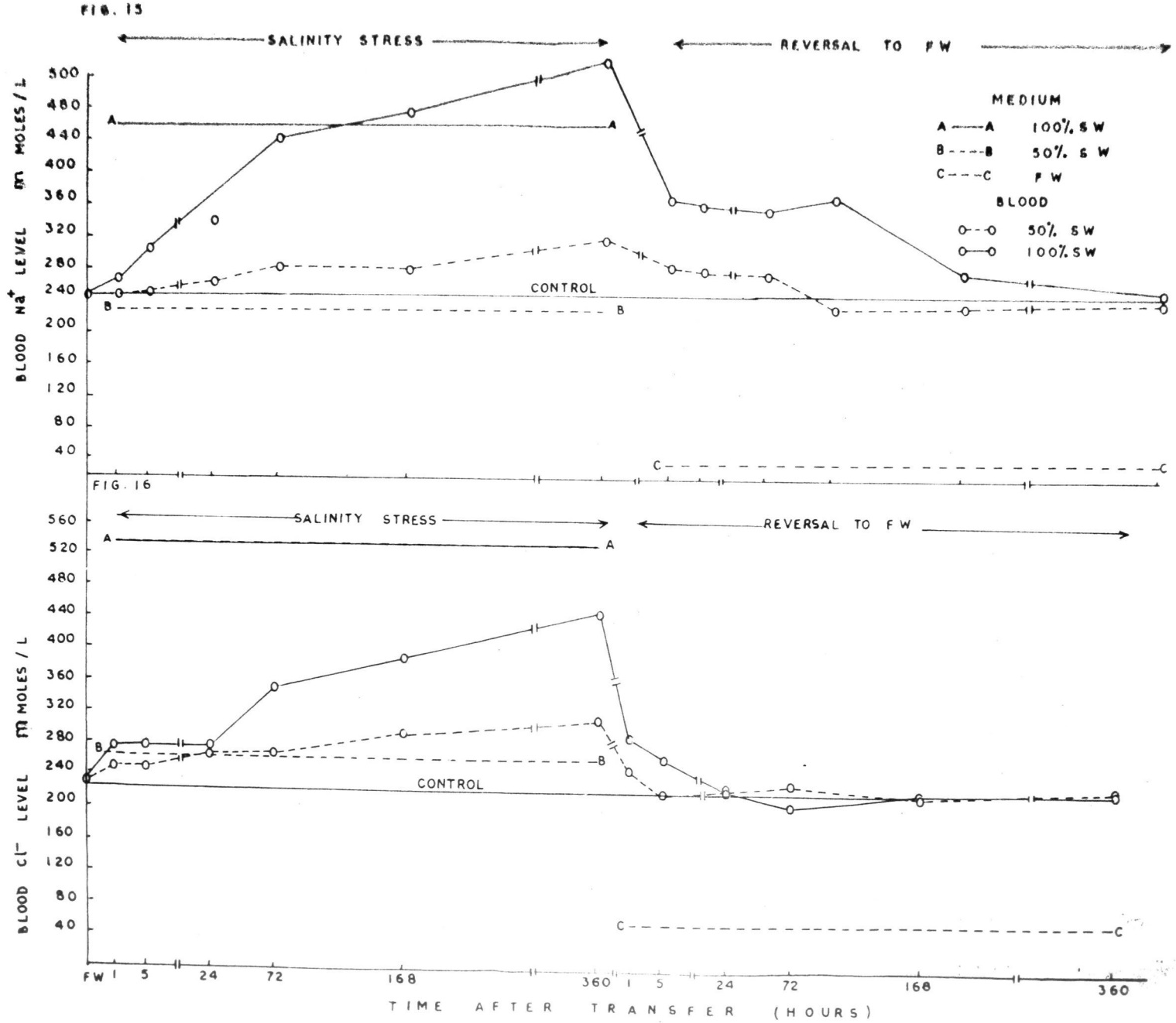
Discussion

Levels of ions in relation to ambient medium

The major inorganic ions of blood viz., Na^+ and Cl^- were elevated to very high levels in 100% SW whereas the increase was not so marked in 50% SW. Figure 15 shows time course of Na^+ levels of blood in relation to ambient medium.

FIGS. 15 & 16: Comparison of the blood Na^+ & Cl^- levels with the medium levels during the period of exposure of the crab to 50% & 100% sea water (SW) and during the reverse acclimation to fresh water (FW).

FIG. 15


SALINITY STRESS
REVERSAL TO FW
MEDIUM
A—A 100% SW
B---B 50% SW
C---C FW
BLOOD
50% SW
100% SW
CONTROL
BLOOD Na+ LEVEL m MOLES / L
500
480
440
400
360
320
280
240
200
160
120
80
40
FIG. 16
SALINITY STRESS
REVERSAL TO FW
560
520
480
440
400
360
320
280
240
200
160
120
80
40
BLOOD Cl- LEVEL m MOLES / L
CONTROL
FW 1 5 24 72 168 360 1 5 24 72 168 360
TIME AFTER TRANSFER (HOURS)

It may be noted that the blood sodium level in 50% SW was more or less iso-ionic with the medium upto 24 hours of acclimation. Subsequently the ion concentration increased to higher levels showing some amount of hyper-ionic regulation. On reversal to FW the ion concentration dropped to normal level only after 72 hours. In 100% SW the Na^+ ion increased steeply and an iso-ionic condition was reached and maintained between 72 and 168 hours (444.9 & 476.4 m moles/litre respectively). Subsequently, the Na^+ ion level increased over that of 100% SW. During this period, the crab strangely seems to show some amount of hyper-ionic regulation. On transfer to FW the Na^+ level fell steeply in the first hour and a further significant decline was recorded 168 hours and then slowly came down to normal level by 360 hours. Still the sodium ion composition of blood was far higher than that of FW. The blood Cl^- levels in relation to ambient medium are shown in figure 16. In 50% SW, the blood Cl^- ion level increased initially and was maintained around that of 50% SW i.e., it was iso-ionic throughout the acclimation period except for some increase around 360 hours showing a slight hyper-ionic regulation. In 100% SW, the blood Cl^- level increased slowly upto 24 hours and subsequently there was a rapid increase reducing the blood-medium gradient to the minimum by about 360 hours. However, the blood Cl^- was hypo-ionic to 100% SW.

On reversal to FW, there was a rapid decline in blood Cl^- level which reached the normal level by 24 hours.

This comparison between the levels of Na^+ and Cl^- in the blood and those of the ambient medium reveals that as far as the Na^+ is concerned the blood was more or less iso-ionic with 50% SW and hypo-ionic with 100% SW to begin with but became hyper-ionic at the end of acclimation period in both 50% and 100% SW. On reversal to FW the levels dropped quickly to normal but were hyper-ionic. The chloride ion showed hyper-ionicity with 50% SW but hypo-ionicity with 100% SW at the end of acclimation period. On return to FW, although there was a drastic and rapid reduction in Cl^-, its level in blood was kept up and was hyper-ionic to the ambient medium. Oziotelphusa is thus found to be a hyper-regulator in FW and 50% SW but nearly a conformer in 100% SW when both Na^+ and Cl^- are taken into account. Similar trends of ionic regulation have been recorded in many recent studies (Venkata Reddy, 1976; Sabourin and Stickle, 1980; Sharp et al, 1980; Greenaway, 1981; Castille & Lawrence, 1981).

Blood-medium ionic gradients and oxygen concumption

Time course of blood-medium ionic gradients are given in table 16 and these values along with oxygen consumption are plotted in figures 17-20. It is seen

FIGS. 17 & 18: Comparison of the blood-medium Na^+ gradient and oxygen consumption of the crab during the period of its exposure to 50% & 100% sea water (SW) and during the reverse acclimation to fresh water (FW).

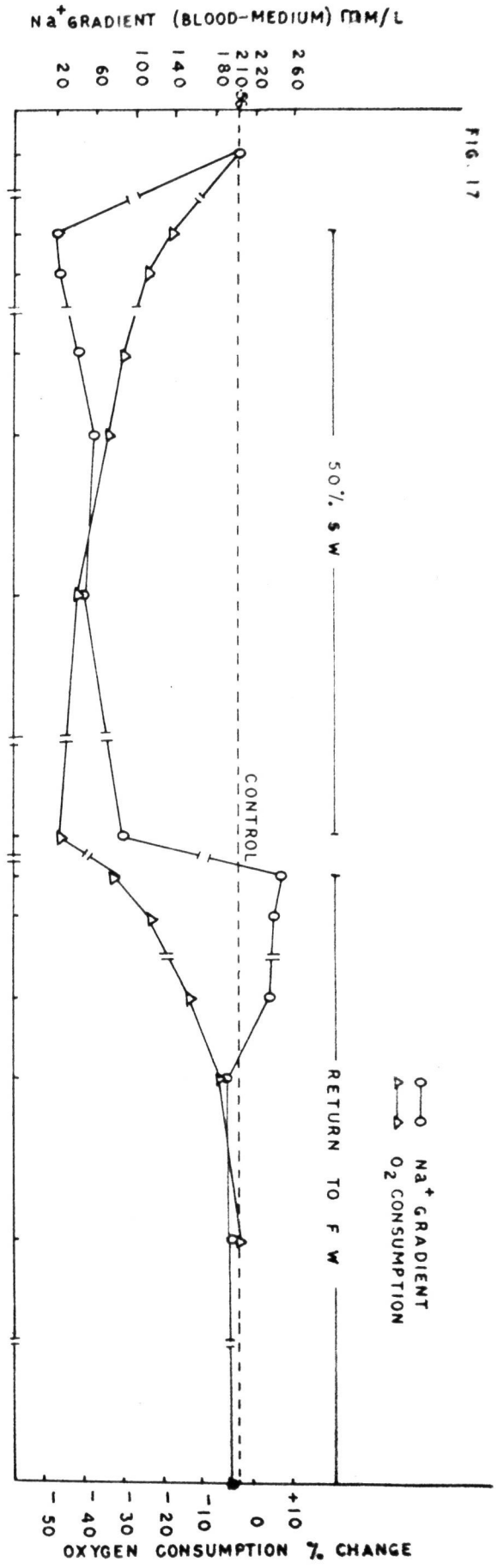

Na+ GRADIENT (BLOOD-MEDIUM) mM/L
20
60
100
140
180
210·56
220
260
CONTROL
50 % SW
RETURN TO FW
Na+ GRADIENT
O2 CONSUMPTION
OXYGEN CONSUMPTION % CHANGE
-50
-40
-30
-20
-10
0
+10

FIG. 17

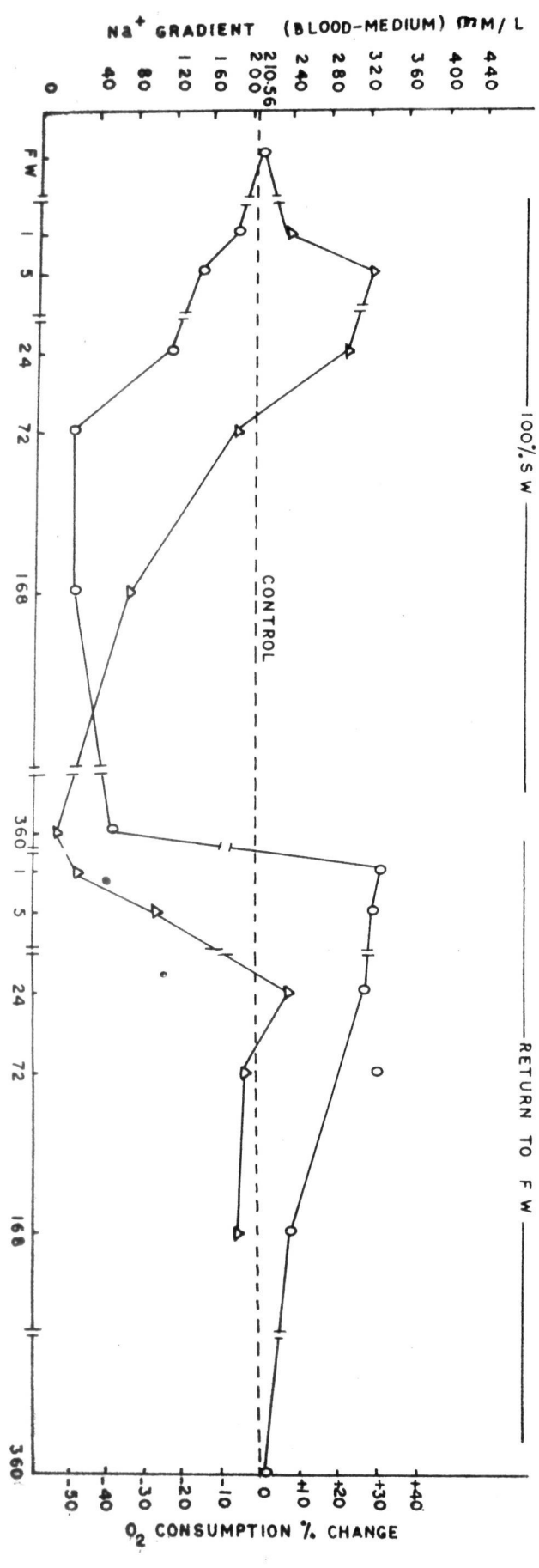

Na+ GRADIENT (BLOOD-MEDIUM) mM/L
0
40
80
120
160
200
210·56
240
280
320
360
400
440
FW
1
5
24
72
168
360
100% SW
RETURN TO FW
CONTROL
TIME IN HOURS
O2 CONSUMPTION % CHANGE
-50
-40
-30
-20
-10
0
+10
+20
+30
+40

FIG. 18

FIGS. 19 & 20: Comparison of the blood-medium Cl^- gradient with the oxygen consumption of the crab during the period of its exposure to 50% & 100% sea water (SW) and during the reverse acclimation to fresh water (FW).

FIG. 19

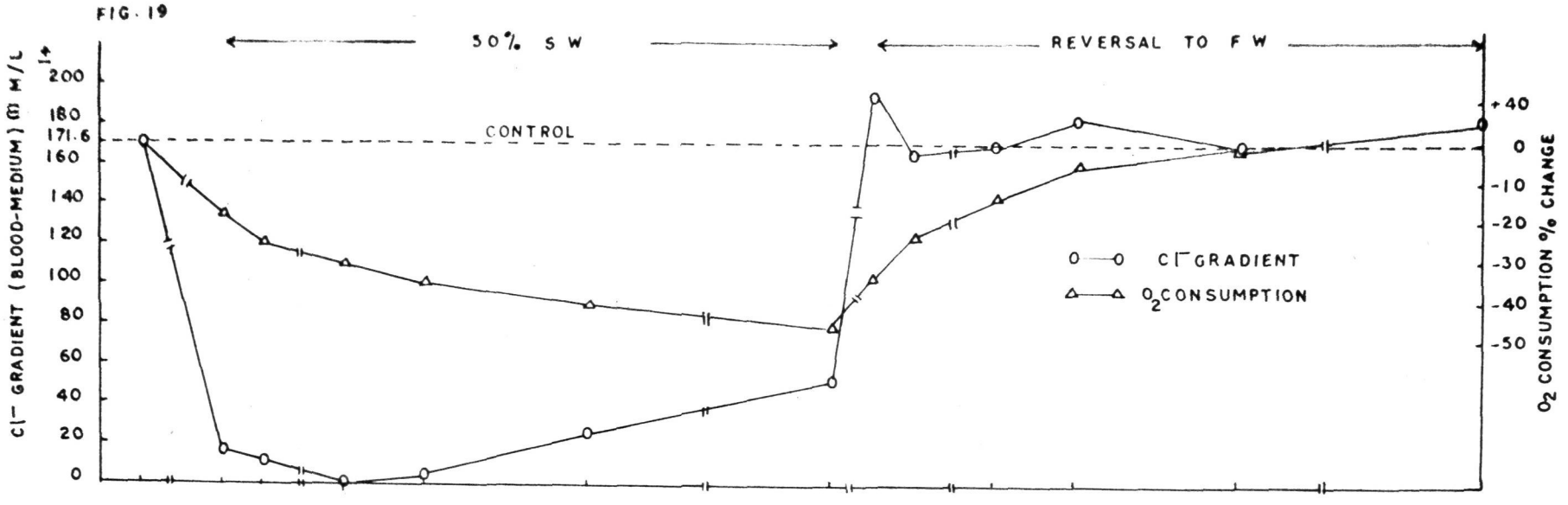
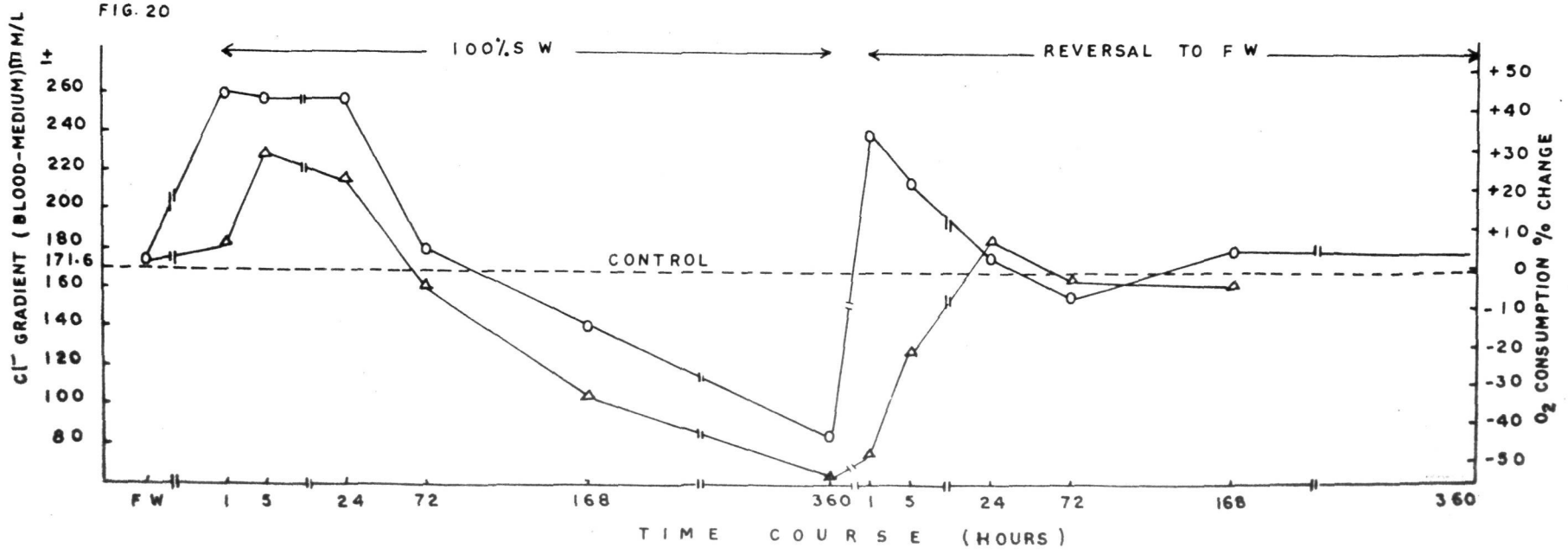

50% S W
REVERSAL TO F W
CONTROL
Cl⁻ GRADIENT (BLOOD-MEDIUM) m M/L
1+
200
180
171.6
160
140
120
100
80
60
40
20
0
+40
0
-10
-20
-30
-40
-50
O2 CONSUMPTION % CHANGE
O—O Cl⁻ GRADIENT
Δ—Δ O2 CONSUMPTION

FIG. 20


100% S W
REVERSAL TO F W
CONTROL
Cl⁻ GRADIENT (BLOOD-MEDIUM) m M/L
1+
260
240
220
200
180
171.6
160
140
120
100
80
+50
+40
+30
+20
+10
0
-10
-20
-30
-40
-50
O2 CONSUMPTION % CHANGE
FW
1
5
24
72
168
360
1
5
24
72
168
360
TIME COURSE (HOURS)

that on transfer of the crabs to 50% SW the Na^+ and Cl^- gradients fell steeply. This reduction in ion-gradients may be responsible for the reduction in oxygen consumption as there is a close parallel between these two lines (figures 17 & 19). On reversal to FW, the ionic gradients increased sharply and the oxygen consumption of animals also showed a corresponding increase after transfer to FW. In 100% SW the Cl^- gradient was higher than that in FW upto 24 hours though in the opposite direction, and it reached the same level as that between normal animals and FW at 72 hours, and thereafter the Cl^- gradient fell steeply. There is a striking similarity between this chloride gradient and oxygen consumption in 100% SW. (Fig. 20) Just as the chloride gradient increased in early stages and returned to the normal level only by about 72 hours, the oxygen consumption was also significantly elevated upto 48 hours and returned to normal level by about 72 hours after transfer to 100% SW. After 72 hours the chloride gradient fell steeply and reached the minimum level by 360 hours. So also the oxygen consumption fell steeply after 72 hours and reached a minimum level by 336 hours. Again, when the animals were returned to FW the chloride gradient increased far above the normal and there was nearly a corresponding increase in oxygen consumption.

Thus, there appears to be a precise relationship between chloride gradient and oxygen consumption. Na^+ gradient also reflects similar relation with oxygen consumption in general. However, this relation does not seem to be apparent only between 1-24 hours in 100% SW.

Na^+ and Cl^- ion gradients necessarily reflect osmotic gradients between blood and medium. Na^+ and Cl^- are the major inorganic osmotic effectors of both blood and ambient medium. Hence, these gradients in ion composition, if not the same, many however correspond to osmotic gradients. It has been shown in several studies that oxygen consumption in various salinities is governed by the osmotic gradients between the blood and medium, the chloride gradient being more important (Schwabe, 1933; Lofts, 1956; Pampapathi Rao, 1958; Pampapathi Rao & Madanmohan Rao, 1963; Ramamurthi, 1965; Venkatachari and Vasantha, 1981). In the crab under study, Ramamurthi has suggested a specific relationship between chloride gradient and oxygen consumption in heterosmotic media ranging from FW to different concentrations of sea water. It can be safely presumed that wherever the osmotic gradients and high, the energy needed for osmotic work, either water or salt balance, may be correspondingly high and where the osmotic gradients are low, the energy requirements are also correspondingly reduced. In the present work it is

clear that at the end of acclimation period the oxygen consumption was lowered to highly significant levels (nearly 50% lower than that in FW) in both 50% & 100% SW, reaching nearly anaerobic conditions. This is because of the significant reduction in osmotic gradients resulting in slight hyper-ionicity in 50% sea water and in more or less iso-ionic conditions in 100% SW. Thus the crabs exposed to higher salinities seem to depend mainly on evasive mechanisms while those that live in FW mainly invoke compensatory mechanisms of osmoregulation. While the crabs living naturally in FW are hyper-osmotic regulators, showing the maximum rate of oxygen consumption, these acclimated to 50% SW seem to show slight hyper-regulation and those exposed to 100% SW appear to become osmo-conformers. This explains the steady state and a slightly higher rate of O_2 consumption in 50% SW and a lower rate in 100% SW at the end of the acclimation period.

The osmoregulatory picture of ECF seen in this crab falls into type I of Lockwood's(1962) classification viz., hyperosmoregulation of ECF concentration in dilute media and iso-osmoticity in higher salinities. Many crustaceans like Carcinus maenas, (Schwabe, 1933; Shaw, 1961a), Hemigrapsus nudus, Cancer magister (Jones, 1941) Marinoganmarus sp (Sutcliffe, 1968) and many freshwater euryhaline forms like Astacus fluviatilis (Schwabe, 1933) Gammarus duebeni

(Beadle and Cragg, 1940; Shaw and Sutcliffe, 1961; Sutcliffe, 1967a), Potamon niloticus (Shaw, 1959c), Asellus aquaticus (Lockwood, 1959, 1962) are some examples showing osmoregulatory capacities more or less resembling the crab under study. The Chinese crab Eriocheir sinensis (Shaw, 1961b) and the freshwater crab Pseudothelphusa jouyi (Thomson, 1970) and many other hyper-regulators also fall into this category. They display isosmoticity of blood with the medium in concentrated media and hyperosmoticity in diluted media. Lockwood's second type includes those that exhibit hypo-osmoticity in concentrated media and hyperosmoticity in dilute media. Palaemonetes varians, Leander serratus (Panikkar, 1941) Artemia salina (Croghan, 1958a) Pachygrapsus crassipes (Jones, 1941) and all grapsoid crabs (Lockwood, 1962; Barnes, 1967) are some examples of this type.

The mechanisms of hyperosmotic regulation have been discussed extensively (Potts and Parry, 1964b; Kirschner, 1979). The reduction in permeability of integument to prevent excessive osmotic influx of water (Gross, 1957a) seems to be one of the evasive mechanisms adopted by hyper-osmoregulators. Permeability to salt and water is remarkably low in most of the freshwater arthropods

compared to marine species (Gross, 1957a; Bryan, 1960; Shaw, 1961b). Permeability rate constants have been shown to decrease in different species of Gammarids from sea water through brackish water to fresh water (Schoffeniels and Gilles, 1970b). The blood-medium osmotic gradients in FW crabs are large inspite of a reduction in blood concentration. However, passive loss of Na^+ across the body surface and in the urine is many times lesser and water uptake is restricted in FW crabs (Shaw, 1959c, 1961b, Thompson, 1970). As a consequence, the active uptake mechanisms are spared a corresponding amount of work.

Weight changes do not seem to occur in Oziotelphusa (Paratelphusa) adapted to different salinities (Ramamurthi, 1967b). Evidently the permeability of the animal to salts and water is comparatively low. This low permeability may be implicated as one of the hyper-regulatory mechanisms. Further, an active salt absorbing compensatory mechanism has been demonstrated in this crab (Pampapathi Rao and Venkata Reddy, 1962; Venkata Reddy and Aravinda Babu, 1969) and in several other hyper-regulators (Krogh, 1939; Ussing, 1960; Maetz, 1973; Kirschner et al, 1973; Payan et al, 1975; Helman & Fisher, 1977; Cala et al, 1978; Neufeld et al, 1980; Greenaway, 1981). The elevated level of oxygen

consumption seen in FW in this crab (fig. 5) is proof of the operation of such active mechanisms. Evidently, such mechanisms may be located primarily in gills of this crab and the antennal glands and the gut may well be the other sites which warrant further investigation. The tendency of this crab to achieve iso-osmoticity in higher salinities by the reduction in osmotic gradients between the blood and medium is an evasive method. A drastic reduction in oxygen consumption in both 50% & 100% SW, measured in later stages of exposure, may well reflect cessation of or great reduction in hyper-osmotic regulation.

Although ions like K^{+}, Ca^{++} and Mg^{++} have not been studied in the present work, it may not be out of place to mention the following correlations between these ions and the activity of the crabs observed in 100% SW. Venkata Reddy (1976) has reported the accumulation of relatively greater amounts of Ca^{++}, Mg^{++} and K^{+} ions in the blood of this crab when exposed to 100% SW. These ions appear to be the causative factors for the following observations:

1. Loss of appetite from the 3rd day in 100% SW leading to an "anorectic" state.
2. Loss of activity on the 3rd day and consequent loss in escape reactions leading to sluggishness or lethargy in 100% SW.

Ca^{++} and Mg^{++} are known to decrease the frequency and amplitude of cardiac rhythmicity (Maynard, 1960) and activity of animals (Robertson, 1960). The K^{+} of the blood is known to increase to 34.61% in 100% SW (Venkata Reddy, 1976). High K^{+} level in blood is reported to affect cardiac activity leading to systolic arrest (Maynard, 1960). In the light of these observations it may be said that 100% SW is perhaps an unfavourable environment on FW crabs and prolonged exposure to this medium brings about cessation of activity and starvation leading to death. This explains why nearly 50% of the crabs exposed to 100% SW die during the course of the experiment.

Ionic composition of tissues

Results

Sodium

Data on time course of Na^+ composition in tissues of crabs exposed to 50% and 100% SW are presented in tables 10 and 11. Tables 10-1 and 11-1 show variance ratios and tables 10-2 and 11-2 give statistical comparison of the data. The mean values are shown as percent deviations from the normal in figure 13b. The mean values of Na^+ in the intracellular tissue water were calculated after making necessary corrections for the extracellular fluid volume of the tissue, and these values are presented in table 12. The method of calculation is given in the section "Materials and Methods". Intracellular fluid concentrations of Na^+ in relation to blood are shown as histograms in figures 21 & 22.

Na^+ concentration in the muscle and hepatopancreas followed in general the trend of changes in blood but the absolute amount of change in tissues was too low compared to that of blood (Figures 21 & 22). The actual concentrations are several times lower in tissues than in blood. However, when percentage

FIGS. 21 & 22: Comparison of the Na^+ level of the blood with that of the intracellular water.

Empty columns = blood (B);
Stippled columns = Hepatopancreas (H).
Striped columns = Muscle (M).

FIG. 21

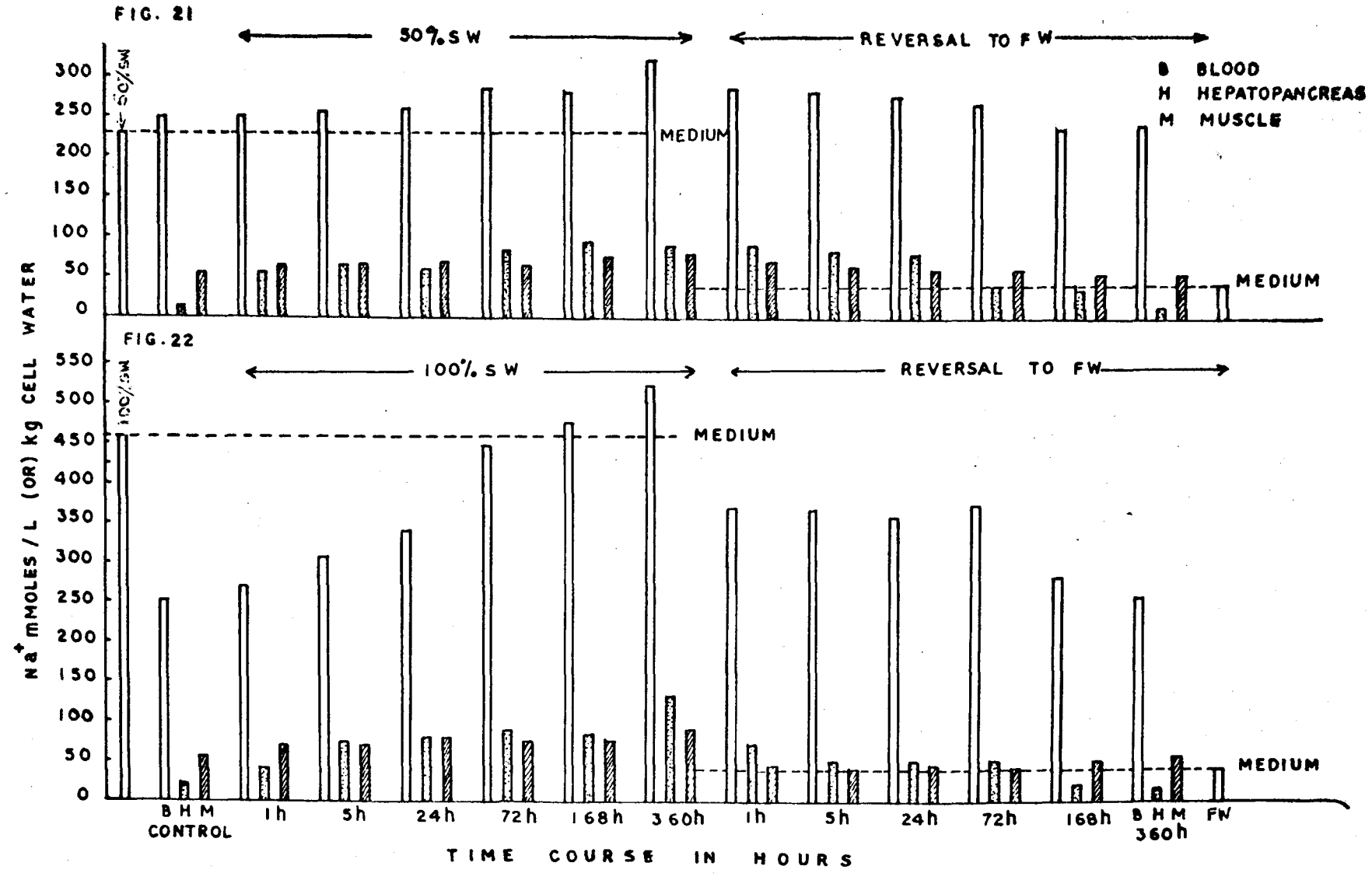

50% SW
REVERSAL TO FW
B BLOOD
H HEPATOPANCREAS
M MUSCLE
MEDIUM
MEDIUM
100% SW
REVERSAL TO FW
MEDIUM
MEDIUM
Na+ mMOLES / L (OR) kg CELL WATER
300
250
200
150
100
50
0
550
500
450
400
350
300
250
200
150
100
50
0
B H M CONTROL
1h
5h
24h
72h
168h
360h
1h
5h
24h
72h
168h
B H M 360h
FW
TIME COURSE IN HOURS

FIG. 22

deviations of Na^+ per Kg wet tissue are considered (see figure 13b) the hepatopancreas showed a disproportionately higher accumulation of this ion in both the salinities (98.72% in 50% SW and 130.14% in 100% SW) whereas the muscle showed a conservative trend (40.53% in 50% SW and 51.18% in 100% SW). It is interesting to note that the intracellular Na^+ concentration increased several fold in hepatopancreas (389.9% in 50% SW and 585.1% in 100%SW) whereas this increase was comparatively low in the muscle (49.23% in 50% SW and 64.54% in 100% SW) (Table 12). Tissue specific differences in Na^+ changes are thus very evident.

Consideration of changes during the course of acclimation reveals that the increase in Na^+ level was significantly high ($P < 0.01$) as early as 1 hour after transfer to 50% and 100% SW in hepatopancreas. In the muscle a significant increase was observed after 1 hour in 100% SW but not in 50% SW where significant elevated level was observed only after 168 hours. In the reverse acclimation, the Na^+ level decreased steeply in both the tissues of crabs exposed to 50% & 100% SW. But normal levels were obtained rather quickly in muscle (i.e., 24 or 72 hours) and slowly in hepatopancreas i.e. 168 hours.

Chloride

Tables 13 and 14 contain data on Cl^- concentration in tissues. Variance ratios are given in tables 13-1 and 14-1. Statistical comparison of the data is presented in Tables 13-2 and 14-2, mean values are plotted as percent deviations from the normal in figure 14b. Intracellular ion concentration is shown in table 15 and these values are shown in relation to blood composition in figures 23 and 24.

There was no ionic parity between blood and tissues. However, Cl^- concentration in the tissues of crabs exposed to 50% and 100% SW showed increasing trend which followed the general increasing trend of the ion in the blood. The percent deviation of the ion per Kg cell water (table 13 & 15) was far below that of blood in both hepatopancreas and muscle except in the hepatopancreas of crabs exposed to 100% SW. The muscle showed a rather conservative trend compared to hepatopancreas. At the end of acclimation period, the percentage increase in muscle was 20.05 in 50% SW and 67.55 in 100% SW but in hepatopancreas the increase recorded was 32.93% and 211.07% respectively. Thus the hepatopancreas seemed to be impregnated with a high Cl^- content especially

FIGS. 23 & 24: Histograms showing comparison of the changes in Cl^- levels in the blood with those of tissues of crabs subjected to salinity stress.

FIG. 23

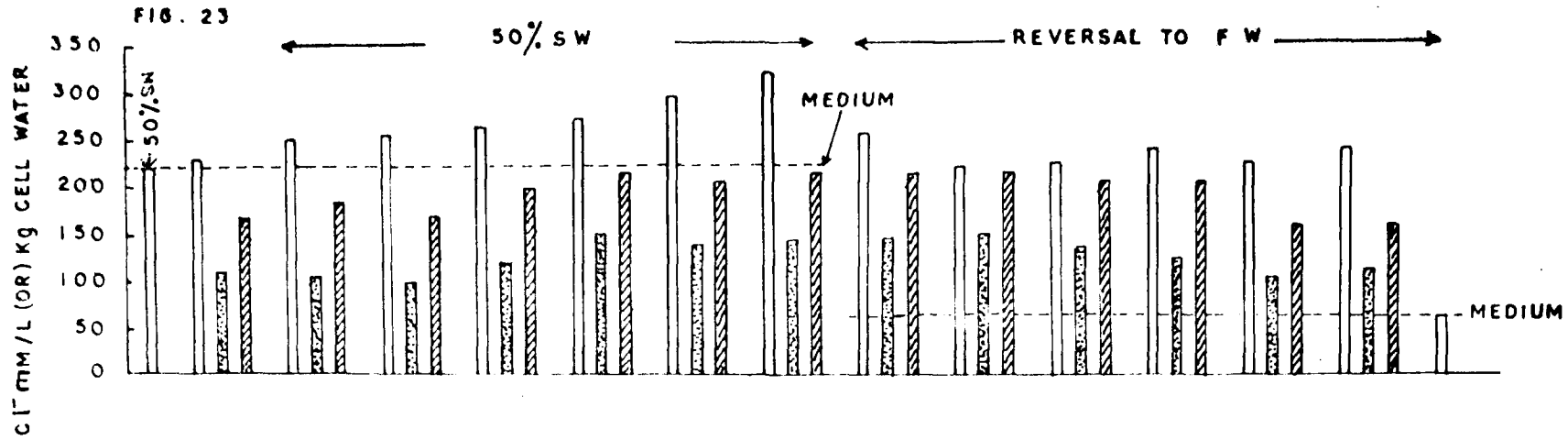
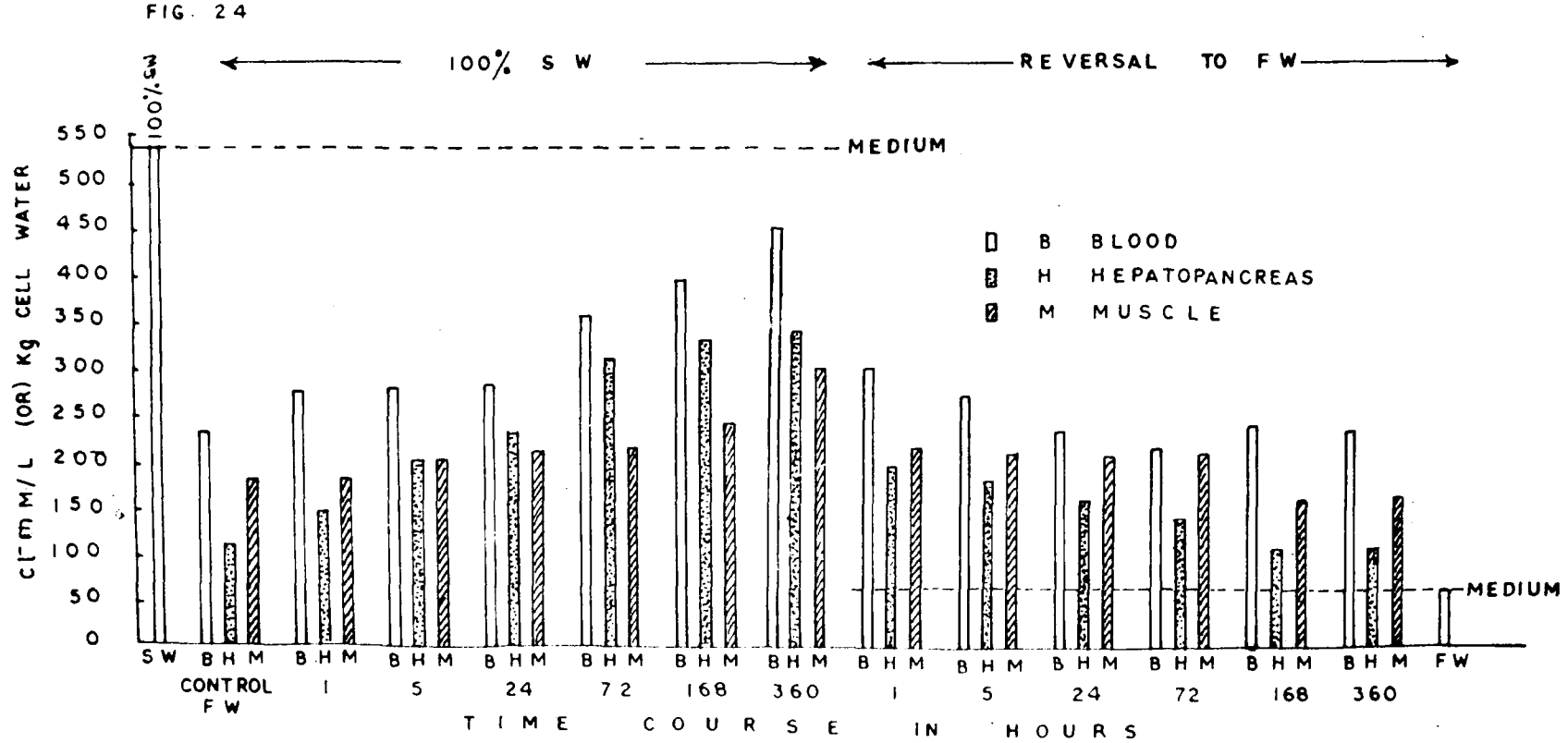

50% SW
REVERSAL TO F W
MEDIUM
50% SW
MEDIUM
Cl⁻ mM/L (OR) Kg CELL WATER
350
300
250
200
150
100
50
0

FIG. 24


100% S W
REVERSAL TO F W
100% SW
MEDIUM
MEDIUM
B BLOOD
H HEPATOPANCREAS
M MUSCLE
Cl⁻ mM/L (OR) Kg CELL WATER
550
500
450
400
350
300
250
200
150
100
50
0
SW
B H M
CONTROL FW
1
5
24
72
168
360
FW
TIME COURSE IN HOURS

in 100% SW. Reversal to FW from SW brought down the chloride ion levels in tissues rather quickly but normal levels were attained relatively slowly.

On the wet tissue weight specific basis, the chloride ion did not increase significantly upto 168 hours in hepatopancreas and upto 72 hours in muscle in 50% SW. Only subsequently significant higher levels were achieved. In the reverse acclimation a gradual decrease was seen in hepatopancreas but significantly lower levels more or less comparable to normal levels were attained only after 168 hours. But in the muscle, there was a significant drop after 5 hours but normal level was attained only around 360 hours. In 100% SW exposed crabs there was a steep increase in Cl^- level of hepatopancreas and the level attained as early as 1 hour after transfer was significantly high. In the muscle this was not so. The increase upto 72 hours was not significantly high but from 168 hours onwards significantly high levels were attained. In the reverse acclimation to FW from 100% SW both tissues showed a steep fall in Cl^- concentration. In hepatopancreas significantly lower Cl^- levels were recorded after 1, 5 and 24 hours and thereafter the lowered levels were not significantly different from that obtained in normal crabs. In the muscle, there was a significant fall

after 1 hour and a further significant decrease after 168 hours. This level was not significantly different from that of normal animals.

Discussion on the ion composition of tissues

Optimum cell function requires an iso-osmotic composition of tissue fluids and blood or atleast a relatively constant composition of blood. (Conway and Mc Cormak, 1953; Appelboom et al, 1958; Maffly and Leaf, 1959; Florkin and Schoffeniels, 1969; Gilles, 1974a, Schoffeniels, 1976). Figures 21-24 show a relative ionic composition of the tissue fluids (intracellular concentrations) in relation to that of blood or the ECF. From these figures it is clear that vast gradients exist in the ion composition, particularly Na+, of blood and tissues in all salinities and during the entire course of acclimation to SW. These differences are minimised only during the first stages of reverse acclimation. There appears to be no ionic parity between ECF and tissues inspite of a tremendous increase in Na^+ and Cl^- concentration in hepatopancreas and a moderate increase of these ions in the muscle. Even in the normal crabs a large gradient in ion concentration exists between blood and tissues. Therefore, this ionic disparity between blood and tissues

necessarily implies that there are other important and major intracellular osmotic effectors. It is a known fact that relatively high K^+ concentration in tissues especially in muscle plays a significant role along with Na^+ and Cl^- in the inorganic osmotic effector profile of the tissues and in cell volume control (Leaf, 1959; Whittam, 1969; Kregenow, 1971a, 1971b; Kleinzeller, 1972; Hendil and Hoffmann, 1974; Venkata Reddy, 1976; Schröck and Hanke, 1979a, 1979b). This ion could not be investigated in the present work for reasons beyond control. That the intracellular osmotic concentration is augmented greatly by organic osmotic effectors particularly free amino acids is well known (Lang and Gainer, 1969; Gerard & Gilles, 1972a, 1972b; Gerard, 1975; Hoffmann & Hendil, 1976; Gilles, 1973a, 1974a, 1979; Schröck & Hanke, 1979a, 1979b). There fore, it can be safely presumed that the deficiencies in total ionic concentration of the tissues in relation to blood would be made good by the major organic osmotic effectors such as free amino acids besides K^+ and other inorganic cations besides Na^+ and Cl^-. Meaningful interpretations are possible only when these are taken into account.

However, the following aspects of ion concentration in tissues are worth describing. Changes in blood osmotic concentration induce concomitant changes in cell osmotic

concentration. This process has been termed by Duchateau and Florkin (1961, 1962) as "intracellular isosmotic regulation". Excessive alterations of tissue hydration and salt concentration may not be conducive for the proper metabolic performance of the tissue. As a matter fact survival of the organisms under a variety of osmotic conditions depends on the ability of the tissues to regulate their cell volume and osmotic pressure in tune with the changes in the ECF or the tissues must be able to tolerate such changes. Under extreme situations, this tolerance may be lost and death ensues.

In the crabs under investigation, the hepatopancreas behaved differently from the muscle. The muscle seemed to show better powers of regulation than the hepatopancreas with respect to the inorganic ions, more so in 50% SW than in 100% SW. When exposed to higher salinities, the hepatopancreas showed excessive dehydration and also excessive uptake of Na^+ and Cl^-. This excessive dehydration during the course of acclimation without significant regulation of water content and probably cell volume at the end of the experimental period coupled with excessive impregnation with salts might be injurious to the animal. This was particularly true in 100% SW exposed crabs in which the hepatopancreas was the most affected tissue. This might have been one of the causes for the loss of appetite in crabs in 100% SW.

Even the muscle was dehydrated to greater levels in 100% SW and it did not show any compensation. This dehydrative phenomenon of muscle which probably results in shrinkage of cells coupled with moderate uptake of salts may atleast be partly responsible for the loss of escape reactions and consequent lethargy in crabs acclimated to 100% SW. As already indicated, divalent ions like Mg^{++} and Ca^{++} may contribute further to such deleterious effects of acclimation to higher salinities particularly to 100% SW. This explains the relatively greater casualities in this medium. However, the muscle maintained a relatively low inorganic ion composition in 100% SW and this conservative trend may probably account for the survival of atleast a smaller percentage (about 50%) of these animals in 100% SW.

The concentration differences between the tissues and blood, with respect to Na^{+} and Cl^{-}, indicate that these ions participate only partially in the intracellular isosmotic regulation. The organic osmotic effectors especially free amino acids are the complementary isosmotic regulation components, and the forthcoming chapter deals with such components.

Table 8

Time course of changes in water content in the tissues of freshwater field crab Oziotelphusa (Paratelphusa) sp. when transferred directly to 50% S.W. and back to F.W. Values expressed as gms/100 gms wet tissue. Mean values are for 6 individuals. S.W. = Sea water; F.W. = Freshwater.

Tissue	Normal (F.W.)	Acclimation to 50% S.W.						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
Hepato pancreas (H.P.)													
Mean	71.37	69.93	67.49	63.77	52.80	56.30	62.94	63.81	63.12	66.21	70.35	71.36	73.12
S.D. ±	6.13	3.28	5.39	5.13	4.63	5.36	4.42	4.71	4.87	4.95	3.58	5.43	5.46
% d from normal		-2.01	-5.43	-10.64	-26.02	-21.10	-11.8	-10.58	-11.55	- 7.22	-1.42	-0.014	+2.45
Muscle													
Mean	76.23	73.0	72.73	70.29	68.55	70.33	74.04	74.85	75.06	78.25	77.55	76.87	77.01
S.D. ±	4.21	3.27	3.86	4.72	5.10	4.17	3.72	3.01	4.25	3.66	3.71	2.38	2.56
% d from normal		-4.23	-4.59	-7.79	-10.07	-7.14	-2.87	-1.81	-1.53	+2.64	+1.73	+0.83	+1.02

Table 8-1

Variance ratios for the data given in table 8.

Tissue	Variance Ratio (F)		Critical difference		
	F. Cal	F. tab at 5% level	5% level	1% level	0.1% level
		50% S.W.			
H.P.	11.54	2.38	6.85	9.70	14.14
Muscle	5.27	2.38	5.21	7.39	10.79
		Reversal to F.W.			
H.P.	4.36	2.26	6.29	8.72	12.42
Muscle	1.57	2.26	3.91	5.43	7.73

Table 8-2

Statistical comparison of the data given in table 8. Mean values are arranged in an ascending order.

Tissue	Treatment							
H.P.	50% S.W.							
	72 h	168 h	360 h	24 h	5 h	1 h	Normal	
	52.80	56.30	62.94	63.77	67.49	69.93	71.37	
	Reversal to F.W.							
	360 h S.S.	5 h	1 h	24 h	72 h	168 h	Normal	360 h
	62.94	63.12	63.81	66.21	70.35	71.36	71.37	73.12
Muscle	50% S.W.							
	72 h	24 h	168 h	5 h	1 h	360 h	Normal	
	68.55	70.29	70.33	72.73	73.0	74.04	76.23	
	Reversal to F.W.							
	360 h S.S	1 h	5 h	Normal	168 h	360 h	72 h	24 h
	74.04	74.85	75.06	76.23	76.87	77.01	77.55	78.25

N.B.:- Values grouped together by a common underline are not significantly different from each other ($P > 0.05$)
S.S.: Salinity Stress

Table 9

Time course of changes in water content in the tissues of the fresh water crab Oziotalphusa (Paratelphusa) sp. when transferred directly to 100% S.W. and back to F.W. values are expressed as gms/100 gms wet tissue. Mean values are for 6 individuals.

Tissue	Normal	100% S.W.						Reversal to F.W.					
	(F.W.)	1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
Hepatopancreas (H.P.)													
Mean	71.37	65.83	57.30	52.20	48.97	47.89	48.59	60.69	59.62	58.54	58.82	68.25	72.31
S.D. ±		5.63	5.64	5.58	5.69	5.63	4.84	4.06	4.15	3.78	7.05	7.26	3.91
% d		-7.76	-19.69	-26.83	-31.36	32.90	-31.91	-14.95	-16.42	-17.97	-17.52	-4.36	+1.32
Muscle													
Mean	76.23	72.59	71.16	67.26	60.71	62.74	62.15	70.73	71.28	71.61	75.46	77.15	77.61
S.D. ±	4.21	4.71	3.58	3.25	3.81	2.77	3.25	3.25	4.17	3.11	3.62	4.65	3.27
% d		-4.77	-6.65	-11.71	-20.36	-17.69	-18.47	-7.21	-6.49	-6.06	-1.01	+1.21	+1.81

Table 9-1

Variance ratios for the data given in table 9.

Tissue	Variance Ratio (F)		Critical difference		
	F. Cal	F. tab at 5% level	5% level	1% level	0.1% level
	100% S.W.				
H.P.	14.57	2.38	7.71	10.94	15.92
Muscle	10.35	2.38	4.75	6.74	9.84
	Reversal to F.W.				
H.P.	7.75	2.26	6.70	9.31	13.23
Muscle	4.70	2.26	4.31	5.98	8.52

Table 9-2

Statistical comparison of the data given in table 9. Mean values are arranged in an ascending order.

Tissue	Treatment							
H.P.	100% S.W.							
	168 h	360 h	72 h	24 h	5 h	1 h	Normal (FW)	
	47.89	48.59	48.97	52.20	57.30	65.83	71.37	
	Reversal to F.W.							
	360 h S.S.	24 h	72 h	5 h	1 h	168h	Normal (FW)	360 h
	48.59	58.54	58.82	59.64	60.69	68.25	71.37	72.31
Muscle	100% S.W.							
	72 h	360 h	160 h	24 h	5 h	1 h	Normal (FW)	
	60.71	62.15	62.74	67.26	71.16	72.59	76.23	
	Reversal to F.W.							
	360 h S.S.	1 h	5 h	24 h	72 h	Normal (FW)	168 h	360 h
	70.15	70.73	71.28	71.61	75.46	76.23	77.15	77.61

N.B.:- Values grouped together by a common underline are not significantly different from each other ($P > 0.05$)
S.S.: Salinity Stress.

Table 10

Time course of changes in sodium ion levels in the tissues of the fresh water field crab, Oziotelphusa (Paratelphusa) sp. when transferred directly to 50% SW and back to FW. Values are expressed in m moles/litre or Kg. wet tissue. Mean values are for 6 individuals. (For intracellular conc. of Na^+, see table 12)

Tissue	Normal (F.W.)	50% S.W.						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
Blood:													
Mean	250.56	250.20	253.86	262.21	286.95	282.66	319.10	287.28	279.66	277.81	236.26	235.76	243.33
S.D. ±	6.41	9.81	12.60	13.75	17.13	17.71	18.40	12.60	9.49	12.13	5.64	8.71	9.88
% d		-0.14	+1.32	+4.64	+14.52	+12.81	+27.37	+14.65	+11.61	+10.87	-5.70	-5.91	-2.88
H.P.:													
Mean	49.17	75.33	75.37	73.00	73.70	85.57	97.71	94.40	91.40	90.60	61.37	61.01	46.71
S.D. ±	4.70	10.41	9.87	6.96	9.36	9.21	8.64	3.13	4.18	5.34	3.21	5.72	4.47
% d		+53.20	+53.28	+48.46	+49.88	+74.02	+98.72	+91.98	+85.88	+84.25	+24.81	+24.07	- 5.00
Muscle:													
Mean	79.36	83.46	83.70	85.28	81.60	100.36	111.53	95.45	29.30	90.10	83.51	77.76	79.56
S.D. ±	7.44	5.55	5.00	7.03	8.61	6.30	10.26	7.17	11.13	5.68	5.62	10.92	8.47
% d		+5.16	+5.47	+7.46	+2.82	+26.46	+40.53	+20.27	+16.30	+13.53	+5.22	-2.02	+0.25

N.B.:- Na^+ level in F.W. = 40.00 m moles/l ; 50% S.W. = 229.71 m moles/l. H.P. – HEPATOPANCREAS

Table 10-1

Variance ratios for data presented **in Table** 10.

Tissue & Treatment	Variance ratio (F)		Critical Difference		
	F. Cal	F. tab at 5% level	5% level	1% level	0.1% level
Blood:					
50% S.W.	30.77	2.38	19.63	27.81	40.67
Reverse	43.40	2.26	13.93	19.35	27.49
H.P.:					
50% S.W.	25.00	2.34	10.06	14.10	20.33
Reverse	117.67	2.26	5.73	7.96	11.30
Muscle:					
50% S.W.:	274.13	2.38	10.11	14.35	20.94
Reverse	10.62	2.26	10.80	15.00	21.31

Table 10-2

Statistical comparison of the data shown in table 10. Mean values arranged in an ascending order.

Tissue	Treatment							
Blood	50% S.W.							
	24 h	1 h	Normal(F.W.)	5 h	168 h	72 h	360 h	
	222.21	250.2	250.56	253.86	282.66	286.95	319.10	
	Reversal to F.W. from 50% S.W.							
	168 h	72 h	360 h	Normal(F.W.)	24 h	5 h	1 h	360 h S.S
	235.76	236.26	243.33	250.56	277.81	279.66	287.28	319.10
H.P.	50% S.W.							
	Normal (F.W.)	24 h	72 h	1 h	5 h	168 h	360 h	
	49.11	73.00	73.70	75.33	75.37	85.57	97.71	
	Reversal to F.W. from 50% S.W.							
	360 h	Normal	168 h	72 h	24 h	5 h	1 h	360 h S.S
	46.71	49.17	61.01	61.37	90.60	91.40	94.4	97.71
Muscle	50% S.W.							
	24 h	Normal	1 h	5 h	24 h	168 h	360 h	
	71.60	79.36	83.46	83.70	85.28	100.36	111.53	
	Reversal to F.W. from 50% S.W.							
	168 h	Normal	360 h	72 h	24 h	5 h	1 h	360 h S.S
	77.56	79.36	79.56	83.51	90.10	92.30	95.45	111.53

N.B.:- Values grouped together by a common underline are not significantly different amongst themselves
S.S: Salinity stress

Table 11

Time course of changes in sodium ion levels in the tissues of the fresh water field crab, Oziotelphusa (Paratelphusa) sp. when transferred directly to 100% S.W. and back to F.W. Values are expressed in m moles/litre or Kg wet tissue. Mean values are for 6 individuals. (For intracellular conc. of Na^+ , see table 12)

Tissue	Normal (F.W.)	100% S.W.						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
Blood:													
Mean	250.56	269.2	305.46	342.08	444.9	476.40	521.00	370.73	366.40	356.46	370.38	279.46	255.08
S.D. ±	6.41	14.09	9.86	10.12	15.20	12.47	12.86	12.93	11.97	9.40	11.05	11.04	5.19
% d		+7.44	+21.91	+36.52	+77.56	+90.13	+107.93	+47.87	+46.23	+42.26	+47.82	+11.53	+1.80
H.P.:													
Mean	49.17	61.88	78.30	78.48	86.83	86.06	113.16	96.65	84.53	82.23	73.23	51.01	49.25
S.D. ±	4.70	3.76	10.08	9.17	6.95	5.69	4.06	12.06	8.42	5.31	7.24	6.88	4.25
% d		+25.84	+59.24	+59.61	+76.59	+75.02	+130.14	+96.56	+71.91	+67.23	+48.93	+3.74	+0.16
Muscle:													
Mean	79.36	90.87	92.81	99.58	101.11	107.96	119.98	99.65	94.11	97.48	82.35	82.90	81.46
S.D. ±	7.44	14.42	9.82	9.50	6.20	5.23	5.56	8.63	10.16	6.46	4.59	6.18	8.58
% d		+14.50	+16.94	+25.47	+27.41	+36.04	+51.18	+25.56	+18.58	+22.83	+3.76	+4.46	+2.64

Na^+ level in F.W. = 40.00 m moles/l ; 100% S.W. = 459.41 m moles/l. H.P. — HEPATOPANCREAS

Table 11-1

Variance ratios for the data given in table 11.

Tissue & Treatment	Variance ratio (F)		Critical difference		
	F. Cal	F. tab at 5% level	5% level	1% level	0.1% level
Blood:					
100% S.W.	481.4	2.38	16.34	23.21	33.85
Reverse	623.1	2.26	13.16	18.28	25.97
H.P.:					
100% S.W.	69.59	2.38	7.93	11.11	16.03
Reverse	79.14	2.26	7.68	10.67	15.15
Muscle:					
100% S.W.	135.31	2.38	11.49	16.32	23.81
Reverse	20.63	2.26	9.31	12.93	18.37

Table 11-2

Statistical comparison of the data shown in table 11. Mean values are arranged in an ascending order.

Tissue	Treatment						
Blood	100%S.W.						
	Normal(F.W.)	1 h	5 h	24 h	72 h	168 h	360 h
	250.56	269.2	305.46	342.08	444.9	476.4	521.00
	Reversal to F.W. from 100% S.W.						
	Normal(F.W.)	360 h	168 h	24 h	5 h	72 h	1 h	360 h S.S
	250.56	255.08	279.46	356.46	366.4	370.73	370.78	521.00
H.P.	100% S.W.						
	Normal(F.W.)	1 h	5 h	24 h	168 h	72 h	360 h
	49.17	61.88	78.30	78.48	86.06	86.83	113.16
	Reversal to F.W. from 100% S.W.						
	Normal	360 h	168 h	72 h	24 h	5 h	1 h	360 h S.S
	49.17	49.25	51.01	73.23	82.23	84.53	96.65	113.16
Muscle	100% S.W.						
	Normal (F.W.)	1 h	5 h	24 h	72 h	168 h	360 h
	79.36	90.87	92.81	99.58	101.11	107.96	119.98
	Reversal to F.W. from 100% S.W.						
	Normal (F.W.)	360 h	72 h	168 h	5 h	24 h	1 h	360 h S.S
	79.36	81.46	82.35	82.90	94.11	97.48	99.65	119.98

N.B.:- Values grouped together by a common underline are not significantly different from each other ($P > 0.05$)
S.S: Salinity stress

Table 12

Time course of changes in the intracellular concentration of Na^+ during acclimation of the crab, Oziotelphusa (Paratelphusa) to 50% & 100% S.W. and upon reverse acclimation to FW after making necessary correction for the ions of the ECF within the tissue. Values are expressed as mosmoles/Kg. Cell water.

Tissue	Normal (F.W.)	Salinity stress						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
				50% S.W.									
H.P.													
Mean	18.78	57.72	60.85	62.06	82.19	95.45	91.44	92.28	88.87	81.26	39.98	38.35	15.22
% d		+207.34	+224.01	+230.45	+337.64	+408.25	+386.90	+391.42	+373.21	+332.69	+112.88	+104.21	-18.95
				100% S.W.									
Mean	18.78	40.15	75.55	81.93	88.33	84.42	128.66	65.10	48.45	49.17	50.42	18.85	17.05
% d		+113.79	+302.28	+336.26	+370.34	+349.52	+585.09	+246.64	+157.98	+161.82	+168.47	+0.37	+9.05
				50% S.W.									
Muscle													
Mean	53.99	64.28	64.31	68.88	61.65	76.69	80.57	70.06	67.04	59.58	60.42	54.01	54.65
% d		+19.05	+19.11	+27.57	+14.18	+42.04	+49.23	+29.76	+24.27	+10.35	+11.90	+ 0.03	+1.22
				100% S.W.									
Mean	53.99	71.34	69.33	79.64	77.56	76.79	88.84	46.73	38.74	44.83	35.06	51.56	53.94
% d		+32.13	+28.41	+47.51	+43.65	+42.23	+64.54	-13.44	-28.24	-16.96	-35.06	-4.50	-0.09

N.B.:- Values for computation are obtained from tables 8, 9, 10, & 11. H.P. — HEPATOPANCREAS

Table 13

Time course of changes in chloride ion levels in the tissues of the fresh water field crab, Oziotelphusa (Paratelphusa) sp. when transferred directly to 50% S.W. and back to F.W. Values are expressed as m moles/litre or Kg wet tissue. Mean values are for 6 individuals. (For intracellular conc. of Cl^-, see table 15)

Tissue	Normal (F.W.)	50% S.W.						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
Blood													
Mean	229.60	249.14	255.35	267.49	272.91	295.12	318.38	254.15	222.22	225.69	240.26	224.98	239.93
S.D. ±	12.50	13.49	7.33	8.73	10.46	8.50	8.17	14.44	13.61	10.00	11.08	7.45	6.24
% d		+8.59	+11.35	+16.67	+19.05	+28.82	+39.06	+10.80	-3.24	-1.72	+4.61	-2.04	+4.54
H.P.													
Mean	111.06	105.51	102.45	111.62	108.86	112.52	131.85	124.69	126.35	120.41	123.02	108.45	118.49
S.D. ±	12.54	6.80	8.41	5.77	5.76	8.72	11.88	9.73	8.18	10.08	5.69	6.65	9.80
% d		-4.95	-7.75	+0.50	-1.98	+1.31	+18.71	+12.30	+13.76	+8.42	+10.76	+2.35	+6.69
Muscle													
Mean	170.79	169.41	165.77	177.73	183.28	184.61	213.80	204.65	198.00	193.84	191.63	185.51	162.98
S.D. ±	12.61	11.35	14.07	12.52	13.90	14.22	11.38	15.27	13.34	11.38	7.45	12.54	6.25
% d		-0.82	-4.52	+4.16	+7.50	+8.29	+25.80	+19.82	+15.93	+13.49	+12.20	+8.62	-4.57

Cl^- level in F.W. = 58.00 m moles/l ; Cl^- level in 50% S.W. = 268.09 m moles/l. H.P. — HEPATOPANCREAS

Table 13-1

Variance ratios for data given in table 13.

Tissue	Variance ratio (F)		Critical difference		
	F. Cal	F. tab at 5% level	5% level	1% level	0.1% level
Blood:					
50%	57.46	2.38	14.15	20.97	29.31
Reverse	191.66	2.26	13.76	19.11	27.15
H.P.:					
50%	53.01	2.38	12.27	17.42	25.41
Reverse	47.82	2.26	12.05	16.74	23.78
Muscle:					
50%	9.43	2.38	17.75	25.20	36.76
Reverse	69.92	2.26	14.62	20.30	28.85

Table 13-2

Statistical comparison of the data given in table 13. Mean values arranged in an ascending order.

Tissue	Treatment							
Blood	50% S.W.							
	Normal	1 h	5 h	24 h	72 h	168h	360 h	
	229.60	249.14	255.40	267.49	272.91	295.12	318.38	
	Reversal to F.W. from 50% S.W.							
	5 h	168 h	24 h	Normal	360 h	72 h	1 h	360 h S.S.
	222.22	224.98	225.69	229.60	239.93	240.26	254.15	318.38
H.P.	50% S.W.							
	5 h	1 h	72 h	Normal	24 h	168 h	360 h	
	102.45	105.51	108.86	111.06	111.62	112.52	131.85	
	Reversal to F.W. from 50% S.W.							
	168 h	Normal	360 h	24 h	72 h	1 h	5 h	360 h S.S.
	108.45	111.06	118.49	120.41	123.02	124.69	126.35	131.85
Muscle	50% S.W.							
	5 h	1 h	Normal	24 h	72 h	168 h	360 h	
	165.77	169.41	170.79	177.73	183.28	184.61	213.80	
	Reversal to F.W. from 100% S.W.							
	360 h	Normal	168 h	72 h	24 h	5 h	1 h	360 h S.S.
	162.98	170.79	185.51	191.63	193.84	198.00	204.65	213.8

N.B.:- Values grouped together by a common underline are not significantly different among themselves ($P > 0.05$).

Table 14

Time course of changes in the chloride ion levels in the tissues of fresh water field crab, Oziotelphusa (Paratelphusa) sp. when transferred directly to 100% S.W. and back to F.W. Values are expressed as m moles/litre or Kg wet tissue. Mean values are for 6 individuals. (For intracellular conc. of Cl^-, see table 15)

Tissue	Normal (F.W.)	100% S.W.						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
Blood													
Mean	229.60	277.78	279.44	279.87	356.38	394.34	451.20	296.36	271.09	232.22	213.87	238.32	236.11
S.D. ±	12.50	9.34	10.20	9.10	12.93	14.64	15.28	13.67	12.79	13.44	12.56	13.12	12.54
% d		+21.20	+21.92	+22.11	+55.16	+72.48	+97.50	+29.37	+18.25	+1.15	-6.92	+3.83	+2.86
H.P.													
Mean	111.06	133.29	145.62	151.18	187.51	195.97	209.63	155.45	138.78	120.80	107.11	106.90	112.44
S.D. ±	12.54	11.78	8.81	9.93	8.70	11.19	6.27	10.23	10.02	8.76	8.66	8.18	8.72
% d		+20.00	+31.10	+36.10	+68.81	+76.42	+88.71	+40.00	+27.72	+8.76	+3.55	-3.74	+1.20
Muscle													
Mean	170.79	171.06	180.80	178.99	174.91	200.96	241.57	195.62	190.95	181.37	188.78	159.71	162.45
S.D. ±	12.61	8.95	8.76	13.53	12.80	5.68	7.45	8.80	11.78	13.36	14.54	8.20	12.67
% d		+0.16	+5.52	+4.92	+2.47	+18.10	+42.46	+14.89	+11.80	+6.19	+10.79	-6.64	-4.88

Cl^- level in F.W. = 58.00 m moles/l; Cl^- level in 100% S.W. = 536.18 m moles/l. H.P. - HEPATOPANCREAS

Table 14-1

Variance ratios for data given in table 14

Tissue	Variance ratio (F)		Critical difference		
	F. Cal	F. tab at 5% level	5% level	1% level	0.1% level
Blood:					
100% S.W.	278.45	2.38	16.14	22.92	33.43
Reverse	184.71	2.26	17.61	24.46	34.75
H.P.:					
100% S.W.	77.43	2.38	13.86	19.68	28.70
Reverse	98.40	2.26	11.73	16.29	23.15
Muscle:					
100% S.W.	36.52	2.38	14.23	20.21	29.47
Reverse	55.66	2.26	14.40	20.01	28.41

Table 14-2

Statistical comparison of data presented in table 14. Mean values arranged in an ascending order.

Tissue	Treatment							
Blood	100% S.W.							
	Normal	1 h	5 h	24 h	72 h	168 h	360 h	
	229.60	277.78	279.44	279.87	356.38	394.34	451.20	
	Reversal to F.W. from 100% S.W.							
	Normal	72 h	24 h	360 h	168 h	5 h	1 h	360 h S.S.
	229.60	213.87	232.22	236.11	238.32	271.09	296.36	451.20
H.P.	100% S.W.							
	Normal	1 h	5 h	24 h	72 h	168 h	360 h	
	111.06	133.29	145.62	151.18	187.51	195.97	209.63	
	Reversal to F.W. 100% S.W.							
	168 h	72 h	Normal	360 h	24 h	5 h	1 h	360 h S.S.
	106.90	107.11	111.06	112.44	120.80	138.78	155.45	209.63
Muscle	100% S.W.							
	Normal	1 h	72 h	24 h	5 h	168 h	360 h	
	170.79	171.06	174.91	178.99	180.80	100.96	141.57	
	Reversal to F.W. from 100% S.W.							
	168 h	360 h	Normal	24 h	72 h	5 h	1 h	360 h S.S.
	159.71	162.45	170.79	181.37	188.78	190.95	195.62	241.57

N.B.:- Values grouped together by a common underline are not significantly different amongst themselves ($P > 0.05$).

Table 15

Time course of changes in the intracellular concentration of Cl^- in the fresh water field crab, Oziotelphusa (Paratelphusa) sp. during acclimation to 50% and 100% S.W. and in the reverse acclimation periods, after making necessary correction for the ECF concentration of ions within the tissue. Values are expressed as mosmoles/Kg cell water.

Tissue	Normal (F.W.)	Salinity stress						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
H.P.				50% S.W.									
Mean	109.69	101.06	100.73	121.53	151.35	140.83	145.81	144.58	149.34	136.72	126.82	106.97	114.07
% d		-7.86	-8.16	+10.79	+37.97	+28.38	+32.93	+31.81	+36.14	+24.64	+15.61	-2.48	+3.99
				100% S.W.									
Mean	109.69	146.97	198.25	233.64	311.63	330.34	341.22	196.86	178.47	159.91	139.32	108.97	108.27
% d		+33.98	+80.73	+113.00	+184.10	+201.15	+211.07	+79.47	+62.70	+45.78	+9.69	-0.65	-1.29
Muscle				50% S.W.									
Mean	178.12	182.25	176.85	199.35	212.54	106.05	213.85	222.58	219.34	202.58	199.05	196.33	163.65
% d		+2.32	-0.71	+11.92	+19.32	+15.68	+20.05	+24.96	+23.14	+13.73	+11.75	+10.22	-8.12
				100% S.W.									
Mean	178.12	180.10	198.19	210.14	216.84	241.44	298.45	217.30	213.66	206.83	207.40	159.35	162.09
% d		+1.11	+11.27	+17.97	+21.74	+35.54	+67.55	+21.99	+19.95	+16.11	+16.43	-10.53	-8.99

N.B.:- Values for computation of intracellular concentration of ions are obtained from tables 8, 9, 13 & 14.
H.P. — HEPATOPANCREAS.

Table 16

Time course of Na^+ and Cl^- gradients between blood and medium during the crabs' exposure to 50% and 100% S.W. and back to F.W. (blood-medium) m moles/l.

Ions	Normal (F.W.)	Salinity stress						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
Cl^-					50% S.W.								
	+171.6	-18.95	-12.74	-0.60	+4.82	+27.03	+50.26	+196.15	+164.22	+167.69	+182.26	+166.98	+181.93
					100% S.W.								
	+171.6	-258.40	-256.74	-256.31	-179.8	-141.84	-84.98	+238.36	+213.09	+174.22	+155.87	+180.32	+178.11
Na^+					50% S.W.								
	+210.56	+20.49	+24.15	+32.5	+57.24	+52.95	+89.39	+247.28	+239.66	+237.81	+196.26	+195.76	+203.33
					100% S.W.								
	+210.56	-190.2	-153.95	-117.33	-14.51	+16.99	+61.59	+330.73	+326.40	+316.46	+330.38	+239.46	+215.08

N.B.:- Ion gradients between blood and medium are calculated from data in tables 10, 11, 13 and 14.
Medium concentration of chloride ion in m moles/L: F.W. = 58.00; 50% S.W. = 268.09; 100% S.W. = 536.18
Medium concentration of sodium ion in m moles/L = F.W. 40.00; 50% S.W. = 229.71; 100% S.W. = 459.41

CHAPTER III

ORGANIC OSMOTIC EFFECTORS

INTRODUCTION

Optimum cell function requires a relatively constant composition of the extracellular fluid (ECF). That "the intracellular fluid is isosmotic or only slightly hyperosmotic to the extracellular fluid" is well known (Conway and Mc Cormack, 1953; Appelboom and coworkers, 1958; Maffly and Leaf, 1959; Florkin and Schoffeniels, 1969; Schoffeniels, 1976) and this aspect has been reviewed by Gilles (1974a). When a euryhaline animal is transferred to media of higher or lower salinities, its ECF establishes a new level of osmoconcentration, which may be anisosmotic or nearly isosmotic with the ambient medium, depending on its osmoregulatory capabilities. Concomitantly the intracellular milieu of the tissues have to establish a new osmotic profile which should usually be isosmotic with the changed level of osmoconcentration of the ECF. Survival of animals may depend on the extent to which tissues tolerate changes in the osmotic and ionic concentration of the ECF during the acclimation period.

The process of intracellular isosmotic regulation is not merely a passive one. It involves active mechanisms of water balance and consequent regulation of cellular volume in view of the fact that animal cells "have easily distensible plasma membranes which necessarily undergo swelling and lysis unless some active mechanisms are present" especially in hypo-

osmotic media (reviews by Gilles, 1974a, 1979; Hoffmann, 1977).

Osmolarity of the body fluids in animals is mostly made up by inorganic ions except in insects and fishes. Fredericq showed as early as 1901, that the tissues contain a lower inorganic ion concentration than the ECF and that the cell's osmotic deficit is compensated by organic substances. In all the cases studied so far amino acids are found to be the most important organic osmotic effectors. Extensive studies have been made on the role of free amino acids as the intracellular osmotic effectors (Bricteux-Gregoire and coworkers, 1962; Florkin, 1962; Lange, 1963; Florkin and coworkers, 1964; Robertson, 1965; Gilles and Schoffeniels, 1969a; Florkin and Schoffeniels, 1969; Schoffeniels and Gilles, 1970, 1972; Gerard and Gilles, 1972a; Gilles and Gerard, 1974; Gilles, 1974a, 1975; Gerard, 1975; Schoffeniels, 1976; Hoffmann, 1977; Gilles, 1979; Schrock and Hanke, 1979a, 1979b; Kevers and coworkers, 1979; Vivers and coworkers, 1980; Tan and Choong, 1981; Koerig and coworkers, 1981). Florkin and coworkers (1962) showed that in the muscle of Eriocheir sinensis, the free amino acids and some other organic constituents account for nearly 40% of the total osmolarity of the tissue when the animal is in freshwater. When the animals are acclimated to sea water, the free amino acids are nearly doubled. Gerard and Gilles (1972a) showed that in the muscle of sea water acclimated blue crab Callinectes

sapidus, the amino acid concentration accounts for about 70% of the total osmolarity of the tissue and the rest can be accounted for mostly by the inorganic ions Na^+, K^+ and Cl^-. When the same crab is acclimated to dilute medium the inorganic ion concentration decreases only slightly but amino acid concentration decreases considerably. In diluted media, the decrease in osmolarity could be mostly accounted for by the decrease in amino acid concentration. It has been argued that the variation in amino acid concentration depends on an active process and "is not simply due to a dilution of the intracellular fluid caused by tissue swelling", although partly this may be so. Many studies indicate that the tissue level of free amino acids, especially of the non-essential ones, is actively regulated in accordance with changes in osmolarity of ECF and this has been so in several invertebrate phyla (reviews by Schoffeniels and Gilles, 1970, 1972; Gilles, 1975, 1979). This has also been evidenced in fishes (Cholette, Cagnon and Germain, 1970; Huggins and Colley, 1971; Lasserre and Gilles, 1971; Venkatachari, 1974; Vislie and Fugelli, 1975) and several other taxonomic groups of animals. The role of amino acids in cell volume regulation has been demonstrated in Ehrlich ascites tumour cells (Hendil and Hoffmann, 1974; Hoffmann and Hendil, 1976) and in elasmobranch fish transferred to dilute sea water (Forster and Goldstein, 1976; Forster et al, 1978; Goldstein and Boyd, 1978) and in the

juveniles and tadpoles of Xenopus exposed to hypertonic salt solution (Schrock and Hanke, 1979a, 1979b) thereby suggesting the possibility of amino acid participation in the intracellular fluid isosmotic regulation as a phenomenon of general occurrence in animals. Various studies have made it clear that the intracellular amino acid concentration invariably increases under hyper-osmotic conditions and decreases under hypo-osmotic conditions (Loc. cit).

Regarding the mechanisms involved in the control of the intracellular amino acid concentration in whole animals subjected to osmotic stresses, the following have been suggested:

i) Increased efflux of amino acids from tissues to blood (Clark, 1968; Binns, 1969; Vincent-Marique and Gilles, 1970a, 1970b; Gerard and Gilles, 1972a; Gilles, 1977, 1979) and increased catabolism of amino acids in cells (Gilles, 1979) have been implicated for the decrease in intracellular concentration of amino acids observed during acclimation to hypo-osmotic stress. Increased catabolism of amino acids is presumed to occur in animals adapting to lower salinities for the following reasons: $^{14}Co_2$ production from injected labelled glutamate has been shown to increase by 50% in Carcinus maenas acclimated to a diluted medium (Chaplin, Huggins and Munday, 1970). Several studies have shown increased oxygen uptake in euryhaline species acclimated to diluted media (Ramamurthi, 1965, 1967b; Lee and Pyung,

1970; Venkata Reddy, 1976; Venkatachari and Vasantha, 1981). Changes in NH_3 excretion of euryhaline species subjected to osmotic stresses have been reported (Needham, 1957; Jeuniaux and Florkin, 1961; Emerson, 1967, 1969; Krishnamoorthy and Srihari, 1973; Mangum and coworkers, 1976). Parallel changes in blood and tissue ammonia levels were reported. An increase in the level of NH_3 in the blood of Callinectes sapidus (Gerard and Gilles, 1972a; Mangum and coworkers, 1976) and in the muscle of Eriocheir sinensis (Vincent-Marique and Gilles, 1970a) was observed during acclimation to dilute media and the gill tissue of Eriocheir sinensis showed ($Na^+ - NH_4^-$) ATPase activity (Pequeux and Gilles, 1977). In other words, hypo-osmotic conditions are known to induce an increase in oxygen consumption, increased $^{14}Co_2$ production from labelled amino acids and an increased release of NH_3 in tissues and blood. Such observations show an increased catabolism of intracellular amino acids and this catabolic activity has been implicated for the decreased levels of amino acids in cells under hypo-osmotic stress (Gilles, 1979).

ii) During hyper-osmotic stress, an increase in the intracellular amino acid content has been observed (Loc. cit). This increase has been ascribed primarily to a decreased intracellular amino acid catabolism and an increased active influx of amino acids from the ECF. The decreased oxygen consumption (Eliassen, 1953;

Ramamurthi, 1965, 1967n; Siebers and coworkers, 1972; Venkata Reddy, 1976; Venkatachari and Vasantha, 1981) and decreased Co_2 production (Siebers, 1972) and decreased NH_3 production (Krishnamoorthy and Srihari, 1973) observed in animals during acclimation to concentrated media lend support to the view that decreased catabolism of amino acids occurs during hyper-osmotic stress. This in turn may result in the augmentation of the intracellular amino acid concentration observed during hyper-osmotic acclimation. The active influx of amino acids from blood into cells under hyper-osmotic conditions is suggested by the works of Siebers and coworkers (1972) and Gilles (1977) but results obtained by Rao and coworkers (1969) and Venkatachari and Keshavan (1973) are at variance. Synthesis of amino acids under hyper-osmotic stress seems to be unlikely as this cannot explain the increase observed in the levels of essential amino acids (Gilles, 1974a, 1977).

iii) Lipids and carbohydrates do not seem to play significant roles during acclimation of animals to hyper- and hypo-osmotic conditions (Siebers, 1972). The role played by modification in the equilibrium between intracellular proteins and amino acids in the regulation of intracellular amino acid content during acclimation to heterosmotic media is doubtful (Florkin and coworkers, 1964; Gilles and Schoffeniels, 1969a; Siebers and coworkers, 1972).

iv) The variations in catabolic activity of amino acids seen under hypo-and hyper-osmotic conditions appear to be triggered mostly by variations in the intracellular content of inorganic ions which are implicated in the modulations of the activity of all oxidative enzymes (Snoke, 1956; Chaplin, Huggins and Munday, 1965; Gilles, 1969; Gilles, Hogue and Kearney, 1971; review articles by Schoffeniels and Gilles, 1970; Gilles, 1974a; 1975, 1979; Schoffeniels, 1976).

v) NH_4^+ or H^+ ions are supposed to act as counter ions for the active Na^+ uptake and HCo_3^- ion has been suggested as the counter ion for the Cl^- uptake. Therefore, a Na^+/NH_4^+ or Na^+/H^+ exchange pump and a Cl^-/HCo_3^- exchange mechanism have been proposed to operate at the exchange surfaces. Both these mechanisms were proposed at first by Krogh (1938) and strengthened subsequently by other investigators (Shaw, 1960; Maetz and Garcia Romeu, 1964; Kerstetter, Kirschner and Rafuse, 1970; Maetz, 1956, 1972, 1973).

Some studies have been made on the role of free amino acids in the blood of Crustaceans in relation to salinity stress (Madanmohan Rao et al, 1969; Siebers and coworkers, 1972; Venkatachari and Ramamani Keshavan, 1973; Vincent Marique and Gilles, 1970a; Venkata Reddy, 1976; Gilles, 1977) but more information is needed in this regard as the resultsobtained so far are confusing and contradictory. Therefofe, an attempt has been made to understand the time course of changes in free amino acids

or total ninhydrin positive substances (TNPS) in the blood along with that of hepatopancreas and muscle of the crab (Oziotelphusa) subjected to 50% and 100% salinity stress and also when returned to freshwater.

Materials and Methods

The method of acclimation of the crabs to sea water and the procedure adopted for the estimation of total ninhydrin positive substances (TNPS) are given in the section "Materials and Methods". The method of calculation of the amount of TNPS in the intracellular fluid, taking into account the levels of hydration of tissues and ECF spaces, is also described.

BLOOD AND TISSUE TNPS

Results

The data on time course of changes in the levels of TNPS in the blood, hepatopancreas and muscle of the crab are presented in tables 17 & 18. Variance ratios for the data are given in tables 17-1 and 18-1 and the statistical comparison is presented in tables 17-2 and 18-2. The mean values for blood and intracellular water (Table-19) are plotted as percent deviations from the normal in figures 25 & 26.

It may be noted from the data that the blood contains negligible amounts of TNPS whereas the tissues are loaded with heavy amounts of these amino acids. This disparity between the

FIGS. 25 & 26: Time course of changes in total ninhydrin positive substances (TNPS) in the blood & tissues of the crab when transferred to 50% & 100% SW and then returned to fresh water.

FIG. 25

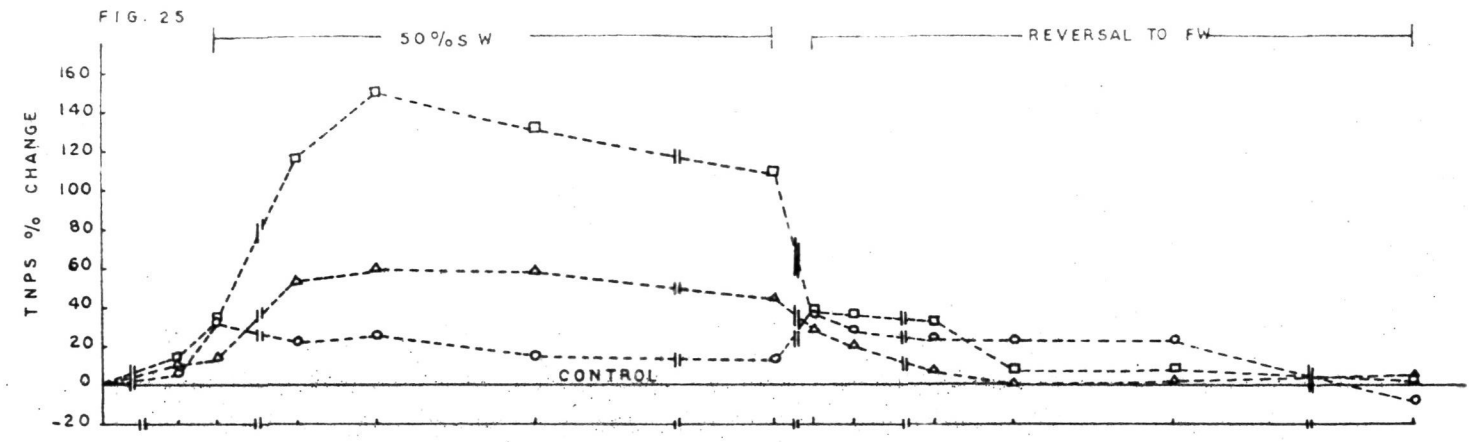
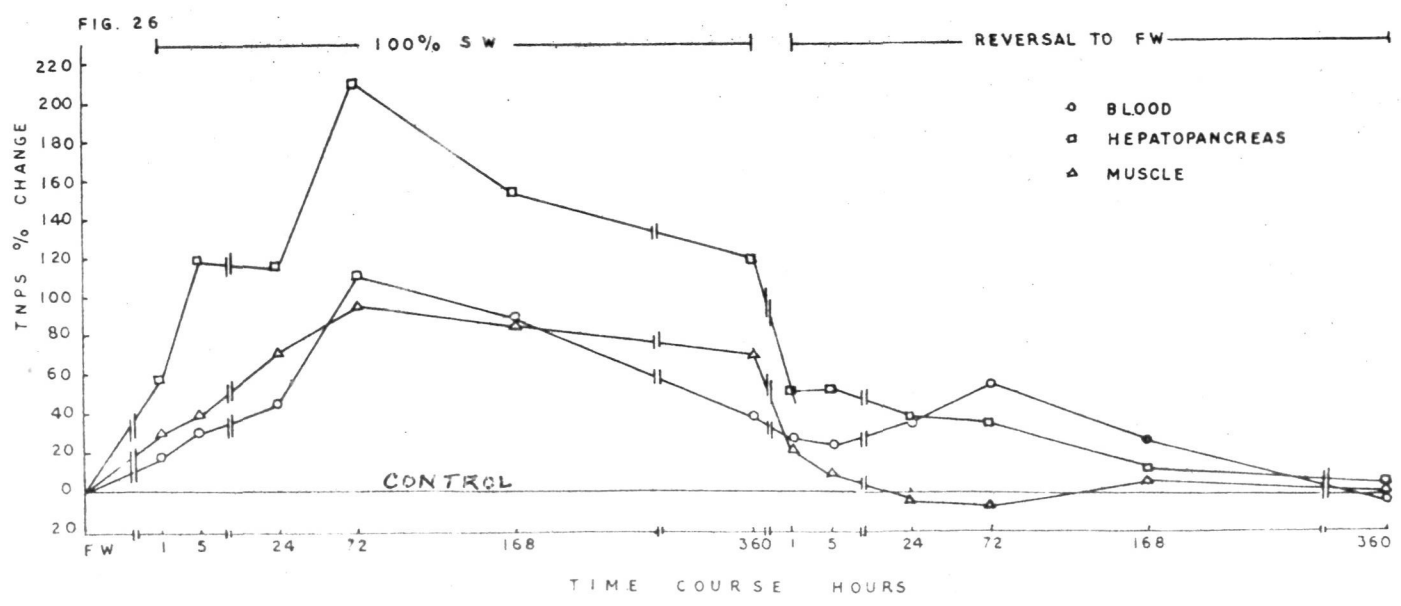

50% S W
REVERSAL TO FW
TNPS % CHANGE
160
140
120
100
80
60
40
20
0
-20
CONTROL

FIG. 26


100% S W
REVERSAL TO FW
BLOOD
HEPATOPANCREAS
MUSCLE
TNPS % CHANGE
220
200
180
160
140
120
100
80
60
40
20
0
20
CONTROL
FW
1
5
24
72
168
360
1
5
24
72
168
360
TIME COURSE HOURS

blood and tissues is maintained throughout the acclimation period to sea water and also in the reverse acclimation process.

Blood TNPS

The blood TNPS level during acclimation to 50% SW showed significant increase between 5 and 72 hours of acclimation ($P < 0.05$). The 5th hour value was highly significant ($P < 0.01$). However, it returned to the control level by 168 hours. Reversal to FW from 50% SW resulted in a significant increase in the 1st hour compared to control value and this increase was maintained for nearly 7 days. The values of the 1st and 5th hours were highly significant ($P < 0.01$). The TNPS level decreased to the control value only by the end of acclimation period (15 days). In the crabs transferred directly to 100% SW, the changes observed were significantly high and reached a maximum level by 72 hours after transfer. Subsequently a decreasing trend was evidnet. However, even after 15 days a significantly higher blood TNPS level was maintained compared to the control value. The values between 24 and 72 hours were highly significant ($P < 0.01$). During the reverse acclimation to FW there was a transient decrease in the 1st and 5th hours but subsequently the level increased significantly and it was maximal after 72 hours of transfer to FW. Only at the end of the acclimation

period it reached the normal level. The levels of TNPS in blood during all the stages of reverse acclimation to FW from 100% SW were highly significant compared to the control value ($P < 0.01$).

To sum up, the TNPS levels of blood showed significant increasing trends in the first stages of acclimation to SW as well as during the reverse acclimation process from SW to FW. But in 50% SW, there was a significant decrease to normal levels from the 7th day of acclimation. In 100% SW, significantly higher levels were maintained even at the end of acclimation period. Significantly high blood TNPS levels were maintained even after transfer to FW from both 50% and 100% SW and it is worth noting that these levels were highly significant compared to controls in the first and fifth hours of reversal to FW in 50% SW acclimated crabs and upto 168 hours in crabs exposed to 100% SW ($P < 0.01$).

TNPS levels in hepatopancreas

The total ninhydrin positive substances of tissues showed a staggering elevation in 50% and 100% SW acclimated crabs but these levels fell steeply in crabs returned to FW. Highly significant ($P < 0.01$) levels of TNPS were evident in hepatopancreas from the 5th hour of acclimation in 50% SW and from the first hour in 100% SW. A further highly significant

increase was observed 24 hours after transfer to 50% SW (+92.51%) and thereafter a steady state was maintained throughout the course of acclimation when the weight specific tissue levels were taken into account. However, when the intracellular concentrations of TNPS per KG tissue water were seen (table 19 & fig. 25) the values increased to a maximum level (+149.09%) about 72 hours after acclimation and thereafter there was considerable decrease to 108.81% at the end of acclimation period. Upon reversal to FW from 50% SW, the TNPS concentration in hepatopancreas dropped significantly ($P < 0.01$) even at the very first stages starting from the 1st hour through 24 hours. There was a further highly significant fall by 72 hours and thereafter the values were not significantly different from the control value. In 100% SW, the significant increase which started in the first hour reached a peak value (111.92%) at 72 hours which was highly significant ($P < 0.001$) and thereafter there was an apparent decreasing trend. But even the lowered level (49.49%) at the end of acclimation period was significantly high ($P < 0.001$) compared to the control value. In the reverse acclimation process to FW from 100% SW, there was a highly significant decrease of the TNPS level even from the first hour. A further significant drop was recorded by 24 hours and the lowered level observed from 168 hours was not significantly different from the control value. The intracellular

TNPS levels expressed as mosmoles/Kg tissue water (table 19 & fig. 26) also showed similar trends but the levels of concentration were nearly doubled compared to the weight specific tissue levels.

TNPS levels in muscle

The concentration of TNPS was generally higher in muscle than in hepatopancreas in all the media studied. But, during acclimation to SW the percent deviation from the control value was much higher in hepatopancreas than in muscle. During acclimation to 50% SW, the TNPS level in muscle increased to a highly significant level ($P < 0.001$) after 24 hours of transfer and thereafter a steady state was achieved and no further significant changes were observed. In the reverse acclimation there was a highly significant drop in TNPS level soon after the first hour and a further significant decrease was observed after 24 hours. From 72 hours after reversal, the values were not significantly different from the control value. In 100% SW, the TNPS showed maximum level of increase after 72 hours of exposure (56.66%) but even after the 1st hour the level was significantly higher ($P < 0.01$) than the control value. There was a downward trend after 72 hours. However, significantly high levels were maintained till the end of the acclimation period ($P < 0.001$). In the reverse acclimation to FW from 100% SW, the TNPS level decreased significantly by the 1st hour and

reached normal level by the 5th hour. However, there was further reduction to significantly lower levels between 24 and 168 hours and only at the end of acclimation period the control level was attained.

The values of TNPS measured as m moles/Kg wet tissue were calculated for Kg tissue water after taking into account the levels of hydration and the ECF volume of the tissues. The method of calculation is described elsewhere. Data on the intracellular TNPS levels thus arrived at are presented in table 19. From the data it is evident that the trend of change during acclimation to sea water as well as in the reverse direction remains nearly the same as the one seen in weight specific wet tissue TNPS levels. But the intracellular concentration levels are much higher. This is understandable as the extracellular fluid of the tissues contain negligible amounts of TNPS compared to the intracellular component. Further, both the tissues loose significant amounts of water during the acclimation to SW and when the ECF volume and hydration of tissues are taken into account, the intracellular concentration of TNPS shows higher levels than what is apparent in Kg wet tissue weight. However, as the trends remain the same, no further elaboration is needed on this data.

Discussion

Blood TNPS

Blood TNPS levels showed a general increase during acclimation to SW as well as during the reverse acclimation process to FW with a later decline to control levels at the end of acclimation period except in 100% SW.

Blood has an important role to play in the physiological responses to stress conditions, since it is in direct permeated communication with the external medium on one hand and with the tissues on the other. Regulation of the osmotic concentration of blood is therefore of paramount importance for optimal cell function. This has been termed as "extracellular anisosmotic regulation" as contrasted to "intracellular isosmotic regulation" (Florkin and Schoffeniels, 1969). The freshwater euryhaline crustaceans which have been classified as strong regulators maintain this anisosmoticity of blood with the external medium. Some of these aspects were discussed earlier (Chapter II). In the marine crustaceans a general isosmoticity exists between the blood and ambient medium. Although this suggests involvement of an evasive mechanism such as a passive equilibrium between blood and external medium, the existence of considerable degree of ionic regulation has been suggested (Florkin, 1960; Potts and Parry, 1964a). The increased levels of blood amino acids immediately after the application of

osmotic stress both in sea water and fresh water with a later decline except in 100% SW may be related to such ionic regulation of the ECF. This ionic regulatory capability assumes greater relevance in fresh water forms which are generally hyper-regulators (Schoffeniels and Gilles, 1970). Reduction of the body wall permeability to water and salts, active uptake of salts mainly through the respiratory surface i.e. the gills and excretion of hypotonic urine are some of the mechanisms operating in these fresh water forms (see Chapter I and II). The active transport of Na^+ and Cl^- against the concentration gradients in the gill involves utilization of energy. The increased levels of amino acids in the blood in the early stages of reversal to FW assumes relevance in this connection. These amino acids are regarded as energy sources in the blood and are made use of in the gills for active uptake of inorganic ions particularly Na^+ and Cl^-. An active salt absorption mechanism has been demonstrated in the crab under investigation (Pampapathi Rao and Venkata Reddy, 1962). That this absorption is an energy requiring process has also been shown in this crab (Venkata Reddy and Aravinda Babu, 1969). It has been shown by Ramamurthi (1965, 1967b) that in the crab under study, oxygen consumption is higher in lower salinities and lower in higher salinities. Similar results have been obtained in the present studies. The oxygen consumption of the crab is generally

elevated in FW and depressed in 50% and 100% SW (Chapter I). The active transport of sodium in isolated amphibian skin and urinary bladder has been shown to be energized by oxidative metabolism (Zerahn, 1956; Leaf and Renshaw, 1957; Viera, Caplan and Essig, 1972). A direct relationship between sodium transport and oxygen consumption has been shown in toad urinary bladder (Lang, Caplan and Essig, 1977). Increased oxygen consumption in dilute media naturally implies concomitant release of Co_2. Hypo-osmotic conditions are known to induce an increased $^{14}Co_2$ production from different labelled amino acids (Chaplin, Huggins and Munday, 1970; Gilles, 1972). This in turn indicates increased catabolism of amino acids in diluted media. The gill tissue of E. sinensis shows increased proline oxidative activity particularly in the posterior gill pairs which are involved in the active uptake of Na^+ and Cl^- (Schoffeniels and Gilles, 1970). Thabrew and coworkers (1973) reported increased oxidation of glutamate and proline by isolated gills of C. maenas subjected to hypo-osmotic stress. A transitory increase in the blood amino acid level particularly of proline, was observed in E. sinensis during acclimation to FW (Vincent-Marique and Gilles, 1970b). The increase observed in blood amino acid level in the early phase of reverse acclimation to FW in the crabs is in agreement with these reports and are thus meaningful.

During hypo-osmotic shocks the amino acids seem to leak out of the cells into the blood (Clark, 1968; Binns, 1969; Vincent-Marique and Gilles, 1970a, 1970b; Gerard and Gilles, 1972a; Gilles, 1977). This perhaps explains the highly significant increase in blood amino acid level in the early stages of reverse acclimation to FW. These amino acids may be stored in the form of blood serum peptides and proteins particularly proteins containing Cu^{2+} (Siebers and coworkers, 1972; Mangum, 1976; Gilles, 1977). Such conversions coupled with increased catabolism of amino acids in gills may account for the decreased blood amino acid levels in the later stages of reverse acclimation to FW. In contrast, hyper-osmotic conditions seem to induce an increased degradation or decreased synthesis of blood serum peptidic material (Siebers and co-workers, 1972; Gilles, 1977; Pequeux, Vallota and Gilles, 1979; Tan and Choong, 1981). This in turn may account for the increased levels of blood amino acids seen initially in 50% and 100% SW. The free amino acids left available in the blood during hyper-osmotic conditions may be actively transported into the cells (Siebers and Coworkers, 1972; Gilles, 1977). This may probably explain the decline in blood amino acid content of crabs in later stages of exposure to 50% and 100% SW. It is interesting to note that proteins containing Cu^{2+}, essentially a respiratory pigment, increase in the blood under

acclimation to diluted media. This increases the efficiency of the oxygen carrier system, which is essential for active transport of ions (Mangum, 1976). The patterns of excretion of NH_3 under conditions of osmotic stress seem to show an interesting correlation with Na^+/NH_4^+ exchange. Similarly, the levels of Co_2 produced in whole animals acclimated to heterosmotic media appear to show correlation with Cl^-/HCo_3^- exchange. Increased levels of NH_3 were observed in the blood of Callinectes sapidus during acclimation to dilute media (Gerard and Gilles, 1972a; Mangum and coworkers, 1976). Similar results were obtained in the muscles of Eriocheir sinensis (Vincent-Marique and Gilles, 1970a). In the crab under investigation, Krishnamoorthy and Srihari (1973) have not shown any changes in the total amount of excreted nitrogen under hyper- and hypo-osmotic conditions. However, a statistical re-evaluation of the same data by Venkata Reddy (1976) has shown significantly higher non-protein, non-amino acid excretory nitrogen levels in FW animals and lower values in SW. Venkata Reddy (1976) has also shown reduction of output of excretory nitrogen from the tissues to the blood in SW acclimated crabs. Such observations agree with the findings of Needham (1957), Jeuniaux and Florkin (1961), Florkin et al. (1964). Production of increased levels of Co_2 under hypo-osmotic conditions has been referred to earlier. The metabolic products of increased amino acid catabolism particularly NH_3 and Co_2 which appear under acclimation

to diluted media seem to participate in the physiological process of inorganic ion transport at the gill level. Gills are mainly known to be involved in the regulation of blood Na^+ and Cl^- levels (Gilles, 1975). The active uptake of Na^+ in the gill under hypo-osmotic conditions seems to be coupled with extrusion of NH_4^+ and H^+. Similarly the active uptake of Cl^- appears to be coupled with the extrusion of HCo_3^- (knogh, 1938) Increased blood ammonia and Co_2 levels coupled with increased oxygen consumption during acclimation to diluted media lend support to these propositions.

In brief, the lowered levels of amino acids seen in the blood of the crab at the end of reverse acclimation to FW may be the result of a greater rate of movement of the amino acids into the gills from the blood for sustaining the uphill task of ion transport. The increased levels of TNPS observed in the blood soon after transfer to FW from SW may indicate a leakage of amino acids out of tissues (Loc. cit). However, their decrease at the end of acclimation to FW also suggests their conversion to blood serum peptides and proteins (Siebers and coworkers, 1972; Gilles, 1977, 1979; Pequeux, Vallota and Gilles, 1979; Tan and Choong, 1981) as well as their mobilization into the gills. In the crabs the TNPS levels of blood show significant increasing trends during the first stages of acclimation to both 50% and 100% SW but the

levels return to control value after 168 hours in 50% SW. In 100% SW, although there is a decrease in TNPS level at the end of acclimation period, it remains significantly high compared to control value. These results are indicative of "increased degradation and/or decreased synthesis of blood serum peptidic material" during the first stages in 50% SW and throughout the period of exposure in 100% SW. Tan and Choong (1981) reported a significant decrease in the hemolymph protein content in Macrobrachium rosenbergii exposed to more concentrated media suggesting hydrolysis of hemolymph proteins to free amino acids. However, they drew the conclusion that the hemolymph NPS is independent of salinity. Venkata Reddy (1976) has reported highly significant decrease ($P < 0.001$) in the total protein content of blood of the same crab after 15 days of exposure to 75% and 100% SW and a non-significant decrease in total protein but a significant decrease ($P < 0.001$) in the insoluble fraction in 50% SW. Further, as the active uptake of ions in the gills is less important in view of the decreased ion gradients, the blood TNPS transport into the gills is perhaps drastically cut down, resulting in the conservation of TNPS in the blood. The free amino acids now available may actively be transported into the cells wherein they contribute to the increased osmolarity in the intracellular isosmotic regulation process. The active influx of TNPS from blood into the cells may also explain the decreased levels of amino acids in the blood during the later stages of acclimation period

(particularly in 50% SW) and the concomitant enormous increase in the intracellular concentration of amino acids under hyper-osmotic conditions.

In a related species, Barytelphusa guerini, Venkatachari and Ramamani Keshavan (1973) reported a slight increase in blood amino acids after acclimation to concentrated media. Similar results were reported in the crab Sesarma plicatum (Madanmohan Rao and Coworkers, 1969). These reports agree with the results obtained in the present work and with those reported by Venkata Reddy (1976) and probably are indicative of the role played by changes in the equilibrium between the blood serum peptidic material and amino acids in the regulation of the amino acid pool during osmotic stress. But, Gilles (1979) interpreted such results to be contradictory in the light of reports (Siebers and coworkers, 1972; Gilles, 1977) showing a "transitory decrease" in the blood amino acid content during acclimation to concentrated media. It appears now that such results which are seemingly contradictory, however, lend to reasonably meaningful interpretations such as the ones given above when the time course of such changes is kept in view. Further, such differences as have been pointed out by Gilles (1979) may be indicative of species specific differences or the blood TNPS variations may be independent of salinity as suggested by Tan & Choong (1981).

The ninhydrin positive substances of blood make a negligible contribution to the total osmolarity of blood. The percent participation of TNPS in the total osmolarity of blood is less than 1 (0.83 to 0.60%) in different increasing salinities (Venkata Reddy, 1976). Therefore, assignment of a role other than contribution to osmotic pressure of blood seems to be relevant for blood TNPS.

The increased blood TNPS levels of whole animals during acclimation to higher salinities, as evident in the present work probably contribute to an important aspect of intracellular isosmotic adjustment. Both the tissues studied, the hepato-pancreas and muscle, show highly elevated levels of amino acids when subjected to hyperosmotic shocks (see table 19). Under such conditions increased active influx of amino acids from the blood into the tissues has been postulated (Siebers and coworkers, 1972; Gilles, 1977, 1979). In such situations, the increasing levels of TNPS in the ECF provide an abundant supply for augmenting the amino acid influx into the cells where they function as organic osmotic effectors in the intracellular isosmotic regulation.

Tissue TNPS

The TNPS levels of tissues (Tables: 17, 18 & 19) showed a staggering increase in 50% and 100% SW exposed crabs and the levels decreased relatively quickly when the sea-water

acclimated crabs were subjected to hypo-osmotic shock in the reverse acclimation to FW. The levels in hepatopancreas were generally lower than those obtained in the muscle throughout the period of exposure to SW and also in FW. This indicates a lesser role of free amino acids in the intracellular osmotic profile of hepatopancreas compared to their importance in the muscle. In the crab Callinectes sapidus, a similar situation has been reported for the hepatopancreas and gill (Gerard and Gilles, 1972a). The general trend of variations of TNPS levels in the tissues of the crab compares well with the trends obtained in euryhaline invertebrates subjected to osmotic stresses (Schoffeniels and Gilles, 1970).

Marine animals have in their tissues generally higher levels of amino acids than fresh water animals. The role of free amino acids as the intracellular osmotic effectors has been well documented (Duchateau-Bosson and Florkin, 1961; Florkin 1962; Florkin et al, 1964; Gilles and Schoffeniels, 1969a, 1969b; Florkin and Schoffeniels, 1969; Schoffeniels and Gilles, 1970; Gerard and Gilles, 1972a,b; Gilles and Gerard, 1974; Schoffeniels, 1976; Gilles, 1979). The intracellular free amino acids play a remarkable role in the establishment of osmotic parity between intracellular milieu and ECF in euryhaline invertebrates during their

adaptive sojourn in altered salinities. For instance, when the blue crab Callinectes sapidus is acclimated to two-times diluted sea water, the inorganic ion levels in tissues remain relatively less altered, but the free amino acid content is greatly reduced (Gerard and Gilles, 1972a). When the animals are acclimated to sea water the free amino acids increase and account for nearly 70% of the total osmolarity of the muscle tissue. A similar trend has been observed in the muscle of Eriocheir sinensis (Florkin and coworkers 1962). Results obtained in the present work are in agreement with such general trends. In the crab under study , Venkata Reddy (1976) showed that contribution of dialyzable N-compounds accounts for nearly 60% of total osmolar concentration of the muscle. Table 21 and figures 29 & 30 show the percentage contribution of TNPS to total concentration of Na^+ , Cl^- and TNPS of the hepatopancreas and muscle in the same crab during the period of exposure to 50% and 100% SW and also during the reverse acclimation to fresh water. It is seen that the percentage participation of free amino acids is generally between 55-70% in the tissues and it is found to be higher in crabs exposed to 50% & 100% SW than in FW crabs with the exception of hepatopancreas of 100% SW exposed animals.

An important difference is noticed in the amino acid concentration between the hepatopancreas and muscle. It is generally low in hepatopancreas and very high in muscle in FW medium (Tables 17, 18 & 19). Upon acclimation to SW, the intracellular concentration of both the tissues increase greatly, which shows clearly that the free amino acids play a definite role as organic osmotic effectors and augment the osmotic concentration of the intracellular fluid in its isosmotic regulation process. However, after a rapid increase upto 72 hours, some amount of decline in amino acid levels in both tissues was evident in later stages with an important reduction in hepatopancreas from the 7th day in 100% SW. This appears to be related to a steep increase in the inorganic ion content of the hepatopancreas and its extreme dehydration with no compensation whatsoever leading to the "Salogenous atrophy" of this tissue reported in this crab (Venkata Reddy, 1976). The steep increase in inorganic ions in the hepatopancreas in 100% SW seems to cause an "ionic injury" to the tissue. The intracellular inorganic ion profile of the muscle is relatively less altered while the organic osmotic effectors fill the gap to provide the necessary isosmotic conditions in 100% SW.

Amino acids are supposed to play an important role in the balancing of high potassium content in tissues, especially in muscle. However, "the retention of K^+ ions

inside the cell seems to be largely electrostatic and only a small part of potassium flux appears to result from the active process (Shaw, 1958a,b). Therefore, the organic effectors constitute "certainly the mechanisms by which sodium and chloride are excluded from the cell" (Schoffeniels and Gilles, 1970).

The intracellular concentrations of free amino acids increase progressively, on the time scale of the experiment, in crabs acclimated to higher salinities and decrease in lower salinities. In the hepatopancreas as well as muscle maximum increase was seen after 72 hours both in 50% & 100% SW and subsequently there was a downward trend. This trend corresponds to that of dehydration of these tissues under salinity stress (see tables 8 & 9). Maximum dehydration in both tissues has been recorded at 72 hours of transfer to SW. This correlation indicates that the changes observed in free amino acid levels are atleast partly due to changes in water content of tissues and concomitant changes in cell volume control (Gilles, 1974a; Hoffmann, 1977). The hepatopancreas, however, shows greater dehydration than muscle and greater penetration of inorganic ions and lesser participation of amino acids which appear to be injurious to the animal in 100% SW. Venkata Reddy (1976) has reported changes in gravimetric status of this tissue in higher salinities. The weight of the hepatopancreas decreases by about 1 gram in

whole animals acclimated to 100% SW and this decrease in weight was considered to be the basis for what he called the "salogenous atrophy" of hepatopancreas. This atrophy, it was argued, was the causative factor for the "anorectic" or "aprandial" state of the animal leading to loss of appetite in 100% SW. The crabs also become sluggish in 100% SW with loss of escape reactions. It has been suggested earlier that extreme dehydration of the muscle in 100% SW resulting perhaps in the shrinkage of the muscle may be an additional factor for the loss of escape reactions and consequent lethargy. However, the "salogenous atrophy" of the hepatopancreas does not seem to cause an irreparable damage to the tissue upto the 15th day of sojourn for, the crabs recovered more or less completely on the 7th day of their return to FW. The crabs resume their normal movements and resort to feeding habits atleast on the 7th day of their return to freshwater. The water content of muscle returns to normal levels by 72 hours in the reverse acclimation process both in 50% and 100% SW exposed crabs. This is also the case in hepatopancreas of 50% SW acclimated crabs but not so in 100% SW exposed animals wherein the hepatopancreas regains its water content by about the 7th day. Thus, loss of appetite and escape reactions in 100% SW acclimated crabs seems to be also partly due to dehydration of the tissues.

Dehydration coupled with lesser participation of free amino acids and heavy impregnation of the hepatopancreas with inorganic ions may result in the "salgenous atrophy" of the pancreas. However, this atrophy does not seem to cause such irreparable damage to the tissue for it regains its normal water content, organic and inorganic osmotic effectors although slowly, by the 7th day. Perhaps, the damage may be irreparable after prolonged sojourn in 100% sea water.

It would be rather interesting and worthwhile if the total osmotic balance of blood and tissues were to be obtained. Meaningful interpretations are possible only when the total osmolar concentrations of blood and tissues are compared. Unfortunately the work suffers from lack of data on other inorganic ions particularly K^+ which plays an important role as one of the inorganic osmotic effectors in tissues, particularly in the muscle. Data on these ions could not be presented as it did not form part of this programme. However, as the osmotic effectors studied viz., Na^+, Cl^-, and amino acids play a major part in the osmotic profile of the blood and tissues, the total concentration of these osmotic effectors is furnished in table 20. The osmolarity of Na^+, Cl^- and TNPS of intracellular fluid has been calculated taking into account the level of tissue hydration and an arbitrary value of 20% as the ECF volume of tissue.

The reasons for considering this arbitrary ECF volume and the method of computation are given in the section on materials & methods of this dissertation. The value assumed for ECF volume, although arbitrary, has yielded data with which some meaningful interpretations could be made. The total osmolar concentrations of Na^+, Cl^- and TNPS in blood, hepatopancreas and muscle are shown in table 20 and in the form of histograms (figures 27 & 28). The following tentative inferences can be made from these data and figures. In FW, the hepatopancreas shows poor osmotic balance. The total osmoconcentration of Na^+, Cl^- and TNPS is lower in hepatopancreas compared to that of ECF. The muscle on the otherhand shows a better osmotic balance. It is nearly isosmotic with blood in FW medium. This indicates that the hepatopancreas does not show isosmotic regulation with ECF in FW. However, isosmotic levels may be visualised in hepatopancreas if other ions like K^+, Ca^{++}, Mg^+ etc are taken into account. In such a situation the muscle would show hyper-osmotic regulation. Under acclimation to SW, progressive elevation of osmolar concentration is observed in the blood and the blood shows hyperosmotic regulation in 50% SW and nearly isosmotic regulation in 100% SW. Hepatopancreas and muscle show increasing osmolar concentrations corresponding to those of blood. However, the rate of increase in osmolar concentration of hepatopancreas is faster upto

FIGS. 27 & 28: Comparison of changes in total osmolarity of Na^+, Cl^- & TNPS in blood with that of tissues in crabs subjected to salinity stress.

TNPS = Total ninhydrin positive substances.

FIG. 27

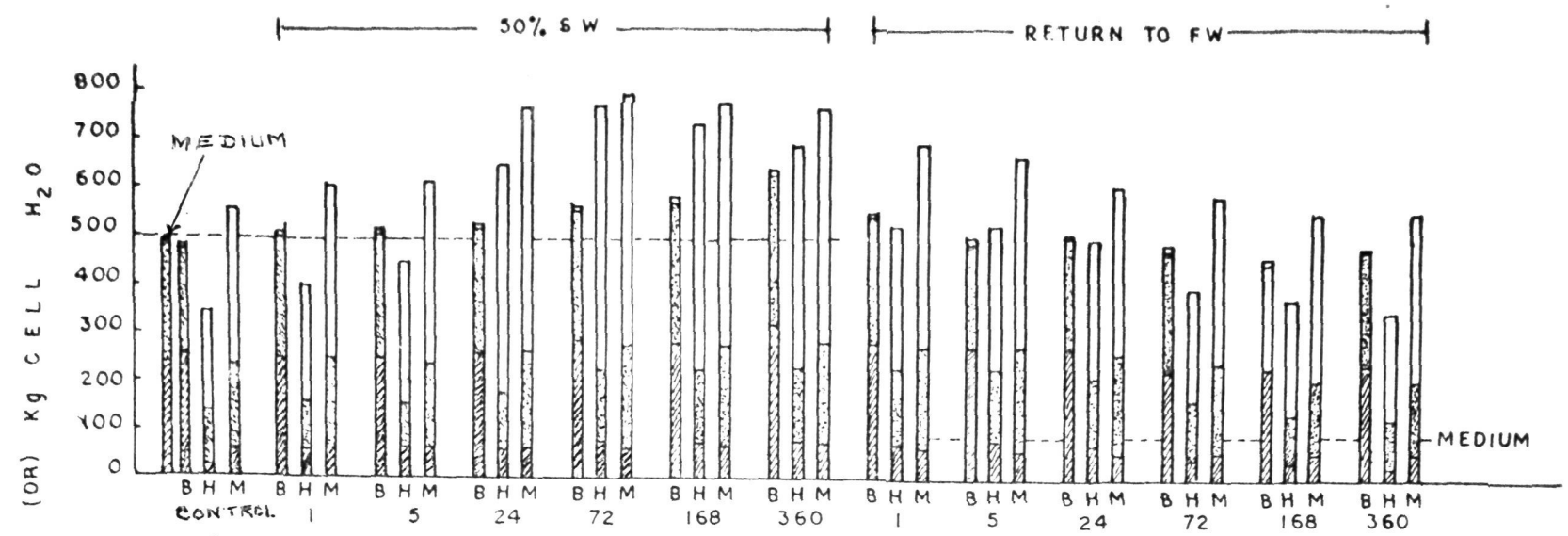
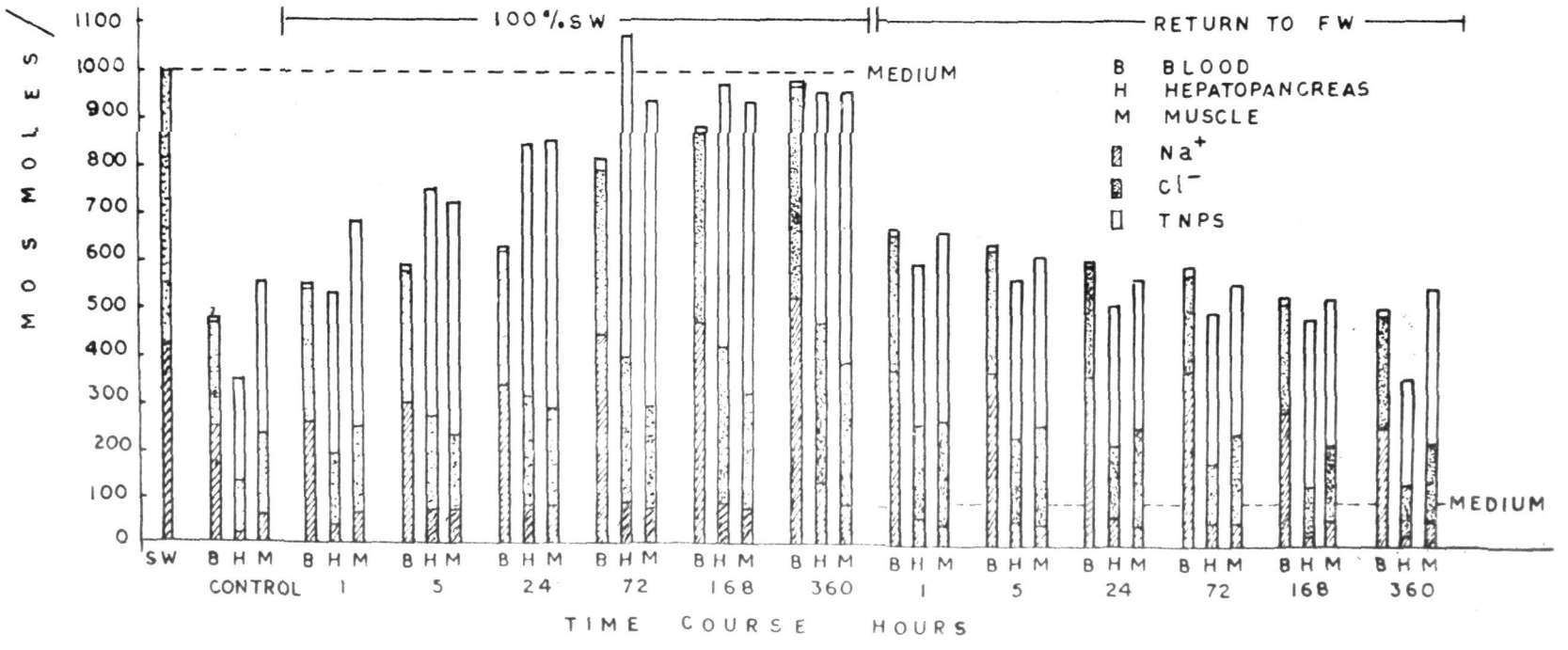

50% S W
RETURN TO FW
MEDIUM
(OR) Kg CELL H2O
800
700
600
500
400
300
200
100
0
B H M
CONTROL
1
5
24
72
168
360
MEDIUM

FIG. 28


100% S W
RETURN TO FW
MEDIUM
B BLOOD
H HEPATOPANCREAS
M MUSCLE
Na+
Cl−
TNPS
MOS MOLES / L
1100
1000
900
800
700
600
500
400
300
200
100
0
SW
B H M
CONTROL
1
5
24
72
168
360
MEDIUM
TIME COURSE HOURS

72 hours after transfer to both 50% & 100% SW and thereafter, the levels show decreasing trends. However, the hepato-pancreass seems to achieve near isosmoticity with blood by about 15 days in 50% SW whereas the muscle retains to hyper-osmotic condition throughout the acclimation period. Both the tissues seem to attain isosmotic levels with ECF by about 15 days in 100% SW. During the reverse acclimation to FW the blood total osmolarity of Na^+, Cl^- and TNPS decreases to almost normal levels in the first stages of reversal in 50% SW acclimated crabs and in 100% SW exposed crabs it falls suddenly by nearly 50% in the first stages and thereafter returns to normal levels by about 7 days. In the reverse acclimation to FW the osmotic concentration of hepatopancreas decreases as fast as it increases in SW. It reaches isosmotic levels in with hemolymph in the first stages but hypo-osmotic levels in the end stages of reverse acclimation from 50% SW. The muscle shows hyperosmotic levels throughout the period of acclimation to 50% SW and also during reverse acclimation. In 100% SW the muscle shows hyperosmotic levels only upto 72 hours and from 168 hours it shows nearly isosmotic condition. In the reverse acclimation from 100% SW the muscle maintains more or less isosmotic condition with ECF. Perhaps, fluctuations in the isosmotic levels of tissues during acclimation process may be encountered in general but nearly isosmotic levels are achieved only at the end stages of acclimation. The poor osmotic balance of hepatopancreas in Fw is rather difficult to

explain and perhaps it shows that tissue specific differences exist in the intracellular isosmotic regulation.

The extent to which free amino acids contribute to total osmolarily can be visualised from table 21 and figures 29 & 30. As already mentioned, the total osmolarity of tissues suffers from insufficiency of data as the data on other inorganic ions, particularly K^+ are not available. However, table 21 shows the percent contribution of TNPS to total concentration of Na^+, Cl^- and TNPS which are the major osmotic effectors. It is seen from this table that the contribution of TNPS to blood osmolarity is negligible (1.00 to 1.6%) whereas it is very high in tissues (55 to 70%) (when K^+ is also taken into account, the figures would perhaps get lowered slightly particularly in tissues). However, it is worth noting that the rate of increase in the TNPS levels in tissues during the sojourn of crabs in 50% & 100% SW and also the rate of decrease in the reverse acclimation process, are in proportion to the rate of increase or decrease in inorganic ions viz., Na^+ and Cl^- although the free amino acids have an edge over the inorganic ions in such variations. As a consequence of this, the percent contribution of free amino acids to total osmolarity increases in general during acclimation to sea water and decreases during reversal to FW with the exception of hepatopancreas of 100% SW exposed crabs. This trend is in agreement with earlier reports

FIGS. 29 & 30: Contribution of total ninhydrin positive substances (TNPS) to the osmolar concentration of the intracellular fluids when only Na^+, Cl^- and TNPS are taken into account.

FIG. 29

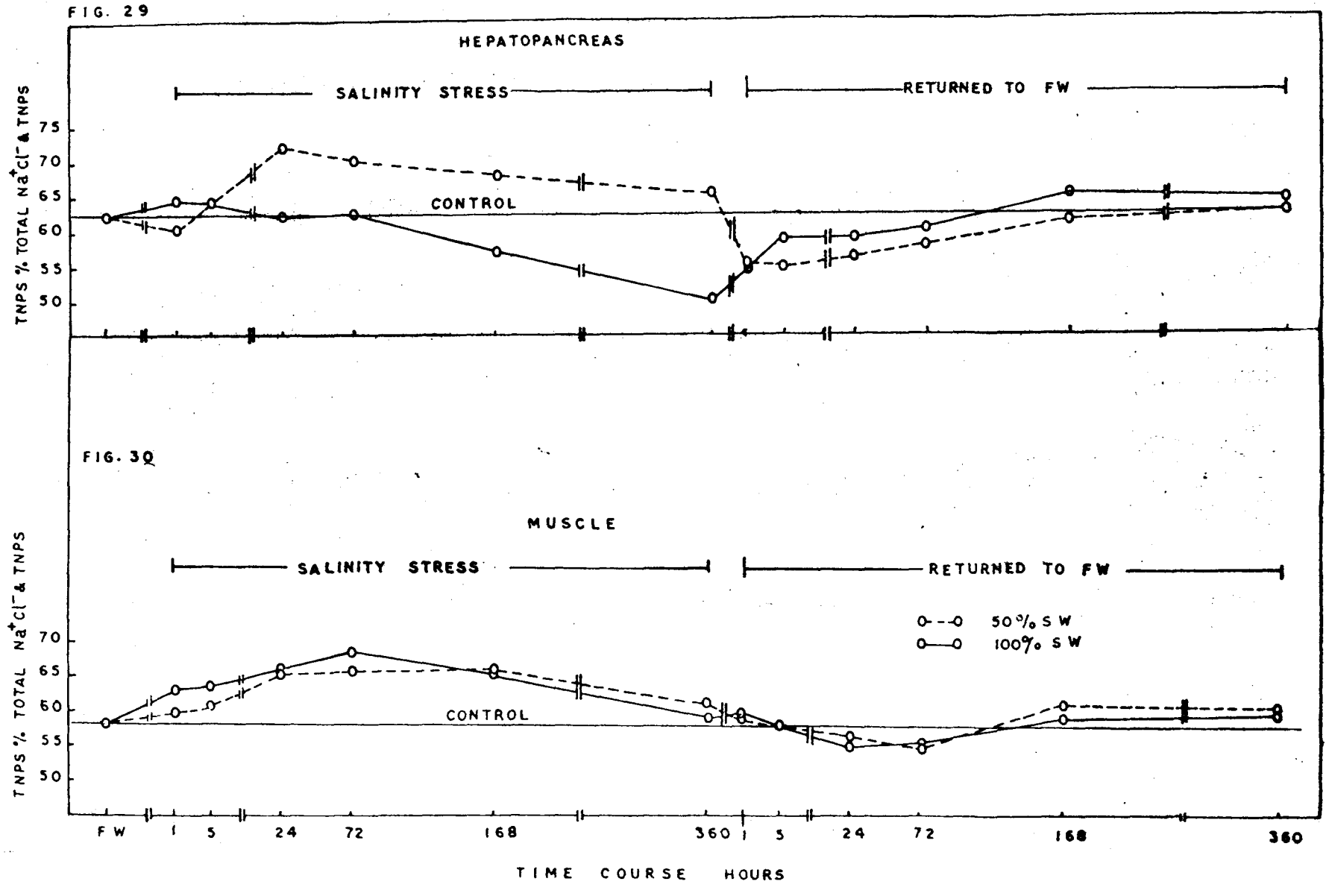

HEPATOPANCREAS
SALINITY STRESS
RETURNED TO FW
TNPS % TOTAL Na+Cl- & TNPS
75
70
65
60
55
50
CONTROL

FIG. 30

MUSCLE
SALINITY STRESS
RETURNED TO FW
50 % S W
100% S W
TNPS % TOTAL Na+Cl- & TNPS
70
65
60
55
50
CONTROL
FW
1
5
24
72
168
360
1
5
24
72
168
360
TIME COURSE HOURS

(Schoffeniels and Gilles, 1970, 1972; Gerard and Gilles 1972a; Gilles, 1974a, 1975, 1979; Tan & Choong, 1981). The reason for the exceptional trend in hepatopancreas is not far to see. This tissue suffers a heavy impregnation of inorganic ions in 100% SW and is unable to mobilise greater amounts of free amino acids. Its metabolic machinery is perhaps geared towards gluconeogenic pathways in order to maintain reasonable levels of glucose in blood (see the forth coming chapter for further elaboration). Progressive increase in the concentration of inorganic ions in the tissues seems to influence corresponding accumulation of organic osmotic effectors. These inorganic ions seem to trigger the process of mobilization of amino acids inside the cells. There is some evidence to show that variations in the inorganic ion content of cells, particularly Cl^-, may cause variations in catabolic activity of amino acids (Snoke, 1956; Schoffeniels and Gilles, 1963, 1964; Chaplin, Huggins and Munday, 1965; Gilles, 1969; Gilles, Hogue and Kearney, 1971). It is known that oxygen consumption is influenced by the osmotic gradient, particularly chloride gradient, that existsbetween the ECF and external medium (Schwabe, 1933; Pampapathi Rao, 1958; Lofts, 1956; Rama-murthi, 1965; 1967b, Venkatachari, 1981). A correlation between oxygen consumption and ionic gradients has been indicated in chapter II of this dissertation. This correlation is particularly striking between Cl^- gradient and oxygen

consumption (see figures 19 & 20). Increased Cl^- gradients between ECF and medium correspond with increased oxygen consumption and as the gradients are lowered, oxygen consumption is also lowered.. The observation that variations in inorganic ion content of cells, particularly Cl^-, are associated with modulations in the catabolism of intracullular amino acids, is further vindicated in this study. Fugires 31 & 32 show regression lines for tissue TNPS and Cl^- levels. The tissue TNPS show positive regression coefficients on the intracellular Cl^- ion concentrations and such correlations indicate some role for Cl^- ions in the metabolism of intracellular amino acids. However, this does not preclude the role of Na^+ from playing a role in such modulations. However, several studies have implicated especially the anionic species in the modulation of enzyme activity related to oxidative metabolism (Chaplin, Huggins and Munday, 1965; Gilles, 1969; Gilles, Hogue and Kearney, 1971).

A brief account of the mechanism involved in the control of intracellular amino acid levels in animals acclimated to heterosmotic media was given earlier. The results obtained in the present work could be interpreted in the light of these mechanisms. For instance, there is a general depression in oxygen consumption of the crabs when

FIGS. 31 & 32: Regression curves of total ninhydrin positive substances (TNPS) and Cl^- in the intracellular fluid.

Muscle

A : 50% SW	: Regression equation	:	y = 1.3961x + 63.79
B : **Reversal** to FW	: Regression equation	:	y = 1.7557x - 6.8
C : 100% SW	: Regression equation	:	y = 1.6508x - 152.54
D : Reversal to FW	: Regression equation	:	y = 0.7429x + 188.96

Hepatopancreas

A : 50% SW	: Regression equation	:	y = 2.8696x -68.81
B : Reversal to FW	: Regression equation	:	y = 1.5555x - 10.68
C : 100% SW	: Regression equation	:	y = 0.9335x + 263.34
D : Reversal to FW	: Regression equation	:	y = 1.2432x + 99.04

FIG. 31

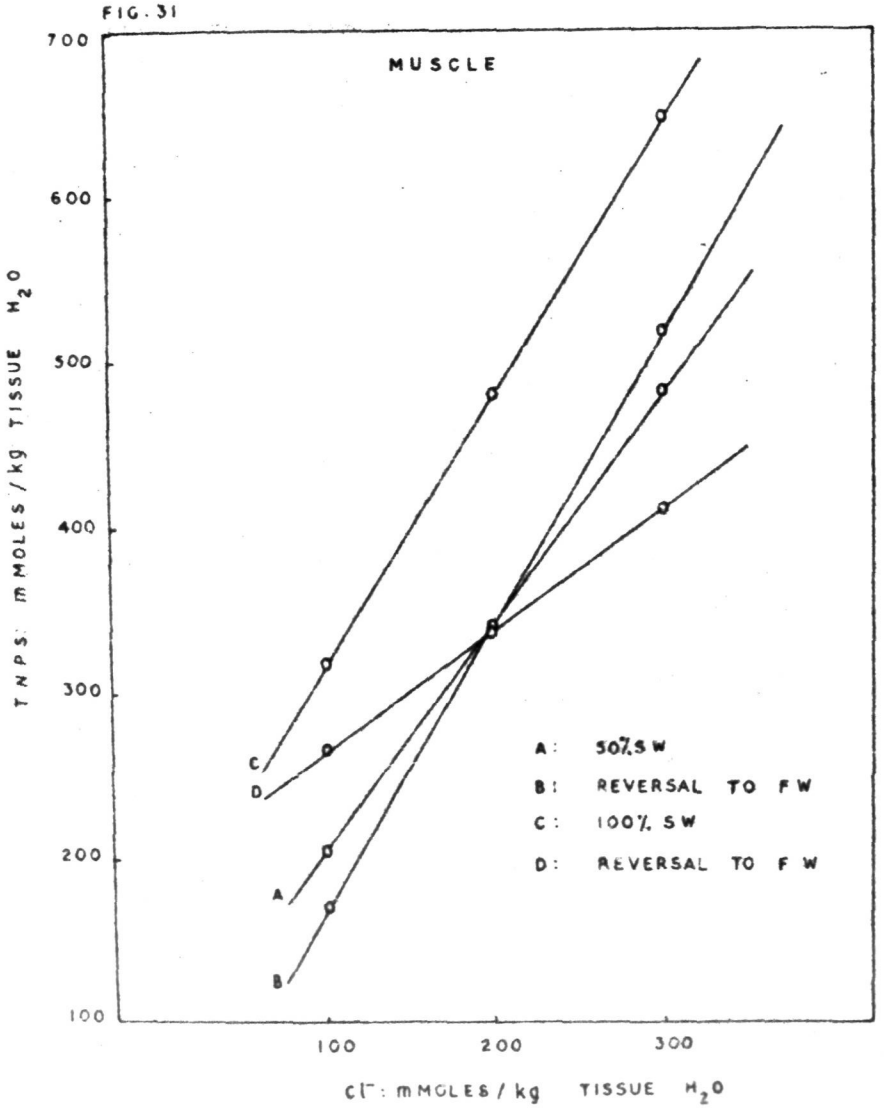
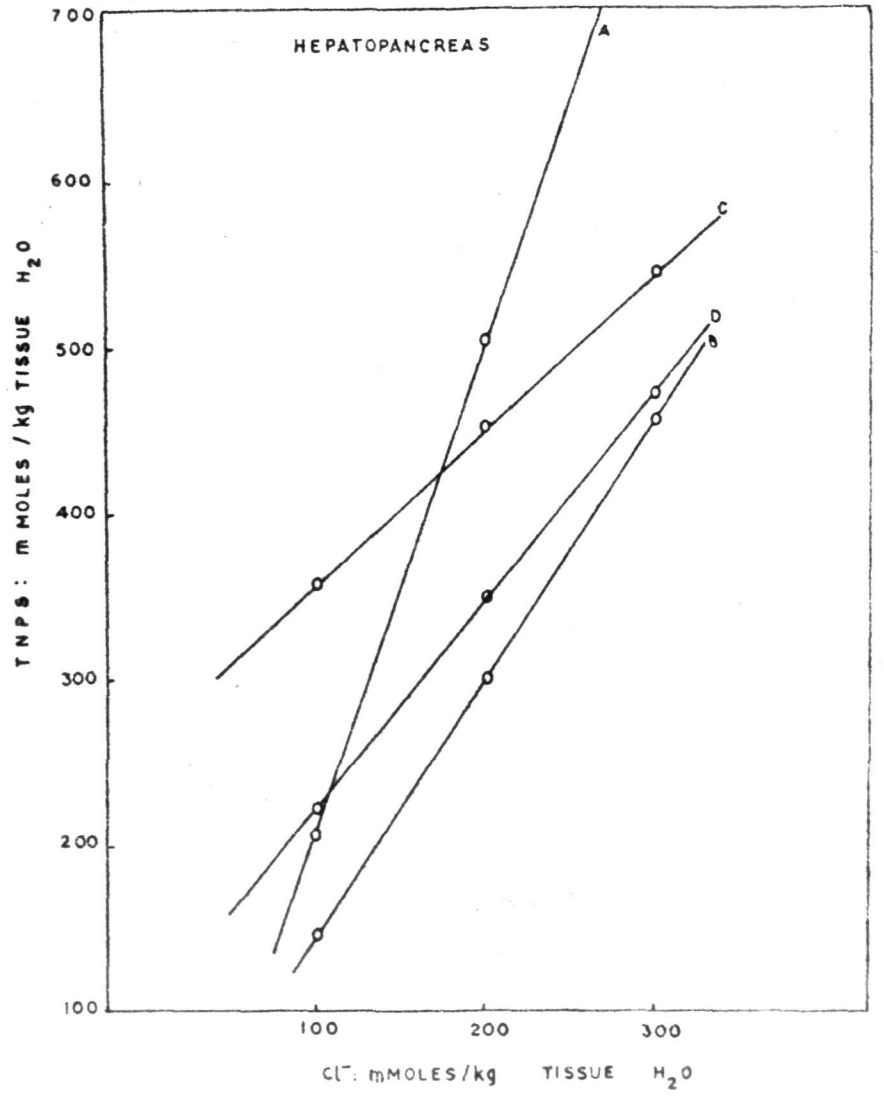

MUSCLE
700
600
500
400
300
200
100
TNPS: mMOLES / kg TISSUE H2O
100
200
300
Cl⁻: mMOLES / kg TISSUE H2O
A: 50% SW
B: REVERSAL TO FW
C: 100% SW
D: REVERSAL TO F W
A
B
C
D

FIG. 32


HEPATOPANCREAS
700
600
500
400
300
200
100
TNPS: mMOLES / kg TISSUE H2O
100
200
300
Cl⁻: mMOLES / kg TISSUE H2O
A
B
C
D

transferred to 50% and 100% SW except for some increase in the initial stages of exposure to 100% SW (Tables 4 & 6). At the end of the acclimation period, the oxygen consumption drops nearly to 50% of that obtained in fresh water (figure 5). Lowered oxygen consumption in higher salinities may be the consequence of decreased catabolism of amino acids which results in the elevation of amino acid content in cells (Siebers and coworkers, 1972; Gilles, 1974a, 1977, 1979). Decreased oxygen consumption implies decreased production of CO_2 and decreased catabolism of amino acids implies decreased production of NH_4^+. CO_2 and NH_3 upon ionization provide the necessary counter ions viz., HCO_3^- and NH_4^+ for the active uptake of Cl^- and Na^+ respectively. Decreased levels of these metabolic products in hyperosmotic conditions implies the absence or reduction in active transport processes which are supposed to involve Na^+/NH_4^+ or H^+ and Cl^-/HCO_3^- exchange mechanisms. Increased levels of intracellular amino acids seen in crabs acclimated to 50% & 100% SW may also be accounted for by the increased influx of amino acids from the ECF. Variations in the amino acid content of blood observed lend support to this possibility. There is a general increase in amino acid levels of blood during the first stages of acclimation to sea water (upto 72 hours)

and subsequently there is decline in the levels. This decline probably suggests influx of amino acids from the blood into the tissues as indicated by Siebers and coworkers (1972) and Gilles (1977, 1979). A third explanation for increased levels of TNPS in the tissues may be dehydration and consequent shrinkage of cells especially in 100% SW. It was mentioned earlier that the tissues generally do not show any compensation for water loss in 50% and 100% SW exposed crabs except the muscle which shows regulation of water content in 50% SW. Levels of dehydration of tissues and intracellular amino acid concentration show rather a perfect correspondence. For instance tissue dehydration was found to be maximal at 72 hours of salinity stress (Figs. 11 & 12). The TNPS levels also reached peak values at 72 hours in both tissues (Figs. 25 & 26) in 50% and 100% SW. Degradation of intracellular proteins is not likely as the total protein content in the tissues of crabs exposed to 75% and 100% SW showed non-significant changes after 15 days of exposure to sea water (Venkata Reddy, 1976) and this is in line with earlier reports (Florkin and coworkers, 1964; Gilles and Schoffeniels, 1969a; Siebers et al, 1972).

In hypo-osmotic conditions the intracellular concentration of amino acids decreases and the oxygen consumption of whole animals increases (Loc. cit). The tissues of the crab

(Oziotelphusa) under investigation showed decreased levels of amino acids during the reverse acclimation process to FW and also in the natural habitat i.e. fresh water (Tables 17, 18 & 19 and figures 25, 26, 27 & 28). During this period there was an increase in oxygen consumption of the crab and it was highest in FW (Tables 5 & 7 and fig. 5). Increased oxygen consumption implies probably increased catabolism of amino acids which in turn results in decreased levels of intracellular amino acid content. Hypo-osmotic conditions are known to induce enhanced production of Co_2 and NH_3 which are the metabolic products of amino acid catabolism (Gerard and Gilles, 1972a; Mangum and coworkers, 1976; Vincent-Marique and Gilles, 1970a; Chaplin, Huggins and Munday, 1965). Venkata Reddy (1976) has shown that the output of NH_3 from cells into the blood is greater in this crab in FW medium than in SW. These products may be useful in providing the counter ions viz., NH_4^+ and H^+ as well as HCo_3^- which are implicated in the active uptake of Na^+ and Cl^- at the exchange surface of the gills (Krogh, 1938; Shaw, 1960a, 1960b; Maetz and Garcia Romeu, 1964; Kerstetter, Kirschner and Rafuse, 1970; Maetz, 1973, 1974). When the crabs were transferred to FW, there was an apparent leakage of solutes from the ECF into the ambient medium followed by an efflux of solutes from tissues to ECF. Table 20 and figures 27 & 28 make this point clear. It is also observed from the data and figures that

in the early stages of return to FW the rate of depletion of amino acids is faster than the inorganic ions. From this it can be inferred that there occurs an efflux of solutes from cells to ECF and concomitantly the intracellular amino acids leak out of the cells into blood at a faster rate than the inorganic ions. This offers another explanation for the decreased levels of free amino acids in cells under hypo-osmotic conditions. A significant increase in the blood amino acid levels during the early phase of reverse acclimation (Tables 17 & 18 and figures 25 & 26) lends support to this view and this is in perfect agreement with earlier reports (Vincent-Marique and Gilles, 1970a,b; Gerard and Gilles, 1972a; Gilles, 1977, 1979). Hydration of cells under hypo-osmotic conditions (Tables 8 & 9 and figures 11 & 12) also seems to contribute partly to the decreased levels of amino acids in tissues. Thus, cell volume regulation which is an integral part of osmoregulation seems to be closely associated with the regulation of water content, organic and inorganic osmotic effectors of the intracellular fluid (Reviews by Gilles, 1974a, 1979; Hoffmann, 1977).

Table 17

Time course of changes in TNPS in the blood and tissues of the fresh water field crab Oziotelphusa (Paratelphusa) senex senex when the animal is transferred directly to 50% S.W. and back to F.W. Values are expressed as m moles/litre of blood or Kg wet tissue. Mean values are for 6 individuals.

Tissue	Control (F.W.)	50% S.W.						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
Blood													
Mean	6.13	6.50	8.04	7.46	7.66	7.07	6.87	8.26	7.80	7.53	7.45	7.50	5.55
S.D. ±	0.62	1.28	1.39	0.47	0.82	0.77	0.89	1.17	0.73	0.61	0.96	0.79	0.51
% d		+6.03	+31.15	+21.69	+24.95	+15.33	+12.07	+34.74	+27.24	+22.83	+21.53	+22.34	-9.46
H.P.													
Mean	155.20	172.32	194.43	298.79	285.14	282.03	284.99	188.66	186.42	189.96	164.26	167.48	161.90
S.D. ±	16.02	10.45	14.60	11.30	14.30	18.12	16.38	18.67	15.08	11.09	18.38	11.46	9.41
% d		+11.03	+25.27	+92.51	+83.72	+81.72	+83.62	+21.56	+20.11	+22.39	+5.83	+7.91	+4.31
Muscle													
Mean	249.34	265.69	271.47	351.35	358.82	362.85	348.94	312.2	292.14	272.92	255.36	258.97	260.76
S.D. ±	20.06	17.14	21.28	15.85	18.85	18.68	20.79	20.57	21.55	16.55	20.92	10.01	8.27
% d		+6.55	+8.87	+40.91	+43.90	+45.52	+39.94	+25.21	+17.16	+9.45	+2.41	+3.86	+4.58

Note:- S.D. - Standard deviation; % d - Percent deviation from the control; H.P. - Hepatopancreas; S.W. - Sea water; F.W. - Fresh water.

Table 17-1

Variance ratios for data given in table 17.

Tissue & Treatment	Variance ratio (F)		Critical difference		
	F. Cal	F. tab at 5% level of significance	at 5% level	at 1% level	at 0.1% level
Blood:					
50% S.W.	3.21	2.38	1.28	1.83	2.65
Reverse	8.12	2.26	1.01	1.41	1.99
H.P:					
50% S.W.	106.93	2.38	20.20	28.69	41.84
Reverse	46.73	2.26	18.76	26.07	37.10
Muscle:					
50% S.W.	42.51	2.38	26.15	37.14	54.16
Reverse	22.40	2.26	22.67	31.50	44.83

NOTE:- H.P. Hepatopancreas

Table 17-2

Statistical comparison of the data presented in table 17. Mean values are arranged in an ascending order.

Tissue	Treatment							
Blood	50% S.W.							
	Control	1 h	360 h	168 h	24 h	72 h	5 h	
	6.13	6.50	6.87	7.07	7.46	7.66	8.04	
	Reversal to F.W.							
	360 h	Control	360 h S.E.	72 h	168 h	24 h	5 h	1 h
	5.55	6.13	6.87	7.45	7.50	7.53	7.80	8.26
H.P.	50% S.W.							
	Control	1 h	5 h	168 h	360 h	72 h	24 h	
	155.20	172.32	194.43	282.03	284.99	285.14	298.79	
	Reversal to F.W.							
	Control	360 h	72 h	168 h	5 h	1 h	24 h	360 h S.E.
	155.20	161.90	164.28	167.48	186.42	188.66	189.96	284.99
Muscle	50% S.W.							
	Control	1 h	5 h	360 h	24 h	72 h	168 h	
	249.34	265.69	271.47	348.94	351.35	358.82	362.85	
	Reversal to F.W.							
	Control	72 h	168 h	360 h	24 h	5 h	1 h	360 h S.E.
	249.34	255.36	258.97	260.76	272.92	292.14	312.20	348.94

N.B.:- Values grouped together by a common underline are not significantly different ($P > 0.05$) amongst themselves.
S.E.: Salinity Exposed.

Table 18

Time course of changes in TNPS in the blood and tissues of the fresh water field crab Oziotelphusa when subjected to salinity stress (100% S.W.) and then returned to F.W. Values are expressed as m moles/litre of blood or Kg wet tissue. Mean values are for 6 individuals.

Tissue	Control (F.W.)	100% S.W.						Reversal to F.W.					
		1 h	5 h	24 h	72h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
Blood													
Mean	6.13	7.26	8.04	8.93	13.05	11.63	8.52	7.86	7.63	8.28	9.54	7.79	5.85
S.D. ±	0.62	0.97	0.52	0.84	1.92	0.93	0.49	0.53	0.63	0.68	1.64	0.81	0.89
% d		+18.43	+31.15	+45.67	+112.88	+89.72	+38.98	+28.22	+24.46	+35.07	+55.62	+27.07	-4.56
H.P.													
Mean	155.20	225.68	273.23	273.60	328.90	265.46	232.01	200.58	197.96	177.14	172.74	165.85	165.37
S.D. ±	16.02	19.17	12.07	14.21	11.82	11.91	9.32	14.38	15.84	10.16	14.03	7.82	16.84
% d		+45.41	+76.05	+76.28	+111.92	+71.04	+49.49	+29.23	+27.55	+14.13	+11.30	+6.86	+6.55
Muscle													
Mean	249.34	310.65	326.21	379.46	390.62	384.92	348.41	285.91	254.11	224.20	229.87	236.79	251.61
S.D. ±	20.06	11.07	11.26	12.15	13.40	10.89	14.49	12.49	7.24	10.19	12.66	10.03	16.95
% d		+24.58	+30.82	+52.18	+56.66	+54.37	+39.76	+14.66	+ 1.91	-10.08	-7.80	-5.03	+0.91

Note:- % d - Percent deviation from the control; H.P. - Hepatopancreas; S.D. - Standard deviation; S.W. - Sea water; F.W.- Fresh water.

Table 18-1

Variance ratios for the data given in table 18.

Tissue & Treatment	Variance ratios (F)		Critical difference		
	F. Cal	F. tab at 5% level of significance	at 5% level	at 1% level	at 0.1% level
Blood:					
100% S.W.	36.00	2.38	1.37	1.95	2.83
Reverse	13.05	2.26	1.05	1.46	2.07
H.P:					
100% S.W.	91.37	2.38	19.02	27.01	39.39
Reverse	21.14	2.26	16.91	23.49	33.44
Muscle:					
100% S.W.	83.29	2.38	18.77	26.65	38.88
Reverse	178.72	2.26	9.65	13.42	19.08

H.P. = Hepatopancreas.

S.W. = Sea Water.

Table 18-2

Statistical comparison of the data given in table 18. Mean values are arranged in an ascending order.

Tissue	Treatment							
Blood	100% S.W.							
	Control	1 h	5 h	360 h	24 h	168 h	72 h	
	6.13	7.26	8.04	8.52	8.93	11.63	13.05	
	Reversal to F.W.							
	360 h	Control	5 h	168 h	1 h	24 h	360 h S.S.	72 h
	5.85	6.13	7.63	7.79	7.86	8.28	8.52	9.54
H.P.	100% S.W.							
	Control	1 h	360 h	168 h	5 h	24 h	72 h	
	155.2	225.68	232.01	265.46	273.23	273.60	328.9	
	Reversal to F.W.							
	Control	360 h	168 h	72 h	24 h	5 h	1 h	360 h S.S.
	155.2	165.37	165.87	172.74	177.14	197.96	200.58	232.01
Muscle	100% S.W.							
	Control	1 h	5 h	360 h	24 h	168 h	72 h	
	249.34	310.65	326.21	348.41	379.46	384.92	390.62	
	Reversal to F.W.							
	24 h	72 h	168 h	Control	360 h	5 h	1 h	360 h S.S.
	224.2	229.87	236.79	249.34	251.61	254.11	285.91	348.41

N.B.:- Values grouped together by a common underline are not significantly different ($P > 0.05$) amongst themselves.
H.P.:- Hepatopancreas, F.W.:- Fresh Water; S.W.:- Sea Water; S.S.:- Salinity Stre

Table 19

Time course of changes in TNPS in the intracellular water of the crab exposed to S.W. Values are expressed as mmoles/Kg cell water. Mean values are for 6 animals.

Tissue	Control (F.W.)	Salinity stress						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
H.P.						50% S.W.							
	216.19	245.12	286.47	467.04	538.51	499.53	451.44	297.24	293.78	285.41	231.98	233.19	220.31
% d		+13.38	+32.51	+116.03	+149.09	+131.06	+108.81	+37.49	+35.88	+32.02	+7.30	+7.86	+1.91
Muscle	325.86	362.65	371.64	498.35	521.91	514.51	469.93	415.45	390.31	347.27	327.78	335.39	337.50
% d		+11.29	+14.04	+52.93	+60.16	+57.89	+44.21	+27.49	+19.78	+ 6.57	+0.58	+2.92	+3.57
H.P.						100% S.W.							
	216.19	341.37	475.23	522.36	671.63	551.86	475.78	328.92	330.40	300.94	291.76	243.00	227.52
% d		+57.90	+119.82	+116.19	+210.66	+155.26	+120.07	+52.14	+52.82	+39.20	+34.95	+12.40	+ 5.24
Muscle	325.86	426.50	456.81	562.38	640.81	611.19	558.89	402.65	354.97	311.43	302.71	305.36	323.03
% d		+30.88	+40.18	+72.58	+96.65	+87.56	+71.51	+23.56	+ 8.93	-4.43	- 7.10	-6.29	-0.87

N.B.: Values for computation are obtained from tables 8, 9, 17 & 18.

% d - Percent deviation from the control.

H.P.- Hepatopancreas.

ECF - Extracellular fluid.

TNPS - Total ninhydrin positive substances.

S.W.- Sea water.

Table 20

Time course of total conc. of Na^+, Cl^- and TNPS in the blood and intracellular fluid of the tissues of the crab during acclimation to 50% and 100% S.W. and in reverse acclimation. Values expressed as mosmoles/litre or Kg. cell water.

Tissue	Control (F.W.)	50% S.W.						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
Blood	486.29	505.84	517.25	537.16	567.52	584.85	644.35	549.69	509.68	511.03	483.97	468.24	488.81
H.P.	344.66	403.90	448.05	650.63	772.05	735.81	688.69	534.10	531.99	503.39	398.78	378.51	349.60
Tissue-Blood gradient	-141.63	-101.94	-69.20	+113.47	+204.53	+150.96	+44.34	-15.59	+22.31	-7.64	-85.19	-89.73	-139.21
Muscle	557.97	609.18	612.80	766.58	796.10	777.25	764.30	702.81	671.01	613.68	595.60	548.75	554.24
Tissue-blood gradient	+71.68	+103.34	+95.55	+229.42	+228.58	+192.40	+119.95	+153.12	+161.33	+102.65	+111.63	+80.51	+65.43
				100% S.W.									
Blood	486.29	554.24	592.94	630.88	814.33	882.37	980.72	674.95	645.12	596.96	593.79	525.57	597.04
H.P.	344.66	528.49	749.03	837.93	1071.59	966.62	945.66	590.88	557.32	510.02	481.50	370.82	352.87
Tissue-blood gradient	-141.63	-25.75	+156.09	+207.05	+257.26	+84.25	-35.06	-84.07	-87.80	-86.94	-112.29	-154.75	-144.17
Muscle	557.97	677.94	724.33	852.16	935.21	929.42	946.18	666.68	607.37	563.09	545.17	516.27	539.06
Tissue-blood gradient	+71.68	+123.70	+131.39	+221.28	+120.88	+47.05	-34.54	-8.27	-37.75	-33.87	-48.62	-9.30	+42.02

N.B.:- Values are calculated from tables 10,11,13,14,17 & 18 for blood and from tables 12, 15 & 19 for tissues

Table 21

Percent contribution of TNPS to osmolarity of blood and intracellular fluid of crabs when only Na^+, Cl^- and TNPS concentrations are taken into account.

Tissue	Control (F.W.)	50% S.W.						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
Blood	1.26	1.28	1.55	1.38	1.35	1.21	1.06	1.50	1.53	1.47	1.54	1.60	1.14
H.P.	62.72	60.68	63.94	71.78	69.75	67.88	65.55	55.65	55.22	56.69	58.17	61.60	63.02
Muscle	58.40	59.53	60.64	65.01	65.56	66.19	61.48	59.11	58.16	56.58	55.03	61.11	60.89

N.B.:- Data for calculations are obtained from tables 17, 19 & 20.
H.P. - Hepatopancreas; S.W. - Sea water; F.W. - Fresh water.

Percent contribution of TNPS to osmolarity of blood and intracellular fluid of crabs when only Na^+, Cl^- and TNPS concentrations are taken into account.

Tissue	Control (F.W.)	100% S.W.						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
Blood	1.26	1.31	1.35	1.41	1.60	1.32	0.86	1.01	1.02	1.18	1.60	1.48	1.18
H.P.	62.72	64.59	63.44	62.33	62.67	57.09	50.31	55.66	59.28	59.01	60.59	65.53	64.47
Muscle	58.40	62.91	63.06	65.99	68.52	65.76	59.06	60.39	58.44	55.30	55.52	59.14	59.92

N.B.:- Data for calculations are obtained from tables 18, 19 & 20.
H.P. - Hepatopancreas; S.W. - Sea water; F.W. - Fresh water.

CHAPTER IV

E N Z Y M E S

INTRODUCTION

Modifications in the catabolic activity of intracellular amino acids during hypo- and hyper-osmotic adjustments seem to involve some key enzymes of amino acid metabolism and the intracellular ionic composition, particularly Na^+ and Cl^-, appears to modify the activity levels of these enzymes. No significant differences were reported in glutamate dehydrogenase activity between tissues of Carcinus maenas acclimated to 50% SW or to 100% SW. (Chaplin, Huggins and Munday, 1965). Similarly, Gilles (1974a) reported "no changes in the specific activity and in the Km for α - ketoglutarate of glutamate dehydrogenase or aspartate aminotransferase in the tissues of Eriocheir sinensis upon acclimation to freshwater or to sea-water. However, "an important increase in specific activity of glutamate dehydrogenase and aspartate amino transferase" has been observed in the oyster Crassostrea virginica when acclimated to concentrated media (Wickes and Morgan, 1976). There is evidence to show that the inorganic ion composition of the incubation medium influences the key enzymes involved in amino acid metabolism such as glutamate dehydrogenase, serine hydrolyase, alanine and aspartate aminotransferases.

The inorganic ions also appear to influence the Krebs cycle enzymes and the fate of reducing equivalents (Review articles by Schoffeniels and Gilles, 1970; Gilles, 1974a, 1975; Schoffeniels, 1976).

Studies made so far indicate that increasing amounts of NaCl in the incubation medium cause a depression in the activity of all enzymes of oxidative metabolism (Katunuma and coworkers, 1966; Weinberg, 1967; Gilles, 1969; Gilles, Hogue and Kearney, 1971). These studies also suggest that increasing concentrations of intracellular NaCl will have a depressing effect on the Krebs cycle and on its anaplerotic pathways, "atleast in the direction Krebs cycle intermediates-3-carbon units of glycolysis". High NaCl concentrations in the incubating medium are reported to inhibit Krebs cycle in different tissues (Tustanoff and Stewart, 1965; Gilles and Schoffeniels, 1968; Huggins and Munday, 1968; Sernka and Jackson, 1976). Decreased oxygen consumption and CO_2 production observed in isolated tissues and whole animals subjected to hyper-osmotic stress are also in agreement with the above findings(Eliassen, 1953; Shaw, 1960; Ramamurthi, 1965, 1967b; Lee and Pyung, 1970; Siebers and coworkers 1972; Venkata Reddy, 1976).

Depression in serine hydrolyase activity has been reported under increasing concentration of NaCl (Gilles, 1969). Several studies have shown that alanine and aspartate amino-

transferase activities remain unaltered under changing concentrations of NaCl (Chaplin, Huggins and Munday, 1967; Huggins and Munday, 1968; Gilles, 1969). The activity of reductive amination of glutamate dehydrogenase has been reported to increase under conditions of increased NaCl or KCl concentrations (Schoffeniels and Gilles, 1963; Chaplin, Huggins and Munday, 1965; Schoffeniels, 1966; Corman and Kaplan, 1967; Measures, 1975). Under similar conditions oxidative deamination of glutamate by glutamate dehydrogenase is reported to be inhibited (Snoke, 1956; Chaplin, Huggins and Munday, 1965) or unaffected (Measures, 1975). These observations indicate that under hyper-ionic conditions glutamate formation from α - ketoglutarate is increased. Decreased serine hydrolyase activity leading to reduced deaminating activity, unaltered aminotransferase activity coupled with decreased oxidative activity of the Krebs cycle, suggest that under hyper-osmotic conditions "the equilibrium between amino acids and their keto-precursors is displaced, leading to a lower rate of reaction in the direction amino acids - ketoprecursors". This should lead to increased levels of intracellular amino acids when considered along with an unaltered or increased influx of amino acids into the metabolic pool (Gilles, 1979). This picture is in agreement with increased amino acid levels in cells (Florkin, 1962; Gerard and Gilles, 1972a; Schoffeniels and Gilles, 1970; 1972; Gilles, 1974a, 1975) decreased

ammonia production (Krishnamoorthy & Srihari, 1973; Mangum & coworkers, 1976) and decreased oxygen consumption and CO_2 production under hyper-osmotic conditions (Ramamurthi, 1965, 1967b; Lee & Pyung, 1970; Siebers and coworkers, 1972; Siebers, 1972; Venkata Reddy, 1976; Venkatachari, 1981). Under hypo-osmotic situations an opposite trend in amino acid metabolism Viz., increased activity of oxidative enzymes and increased catabolism coupled with increased efflux, of amino acids from cells might be envisaged and this should result in the decreased amino acid concentration in cells. Increased oxygen consumption and CO_2 production and increased NH_3 formation during acclimation to hypo-osmotic media seem to lend support to this view (Ramamurthi, 1965, 1967b; Chaplin, Huggins and Munday, 1970; Lee and Pyung, 1970; Vincent-Marique and Gilles, 1970a, 1970b; Schoffeniels and Gilles, 1970, 1972; Gerard and Gilles, 1972a; Gilles, 1974a, 1977, 1979).

In the present study changes in the activity levels of three important enzymes viz: aspartate amino transferase (AAT), alanine amino transferase (AlAT) and glutamate dehydrogenase (GDH) have been followed in the tissues during the course of acclimation of the crabs to 50% and 100% sea water and also in the reverse acclimation process on the time scale planned. Such a study is undertaken to understand the modulations in the activity of these enzymes in relation to changes in amino acid and inorganic ion concentrations during acclimation to heterosmotic media (100% SW, 50% SW and FW).

Material and methods

The procedure followed for the acclimation of crabs to sea water and back to freshwater (reverse acclimation) is given in the section "Materials and Methods". The method for the estimation of the activity levels of enzymes is described in the same section.

Results

Transaminases

Data obtained on changes in AAT and ALAT activity levels in tissues are presented in tables 23-26 and the % changes from the control values are plotted in figures 33 & 34. The data were subjected to statistical analyses of variance ratios and the significance levels were calculated by the method of critical difference or least significant difference (Pillai & Sinha, 1968). Variance ratios, critical difference at different levels of significance and statistical comparison of the data for each enzyme and tissue are presented in the corresponding tables, such as 23-1 & 23-2 etc.,

Aminotransferase levels in tissues

The aminotransferase (AAT & AlAT) levels (tables 23-26 and figures 33 & 34) in the muscle were higher than those of the hepatopancreas in all the acclimation media (FW,50% &

FIGS. 33 & 34: Time course of changes in the levels of AAT & AlAT activities in tissues when the crabs are exposed to 50% & 100% sea water (SW) and then returned to fresh water (FW).

Fig. 33: AAT

Fig. 34: AlAT

FIG. 33

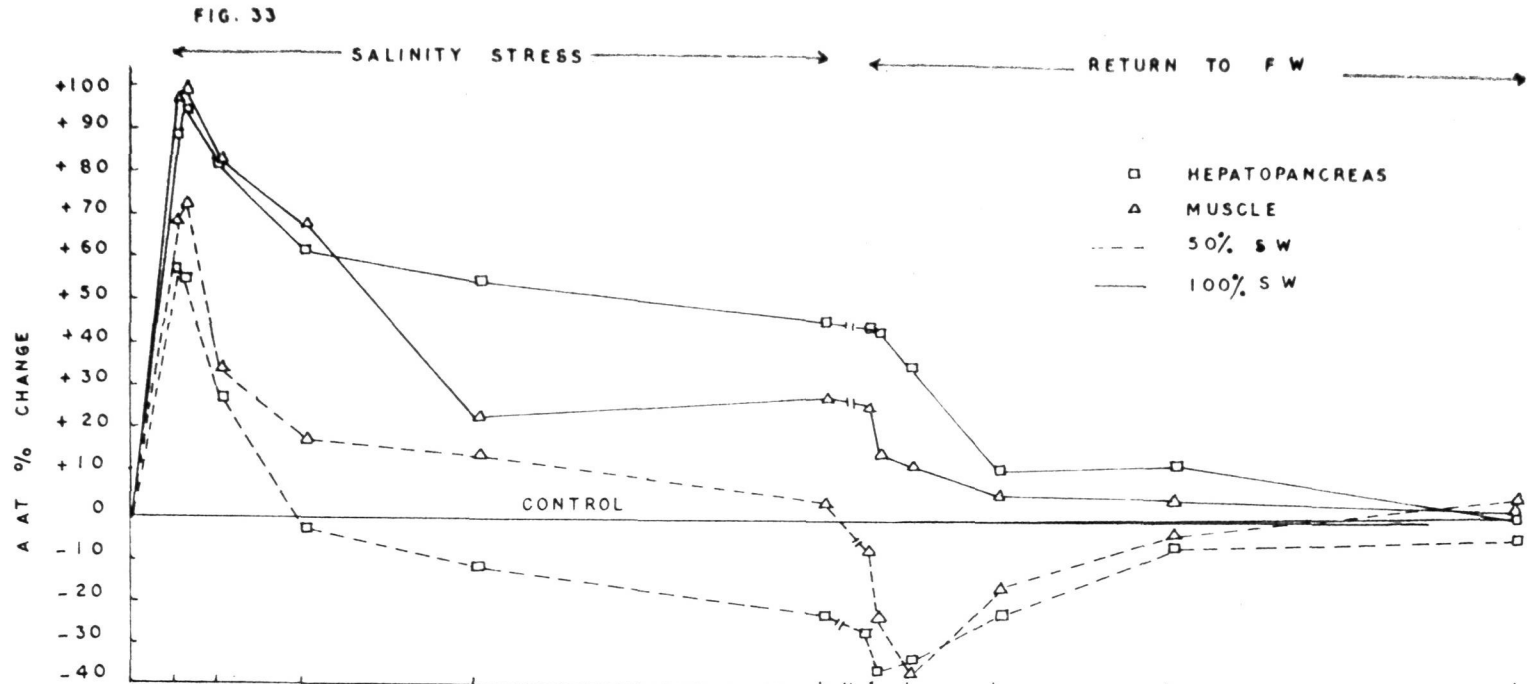
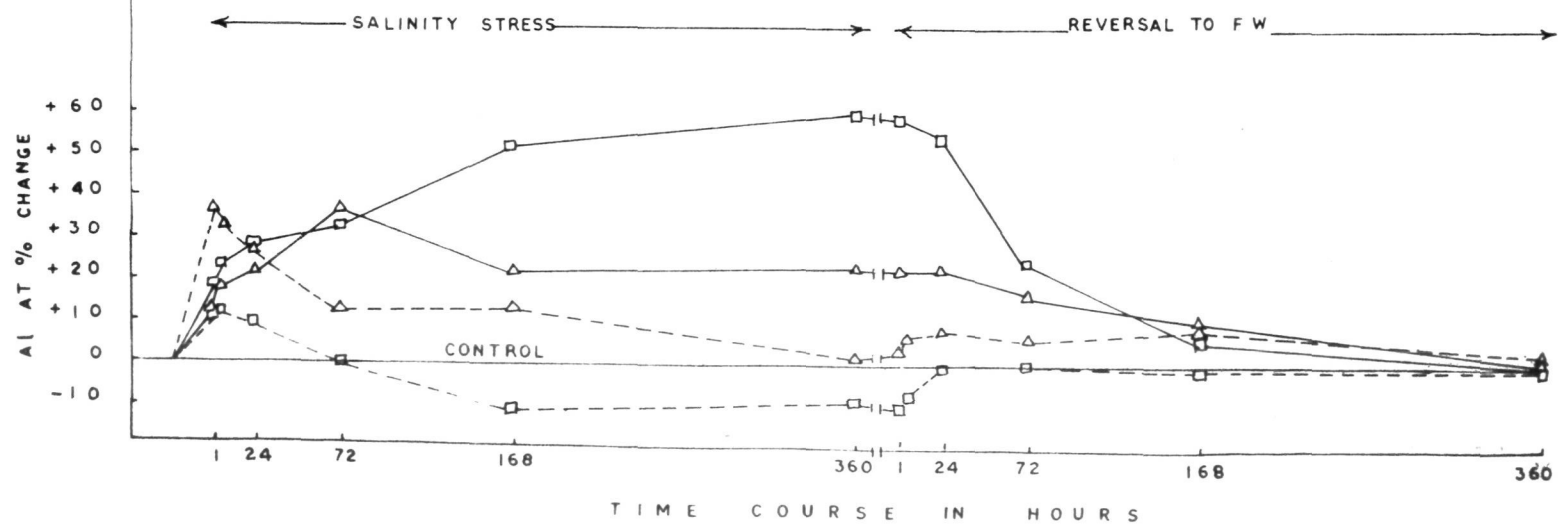

SALINITY STRESS
RETURN TO F W
HEPATOPANCREAS
MUSCLE
50% S W
100% S W
A AT % CHANGE
+100
+ 90
+ 80
+ 70
+ 60
+ 50
+ 40
+ 30
+ 20
+ 10
0
- 10
- 20
- 30
- 40
CONTROL
1 24 72 168 360 1 24 72 168 360

FIG. 34


SALINITY STRESS
REVERSAL TO F W
AI AT % CHANGE
+ 60
+ 50
+ 40
+ 30
+ 20
+ 10
0
- 10
CONTROL
1 24 72 168 360 1 24 72 168 360
TIME COURSE IN HOURS

100% SW). They show significantly increasing trends in the early phase of acclimation to SW but decrease in later stages to either normal levels, or lower than normal levels or higher than normal levels.

Hepatopancreas

Transfer to 50% SW

The AAT and AlAT activities in hepatopancreas increased to significantly high levels upto 24 hours and thereafter there was a significant downward trend which continued upto 15 days. The values seen at the end of acclimation period were significantly lowerover the control value ($P < 0.01$).

Return to FW from 50% SW

Reversal to FW showed a significant depression in AAT activity at 5th hour but there was a subsequent elevation and it reached the control level by the 7th day. The AlAT activity increased quickly to the control level at the end of 24 hours of return to FW.

Transfer to 100% SW

The hepatopancreas showed highly elevated levels ($P < 0.001$) of AAT and AlAT activity immediately after transfer. Whereas the AAT activity thereupon decreased to comparatively low level, the AlAT activity continued to

increase even after 15 days of acclimation. The decreased level of AAT, however, was significantly higher over the control value ($P < 0.001$). In other words, the levels of both AAT and AlAT were significantly higher in 100% SW at the end of the experimental period (15 days) compared to FW values.

Return to FW from 100% SW

Upon reverse acclimation from 100% SW to FW, the AAT and AlAT levels decreased significantly at 24 hours and 72 hours respectively. However, control levels were reached only around 15 days of return with respect to AAT and around 7 days with respect to AlAT.

Muscle

Transfer to 50% SW

The AAT and AlAT levels of muscle increased to highly significant levels during the first stages of acclimation to 50% SW but thereafter the activity levels started falling steeply and returned to the control levels by the end of acclimation period.

Return to FW from 50% SW

The AAT activity decreased significantly upon transfer to FW and this trend was seen upto 24 hours of transfer. Henceforth, the level increased and reached normal level

on the 7th day. The AlAT level increased slightly on transfer to FW and was found to be significantly elevated over the normal ($P < 0.05$) between 24 and 168 hours of transfer. However, it returned to normal level on 15 days of reverse acclimation to FW.

Transfer to 100% SW

Both the AAT and AlAT levels of muscle became elevated to highly significant levels ($P < 0.001$) even after 1 hour of transfer to 100% SW. After the initial shoot up, the AAT level decreased significantly but the lowered level seen after 15 days was still significantly higher ($P < 0.001$) over the control value. The AlAT level continued its increasing trend upto 72 hours and thereafter there was a slight decrease. However, it was also maintained at a significantly higher level compared to the control ($P < 0.001$)

Return to FW from 100% SW

Upon return to FW from 100% SW, the levels of AAT and AlAT showed decreasing trends frm the SW acclimated values. Significant decrease in the level of AAT was recorded after 5 and 72 hours of transfer and by now the level reached the control value. With respect to AlAT, a significant drop occurred at 72 hours and at 168 hours, the value decreased to normal level.

Glutamate dehydrogenase activity levels in tissues

The GDH activity levels in tissues were determined by the method described in the section "Methods & Materials" and the data are presented in tables 27 & 28. Analyses of variance ratios and statistical comparison of the data are presented in tables 27-1, 27-2, 28-1, and 28-2. Figure 35 shows GDH levels in the tissues as percent deviations from the control values.

Results

Transfer to 50% SW

The GDH level in hepatopancreas was found to be lower than that in muscle in all the acclimation media. The level in hepatopancreas decreased significantly in the early stages of acclimation upto 72 hours ($P < 0.001$) and later there was an elevation but the elevated value was significantly lower compared to the normal ($P < 0.01$). In the muscle the GDH activity level became elevated during the early phase of acclimation upto 72 hours ($P < 0.01$) but decreased to the control level in later stages i.e., between 168 and 368 hours.

Return to FW from 50% SW

On return to FW from 50% SW, the GDH activity in the hepatopancreas decreased significantly to lower level by the 5th hour ($P < 0.001$) and later there was a slow and gradual

FIG. 35: Time course of changes in the levels of GDH activity in tissues exposed to 50% & 100% sea water (SW) and then returned to fresh water (FW).

FIGS. 36 & 37 Changes in GDH/total aminotransferase (TAT) ratios in tissues during acclimation to 50% & 100% SW & during the reverse acclimation to fresh water.

FIG. 35

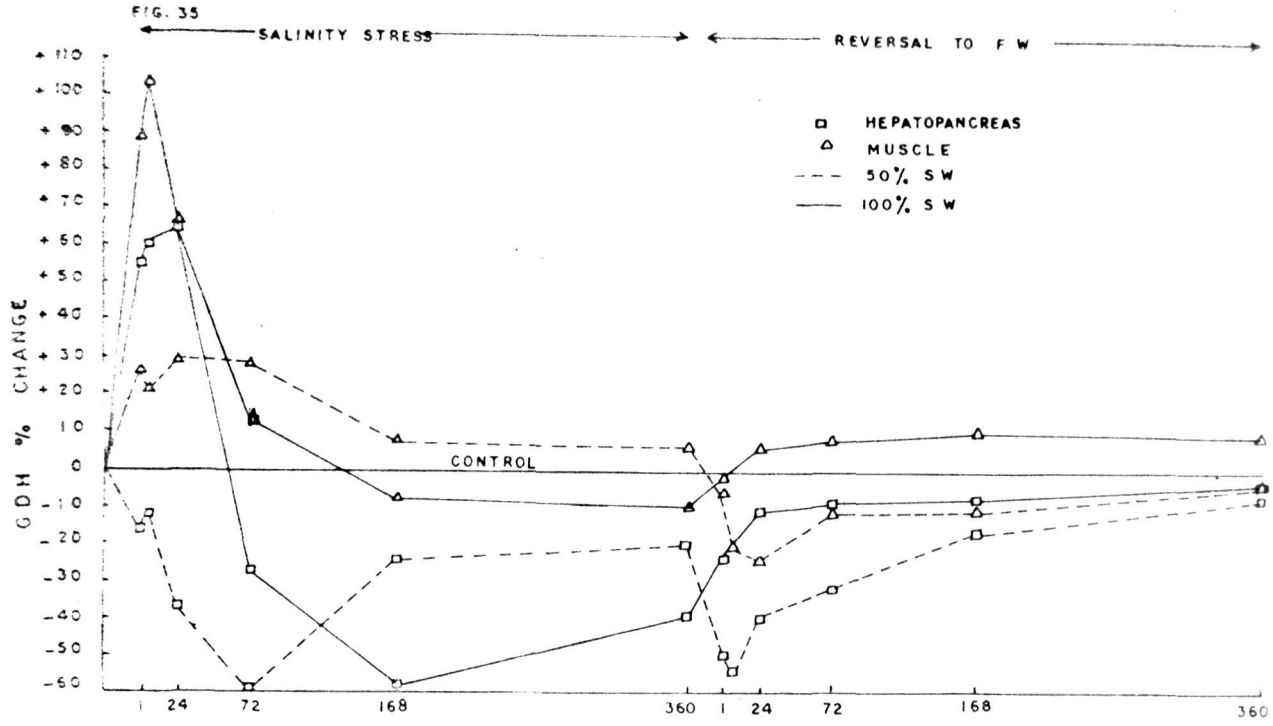


FIG 36

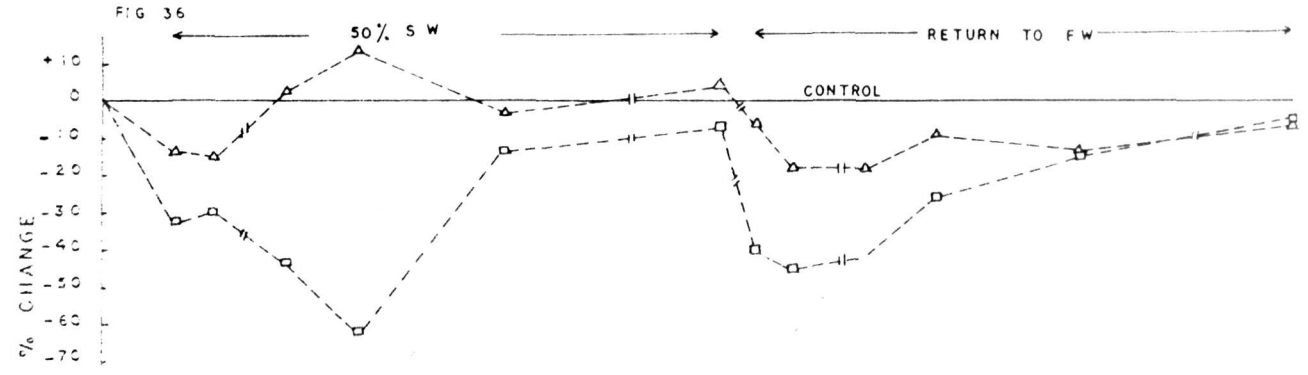
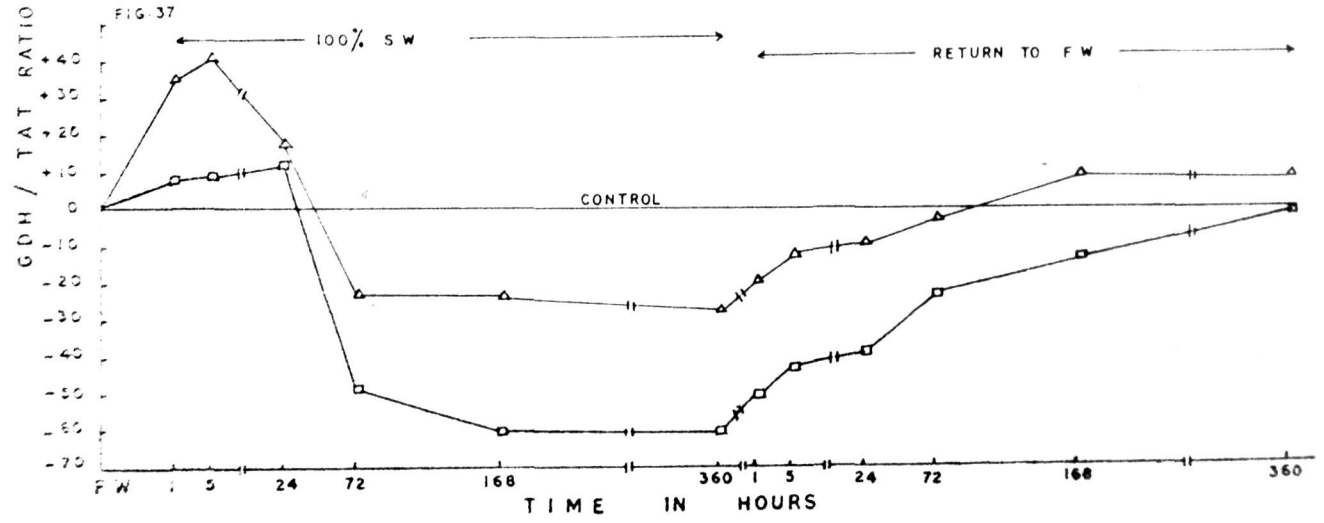


FIG. 37



increase to normal level around 15 days of return to FW. In the muscle, the GDH level showed a transient but significant drop from the normal as well as acclimated values ($P < 0.01$) between 5 and 24 hours of return to FW but by 72 hours it increased to normal level.

Transfer to 100% SW

The GDH activity in the muscle and hepatopancreas increased to very high levels ($P < 0.001$) in the early phase (upto 24 hours) of acclimation but by 72 hours the levels fell steeply and reached nearly normal level in muscle and significantly below the normal level in hepatopancreas ($P < 0.05$). In later stages the activity of GDH in the muscle remained unaltered but in the hepatopancreas it decreased further steeply at 168 hours ($P < 0.01$) and then became elevated. However, the level of GDH activity in hepatopancreas remained significantly low compared to the normal at 360 hours ($P < 0.01$) of transfer to 100% SW.

Return to FW from 100% SW

During reverse acclimation to FW from SW, the GDH level in hepatopancreas increased and reached the control level by 24 hours whereas in the muscle no significant changes were recorded.

Changes in GDH/total aminotransferase ratios in tissues (GDH % TAT)

Table 29 shows GDH % TAT ratios in the tissues of crabs during acclimation to SW and in the reverse process. The values are plotted as percent deviation from the normal in figures 36 & 37. The ratios were generally higher in the crabs in fresh water compared to those acclimated to sea water. Upon transfer of the crabs to sea water, both the tissues studied showed general decrease in GDH/TAT ratios. However, in 50% SW, the muscle showed an initial decrease but a subsequent increase at 72 hours. However, between 168 and 360 hours nearly normal level was maintained. In 100% SW, both hepatopancreas and muscle showed higher GDH/TAT ratios upto 24 hours of transfer and thereupon the ratios fell far below the normal levels. During reverse acclimation to FW both the tissues of 50% SW acclimated crabs showed an initial decrease with a later gradual increase to normal levels and in 100% SW exposed crabs there was a gradual increase to control levels by about 72 hours in the muscle and by about 15 days in the hepatopancreas.

Changes in GDH/FAA ratios in tissues

Table 30 and figures 38-41 show the ratios between glutamate dehydrogenase activity and free amino acid concentration levels in tissues. It is evident that the

GDH/FAA ratios decreased greatly in hepatopancreas upon transfer of the crabs from fresh water to 50% and 100% sea water (Figures 38 & 39). In the reverse acclimation process, there was a gradual increase to nearly normal levels after 15 days of reversal. In the muscle, the GDH/FAA ratios showed a sudden and transient increase over the normal in the early phase (upto 5 hours) of transfer to both 50% and 100% SW and thereafter the ratios decreased to lower than normal levels and this trend continued till the end of acclimation period (Figures 40 & 41). Upon reversal to FW, the GDH/FAA ratios in the muscle showed a general increasing trend reaching more or less normal levels at the end of reverse acclimation period. Between 24 and 360 hours, this ratio was however, found to be far higher than the control ratio in 100% SW exposed crabs (Figures 41).

Changes in AAT/FAA ratios

Table 31 and figures 38 to 41 indicate the trend of changes in AAT/FAA ratios in the tissues of the crabs acclimated to 50% & 100% SW and also in the reverse acclimation process. It is clear from the data that the ratios increased immediately after transfer to both 50% and 100% SW. The muscle showed greater increase in AAT/FAA ratio than

FIGS. 38 & 39: Time course of changes in GDH/FAA, AAT/FAA, and AlAT/FAA ratios in the hepatopancreas of crabs exposed to 50% SW (Fig. 38) and 100% SW (Fig. 39) and later returned to FW.

FIG. 38

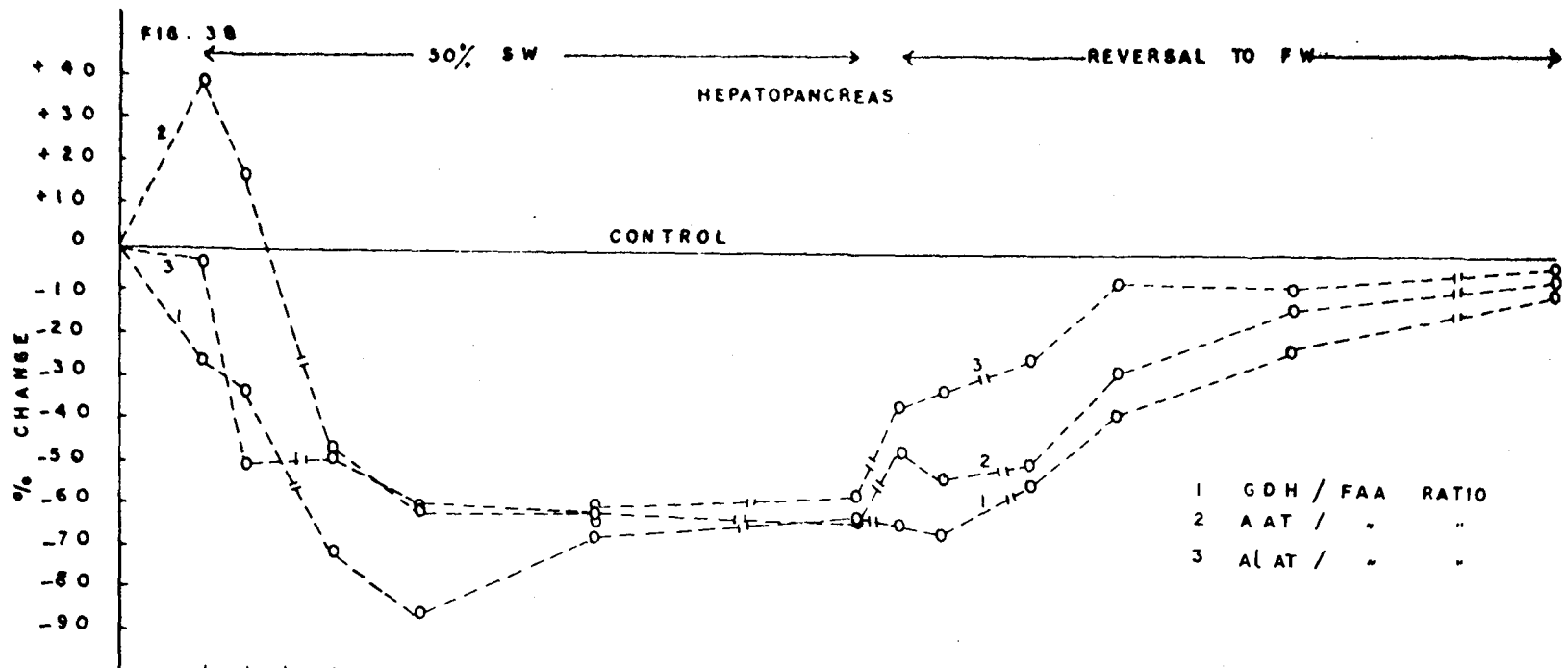
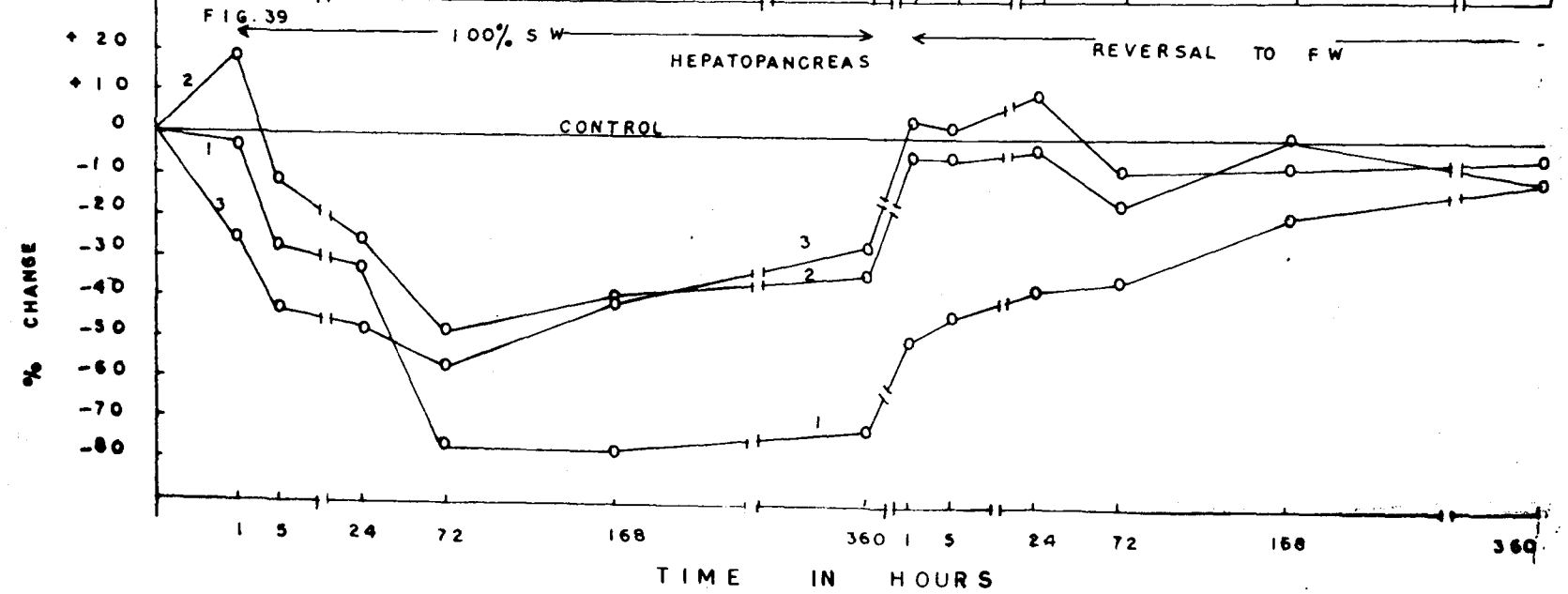
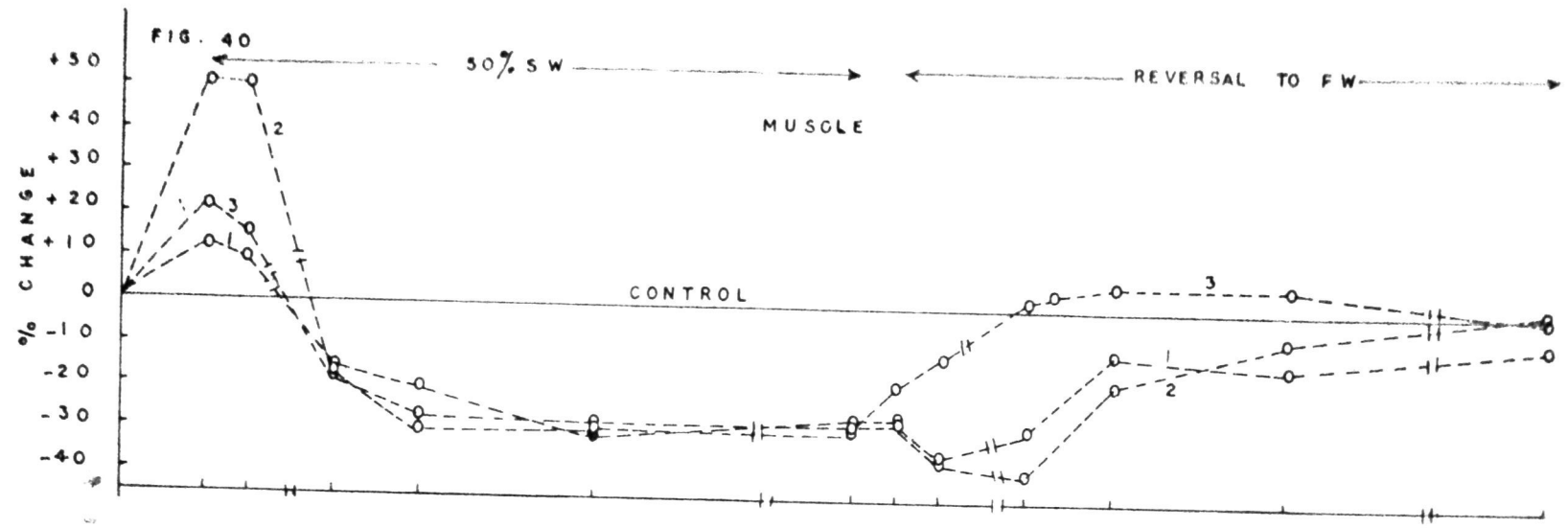
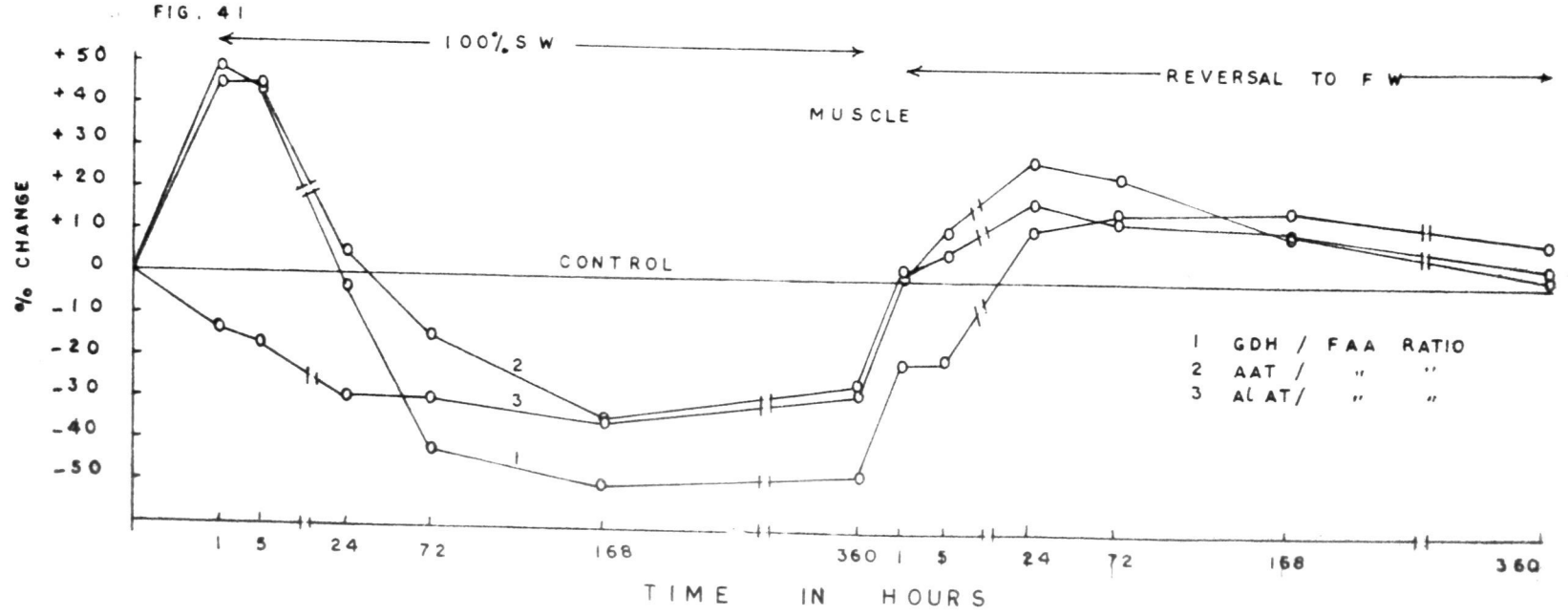

50% SW
REVERSAL TO FW
HEPATOPANCREAS
CONTROL
+40
+30
+20
+10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
% CHANGE
1 GDH / FAA RATIO
2 AAT / " "
3 ALAT / " "

FIG. 39


100% SW
REVERSAL TO FW
HEPATOPANCREAS
CONTROL
+20
+10
0
-10
-20
-30
-40
-50
-60
-70
-80
% CHANGE
1 5 24 72 168 360 1 5 24 72 168 360
TIME IN HOURS

FIGS. 40 & 41: Time course of changes in GDH/FAA, AAT/FAA, & AlAT/FAA ratios in the muscle of crabs exposed to 50% SW (Fig. 40) and 100% SW (Fig.41) and then returned to FW.


FIG. 40
50% S W
REVERSAL TO F W
MUSCLE
% CHANGE
+50
+40
+30
+20
+10
0
-10
-20
-30
-40
CONTROL
1
2
3


FIG. 41
100% S W
REVERSAL TO F W
MUSCLE
% CHANGE
+50
+40
+30
+20
+10
0
-10
-20
-30
-40
-50
CONTROL
1 GDH / FAA RATIO
2 AAT / " "
3 ALAT / " "
1 5 24 72 168 360 1 5 24 72 168 360
TIME IN HOURS

the hepatopancreas. Subsequently the ratios fell steeply. The decline in the ratios was nearly-30% except in hepatopancreas of 100% SW exposed crabs where it was nearly-60%. In the reverse acclimation to FW, the AAT/FAA ratios showed a general increasing trend till they reached nearly normal levels by about 15 days. In the muscle of 100% SW exposed animals, this ratio increased immediately to normal level in the early stages of reversal and thereafter increased to further higher level, far above the normal, until 168 hours and only after 15 days returned to normal level.

Changes in AlAT/FAA ratios

The AlAT/FAA ratios in the tissues of crabs acclimated to 50% SW and 100% SW are presented in table 32. These ratios are plotted in figures 38 to 41 as percent deviations from the normal. The hepatopancreas and muscle showed a general decreasing trend in these ratios in sea water and increasing trend in FW. The muscle showed an exception in that it showed an increase in this ratio in the early phase (upto 5 hours) of transfer to 50% SW. However, the ratio declined subsequently. In the reverse acclimation to FW, a gradual increase to the control ratio was observed in both the tissues of 50% and 100% $\overset{SW}{\wedge}$ exposed crabs, but in the muscle of 100% SW exposed crabs , the ratio increased to more than the normal between 5 and 168 hours of reversal to FW.

GDH/Cl^-, AlAT/Cl^- and AAT/Cl^- ratios

Tables 33, 34 and 35 show the activity levels of GDH, AlAT and AAT respectively as represented per cent intracellular chloride content in the tissues of crabs during their acclimation to 50% and 100% sea water and in the reverse acclimation to fresh water. These ratios are plotted in figures 46-51.

GDH% Cl^- ratios

It is observed that the ratios increased in the muscle upto 24 hours of transfer to 50% and 100% sea water with a later fall (Figures 46 & 47). In 50% sea water the ratio remained close to the control value between 72 and 168 hours and subsequently decreased slightly below the control value. In 100% sea water, the ratio fell steeply after 24 hours. In the hepatopancreas, however, there was a general decreasing trend upto the end of acclimation period in 100% sea water and upto 72 hours in 50% sea water with a later increase reaching a steady level between 168 and 360 hours.

In the reverse acclimation to fresh water there was a gradual increase to normal levels in both the tissues. However, the ratio increased far above the control value in the muscle between 168 and 360 hours of return from 100% SW to fresh water. It may be noted that in 50% sea water -

FIGS. 42 & 43: Comparison of changes in GDH, AAT & AlAT enzyme activities in relation to Cl^- in muscle (Fig. 42) and hepatopancreas (Fig. 43) during acclimation of the crabs to 50% SW and reversal to FW.

FIG. 42

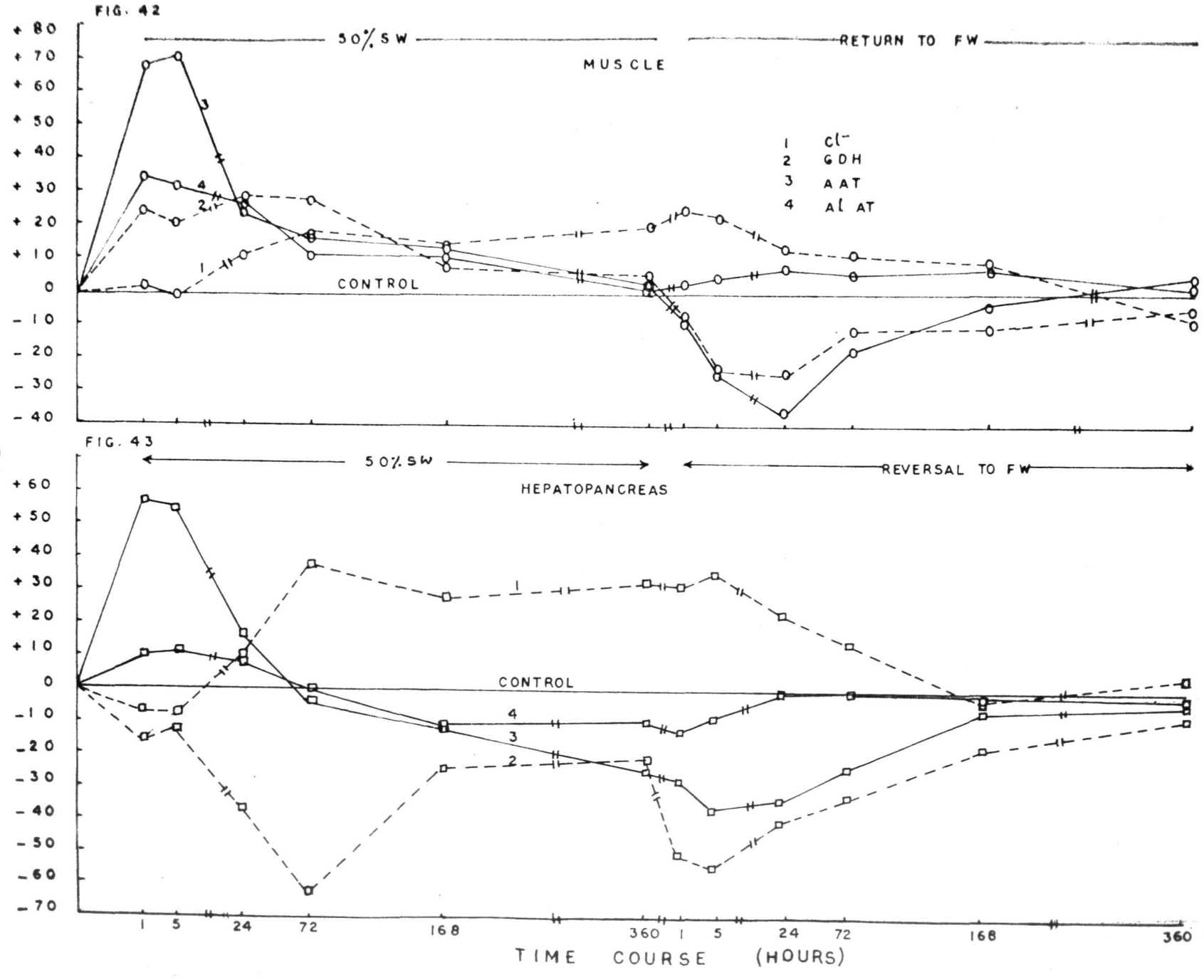

50% SW
RETURN TO FW
MUSCLE
1 Cl⁻
2 GDH
3 AAT
4 Al AT
CONTROL
+80
+70
+60
+50
+40
+30
+20
+10
0
-10
-20
-30
-40
50% SW
REVERSAL TO FW
HEPATOPANCREAS
CONTROL
+60
+50
+40
+30
+20
+10
0
-10
-20
-30
-40
-50
-60
-70
% CHANGE
1 5 24 72 168 360 1 5 24 72 168 360
TIME COURSE (HOURS)

FIG. 43

FIGS. 44 & 45: Comparison of changes in GDH, AAT & AlAT enzyme activities in relation to Cl^- in muscle (Fig. 44) and hepatopancreas (Fig. 45) when the crabs are exposed to 100% SW and returned to FW.

FIG. 44

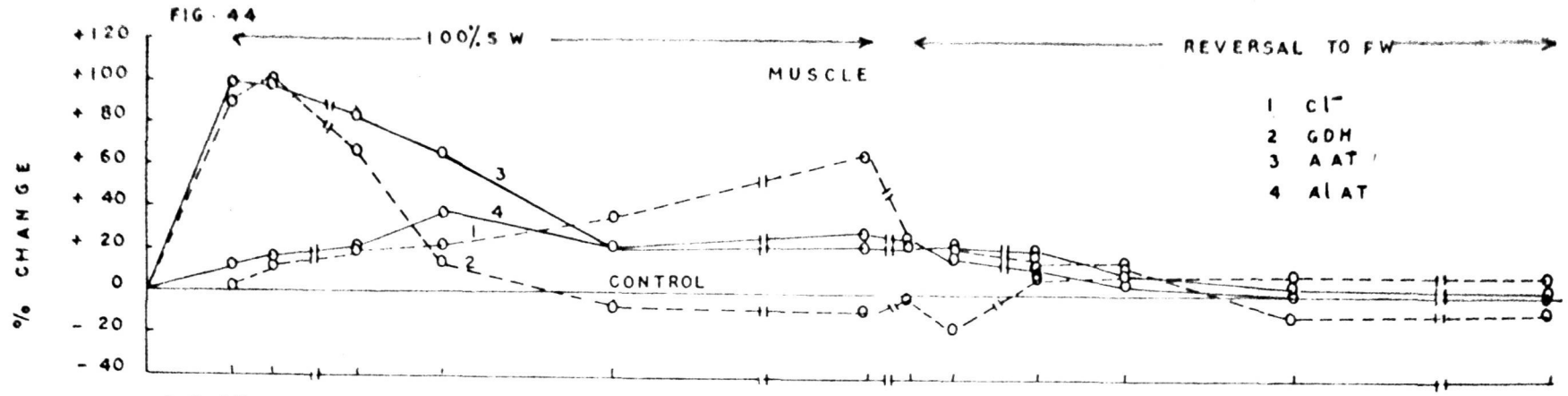
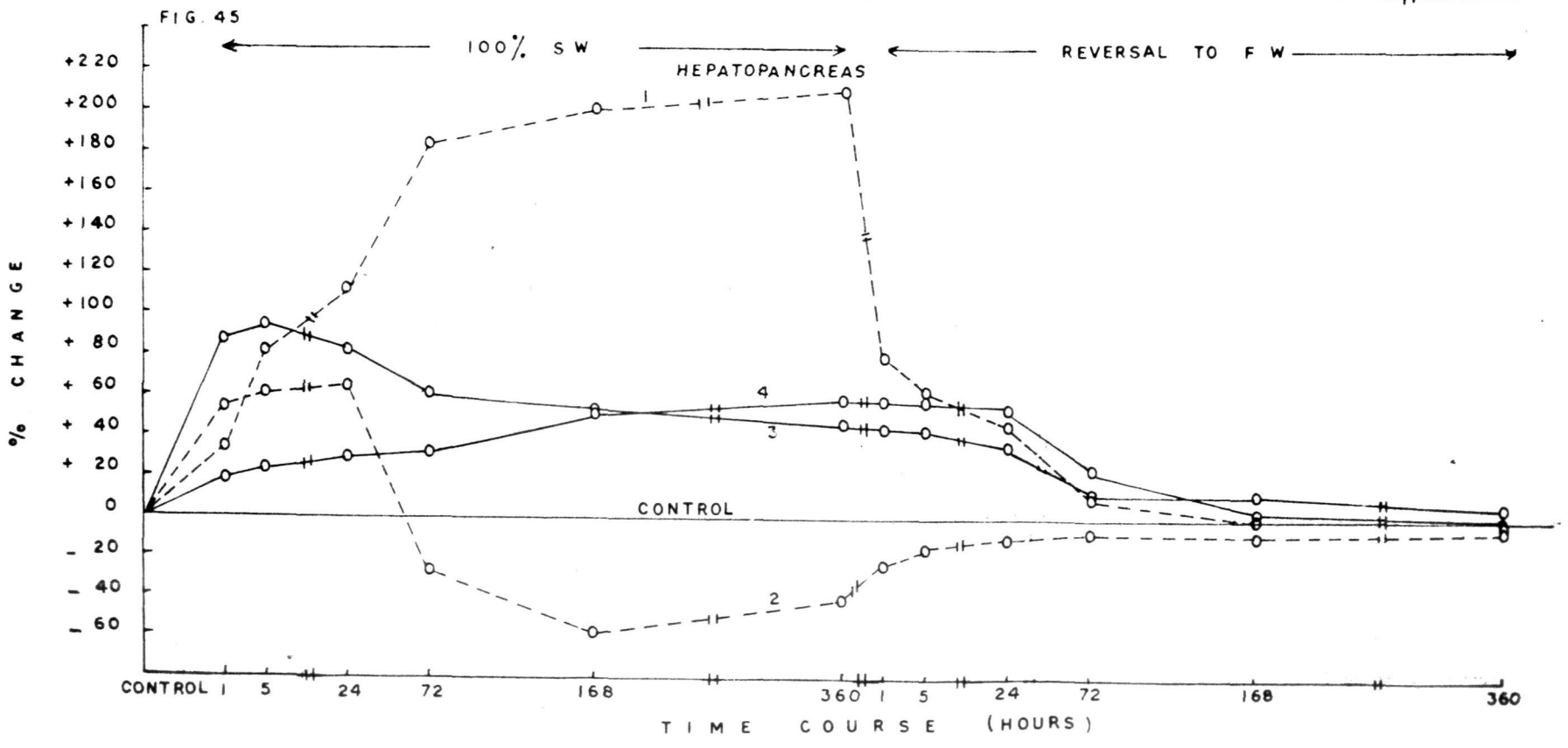

100% S W
REVERSAL TO FW
MUSCLE
1 Cl⁻
2 GDH
3 AAT
4 AlAT
% CHANGE
+120
+100
+ 80
+ 60
+ 40
+ 20
0
- 20
- 40
CONTROL

FIG. 45


100% S W
REVERSAL TO F W
HEPATOPANCREAS
% CHANGE
+220
+200
+180
+160
+140
+120
+100
+ 80
+ 60
+ 40
+ 20
0
- 20
- 40
- 60
CONTROL
CONTROL 1 5 24 72 168 360 1 5 24 72 168 360
TIME COURSE (HOURS)

FIGS. 46 & 47: Time course of changes in GDH/Cl^- ratio in the muscle (Fig. 46) and hepatopancreas (Fig. 47) of crabs transferred to 50% & 100% SW and later brought back to fresh water.

FIG. 46

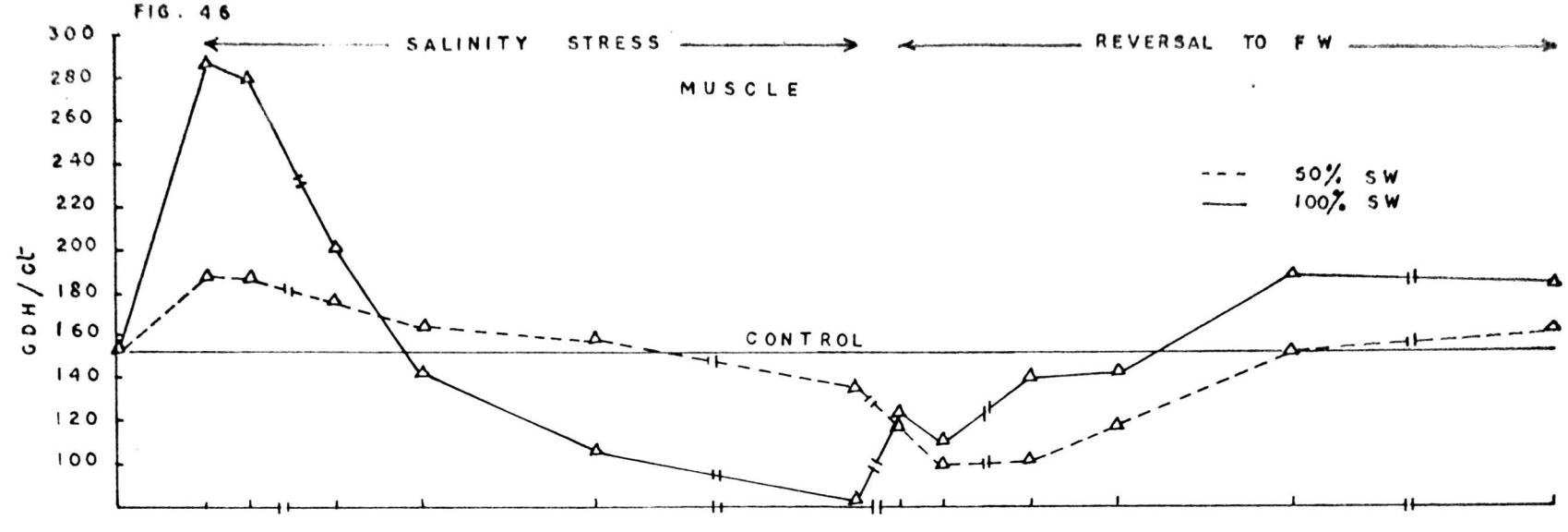
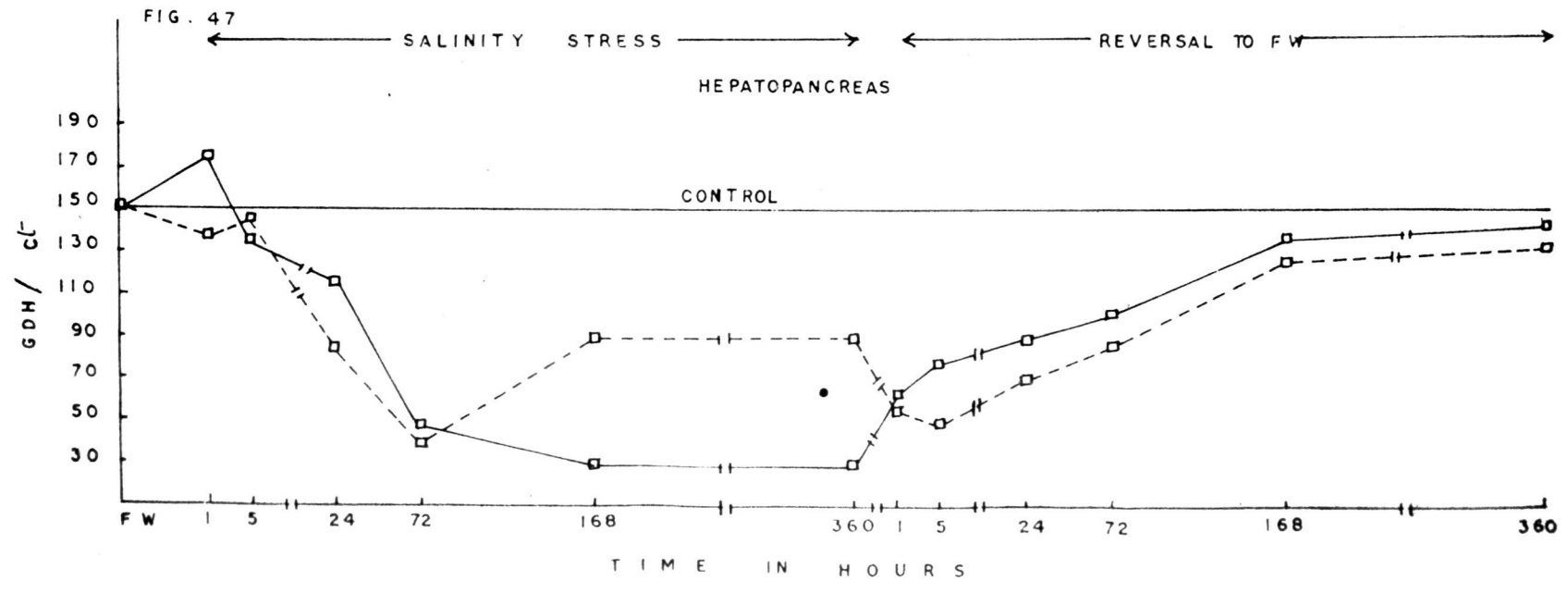

SALINITY STRESS
REVERSAL TO F W
MUSCLE
50% SW
100% SW
CONTROL
GDH/cl⁻
300
280
260
240
220
200
180
160
140
120
100

FIG. 47


SALINITY STRESS
REVERSAL TO F W
HEPATOPANCREAS
CONTROL
GDH / cl⁻
190
170
150
130
110
90
70
50
30
FW
1
5
24
72
168
360
1
5
24
72
168
360
TIME IN HOURS

acclimated crabs, the ratios showed an initial decreasing trend in both the tissues with a later increase to normal levels. (Tables 33 and figures 46 & 47).

AlAT/Cl^- ratios

It is seen from table 34 and figures 48 & 49 that the AlAT/Cl ratios increased in the muscle upto 24 hours in 50% sea water and upto 72 hours in 100% sea water, subsequently the ratio decreased gradually in 50% sea water and steeply in 100% sea water. In the hepatopancreas an increase in the ratio was seen upto 5 hours of transfer to 50% SW with a later fall. In 100% sea water, there was an immediate fall which continued till the end of acclimation period. The decrease in this ratio was greater in 100% sea water than in 50% sea water.

In the reverse acclimation to fresh water an immediate increase in the ratios was recorded in both tissues in 100% sea water exposed crabs but only a gradual increase in 50% sea water acclimated crabs. The ratios showed an 'over shoot' effect in the muscle of both 50% & 100% SW exposed animals in the later stages of reversal to FW.

AAT% Cl^- ratios

The trends are almost similar in both the tissues studied and in both salinities. There was an initial increase in the ratios with a later decreasing trend.

FIGS. 48 & 49: Changes in AlAT/Cl^- ratios in muscle (Fig. 48) and hepatopancreas (Fig.49) when the crabs are exposed to 50% & 100% SW and then returned to fresh water.

FIG. 48

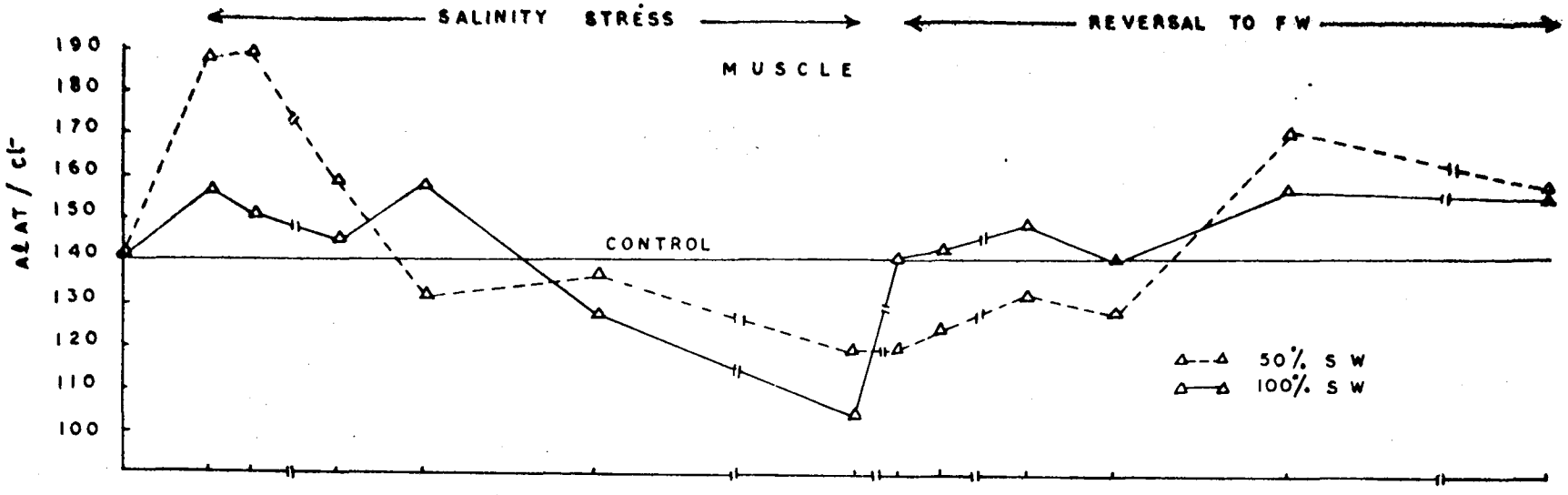
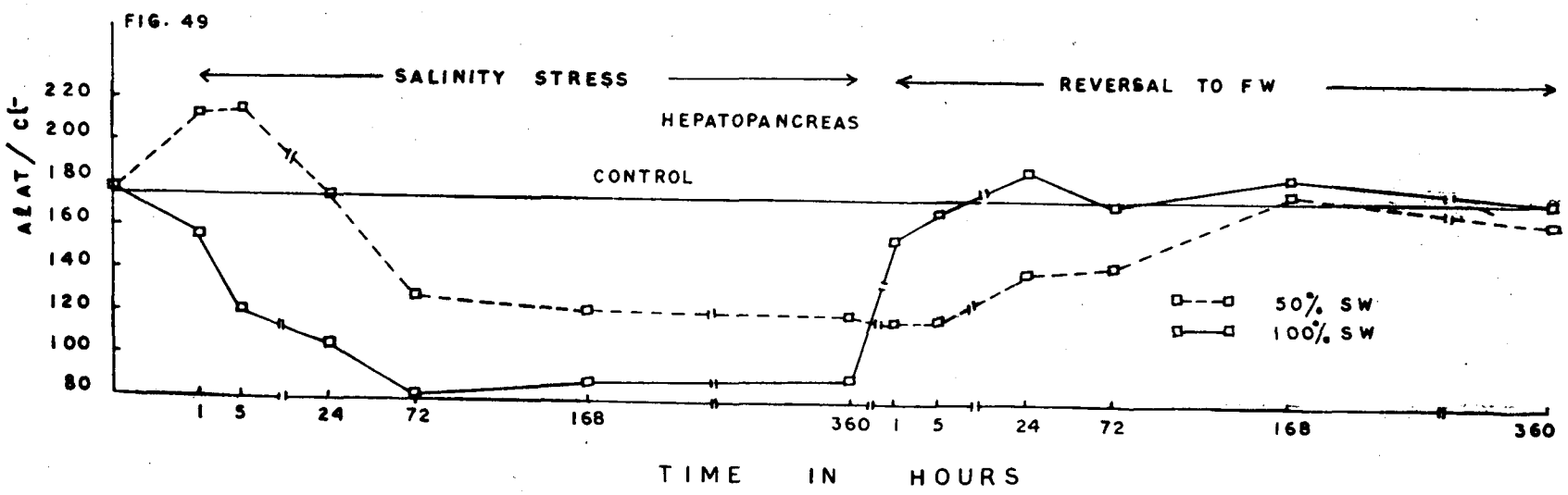

SALINITY STRESS
REVERSAL TO FW
MUSCLE
ALAT / cl-
190
180
170
160
150
140
130
120
110
100
CONTROL
50% S W
100% S W

FIG. 49


SALINITY STRESS
REVERSAL TO FW
HEPATOPANCREAS
ALAT / cl-
220
200
180
160
140
120
100
80
CONTROL
50% SW
100% SW
1
5
24
72
168
360
1
5
24
72
168
360
TIME IN HOURS

However, the hepatopancreas showed a greater suppression of AAT activity per cent chloride ion content than the muscle in both 50% & 100% SW. In the reverse acclimation to fresh water, both the tissues of 50% sea water acclimated crabs showed a decreasing trend in the ratios in the early stages with a subsequent gradual increase to normal levels. However, in 100% sea water exposed crabs, there was an immediate increase to normal levels in both the tissues with a subsequent overshoot effect (Table 35 and figures 50 & 51).

FIGS. 50 & 51: Time course of changes in AAT/Cl^- ratio in the muscle (Fig. 50) and hepatopancreas (Fig. 51) of crabs exposed to 50% & 100% SW and thence returned to FW.

FIG. 50

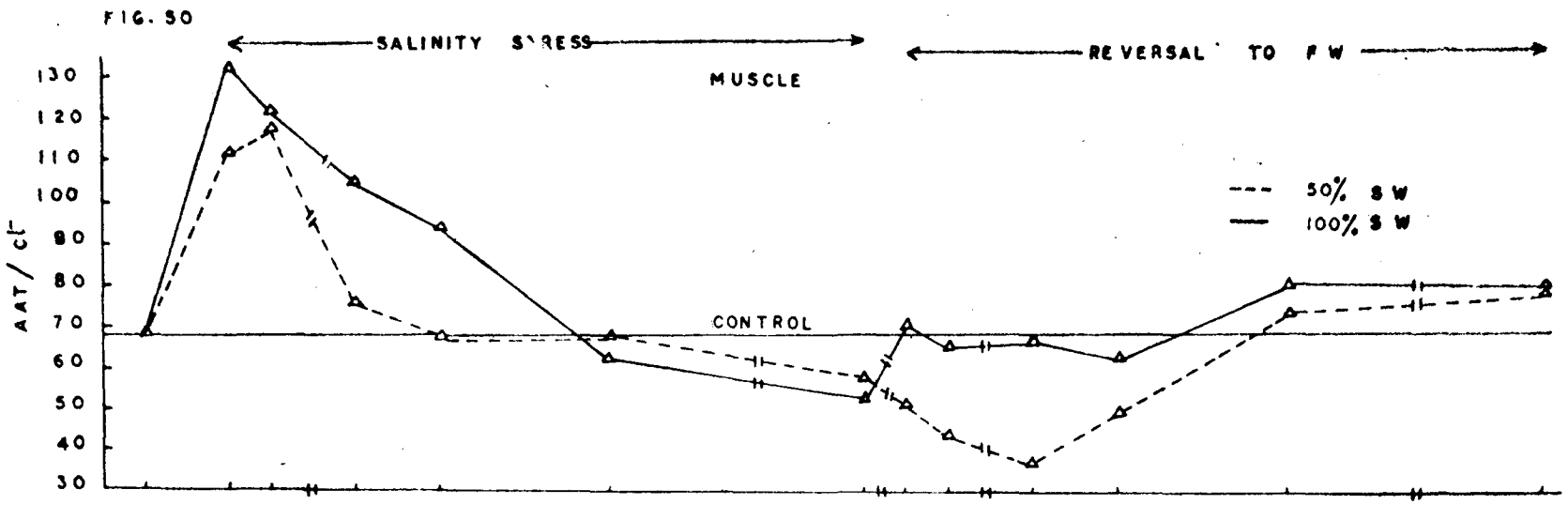
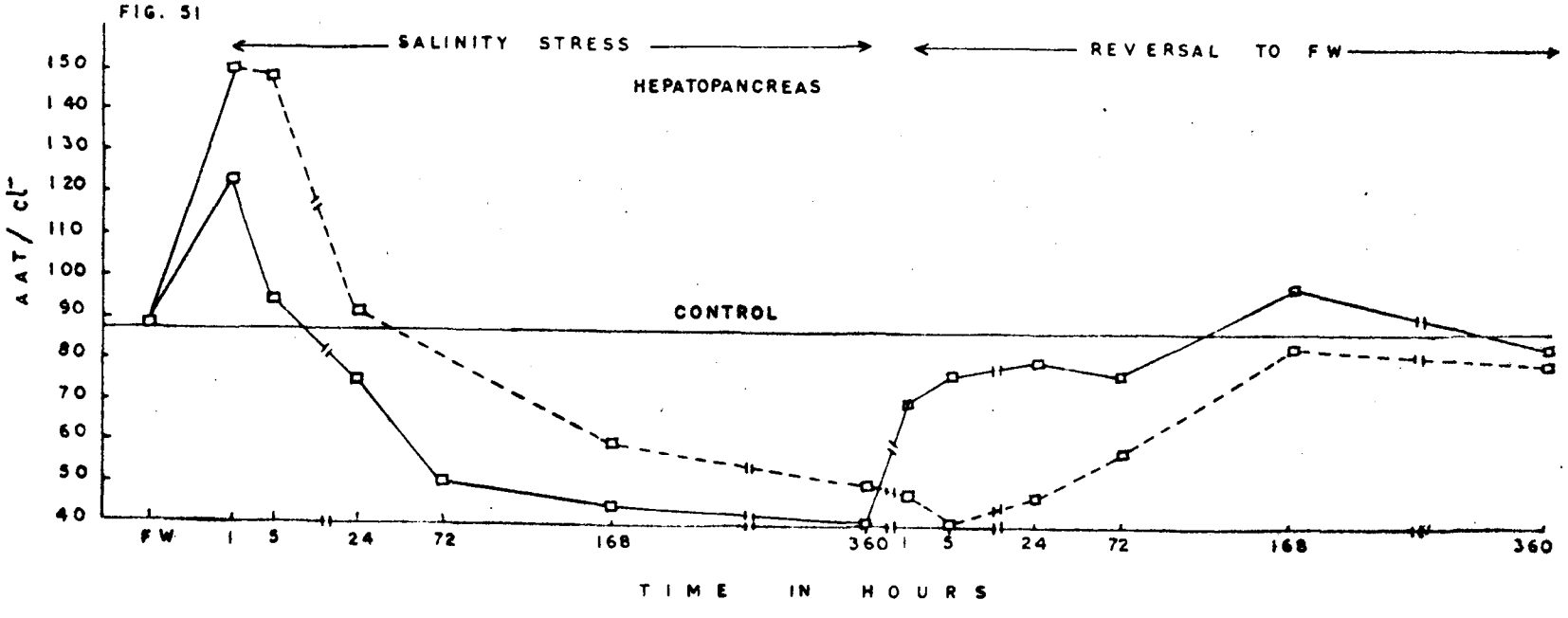

SALINITY STRESS
REVERSAL TO F W
MUSCLE
50% S W
100% S W
CONTROL
AAT / cl⁻
130
120
110
100
90
80
70
60
50
40
30

FIG. 51


SALINITY STRESS
REVERSAL TO FW
HEPATOPANCREAS
CONTROL
AAT / cl⁻
150
140
130
120
110
100
90
80
70
60
50
40
FW
1
5
24
72
168
360
1
5
24
72
168
360
TIME IN HOURS

DISCUSSION

Hypo- and hyper-osmotic media exert potent stress conditions on the inhabitants, both at the animal and cellular levels of organization. The animals have to develop regulatory mechanisms for successful survival in such media, failing which mortality occurs. In order to maintain a constant internal milieu, the organisms tend to regulate the influx of water and ions from the external medium into the blood and also from the blood to the intracellular environment. Electrostatic equilibrium at the cellular level is essential for the normal and efficient functioning of the metabolic activities (Florkin, 1962). The concentration of electrolytes at the inter-and intra-cellular media play dominant role on the influx and efflux of the essential organic constituents and metabolic end products from cell exterior and interior respectively. In hyper-ionic media, the cells are posed with the problem of regulating the influx of ions from the blood. Under these conditions, the cellular medium mobilizes the amino acids which participate in preventing the entry of the ions from the extracellular medium to cell interior (Robertson, 1965; Florkin and Schoffeniels, 1969; Gilles and Schoffeniels, 1969a; Schoffeniels and Gilles, 1970;

Gerard and Gilles, 1972a; Gilles and Gerard, 1974; Gerard, 1975; Schoffeniels, 1976; Gilles, 1975, 1979). Mobilization of amino acids towards the maintenance of electrostatic equilibria across the cell membranes becomes vital under hyper-osmotic stress. Hence it will be worthwhile to understand the mechanisms of the amino acid shuttle into the metabolic activities in hypo- and hyper-osmotic media in order to elucidate the impact of environmental ionic composition on the cellular metabolism.

The amino acid metabolism is linked to other metabolic pathways in view of their potential to provide the amphibolic substances (Harper, 1979). The keto-derivatives of the amino acid metabolism participate in a variety of metabolic functions of cells leading to flexible interconversions in the metabolic pathways. In view of stress conditions on the animals under hypo- and hyper-osmotic media, extreme alterations in the operation of metabolic pathways can be expected, since a variety of stress conditions are known to alter the pattern of metabolic pathways (Wu, 1954; Freeland, 1967; Corman and Kaplan, 1967; Owen and coworkers, 1969; Chaplin and coworkers, 1965, 1967, 1970; Schoffeniels and Gilles, 1970; Felig and coworkers, 1969, 1970; Gilles, 1974a, 1979; Measures, 1975; Schoffeniels, 1976; Snehlata, 1978; Reddanna and Govindappa, 1979; Bhaskara Haranath, 1979). The stress conditions always lead to conditions for the formation of carbohydrates in view

of prevalence of hypoglycemic conditions (Inui and Ohishima, 1966; Inui and Egusa, 1967; Larsson and Lewander, 1973; David and Katherine, 1975; Snehlata, 1978; Reddanna and Govindappa, 1979; Assem and Hanke, 1979). Since the stress conditions contribute to hypoglycemic conditions (Black et al, 1960; Dean and Goodnight, 1964; Masoro, 1966; Umminger, 1971; Dave et al, 1975; Ince and Thorpe, 1976) and under carbohydrate deficiencies amino acids may participate in energy metabolism through the catabolic reactions leading to the formation of nitrogenous end products (Chaplin and coworkers, 1965, 1970; Schoffeniels and Gilles, 1970; Gilles, 1974a; Schoffeniels, 1976). The amino acid metabolism under such conditions can be envisaged to extrapolate into activities like oxygen consumption, excretory patterns and electrostatic equilibria at the cellular level. Hence the study has been extended to amino acids in order to understand the role played by them in metabolic activities of the animals under hyper- and hypo-osmotic stress conditions.

The free amino acid concentration was subjected to alterations at the blood and intracellular levels of the animal in hypo- and hyper-osmotic media as already described in the previous chapter. During the initial stages of exposure to 50% and 100% SW, the amino acid concentration of the blood

was elevated. This observation indicates increased degradation of plasma peptidic material as indicated by previous workers (Siebers and coworkers, 1972; Gilles, 1977). Various stress conditions are known to result in the active efflux of intracellular amino acids into the blood, leading to elevation in blood amino acid content (Balinsky et al, 1967; George et al, 1968; Exton et al, 1970; Adibi, 1971; Adibi et al, 1975; Shakoori et al, 1976; Paul and Adibi, 1976; Paul et al, 1978; Bhaskara Haranath, 1979). Under hyperosmotic conditions, the elevation in the blood amino acid level can be looked upon as a regulatory event to prevent the entry of external ions. During the early period of stress (100% SW and FW) there was an elevation in the oxygen consumption of the animal. Under these conditions of over all elevation in the general metabolism of the animal, active utilization of cellular amino acids into the metabolic activities can also be expected. Thus in the early period of stress a dynamic shuttle of amino acids into the blood and also into metabolic activities can be expected. But a prolonged exposure to higher salinities resulted in the reversal of this situation particularly in 50% SW where the increased amino acid level of early periods in the blood was decreased with a simultaneous elevation in the intracellular media. This elevation in the intracellular media might be due

to the active uptake of amino acids from the blood into the cells on one hand and decreased mobilization of amino acids into the metabolic activities on the other. Hence in view of the changes in the amino acid levels, it will be interesting to study the catabolic events of the amino acid metabolism in order to elucidate the mechanisms involved in the alterations in the concentration of amino acids at the intracellular environment, and to deduce the significance of participation of amino acids in the cellular metabolism under hyper-and hypo-osmotic stress conditions.

The pattern of key enzymes of amino acid metabolism, viz., GDH and aminotransferases (AAT and AlAT) has been studied on acclimation of the animals to 50% and 100% sea water and reverse acclimation to freshwater. Glutamate dehydrogenase (GDH) activity forms the marker of amino-acid oxidations in the cells (Harper, 1979) and it catalyzes the oxidative deamination of glutamic acid leading to the formation of an important TCA cycle intermediary product, viz., α - ketoglutaric acid. Aspartate aminotransferase (AAT) activity indicates the formation of another important TCA cycle intermediary product oxaloacetic acid from aspartic acid. Alanine amino-transferase (AlAT) activity marks the formation of pyruvate from alanine. In general, the activities of AAT and AlAT are taken as markers towards the operation of gluconeogenesis (Harper, 1979) in the cells where

the carbohydrates are formed from non-carbohydrate precursors. Hence, the activity patterns of AAT and AlAT represent the mobilization of amino acids into the formation of TCA cycle intermediaries or into the carbohydrates.

The ratio between the activities of glutamate dehydrogenase and total aminotransferases (GDH/TAT) revealed interesting correlations with the oxygen consumption of the animal. This ratio indicates the extent of mobilization of amino acids into oxidative reactions over that of mobilization into formation of amphibolic substances. In 50% sea water the ratio showed a lower level than control within one hour of exposure both in the muscle and hepatopancreas (table 29 and figure 36). This observation indicates that the amino acid oxidations were decreased in the cells with probable increase in the amino acid concentration. Similarly, the oxygen consumption of the animal was also highly suppressed within the first hour of transfer to 50% sea water (Chapter I; Fig: 5). This observation indicates that the amino acid oxidations represent the general oxidative pattern prevailing at the cellular level and decreased cellular oxidation might have reflected on the oxygen consumption of the whole animal. The further periods of exposure resulted in a gradual increase in the ratio of GDH/TAT which reflected in the gradual attainment

of a steady state of oxygen consumption upon acclimation to 50% sea water. It is rather interesting to recollect here that the muscle water content showed complete compensation whereas the hepatopancreas exhibited partial compensation for water loss in 50% sea water. The increased levels of GDH/TAT ratios in later stages compared to the initial lowered levels in both the tissues suggest the operation of such a compensatory mechanism of water and salt regulation towards the later phase of acclimation to 50% sea water. It is also worthwhile to note that both the tissues studied showed hyper-osmotic regulation (see figure 27) after 24 hours of exposure to 50% sea water. This inturn indicates that the influx of free amino acids from the blood into the cells is an active process as reported by several workers. (Vincent-Marique and Gilles, 1970a, Gerard and Gilles, 1972a; Gilles, 1977, 1979) and the higher levels of GDH/TAT ratios after an initial lowered level point towards the operation of some such compensatory mechanism at the tissue level involving catabolism of amino acids. In fact the amino acid content was found to decrease slightly from the elevated levels after 72 hours of acclimation to 50% sea water, suggesting the onset of amino-acid catabolism. The reverse acclimation to fresh water showed an increasing trend in GDH/TAT ratio during the early phase with a concomitant elevation in the whole animal oxygen consumption

suggesting increased catabolism of amino acids in hypo-osmotic conditions.

However, the response of the animal in oxygen consumption and GDH/TAT ratio was one of elevation over the normal in the early phase of exposure to 100% sea water with a later gradual decline. Since 100% sea water exerts extreme stress conditions on the animal, being highly hyper-osmotic and resulting in large osmotic and ionic gradients between blood and ambient medium (see figures 18 & 20) the oxidative activities might have been elevated initially with a later suppression consequent on reduction of osmotic gradients. Hence GDH/TAT ratio directly reflects the cellular oxidations which will be expressed at the animal level as oxygen consumption. In the reverse acclimation to fresh water from 100% sea water, the GDH/TAT ratio showed a general increase corresponding to the increase in oxygen consumption of the whole animal suggesting increased catabolism of amino acids under hypo-osmotic conditions. This picture fits well into the general trends reported by other investigators (Siebers and coworkers, 1972; Gilles, 1974a, 1977, 1979). Thus amino acid metabolism seems to exert direct influence on the general metabolism of the animal under both hyper-and hypo-osmotic conditions.

The foregoing account suggests that the amino acid mobilization into oxidations and transaminations was variable during the course of acclimation. These observations are also suggestive of modulations in the cellular requirements in the amino acid catabolism under stress conditions. Hence, the time course study of the activity of glutamate dehydrogenase and aminotransferases has been undertaken in the tissues of the animal during acclimation to hyper- and hypo-osmotic media.

In 50% sea water glutamate dehydrogenase activity showed differential trend in muscle and hepatopancreas (fig: 35) and thereby tissue specificity was expressed in the oxidative deamination of the amino acids. While the muscle recorded an initial rise in GDH activity upto 72 hours of exposure with a later reversal to the control level, the enzyme activity was inhibited in the hepatopancreas throughout the period of exposure. Hence, the muscle metabolism was directed towards the mobilization of amino acids into oxidative metabolism. Such a differential pattern in the activity of GDH between these tissues can be explained on the basis of intracellular chloride content. Hepatopancreas had higher accumulation of chloride ions when compared with the muscle(See figures 42 & 43). Hence chloride seems to play an important role on the expression

of GDH activity. This observation is in perfect agreement with the earlier reports where, anions, particularly Cl^- have been demonstrated to inhibit oxidative enzymes of amino acid metabolism (Chaplin, Huggins and Munday, 1965; Gilles, 1969; Gilles, Hogue and Kearney, 1971). The muscle tissue had elevated GDH activity upto ~~24~~ 72 hours with a later gradual decline to the control level on acclimation. The initial increase in the enzyme activity seems to be due to regulation imposed on the influx of chloride ions. As the time course of acclimation continued, there was elevation in the chloride content with subsequent inhibition on GDH activity. In the hepatopancreas there was a greater increase in the intracellular chloride content than that of muscle with greater inhibition of the GDH activity. In view of these observations correlation can be drawn between glutamate dehydrogenase activity with intracellular chloride content. As the intracellular chloride content increased there was a corresponding inhibition on the activity of glutamate dehydrogenase.

However the pattern of activities of aminotransferases was different from GDH activity in crabs exposed to 50% sea water (Figs. 33 & 34 and 42 & 43). In both the tissues there was significant increase in the activities of both AAT and AlAT in the early periods of acclimation,

maximum elevation being in the activity of AAT. The activity level of AAT was highly elevated in both hepatopancreas and muscle tissues upto 5 hours duration of exposure. . Aspartate aminotransferase is concerned with the active mobilization of aspartic acid into the formation of oxaloacetic acid. Such an increase in the oxaloacetic acid content, in the light of decreased oxygen consumption, envisages mobilization of oxaloacetic acid towards the formation of malate and then to the carbohydrate synthesis through gluconeogenesis (Harper, 1979). Similarly, there was an elevation in the activity of AlAT during the early period of exposure of the animals to 50% sea water. Since the activities of both AAT and AlAT form the markers towards the operation of gluconeogenesis their increase in the tissues indicate the active shuttle of amino acids into the formation of carbohydrates. Since the animals under stress conditions exhibit hypoglycemia (Blazka, 1958; Miller et al, 1959; Black et al, 1960; Dean and Goodnight, 1964; Masoro, 1966; Umminger, 1971; Dave et al, 1975; Ince and Thorpe, 1976). and carbohydrate deficiencies promote the operation of gluconeogenesis (Freeland, 1967; Inui and Egusa, 1967; Larsson and Lewander, 1973; David and Katherine, 1975; Dave et al, 1975; Sakamoto and yone, 1978; Snehlata, 1978), the elevated activities of AAT and AlAT in the tissues of

the crabs exposed to 50% sea water are suggestive of such mobilization of amino acids into carbohydrate formation. It is desirable to extend studies in the direction of understanding changes in the carbohydrate levels of tissues during the course of acclimation as data in this respect are wanting. However, this aspect does not form part of this dissertation.

The muscle tissue maintained fairly higher levels of the activities of AAT and AlAT upto 168 hours of acclimation with a later decline to normal levels, while hepatopancreas suppressed the activities of these enzymes over the control level beyond 72 hours of exposure to 50% SW. Hence on acclimation, hepatopancreas exhibited highly suppressed activities of GDH and aminotransferases while the muscle tissue maintained the control level of activities. Hence tissue specificities prevailed during the course of acclimation to 50% sea water in the catabolism of amino acids. Evidently, hepatopancreas showed greater accumulation of amino acids than the muscle (table 17 and 19; Fig: 25) in as much as this tissue showed greater suppression of amino acid catabolism than the muscle.

The ratios like GDH/FAA, AAT/FAA and AlAT/FAA represent the relative mobilization of amino acids into the activities of glutamate dehydrogenase and aspartate and alanine aminotransferases (Fig: 38 & 40). GDH/FAA ratio

was higher in muscle tissue upto 5 hours of exposure to 50% sea water with lesser values relative to the level of controls on the subsequent period upto 15 days. This observation indicates that mobilization of free amino acids into GDH activity was increased upto 5 hours with a later decline. In hepatopancreas the ratio was lesser compared to the control level from the beginning of exposure suggesting that the amino acid oxidations were inhibited immediately after transfer of the animals to 50% sea water. Both muscle and hepatopancreas expressed higher ratio of AAT/FAA in the initial periods of exposure to sea water medium, suggesting higher level of mobilization of tissue amino acids into oxalo acetic acid formation. The AlAT/FAA ratio was higher than the control value in the muscle upto 5 hours, while the same was lower in the hepatopancreas. This observation indicates that the mobilization of amino acids into the activities of glutamate dehydrogenase and aminotransferases was prevalent in the early periods of exposure in the muscle tissue but in the hepatopancreas the amino acids were mobilized preferentially into aspartate aminotransferase activity with a suppression in GDH and AlAT activities.

In general it can be concluded that the tissues in the animal exposed to 50% sea water resorted to amino acid catabolism leading to their mobilization into the formation of TCA cycle intermediaries oriented towards the formation of carbohydrates initially upto with a later suppression in the mobilization of amino acids into catabolic activities, probably leading to the elevation in amino acid content of the tissues. Since amino acids actively participate in the intracellular isosmotic regulation through electrostatic equilibrations, (Jeuniaux and Florkin, 1961; Florkin, 1962; Bricteux-Gregoire et al, 1962; Florkin et al, 1964; Florkin and Schoffeniels, 1969; Schoffeniels, 1976; Huggins & Munday, 1968, 1971; Schoffeniels and Gilles, 1970, 1972; Vincent-Marique and Gilles, 1970a,b; Gerard and Gilles, 1972a,b; Gilles, 1972, 1974a, 1975; Hoffmann and Hendil, 1976), at later stages of acclimation amino acids seem to have been spared for the regulation of ionic influxes from the hyperionic blood. Hence on acclimation to 50% SW the tissues of the crab resorted to utilization of amino acids to maintain isosmotic condition with blood through electrostatic equilibrium.

The process of reverse acclimation to fresh water resulted in a gradual decline in the chloride content of the tissues. Such an event in chloride fluxes resulted

into changes in the amino acid metabolism. GDH/TAT ratio was initially lowered suggesting a decreased mobilization of amino acids into oxidative activities. This may be attributed to the passive efflux of inorganic ions and free amino acids from the cells into the blood initially. However in later stages this ratio increased gradually in both tissues, suggesting an entirely reverse situation to exist in the tissue amino acid catabolism. This observation indicates that the amino acid mobilization into glutamate dehydrogenase activity was preferentailly increasing over the mobilization into aminotransferases. Consequent on such change in the ratio, there was a gradual increase in the whole animal oxygen consumption. In view of such a possibility of reversal of oxygen consumption of the animal and GDH/TAT ratio, ectopic condition of the amino acid metabolism even after acclimation to higher salinities can be envisaged. Hence, amino acid mobilization pattern into the catabolic activities depends on the osmotic stress in the medium. In higher salinities the mobilization of amino acids preferentially into the aminotransferases was favoured while in lower salinities, the mobilization was shifted towards the oxidations of the amino acids. GDH activity level increased and GDH/FAA ratio was higher during reverse acclimation to the fresh

water indicating mobilization of amino acids into the oxidative metabolism than into the formation of carbohydrates. Thus the chloride concentration in the medium seems to shift the amino acid catabolic pattern. Higher salinities shift the amino acid catabolic equilibrium between amino acid oxidation and transaminations towards transaminations and lower salinities favour the equilibrium towards the oxidations.

The pattern of amino acid catabolism was distinctly different in the tissues of the crabs exposed to 100% SW in comparison to those exposed to 50% SW, during the course of acclimation. GDH/TAT ratio (Fig: 37) was higher initially (upto 24 hours) in both hepatopancreas and muscle, which indicates increased oxidative metabolism in the tissues. Such an increase in oxidative metabolism of the amino acids is in perfect correlation, with the whole animal oxygen consumption, in that, the oxygen consumption was also elevated during the period of higher GDH/TAT ratio (see chapter I and figure 5). This type of response of the animal in 100% sea water was quite reverse to that in 50% sea water where GDH/TAT ratio was lower in both tissues during the early period of acclimation. In view of elevation in the tissue oxidations, prevalence of extreme stress conditions on the animal can be expected in 100% sea water. The external

medium, being highly hyper-ionic to the animal, it has to invoke regulatory mechanisms through active transport for the maintenance of a constant composition of the internal milieu. In view of extreme concentration gradient, Na^+ and Cl^- tend to penetrate into the body. Since Cl^- ions were found to be inhibitors of oxidative metabolism (Schoffeniels and Gilles, 1963; Warren and Cheatum, 1966; Warren, Stowring and Morales, 1966; Chaplin, Huggins and Munday, 1965; Gilles, 1969; Gilles, Hogue and Kearney, 1971) and Na^+ and Cl^- ions being injurious to the tissue organization (Von Hippel and Wong, 1964; Warren and Cheatum, 1966; Warren, Stowring and Morales, 1966; Chimoskey and Gergely, 1968; Gilles, 1974a, 1975), the body was probably resorting to active transport mechanisms to prevent the entry of external ions into the system. Since the active transport mechanisms need expenditure of energy, oxidative metabolism of the animal might have been switched on to release energy. Thus the animal on sudden exposure to 100% sea water was switching over to the geared up oxidative metabolism to regulate the ionic influxes. Consequent on such steps, the intracellular Cl^- concentration was found to be at lower levels in the tissues in the early stages of transfer to 100% SW. Among the two tissues studied, muscle expressed a better

regulatory capabilities in the early phases of exposure to the entry of chloride ions than the hepatopancreas (see figures 44 & 45). In hepatopancreas, within one hour of exposure the chloride ions were elevated over the control level, indicating that the tissue permeated the chloride ions with least regulation (Fig:45). In contrast, the muscle tissue had slight elevation in intracellular chloride content even after 5 hours of exposure (Figure 44), suggesting that this tissue was utilising the regulatory mechanisms for the prevention of ionic influxes from the ECF. Since GDH activity pattern was different in both the tissues, this enzyme seems to be directly responsive towards the chloride influxes. In hepatopancreas, the GDH activity was inhibited over the control level within 72 hours of exposure, while the same was higher than the control level beyond this period in muscle. Whereas the maximum elevation in GDH activity was 64% in the hepatopancreas, it was 103% in the muscle in the early phase of acclimation. However, in later stages, the GDH activity was suppressed over the normal level. The hepatopancreas showed greater inhibition of GDH activity (-58%) than the muscle (-10%) in later stages of acclimation to 100% sea water (Fig: 35, 44 & 45). Such a response in the enzyme activity might be due to the accumulation of chloride ions in the tissues. Greater accumulation of chloride ions

in the hepatopancreas might be responsible for the greater inhibition of this enzyme activity compared to a lesser accumulation of chloride ions in the muscle with a lesser degree of suppression of GDH activity in it. Since chloride ions were demonstrated to be the inhibitors of glutamate dehydrogenase activity in particular and oxidative metabolism in general (Snoke, 1956; Schoffeniels and Gilles, 1963; Chaplin, Huggins and Munday, 1965; Gilles, 1969; Gilles, Hogue and Kearney, 1971) gradual accumulation of these ions in the intracellular environment might have been directly responsible for the observed inhibition in the GDH activity. It was interesting to note quite opposing trends in the activity pattern of GDH and intracellular chloride penetration in both the tissues. Hence the GDH activity seems to exhibit an inverse relationship with chloride accumulation in the tissues. It is interesting to note that the pattern of GDH activity in the tissues corresponds to oxygen consumption of whole animals exposed to 100% SW.

GDH/FAA ratio (Figures: 39 & 41) which denotes the mobilization of amino acids into glutamate dehydrogenase activity particularly and into oxidative deaminations in general showed higher level initially in the muscle with a later decrease over the control level (figure 41). This observation is also suggestive of active involvement of

amino acid catabolism towards oxidative deaminations during the early phase of exposure to 100% SW. The fact that the oxidative metabolism of the tissues were elevated with consequent elevation in oxygen consumption of the whole animal initially, indicates that the animal was gearing up the regulatory mechanisms for preventing the entry of ions from the ambient medium in the early phases of exposure. But, on chronic exposure to this stress condition, the animal was turning as an osmoconformer with free penetration of ions from the exterior with particular reference to sodium and chloride ions. Hence, from these patterns it can be generally predicted that the crab in 100% sea water was a regulator in the early phases with a later transformation to an osmoconformer. Hence, oxidative metabolism of amino acids in the muscle can be taken as a general marker towards the regulation of ionic fluxes in the muscle, in the early phase of exposure to extreme hyper-ionic stress GDH/FAA ratio in the hepatopancreas (figure 39) showed a different picture. The ratio was lower than control immediately after exposure to sea water suggesting decreased catabolism of amino acids and their consequent initial conservation in the hepatopancreas in the phase of greater penetration of chloride ions (figure 45). The GDH/TAT ratios (figure 37) in both the tissues showed an initial elevation in 100% SW corresponding to the elevation in the oxygen

consumption of the whole animal. However, this ratio was found to be greater in the muscle than in hepatopancreas. This observations indicates that the oxidative deamination of amino acids by glutamate dehydrogenase activity is prodominant over transamination activities particularly in the muscle in the early phase of exposure to 100% sea water. However, the GDH/FAA and GDH/TAT ratios (Figures: 37, 39 & 41) were gradully decreasing during the later phase of exposure to 100% sea water, suggesting that there was a gradual shift in the mobilization of amino acids from oxidative metabolism to the formation of amphibolic substances through transamination reactions. Since the activities of aminotransferases particularly AAT and AlAT are generally considered as the markers of gluconeogenesis (Harper, 1979), the pattern of activity of aminotransferases can be taken as an index of carbohydrate formation in the tissues. In the light of this information, it can be suggested that the tissues of the animals subjected to extreme hyperionic stress were tending towards mobilizing the amino acids into carbohydrate formation from the oxidative metabolism. Since total aminotransferase activity was gradually increasing, active formation of intermediary amphibolic substances like pyruvic acid and oxaloacetic acid in the tissues can be

expected. In the light of decreased oxygen consumption mobilization of these amphibolic substances into the formation of carbohydrates through the gluconeogenesis can be expected. Since the animals under extreme stress conditions develop carbohydrate deficiency with severe hypoglycemic conditions, (Black and coworkers, 1960; Dean and Goodnight, 1964; Masoro, 1966; Umminger, 1971; Dave et al, 1975; Ince and Thorpe, 1976; Venkata Reddy, 1976; Bhaskara Haranath, 1979) there was necessity on the part of the tissue metabolism to form the carbohydrates for homeostatic mechanisms. Since maintenance of glucose concentration of blood forms an important homeostatic factor (Ruderman et al, 1976; Harper, 1979) and the nervous tissue metabolism depends on the carbohydrates (Ruderman et al, 1976; Harper, 1979) the tissue metabolic pattern must be oriented for providing basal requirements of carbohydrates. Moreover, in 100% sea water starvation effect was superimposed on the metabolic patterns, since the animals remained without feeding after 3 days of transfer. Since starvation forms a potent stress condition with induction of hypoglycemia in the body (Owen et al, 1967; Larsson and Lewander, 1973; Dave et al, 1975; Ince and Thorpe, 1976; Bhaskaraharanath, 1979) such a possibility of carbohydrate deficiency might have developed in the

tissues of crabs exposed to 100% sea water. The observation on the drastic decrease in the carbohydrate content of the tissues of the crabs exposed to 100% sea water at the end of acclimation period (Venkata Reddy, 1976) was indicative of prevalence of hypoglycemia in the tissues of crabs exposed to 100% SW. Hence, the tissue metabolic pattern was shifting towards the mobilization of amino acids towards transamination reactions from oxidations.

The peculiar event of tissue amino acid metabolism in the crabs exposed to 100% sea water was to maintain higher levels of AAT and AlAT activities over the controls throughout the period of acclimation. This observation suggests that the tissue metabolism was continuously mobilising the amino acids into the carbohydrate formation probably owing to extreme carbohydrate deficiency in the body of the animals under extreme salinity stress. In view of such mobilization of amino acids the tissue amino acid concentration might be depleted. The pattern of changes in the free amino acid content was also suggestive of gradual decline in the amino acid concentration from the levels of early period i.e., after 72 hours onwards (Table: 19; Fig: 26). However, the amino acid level was always higher over the control level. The gradual decline in the tissue amino acid concentration from the peak

levels can be attributed to the continuous utilization of them into the catabolic activities of the tissue. Consequent on such gradual decline in the amino acid concentration of the tissues and amino acids being one of the active factors responsible for the regulation of ionic fluxes, the intracellular chloride concentration was gradually elevated during the course of acclimation of the crabs to 100% sea water (Fig: 44 & 45). Hence, the animal's behaviour as an osmoconformer can be correlated to the tissue level at the amino acid metabolism.

Hepatopancreas was maintaining higher percent elevation in the activities of AAT and AlAT than the muscle (Fig: 33 & 34). Hence, transamination seems to be at a higher level in the hepatopancreas than in the muscle. Such a response might be essential in view of its participation in keeping up the blood glucose concentration which showed a drastic decrease in crabs acclimated to 100% sea water, (Venkata Reddy, 1976). In view of such an active mobilization of amino acids towards transamination activities, the intracellular chloride concentration was drastically elevated in hepatopancreas (Figure: 45). Since chloride ions inhibit the oxidative metabolism of the tissues (Chaplin, Huggins and Munday, 1965; Gilles, 1969; Gilles, Hogue and Kearney, 1971) and oxidative metabolism is essential for the integrity of the tissue

organization, such a drastic elevation of chloride ion concentration can be attributed to be responsible for the tissue degradation observed earlier (Venkata Reddy, 1976). The hepatopancreas seems to have been subjected to degradation activities leading to atrophy of the tissue, which might be the reason for an overall decrease in the weight of the organ which has been termed as "Salogenous atrophy (Venkata Reddy, 1976). Hence, under the stress of 100% sea water, hepatopancreatic histopathological condition might be developed owing to disturbances in the amino acid catabolism which reflects as a deranged metabolic pattern leading to "chloride injury" of the tissue. Such an inference is also in perfect agreement with reports that anions disrupt the structure of various macromolecules (Von Hippel and Wong, 1964; Warren and coworkers, 1966; review articles by Gilles, 1974a, 1975).

However, in the muscle, the activities of amino-transferases were maintained at a fairly lower level than that of hepatopancreas. But the activities of these enzymes were always higher than the controls. Probably, owing to such regulation on the activities of these enzymes, the intracellular chloride concentration was also regulated at a fairly lower level than the hepatopancreas. Hence, in 100% sea water the muscle of the crab was exhibiting

regulation on the influx of chloride ions, while the same was deranged in the hepatopancreas.

Therefore, it can be concluded in general that the tissues of the crabs exposed to 100% sea water were continuously mobilizing the amino acids by decreased catabolism as well as active influx from the blood. These free amino acids served as the major organic osmotic effectors, but a protion of these amino acids were also utilised towards the formation of carbohydrates through gluconeogenesis which was essential for the maintenance of basal metabolism of the body. Probably in view of extreme hypoglycemia, there was high mortality in crabs exposed to 100% sea water. The mortality rate was observed to be nearly 50% at different periods of acclimation, the mortality rate after 3 days being the highest. Such of those crabs which shuttled the precursors like amino acids into carbohydrate formation alone might have survived, for they were perhaps capable of maintaining minimum level of carbohydrate concentrations in the body. Hence, continuous operation of the activities of AAT and AlAT seems to be vital for the survival of animals in 100% sea water and the same can be suggested to result in the gradual accumulation of chloride ions in the intracellular

medium particularly in the hepatopancreas.

Another remarkable feature of tissue amino acid metabolism was the amino acid content of the tissues during the course of acclimation to 100% sea water and the pattern of its mobilization into the catabolic activities. The tissue amino acid content (Figure 26) was maintained at a higher level than the control throughout the period of exposure to the sea water medium inspite of the utilization of amino acids for the transamination activities indicated earlier. Such a possibility may be due to a continuous supply of free amino acids fron the blood. This view is strengthened by the works of Siebers and Coworkers (1972) and Gilles (1977) who observed transitory decrease in blood amino acid content in Carcinus maenas and Eriocheir sinensis during acclimation to concentrated media. In the crab under investigation a similar decrease in blood amino acid level was observed after an initial elevation. Such changes in amino acid content of blood might indicate changes in the blood serum content of peptidic material. Blood serum peptides might be degraded into amino acids under hyperosmotic conditions involving proteolysis (Siebers and coworkers, 1972; Gilles, 1977; Pequeux and coworkers, 1979). The free amino acids thus

accumulating in the blood might be actively transported into the tissues. Thus a continuous supply of free amino acids from the blood can be envisaged and the same could be responsible for the higher levels of amino acid content in tissues throughout the period of exposure to sea water. Thus, higher levels of amino acids in the tissues might be due to their active uptake from the blood. Besides, there might be the possibility of certain extent of proteolysis in the tissues since all the stress conditions including starvation stress induce tissue proteolysis (Tappel et al, 1962; Owen et al, 1969; Felig et al, 1969; Weinstock and Iodice, 1969; Love, 1970; Felig et al, 1970; Pozefsky et al, 1976; Goldspink, 1976; Narayana Reddy, 1977; Bhaskara Haranath, 1979)and 100% sea water medium forms a potent stress condition. Another alternative for maintenance of higher amino-acid level in the tissues might also be due to the relative extent of mobilization of amino acids into the amino acid catabolism. This aspect can be visualized in the analysis of AAT/FAA, AlAT/FAA and GDH/FAA ratios. These ratios were gradually lowered over the control level in the tissues throughout the course of exposure to sea water, excepting the initial periods. Such lower levels of the ratios suggest a relative decrease in the mobilization of amino acids into the activities of catabolic enzymes leading to their

accumulation inside the cells as organic osmotic effectors. Lowered oxygen consumption observed in this crab in 100% SW and decreased NH_3 production (Krishna moorthy and Srihari, 1973) lend support to this view and this fits well into the general trends reported earlier (Ramamurthi, 1965, 1967b, Chaplin, Huggins and Munday, 1970; Siebers, 1972; Siebers and coworkers, 1972; Gilles, 1974a, 1979, Venkata Reddy, 1976). Hence, the conservation of free amino acids in the cells seems to be at a higher level than their mobilization into the activities of GDH, AAT and AlAT, though there was an apparent elevation in the activities of aminotransferases. Hence, the tissues under salinity stress were maintaining two important phases of responses, one being the maintenance of higher activities of aminotransferases and thereby leading to the maintenance of carbohydrate content on one side and the other being maintenance of higher amino acid content for electrostatic equilibrium to check the ionic influxes. Hence from this analysis, it can be predicted that the tissues were still capable of certain extent of regulation of ionic influxes, which could have been responsible forthe adaptive behaviour with survival value. Therefore, it can be suggested that the tissues of the crabs under salinity stress were capable of regulating the free amino acid content with relative

restrictions on the mobilization of amino acids into the catabolic activities. Thus the salinity stress seems to modulate the tissue metabolism for the maintenance of homeostasis, which provides the survival value leading to the acclimation of the animals to stress conditions.

During the reverse acclimation of the crabs to fresh water from 100% sea water the chloride ions seem to leak out more rapidly than Na^+ from both the tissues. There was a steep fall in this ion concentration during the first hour of reversal with a later gradual return to normal levels. The amino acid content of the muscle tissue also decreased to normal levels relatively quickly by about 72 hours whereas in the hepatopancreas a slow and gradual decrease was observed. The blood amino-acids showed elevated levels in the early periods. While the GDH activity increased immediately after transfer to fresh water in both the tissues, the AlAT and AAT activities decreased to nearly normal levels in the early phase of transfer in the muscle and in later periods in hepatopancreas. These observations suggest the following probable trends in reverse acclimation to fresh water. There was a passive leakage of inorganic ions, particularly Cl^-, from the tissues to blood and from there

to the ambient medium immediately after the transfer of crabs from 100% sea water to fresh water. Concomitant with the decrease in the Cl^- content of cells there was a decrease in the free amino-acid content of the tissues with probable elevation in the influx of water into tissues and consequent regulation of cell volume (see chapter III). Decreased amino-acid content of the cells could be explained on the basis of efflux of amino acids into the blood as well as increased catabolism of intracellular amino acids. The increased amino acid levels observed in the blood in the early periods of reverse acclimation (figure 26) lends support to the view that the hypo-osmotic shocks result in the efflux of amino acids from the tissues into the ECF and this observation is in perfect agreement with earlier findings on isolated cells (Fugelli, 1967; Lang and Gainer, 1969; Gilles and Schoffeniels, 1969a; Pierce and Greenberg, 1970; Gerard and Gilles, 1972b; Hendil and Hoffmann, 1974; Gerard, 1975; Kevers, Pequeux and Gilles, 1979) and on whole animals (Clark, 1968; Binns, 1969; Vincent-Marique and Gilles, 1970a, 1970b; Gerard and Gilles 1972a; Gilles, 1977). Higher level in GDH/FAA and GDH/TAT ratios observed in tissues of crabs transferred to fresh water implies increased mobilization of amino acids into catabolic activities such as oxidative deamination. The oxygen consumption in the

whole animals which was tending to anaerobic level in 100% SW was found to shoot up over the normal level (see figure: 5) soon after return to fresh water suggesting increased oxidative metabolism in hypo-osmotic media. Such observations on increased GDH activity in tissues and increased oxygen consumption of whole animals brought back to fresh water fit well into the general trends of increased oxidation of amino acids resulting in the increased production of CO_2 and NH_3 observed under hypo-osmotic stress conditions (Ramamurthi, 1965; 1967b; Chaplin, Huggins and Munday, 1970; Lee & Pyung, 1970; Vincent-Marique and Gilles, 1970a; Siebers and coworkers, 1976). The AAT/FAA and AlAT/FAA ratios also were higher in the tissues of the crabs in the reverse acclimation showing increased aminotransferase activities leading to the depletion of intracellular amino acid content. However, the relative extent of depletion of amino acids due to efflux and due to catabolism cannot be visualised in view of the complex nature of events occurring simultaneously. However, the activities of AlAT and AAT continued to remain at higher levels beyond 72 hours of transfer to freshwater particularly in the hepatopancreas. This implies a continued utilization of amino acids for the formation of TCA cycle intermediaries and for gluconeogenic activities. The crabs were observed to resume normal feeding behaviour

only after 7 days of return to fresh water. In other-words the crabs continued to remain under starvation stress. This probably explains the higher levels of AlAT and AAT activities in tissues and continued mobilization of amino acids into the formation of carbohydrates in the hepato-pancreas inorder to prevent hypoglycemic shocks in the animals. This aspect seems to account for the observed absence of mortality in the crabs in the reverse acclimation process to fresh water medium. GDH, AlAT and AAT activities were found to return to normal levels sooner or later during the reverse acclimation to FW. Hence, ecotopic conditions in the animal metabolism when returned from 100% sea water to fresh water can be envisaged. The enzyme patterns observed in the tissues of crabs returned to fresh water from 100% sea water were just the reverse of what were generally observed during acclimation to higher salinities, probably, indicating that the animals had not suffered an irreparable damage during their sojourn for 15 days in 100% sea water and that all the metabolic events leading to hyperosmotic adjustments were reversible under hypo-osmotic conditions. It is worth noting here that adaptation to lower salinities was a quicker process than adaptation to higher salinities as shown in the present work with respect to the regulation of water content in tissues, regulation of inorganic and organic osmotic affectors at the tissue level and oxygen consumption at whole animal level.

Another interesting feature which can be elaborated by the present study is the chloride-sensitivity of enzyme activities concerned with amino-acid catabolism. When these enzymes are represented per unit intracellular chloride content (figures 46-51) there is an interesting trend during the temporal sequence of exposure of the crab to the sea water media. The sensitivity of the enzyme activitiesto intracellular chloride content varies at different periods of exposure to sea water. In the muscle, activities of GDH, AAT and AlAT per unit intracellular chloride content were elevated over the control upto 24 hours or 72 hours of exposure with a later gradual decline both in 50% and 100% sea water. This observation indicates that these enzyme activities were highly sensitive in the initial periods and thereby any entry of chloride ions seems to activate the enzymes of amino-acid catabolism leading to its regulation. However, on prolonged exposure the chloride ions seem to inhibit the enzyme activities. Thus the results of the present study clearly demonstrate that the chloride effect on these enzyme activities is dependent on the length of exposure of the animal to the ambient medium. Hence However, the study on the temporal sequence of exposure to the ambient medium is highly essential to draw meaningful conclusions on the effect of ions of external medium on the cellular activities. Unfortunately, several

investigators have employed either initial periods of exposure or the end stages of acclimation to relate the metabolic activities with the ions of the medium (Ramamurthi, 1965; 1967b; Madanmohan Rao and Coworkers, 1969; Venkatachari and Ramamani Keshavan, 1973; Venkatachari, 1974; Venkata Reddy, 1976). In the light of the present study, selecting any particular period of exposure leads to erroneous conclusions as far as the effect of external ions on the activities of enzymes is concerned. Hence, studies involving temporal sequence are vital to explain changes in enzyme activities in relation to ions of the external medium.

The pattern of enzyme activities per unit intracellular chloride during the temporal sequence also depends on the tissue employed for the study. The hepatopancreas of the crab expressed a general decreasing trend in GDH and AlAT activities from the beginning of the exposure period, unlike the muscle where there was elevation in the early period with a later decline. Thus the sensitivity of enzyme activities seems to depend on the tissue specificity also besides the time of exposure. But AAT activity per unit chloride content recorded a different trend from the other enzymes in the hepatopancreas in that it showed elevation upto 5 hours of exposure, tending towards the pattern of muscle.

It is also worth noting here that the activity pattern of each enzyme when expressed per unit intracellular chloride was generally the same both in 50% sea water and 100% sea water acclimated crabs, the differences, if any, were only in the degree of elevation or suppression but not in the pattern. Changes in the pattern of enzyme activities were thus found to be essentially a function of time. This observation also suggests that the time of exposure to the new medium is of paramount importance in enzyme studies rather than the medium itself.

The pattern of chloride sensitivity of these enzymes is also interesting during reverse acclimation to freshwater. All the enzymes studied showed a general increasing trend indicating increased catabolism of amino acids when the crabs were transferred from sea water to fresh water. The enzymes of ~~both~~ the muscle, however, recorded 'Overshoot' sensitivity on reverse acclimation since the activity levels were far higher than the controls especially in crabs reversed from 100% sea water (figures 46, 48 & 50). The hepatopancreas on the other hand, did not record any 'overshoot' response in GDH activity but the same was evident in a samller measure in the AlAT and AAT activities.

Thus the muscle tissue after reversal to fresh water from 100% sea water seemed to show quicker and more efficient regulatory mechanisms than the hepatopancreas with respect to free amino acids. This view is in close agreement with the regulation of water and ionic content of the muscle discussed elesewhere. It is interesting to note that the 'overshoot' response in the enzyme activities per unit chloride content observed at the tissue level was also reflected in the oxygen consumption of the whole animals during the reverse acclimation to fresh water from 100% sea water (figure 5). In the crabs reversed to fresh water from 50% sea water no such 'overshoot' effects were recorded both in the enzyme activities of tissues (excepting the AlAT activity of muscle) and oxygen consumption of the whole animals. Such differences between 100% and 50% sea water acclimated crabs may be attributed to differences in the amplitude of change in the medium, the amplitude of change being greater from 100% sea water rather than from 50% sea water to fresh water. Thus the present study indicates that the chloride sensitivity of the activity levels of GDH and aminotransferases is dependent on the time scale of exposure of the animal to the ambient medium, tissue specificity and the direction and amplitude of salinity change.

Time course of changes in the levels of Aspartate aminotransferase (AAT) activity in the tissues of the fresh water field crab Oziotelphusa acclimated to 50% S.W. and then returned to F.W. Values are expressed as μ moles of pyruvate/gm protein/hour. Mean values are for 6 individuals.

Tissue	Control (F.W.)	50% S.W.						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
H.P.													
Mean	96.11	151.40	149.21	112.17	91.93	84.81	73.38	69.93	61.31	64.30	74.24	89.96	92.00
S.D. ±	8.75	9.69	5.48	9.80	8.74	7.40	4.42	6.39	9.29	7.79	7.86	10.22	5.72
% d		+57.52	+55.24	+16.71	-4.35	-11.75	-23.64	-27.23	-36.21	-33.27	-22.75	-6.39	-4.27
Muscle													
Mean	121.02	203.33	208.53	150.17	142.20	138.17	125.03	111.98	92.75	77.30	100.84	116.88	127.05
S.D. ±	8.70	4.22	8.45	12.14	10.56	11.63	7.04	8.99	8.34	9.71	11.37	8.36	6.74
% d		+68.01	+72.31	+24.08	+17.50	+14.17	+3.31	-7.46	-23.35	-36.12	-16.67	-3.42	+4.98

H.P. : Hepatopancreas

Table 23-1

Variance ratios for the data given in table 23.

Tissue and Treatment	Variance ratios (F)		Critical difference		
	F. Cal	F. tab at 5% level of significance	at 5% level	at 1% level	at 0.1% level
H.P.:					
50% S.W.	89.76	2.38	10.99	15.60	22.76
Reverse	17.40	2.26	9.77	13.57	19.28
Muscle:					
50% S.W.	192.70	2.38	12.90	18.32	26.72
Reverse	23.94	2.26	11.09	15.40	21.88

NOTE: H.P. - Hepatopancreas

Table 23-2

Statistical comparision of data given in table 23. Mean values are arranged in an ascending order.

Tissue	Treatment							
H.P.				50% S.W.				
	360 h	168 h	72 h	Control	24 h	5 h	1 h	
	73.38	84.81	91.93	96.11	112.17	149.21	151.40	
				Reversal to F.W.				
	5 h	24 h	1 h	360 h S.E.	72 h	168 h	360 h	CONTROL
	61.31	64.13	69.93	73.38	74.24	89.96	92.00	96.11
Muscle				50% S.W.				
	Control	360 h	168 h	72 h	24 h	1 h	5 h	
	121.02	125.03	138.17	142.20	150.17	203.33	208.53	
				Reversal to F.W.				
	24 h	5 h	72 h	1 h	168 h	Control	360 h S.E.	360 h
	77.30	92.75	100.84	111.98	116.88	121.02	125.03	127.05

N.B.:- Values grouped together by a common underline do not differ significantly amongest themselves ($P > 0.05$)

H.P.: Hepatopancreas.
S.E.: Salinity Exposed.

Table 24

Time course of changes in the levels of Aspartate aminotransferase (AAT) activity in the tissues of the fresh water crab Oziotelphusa exposed to 100% S.W. and then returned to F.W. Values are expressed as μ moles of pyruvate/ gm protein/ hour. Mean values are for 6 animals.

Tissues	Control (F.W.)	100% S.W.						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
H.P.													
Mean	96.11	180.40	186.90	175.04	155.18	148.68	140.48	139.00	138.19	130.16	107.34	107.83	90.93
S.D. ±	8.75	10.88	10.98	8.72	8.17	11.56	9.48	9.57	8.25	6.50	5.55	5.06	8.64
% d		+87.70	+94.46	+82.12	+61.46	+54.69	+46.16	+44.62	+43.78	+35.42	+11.68	+12.19	-5.39
Muscle													
Mean	121.02	238.30	240.72	220.00	202.94	149.28	155.42	152.72	139.33	136.54	127.88	127.38	124.40
S.D.±	8.70	11.78	11.24	6.63	3.98	11.17	6.22	4.49	6.16	4.87	4.72	6.70	6.55
% d		+96.91	+98.90	+81.78	+67.69	+23.35	+28.42	+26.19	+15.13	+12.82	+ 5.66	+5.25	+2.79

H.P. : Hepatopancreas

Table 24-1

Variance ratios for the data given in table 24.

Tissue and Treatment	Variance ratios (F)		Critical difference		
	F. Cal	F. tab at 5% level of significance	at 5% level	at 1% level	at 0.1% level
H.P:					
100% S.W.	59.79	2.38	13.57	19.27	28.10
Reverse	35.63	2.26	9.94	13.81	19.61
Muscle:					
100% S.W.	166.23	2.38	12.42	17.63	25.72
Reverse	27.63	2.26	7.88	10.94	15.55

Table 24-2

Statistical comparison of the data presented in table 24. Mean values are arranged in an ascending order.

Tissue	Treatment							
H.P. :				100% S.W.				
	Control	360 h	168 h	72 h	24 h	1 h	5 h	
	96.11	140.48	148.68	155.18	175.04	180.40	186.90	
				Reversal to F.W.				
	360 h	Control	72 h	168 h	24 h	5 h	1 h	360 h S.E.
	90.93	96.11	107.34	107.83	130.16	138.19	139.00	140.48
Muscle				100% S.W.				
	Control	168 h	360 h	72 h	24 h	1 h	5 h	
	121.02	149.28	155.42	202.94	220.00	238.30	240.72	
				Reversal to F.W.				
	Control	360 h	168 h	72 h	24 h	5 h	1 h	360 h S.E.
	121.02	124.4	127.38	127.88	136.54	139.33	152.72	155.42

N.B.:- Values grouped together by a common underline are not significantly different from each other ($P > 0.05$)
H.P.: Hepatopancreas.
S.E.: Salinity Exposed.

Table 25

Time course of changes in the levels of alanine aminotransferase (AlAT) activity in the tissues of the fresh water field crab Oziotelphusa acclimated to 50% S.W. and then returned to F.W. values are expressed as μ moles of pyruvate/gm protein/hour. Mean values are for 6 animals.

Tissue	Control (F.W.)	50% S.W.						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
H.P.													
Mean	195.08	215.57	216.94	214.12	194.17	173.19	176.35	171.84	178.95	193.47	193.92	192.58	191.38
S.D. ±	8.33	11.62	3.38	10.64	8.42	9.77	7.38	8.72	9.01	8.10	12.60	12.32	10.66
% d		+10.50	+11.20	+9.76	-0.42	-11.22	-9.60	-11.91	-8.26	-0.82	-0.59	-1.95	-1.89
Muscle													
Mean	251.92	341.47	333.49	318.02	282.07	283.37	255.78	259.58	265.52	271.44	266.44	271.79	257.58
S.D. ±	9.94	9.87	9.19	8.18	5.92	11.43	11.68	10.72	10.03	12.04	11.06	11.28	10.55
% d		+35.54	+32.37	+26.23	+11.96	+12.48	+1.53	+3.04	+5.39	+7.74	+5.76	+7.88	+2.24

H.P. : Hepatopancreas

Table 25-1

Variance ratios for the data shown in table 25.

Tissue & Treatment	Variance ratios (F)		Critical difference		
	F. Cal	F. tab at 5% level of significance	at 5% level	at 1% level	at 0.1% level
H.P. :					
50% S.W.	26.38	2.38	12.18	17.29	25.23
Reverse	5.76	2.26	12.34	17.14	24.35
Muscle:					
50% S.W.	84.97	2.38	13.94	19.79	28.87
Reverse	3.05	2.26	13.74	19.09	27.11

Table 25-2

Statistical comparison of the data presented in table 25. Mean values are arranged in an ascending order.

Tissue	Treatment							
H.P.	50% S.W.							
	168 h	360 h	72 h	Control	24 h	1 h	5 h	
	173.19	176.35	194.17	195.08	214.14	215.57	216.94	
	Reversal to F.W.							
	1 h	360 h S.E.	5 h	360 h	168 h	24 h	72 h	Control
	171.84	176.35	178.95	191.38	192.58	193.47	193.92	195.08
Muscle	50% S.W.							
	Control	360 h	72 h	168 h	24 h	5 h	1 h	
	251.92	255.78	282.07	283.37	318.02	333.49	341.47	
	Reversal to F.W.							
	Control	360 h S.E.	360 h	1 h	5h	72 h	24 h	168 h
	251.92	255.78	257.58	259.58	265.52	266.44	271.44	271.99

N.B.:- Values grouped together by a common underline are not significantly different amongst themselves ($P > 0.05$).

H.P.:- Hepatopancreas; S.E.:- Salinity Exposed.

Table 26

Time course of changes in the levels of alanine aminotransferase (AlAT) activity in the tissues of the fresh water field crab Oziotelphusa exposed to 100% S.W. and then returned to F.W. values are expressed as μ moles of pyruvate/gm protein/ hour. Mean values are for 6 animals.

Tissue	Control (F.W.)	100% S.W.						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
H.P.													
Mean	195.08	230.02	241.08	249.14	258.35	295.55	310.97	309.58	303.95	301.18	242.45	204.35	194.63
S.D. ±	8.33	12.11	14.47	12.86	9.88	10.92	8.89	10.23	6.76	10.03	13.11	10.37	10.17
% d		+17.91	+23.58	+27.72	+32.43	+51.50	+59.40	+58.69	+55.80	+54.38	+24.28	+4.75	-0.23
Muscle													
Mean	251.92	281.99	295.60	305.75	344.76	308.36	309.95	307.10	306.72	308.60	291.76	250.77	252.08
S.D. ±	9.94	12.28	6.38	9.30	10.70	12.18	10.18	11.19	12.29	13.12	10.24	10.23	9.83
% d		+11.93	+17.33	+21.36	+36.85	+22.40	+23.05	+21.90	+21.75	+22.49	+15.81	-0.45	+0.06

H.P. : Hepatopancreas

Table 26-1

Variance ratios for the data presented in table 26.

Tissue & Treatment	Variance ratios (F)		Critical difference		
	F. Cal	F. tab at 5% level of significance	at 5% level	at 1% level	at 0.1% level
H.P. :					
100% S.W.	73.57	2.38	15.47	21.97	32.04
Reverse	180.02	2.26	12.44	17.28	24.54
Muscle:					
100% S.W.	194.60	2.38	14.16	20.11	29.33
Reverse	320.27	2.26	13.76	19.11	27.15

Table 26-2

Statistical comparison of the data shown in table 26. Mean values are arranged in an ascending order.

Tissue	Treatment						
H.P.	100% S.W.						
	Control	1 h	5 h	24 h	72 h	168 h	360 h
	195.08	230.02	241.08	249.14	258.35	295.55	310.97
	Reversal to F.W.						
	360 h	Control	168 h	72 h	24 h	5 h	1 h	360 h S.E.
	194.63	195.08	204.35	242.45	301.18	303.95	309.58	310.97
Muscle	100% S.W.						
	Control	1 h	5 h	24 h	168 h	360 h	72 h	
	251.92	281.99	295.60	305.75	308.36	309.95	344.76	
	Reversal to F.W.							
	168 h	Control	360 h	72 h	5 h	1 h	24 h	360 h S.E.
	250.77	251.92	252.08	291.76	306.72	307.10	308.60	309.95

N.B.:- Values grouped together by a common underline are not significantly different amongst themselves ($P > 0.05$).

H.P.:- Hepatopancreas; S.E.:- Salinity Exposed.

Table 27

Time course of changes in the levels of glutamate dehydrogenase (GDH) activity in the tissues of fresh water field crab Oziotelphusa when acclimated to 50% S.W. and then returned to F.W. values are expressed as μ moles of formazan/gm protein/hour. Mean values are for 6 individuals.

Tissue	Control (F.W.)	50% S.W.						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
H.P.													
Mean	16.63	13.98	14.69	10.40	6.21	12.70	13.25	8.22	7.53	9.90	11.22	13.75	15.30
S.D. ±	0.86	1.38	1.59	1.52	1.05	1.37	1.51	1.21	1.43	1.83	0.78	1.71	1.30
% d		-15.93	-11.66	-37.46	-62.65	-23.63	-20.32	-50.57	-54.72	-40.46	-32.53	-17.31	-7.99
Muscle													
Mean	27.33	34.33	33.16	35.25	34.99	29.53	29.01	25.66	21.34	20.61	24.33	24.45	26.21
S.D. ±	2.26	2.08	4.40	5.44	5.51	3.72	3.34	2.62	2.29	2.17	3.32	4.13	3.52
% d		+25.61	+21.33	+28.97	+28.02	+8.04	+6.14	-6.11	-21.91	-24.58	-10.97	-10.53	-4.09

H.P. : Hepatopancreas

Table 27-1

Variance ratios for the data given in table 27

Tissue and Treatment	Variance ratios (F)		Critical difference		
	F. Cal	F. tab at 5% level of significance	at 5% level	at 1% level	at 0.1% level
H.P. :					
50% S.W.	41.90	2.38	1.83	2.59	3.79
Reverse	37.74	2.26	1.73	2.40	3.41
Muscle:					
50% S.W.	4.41	2.38	5.54	7.86	11.47
Reverse	5.17	2.26	3.81	5.29	7.51

Table 27-2

Statistical comparison of the data presented in table 27. Mean values are arranged in an ascending order.

Tissue	Treatment						
H.P.	50% S.W.						
	72 h	24 h	168 h	360 h	1 h	5 h	Control
	6.21	10.40	12.70	13.25	13.98	14.69	16.63
	Reversal to F.W.						
	5 h	1 h	24 h	72 h	360 h S.E.	168 h	360 h	Control
	7.53	8.22	9.90	11.22	13.25	13.75	15.30	16.63
Muscle	50% S.W.						
	Control	360 h	168 h	5 h	1 h	72 h	24 h	
	27.33	29.01	29.53	33.16	34.33	34.99	35.25	
	Reversal to F.W.							
	24 h	5 h	72 h	168 h	1 h	360 h	Control	360 h S.E.
	20.61	21.34	24.33	24.45	25.66	26.21	27.33	29.01

N.B.:- Values grouped together by a common underline are not significantly different from each other ($P > 0.05$).

Table 28

Time course of changes in the levels of glutamate dehydrogenase (GDH) activity in the tissues of the fresh water field crab Oziotelphusa during acclimation to 100% S.W. and in the reverse acclimation to F.W. values are expressed as μ moles of formazan/gm protein/hour. Mean values are for 6 individuals.

Tissue	Control (F.W.)	100% S.W.						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
H.P.													
Mean	16.63	25.75	26.61	27.27	12.09	9.79	10.12	12.57	14.11	14.75	15.09	15.28	15.68
S.D. ±	0.86	3.32	2.92	3.57	1.93	1.32	2.52	2.39	1.36	1.85	1.57	1.61	2.92
% d		+54.84	+60.01	+63.98	-27.30	-58.86	-39.14	-24.41	-15.15	-11.30	-9.26	-8.11	-5.71
Muscle													
Mean	27.33	51.72	55.61	45.48	30.99	25.35	24.51	26.77	23.44	29.23	29.53	30.13	29.87
S.D. ±	2.26	5.94	5.75	5.86	2.69	2.63	5.49	2.16	3.31	4.23	3.88	3.32	2.26
% d		+89.24	+103.47	+66.41	+13.39	-7.24	-10.32	-2.05	-14.23	+6.95	+8.05	+10.24	+9.29

H.P. : Hepatopancreas

Table 28-1

Variance ratios for the data given in table 28.

Tissue and Treatment	Variance ratios (F)		Critical difference		
	F. Cal	F. tab at 5% level of significance	at 5% level	at 1% level	at 0.1% level
Hepato-pancreas					
100% S.W.	22.47	2.38	3.83	5.44	7.93
Reversal F.W.	2.65	2.26	2.50	3.49	4.95
Muscle					
100% S.W.	26.91	2.38	6.41	9.10	13.27
Reversal F.W.	2.01	2.26	4.45	6.18	8.78

Table 28-2

Comparison of the data presented in table 28.

Tissue	Treatment							
H.P.	100% S.W.							
	168 h	360 h	72 h	Control	1 h	5 h	24h	
	9.79	10.12	12.09	16.63	25.75	26.61	27.27	
	Reversal to FW.							
	360 hS.S.	1 h	5 h	24 h	72 h	168 h	360 h	Control
	10.12	12.57	14.11	14.75	15.09	15.28	15.68	16.63
Muscle	100% SW.							
	360 h	168 h	Control	72 h	24 h	1 h	5 h	
	24.51	25.35	27.33	30.99	45.48	51.72	55.61	
	Reversal to FW.							
	5 h	360h SS	1 h	Control	24 h	72 h	360 h	168 h
	23.44	24.51	26.77	27.33	29.23	29.53	29.87	30.13

N.B.:- Values grouped together by a common underline are not significantly different from each other ($P > 0.05$).

Table 29

The ratio of glutamate dehydrogenase to total aminotransferases (TAT) in the tissues of the Oziotelphusa during acclimation to S.W. and reverse acclimation to F.W.(GDH % TAT)

Tissue	Control	Salinity Stress						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
						50% S.W.							
H.P.	5.71	3.8	4.01	3.18	2.13	4.92	5.30	3.39	3.13	3.84	4.18	4.86	5.40
% d		-33.45	-29.77	-44.31	-62.69	-13.83	-7.18	-40.63	-45.18	-32.75	-26.79	-14.88	-5.42
Muscle	7.32	6.30	6.12	7.50	8.26	7.04	7.60	6.90	6.00	5.90	6.62	6.29	6.81
% d		-13.93	-16.39	+2.46	+12.84	-3.82	+3.82	-5.73	-18.03	-19.39	-9.56	-14.07	-6.96
						100% S.W.							
H.P.	5.71	6.20	6.21	6.42	2.92	2.20	2.23	2.80	3.19	3.41	4.31	4.89	5.57
% d		+8.58	+8.75	+12.43	-48.86	-61.47	-60.94	-50.96	-44.13	-40.28	-24.52	-14.36	-2.45
Muscle	7.32	9.90	10.30	8.65	5.60	5.53	5.26	5.82	6.37	6.56	7.03	7.96	7.93
% d		+35.24	+40.71	+18.17	-23.49	-24.45	-28.14	-20.49	-12.98	-10.38	-3.96	+8.74	+8.33

N.B. :- Values for calculation were taken from tables 23 to 28.
% d : Percent deviation from the control.

Table 30

Time course of GDH/FAA, ratios in the tissues of the crab Oziotelphusa when transferred to 50% and 100% S.W. and in reverse acclimation. % d = % deviation from control.

Tissue & Treatment	Control	Salinity Stress						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
						50% S.W.							
H.P.	.0769	.0570	.0512	.0223	.0115	.0254	.0293	.0276	.0256	.0346	.0483	.0589	.0694
% d		-5.88	-33.42	-71.00	-85.04	-66.97	-61.90	-64.11	-66.71	-55.01	-37.19	-23.41	-9.75
Muscle	.0838	.0946	.0923	.0707	.0670	.0573	.0617	.0617	.0546	.0593	.0742	.0729	.0776
% d		+12.88	+10.14	-15.63	-20.05	-31.62	-26.37	-26.37	-34.84	-29.24	-11.45	-13.01	-7.40
						100% S.W.							
H.P.	.0769	.0754	.0559	.0552	.0180	.0177	.0212	.0382	.0427	.0476	.0496	.0620	.0689
% d		-1.95	-27.31	-32.12	-76.59	-76.98	-72.43	-50.32	-44.47	-38.10	-35.50	-19.37	-10.40
Muscle	.0838	.1212	.1217	.0808	.0483	.0414	.0438	.0634	.0660	.0938	.0977	.0986	.0924
% d		+44.63	+45.23	-3.58	-42.36	-50.59	-47.73	-20.76	-21.24	+11.93	+16.58	+17.66	+10.26

N.B.:- Values for calculation are taken from tables 19, 27 & 28.

Table 31

Time course of AAT/FAA ratios in the tissues of the crab Oziotelphusa when transferred to 50% and 100% S.W. and in reverse acclimation. % d = Percent deviation from the control.

Tissue	Control	Salinity stress						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
					50% S.W.								
H.P.	.4445	.6176	.5208	.2401	.1707	.1697	.1625	.2352	.2087	.2246	.3200	.3857	.4175
% d		+38.94	+17.16	-45.98	-61.59	-61.82	-63.44	-47.08	-53.05	-49.47	-28.01	-13.22	-6.07
Muscle	.3713	.5606	.5611	.3013	.2724	.2685	.2660	.2695	.2376	.2225	.3076	.3485	.3764
% d		+50.98	+51.12	-18.85	-26.63	-27.68	-28.36	-27.42	-36.01	-40.07	-17.15	-6.14	+1.37
					100% S.W.								
H.P.	.4445	.5284	.3932	.3350	.2310	.2694	.2952	.4225	.4182	.4325	.3679	.4437	.3992
% d		+18.87	-11.54	-24.63	-48.03	-39.39	-33.59	-4.95	-5.91	-2.69	-17.23	-0.18	-10.19
Muscle	.3713	.5587	.5269	.3911	.3166	.2442	.2780	.3792	.3925	.4284	.4224	.4171	.3851
% d		+50.47	+41.91	+5.33	-14.73	-34.23	-25.12	+2.13	+5.71	+18.07	+13.76	+12.33	+3.71

N.B.:- Values for calculation are obtained from tables 19, 23 & 24.

Table 32

Time course of AlAT/FAA ratios in the tissues of the crab Oziotelphusa when acclimated to 50% and 100% S.W. and in reverse acclimation. % d = Percent deviation from the control.

Tissue	Control	Salinity Stress						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
						50% S.W							
H.P.	.9023	.8794	.4431	.4584	.3605	.3467	.3906	.5781	.6091	.6778	.8353	.8258	.8686
% d		-2.54	-50.89	-49.19	-60.04	-61.57	-56.71	-35.93	-32.49	-24.86	-7.425	-8.47	-3.73
Muscle	.7731	.9415	.8973	.6381	.5404	.5507	.5442	.6248	.6804	.7816	.8128	.8103	.7632
% d		+21.78	+16.06	-17.46	-30.09	-28.76	-29.60	-19.18	-11.99	+1.09	+5.13	+4.81	-1.28
						100% S.W.							
H.P.	.9023	.6738	.5072	.4769	.3846	.5351	.6536	.9412	.9199	1.001	.8309	.8409	.8554
% d		-25.32	-43.79	-47.14	-57.37	-40.69	-27.56	+4.31	+1.95	+10.94	-7.91	-6.80	-5.19
Muscle	.7731	.6611	.6471	.5336	.5380	.5045	.5546	.7626	.8641	.9909	.9638	.8212	.7803
% d		-14.48	-16.29	-29.68	-30.41	-34.74	-28.26	-1.36	+11.77	+28.17	+24.66	+6.22	+0.93

N.B.:- Values for calculation are obtained from tables 19, 25 & 26.

Table 33

Time course of GDH/Cl^- ratios in the tissues of the crab Oziotelphusa during acclimation to 50% and 100% sea water and in reverse acclimation to fresh water. Ratios are expressed as GDH % Cl^-.

Tissue	Control (F.W.)	Salinity Stress						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
						50% Sea Water							
H.P.	15.16	13.83	14.58	8.55	4.50	9.02	9.08	5.68	5.04	7.26	8.85	12.85	13.41
Muscle	15.34	18.83	18.75	17.65	16.46	15.87	13.56	11.80	9.98	9.96	11.73	15.34	16.20
						100% Sea Water							
H.P.	15.16	17.52	13.42	11.67	3.87	2.96	2.96	6.38	7.90	8.97	10.30	13.83	14.48
Muscle	15.34	28.71	28.06	21.64	14.29	10.49	8.21	12.31	10.97	14.13	14.23	18.90	18.42

N.B.: Values for calculation were obtained from tables 15, 27 & 28.

Table 34

Time course of AlAT/Cl^- ratios in the tissues of the crab Oziotelphusa during acclimation to 50% and 100% sea water and in the reverse acclimation to fresh water. Ratios are expressed as AlAT % Cl^-.

Tissue	Control (F.W.)	Salinity Stress						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
					50% Sea Water								
H.P.	177.84	213.30	215.36	176.18	128.29	122.97	120.94	118.85	119.82	141.50	145.02	180.03	167.77
Muscle	141.43	187.36	188.57	159.52	132.71	137.52	119.63	119.45	124.30	131.23	128.46	170.56	158.91
					100% Sea Water								
H.P.	177.84	156.50	121.60	106.63	82.90	89.46	91.13	157.25	170.30	188.84	174.02	187.52	176.06
Muscle	141.43	156.57	149.14	145.49	158.99	127.71	103.85	141.32	143.55	149.30	140.67	157.37	155.51

N.B.: Values for calculation were obtained from tables 15, 25 & 26.

Table 35

Time course of AAT/Cl^- ratios in the tissues of the crab Oziotelphusa during acclimation to 50% and 100% sea water and in the reverse acclimation to fresh water. Ratios are expressed as AlAT% Cl^-.

Tissue	Control (F.W.)	Salinity Stress						Reversal to F.W.					
		1 h	5 h	24 h	72 h	168 h	360 h	1 h	5 h	24 h	72 h	168 h	360 h
						50% Sea Water							
H.P.	87.61	149.81	148.12	92.29	60.74	62.22	50.32	48.36	41.06	46.90	58.53	84.09	80.65
Muscle	67.94	111.56	117.91	75.32	66.90	67.05	58.47	51.53	43.41	37.37	48.62	73.34	78.38
						100% Sea Water							
H.P.	87.61	122.74	94.27	74.91	49.83	45.00	41 .16	70.60	77.43	81.39	77.04	98.95	83.89
Muscle	67.94	132.31	121.45	104.69	93.58	61.82	52.07	70.28	65.21	66.01	61.65	79.93	76.74

N.B. : Values for calculation were obtained from tables 15, 23 & 24.

GENERAL CONCLUSIONS

Problems of metabolic response to salinity stress and consequent regulation of inorganic and organic osmotic effectors and enzyme modulations have not been investigated on any specific time-scale in the enryhaline fresh water field crab OZIOTELPHUSA (PARATELPHUSA) SENEX SENEX (FABRICIUS). The present work was aimed in this direction.

A new technique has been employed for the determination of the oxygen consumption viz., "the continuous flow method", which enables us to draw water samples at different intervals without dismantling the set up of or disturbing the animal in the respiration chambers. This method has several advantages over the method described by Saroja (1959) especially for studying the time-course of oxygen consumption at several intervals soon after exposing the animal to new media or temperatures. Using this method the following aspects have been studied:

(1) Oxygen consumption of the crab in relation to sex and size.

(2) Oxygen consumption of the crab in relation to diurnal rhythms in winter and summer.

(3) Time course of oxygen consumption of the crab in response to salinity stress viz., 50% & 100% sea water for a period of 15 days, with intervals of 1, 2, 3, 5, 8, 12 & 24 hours during the first day and 24 hour intervals thereafter.

(4) Time course of oxygen consumption of the crabs during their reverse acclimation from 50% sea water and 100% sea water to fresh water for a period of 7 days, keeping the time course as for the forward acclimation.

Studies on oxygen consumption in relation to sex and size and also diurnal rhythms in winter and summer were undertaken as a preliminary step for further studies. Results of such studies fit well into the general patterns of metabolism already reported (Orton, 1936; Prosser et al, 1950; Zeuthen, 1953; Bertalanffy, 1953; 1957; Ramamurthi and Sainath, Janak, 1973). The unit oxygen consumption in the crabs was found to be greater in males than in females and it was greater in smaller crabs than in larger ones. The 'b' values were close to 2/3 rule indicating relationship between surface area and metabolic rate. In view of differences in the metabolic activity between the sexes, equal number of males and females were chosen for each experiment. In order to avoid influence of size on metabolism, crabs generally ranging from 20 to 40 gms were used avoiding crabs of extreme sizes. Diurnal rhythms of oxygen consumption were studied in winter and summer in the two sexes separately. In the summer, (May, June & July) both sexes showed two peaks, viz., one at 10 A.M and the other at 8 P.M. and these maximal values were significantly

different from most of the other values. But in the winter months (December, January and February) the picture was different. The males showed peak activity at 2 P.M. and at 12 midnight and the females at 2 A.M. only. However, these maximal values of oxygen consumption in both males and females were not significantly different from the other values. Therefore, the time of sampling during any part of the day in winter may not have any significant influence on the metabolism of this crab. Hence, most of the work was carried out during winter months.

Time course of oxygen consumption in response to salinity stress.

Using the new method, viz., 'continuous flow method', the oxygen consumption of the fresh water crabs upon direct transfer to 50% and 100% sea water was studied for a period of 15 days at various intervals indicated earlier. After their sojourn in these two media for 15 days, the crabs were directly brought back to fresh water (their natural medium) and their oxygen consumption was studied at similar intervals for 7 days or until a steady state was achieved.

A well recognised pattern of matabolism in most enryhaline crustaceans is decreased levels of oxygen consumption in higher salinities and increased levels in lower

salinities. However, there are several conflicting reports in this matter (Gilles, 1973b; 1974a). In the case of Eriocheir sinensis (Schwabe, 1933), Artemia salina (Gilchrist, 1956) and Palaemonetes varians (Mc Farland and Pickens, 1965) the oxygen consumption was reported to be unaffected when transferred to different salinities. However, Lee and Pyung (1970) showed higher oxygen consumption in lower salinities in Eriocheir sinensis and Eliassen (1953) showed lower oxygen consumption in higher salinities in Artemia salina. In the crab under investigation, Ramamurthi (1965) showed minimal oxygen consumption in 50% sea water and higher oxygen consumption in salinity concentrations on either side of 50% sea water, immediately after exposing the crabs to new media. Venkata Reddy (1976), however, showed a decreasing trend in oxygen consumption in increasing salinities in the same crabs after 15 days of acclimation. In the absence of time-course studies it is rather difficult to resolve such conflicting reports. Hence, the present study was directed towards the understanding of the temporal sequence of metabolic response to salinity stress in the same crab Oziotelphusa (Paratelphusa) senex senex. The results obtained in the present work seem to be meaningful in this connection.

The oxygen consumption of the crab was found to be lower in 50% sea water than in fresh water or 100% sea water in the early stages of acclimation. This agrees well with the findings of Ramamurthi (1965). But, in 100% sea water, an initial shoot up of oxygen consumption was observed which was far above that found in fresh water and this significantly higher level was maintained upto 48 hours with a later decline compared to the normal level. Three probable interpretations were suggested for the initial "Over short" in oxygen consumption in 100% sea water. This might primarily be due to the osmotic gradient between the blood and external medium and consequent utilization of metabolic energy for osmotic work. (Schwabe, 1933; Lofts, 1956; Pampapathi Rao, 1958). It may also be attributed to the chloride gradient (Flemister and Flemister, 1951; Pampapathi Rao and Madanmohan Rao, 1963; Ramamurthi, 1965). Finally, the initial increase in oxygen consumption reaching a peak value at the 8th hour may be suggestive of "escape reactions" (Krogh, 1939) and consequent increase in the locomotor activity (Gross, 1957).

The significant depression of oxygen consumption immediately after transfer from fresh water to 50% sea water may clearly be attributed to a drastic reduction in osmotic gradient as well as in chloride gradient between the blood and external medium, since 50% sea water is nearly isosmotic to the internal medium (Ramamurthi, 1965).

A similar situation seems to be achieved in 100% sea water only slowly. Upto 11 days of exposure to 100% sea water, the oxygen consumption of the crab remained higher than that in 50% sea water but lower than that in fresh water, excepting in the early stages i.e., upto 48 hours, when the metabolic rate was higher than that in fresh water. Beyond 11 days, the oxygen consumption in 100% sea water was lower than that in 50% sea water and this is in agreement with the trends indicated (Venkata Reddy, 1976). Thus, the duration of exposure to sea water was found to be important and as such it may be said that the metabolic response to salinity stress is also a function of time besides many other factors.

The reasons for the shoot up in oxygen consumption of crabs in the early stages of exposure to 100% sea water have been explained. The decreased trend in oxygen consumption in 100% sea water from 72 hours onwards may be explained on the basis of decreasing osmotic gradients between blood and ambient medium on one hand and between the blood and intracellular fluid on the other. Such osmotic adjustments have probably been achieved more quickly in 50% sea water and rather slowly in 100% sea water. This perhaps explains the reason why in 100% sea water the oxygen consumption is higher than in 50% sea water upto 11 days. Beyond 11 days,

the oxygen consumption in 100% sea water fell below that in 50% sea water, indicating perhaps the attainment of isosmotic conditions, whereas in 50% sea water the crabs seemed to have achieved some amount of hyper-osmotic regulation during the later stages of acclimation. This also explains why the crabs showed steady state in oxygen consumption from the 10th day of exposure to 50% sea water whereas no such steady state seemed to have been achieved in 100% sea water till the end of exposure period. The decreasing trend in oxygen consumption in 100% sea water, far below that in 50% sea water, after 11 days may also be due to excessive penetration of chloride ions into the hepatopancreas and consequent 'ionic injury' to the tissue leading to its atrophy (Venkata Reddy, 1976).

In general it can be said that the crabs under study showed higher levels of oxygen consumption in fresh water its natural habitat, and lowered levels in higher salinities. This generalisation is in perfect agreement with earlier reports (Gilles, 1973b, 1974a, 1979).

The increased levels of oxygen consumption in fresh water may primarily be due to the increased metabolic energy required for 'osmotic work' in view of the retention of a large osmotic gradient (Schwabe, 1933; Lofts, 1956; Pampapathi Rao, 1958) and particularly of a large chloride

gradient between the blood and ambient medium (Flemister and Flemister, 1951; Pampapathi Rao and Madanmohan Rao, 1963; Ramamurthi, 1965) and consequent increased catabolism of amino acids (Schoffeniels and Gilles, 1970; Siebers and coworkers, 1972; Gilles, 1973b, 1974a, 1979). The decreased levels of oxygen consumption in 50% and 100% sea water after prolonged exposure may similarly be attributed to the decreased metabolic energy required for osmotic work as against decreased osmotic and chloride gradients between the blood and medium and consequent decreased catabolism of amino acids which are the major intracellular organic osmotic effectors. When the amplitude of salinity change is very great as between fresh water and 100% sea water two other important factors are superimposed over those already mentioned viz., (1) "escape reactions" (Krogh, 1939) and consequent increase in locomotor activity (Gross, 1957) immediately after transfer to the new medium which explains the 'over shoot effect' (Grainger, 1958) in oxygen consumption upto 8 hours and (2) starvation effect resulting in decreased oxygen consumption tending toward anaerobic conditions in the later periods of exposure to 100% sea water (Venkata Reddy, 1976).

Reverse acclimation to fresh water from 50% and 100% sea water

Fresh water is the natural medium of the crabs under investigation. Transfer of 50% and 100% sea water acclimated crabs to FW resulted in increased oxygen consumption. In 50% sea water acclimated crabs, the oxygen consumption increased gradually to normal levels by about 72 hours and thereupon a steady state was maintained upto 7 days. In 100% sea water 'acclimated' crabs, the oxygen consumption shot up to more than normal by about 8 hours after transfer to fresh water and thereupon the values were not significantly different from the control value. There appears to be an 'over shoot effect' (Grainger, 1958) in oxygen consumption in 100% sea water acclimated crabs between 8 and 24 hours of transfer to fresh water which resembles the one observed when these animals were transferred from fresh water to 100% sea water. This overshoot in oxygen consumption may be due to the greater amplitude in salinity change from 100% sea water to fresh water and greater osmotic and ionic gradient between the blood and medium. The reasons for increased metabolism of crabs in fresh water have been explained. In fresh water medium the crabs are hyper-regulators. They have to employ compensatory mechanisms to retain their hyper-osmoticity in hypo-osmotic medium which means absorption of salts against the concentration gradients and this

is an active mechanism involving expenditure of energy. This explains the reason for the increased oxygen consumption of the crabs in the reverse acclimation to fresh water from sea water. Increased catabolism of amino acids is envisaged in organisms adapting to lower salinities, thereby decreasing the intracellular amino acid content inorder to achieve isosmoticity of the intracellular fluid with the extracellular fluid (Florkin, 1962). This increased catabolism of amino acids was perhaps reflected in the increased oxygen consumption of the crabs which were returned to fresh water from sea water.

It is interesting to note here that a steady state in oxygen consumption of the crabs was achieved rather quickly i.e. by 72 hours in the reverse acclimation to fresh water whereas a steady state was attained only after 10 days in 50% sea water and no such steady state was reached till the end of acclimation period in 100% sea water. This indicates that adaptation to diluted media is quicker than adaptation to concentrated media and this is in perfect agreement with earlier reports (review by Gilles, 1979, Schrock and Hanke, 1979). The quickness and ease with which these crustaceans adapt to lower salinities must have had some bearing in their evolutionary history. Perhaps, such a

mechanism must have enabled marine forms to colonise relatively quickly and easily brackish and estuarine waters and later fresh waters whereas the reverse invasion of seas by fresh water and brackish water froms was perhaps rare, difficult and time-consuming process with the exception of the homeosmotic ones.

Results obtained in this investigation on time course of changes in (1) water content of the tissues, (2) major inorganic ions viz., Na^+ and Cl^- (3) organic osmotic effectors viz., free aminoacids and (4) the key enzymes relating to amino acid metabolism fully substantiate the arguments so far put forth.

Time course of changes in water content of tissues

Upon transfer of whole animals from fresh water to 50% and 100% sea water, both the tissues studied, viz., the hepatopancreas and muscle showed dehydration. Water loss was found to be greater in 100% sea water than in 50% sea water in both the tissues. In both salinities, water loss was greater in hepatopancreas than in muscle. Maximum dehydration was recorded around 72 hours except in the hepatopancreas of 100% sea water acclimated crabs which continued to lose water upto 168 hours. In 50% sea water, the muscle showed full compensation for water loss by the

end of acclimation, whereas in the hepatopancreas compensation was only partial. In 100% sea water, both tissues failed to show compensation for water loss.

Although 50% sea water is nearly isosmotic to the body fluids of the crab (Ramamurthi, 1965), it seems to act as an hyperosmotic stress on the crabs whose natural habitat is fresh water. This may be the probable reason for water loss in tissues in the early stages of acclimation to 50% sea water. However, some homeostatic mechanisms seem to come into operation sooner or later and as a consequence, the muscle regained its water content fully and the hepatopancreas partially. The steady state in oxygen consumption observed after 9 days of exposure to 50% sea water is further proof of the operation of some such homeostatic mechanism. The crabs showed hyper-ionic regulation in later stages of acclimation to 50% sea water which implies the operation of compensatory mechanisms. In 100% sea water greater dehydration of the tissues was observed as the medium was highly hyper-osmotic and the osmotic gradient was too large. Diffusive loss of water from the body fluids and tissues could be envisaged in an external medium that is highly hyperionic to the body fluids. However, in the early stages, some homoeostatic mechanisms seem to operate to prevent this heavy leakage of water from and entry of ions into the body fluids and tissues. An "overshoot" in the uptake of

oxygen was noticed in the crabs upto 48 hours of transfer to 100% sea water and this is suggestive of such compensatory mechanisms coming into play in the early periods of acclimation to 100% sea water. However, these mechanisms seem to fail upon continued exposure to 100% sea water. The free influx of ions and efflux of water seems to be the consequence of failure of compensatory mechanisms and consequently the crabs tend to become osmoconformers in higher salinities and this is evidently an evasive mechanism which cuts down the energy requirements. This explains why the oxygen consumption of the crabs fell steeply after 48 hours of exposure to 100% sea water and no compensation for water loss was observed in the tissues.

In the reverse acclimation to fresh water, tissue hydration resumed its normal level relatively quickly i.e. immediately after transfer to fresh water in the muscle of 50% sea water acclimated crabs, within 24 hours in the muscle of 100% sea water acclimated crabs, before 72 hours and 168 hours in the hepatopancreas of 50% and 100% sea water acclimated crabs respectively. The oxygen consumption also increased to normal levels around 72 hours of transfer to fresh water from sea water. Thus, there appears to be some relationship between oxygen consumption and hydration of tissues. Remane and Schlieper (1958) have implicated

hydration of tissues as the causative factor for the elevated metabolism in animals transferred from a marine habitat to diluted media. Gilles (1973a) observed in the isolated axons of Eriocheir sinensis a quicker regulation of water content and consequent volume regulation in hypo-osmotic medium where as in hyper-osmotic conditions there was shrinkage of tissue and no regulation of water content during the time course of the experiment. The results obtained in the present investigation are comparable with such observations. Oxygen consumption, tissue hydration and consequent volume control appear to be related phenomena. Whereas oxygen consumption, and tissue hydration were found to be regulated rather quickly in the crabs returned to freshwater from sea water such regulation was found to be slow in 50% sea water and it was completely absent in 100% sea water. The absence of regulation of oxygen consumption and tissue hydration in 100% sea water was also reflected in the absence of locomotor activity and feeding behaviour of the crabs after about 3 days of exposure leading to lethargy and anorexia. The absence of escape reactions in the crabs exposed to 100% sea water may probably be attributed to the shrinkage of muscle and nerve stissues due to extreme dehydration as was reported earlier (Gilles, 1973a) in the isolated axons of Eriocheir sinensis subjected to hyper-osmotic shock.

Inorganic ions of blood

Changes in two inorganic ions viz: Na^+ and Cl^- were investigated as these are the two major inorganic ions common to most natural waters and body fluids of all animals. Upon transfer of fresh water crabs to 50% sea water, Na^+ level of blood showed a non-significant increase upto 72 hours and this level was nearly iso-ionic to the medium. Subsequently the blood Na^+ level increased significantly showing some amount of hyper-ionic regulation. In 100% sea water the blood Na^+ level increased rapidly but remained hypo-ionic to the external medium upto 72 hours, but later became iso-ionic and beyond 168 hours it was hyper-ionic to 100% sea water. In the reverse acclimation to fresh water from 50% and 100% sea water the Na^+ ion levels fell steeply by 72 hours.

The blood Cl^- level remained iso-ionic with 50% sea water throughout the acclimation period except for a slight increase around 360 hours. In 100% sea water the blood chloride level increased steeply reducing the blood-medium gradient to the minimum by about 360 hours. However, the blood chloride remained hypo-ionic to 100% sea water. On reversal to fresh water, a steep fall in blood chloride level was observed touching the normal levels by about 24 hours.

However, these normal levels of Na^+ and Cl^- were always hyper-ionic to fresh water.

Blood-medium ionic gradients and oxygen consumption

A close relationship was observed between blood-medium ionic (Na^+ and Cl^-) gradients and oxygen consumption. When the animals were transferred to 50% sea water, these gradients were reduced to the minimum and so also the oxygen consumption was lowered. When transferred to 100% sea water the gradients were very high to begin with and so also the oxygen consumption was elevated upto 48 hours. As the gradients were minimised gradually during the course of acclimation to 100% sea water, the uptake of oxygen also decreased correspondingly. Upon reversal to fresh water from 50% and 100% sea water, the blood-medium ionic gradients became higher once again and so also there was a steep elevation in metabolic rate. This correlation between ionic gradients and oxygen consumption was particularly striking in respect of Cl^-, suggesting that Cl^- played some role in the metabolic modulation of animals acclimating to different salinities. These observations are in perfect agreement with earlier reports which have indicated relationship between oxygen consumption and osmotic gradients, particularly Cl^- gradients (Schwabe, 1933; Flemister and Flemister, 1951; Lofts, 1956; Pampapathi Rao, 1958; Pampapathi Rao and Madanmohan Rao, 1963; Ramamurthi,

1965). Therefore, it can safely be presumed that wherever the osmotic gradients between body fluid and ambient medium are very high the energy needed for osmotic balance would also be correspondingly high and when the osmotic gradients become reduced the energy requirements would also be reduced. In the present work the oxygen consumption of the crabs was found to be higher in fresh water and lowered to a steady state in 50% sea water and further depressed to nearly anaerobic conditions at the end of exposure to 100% sea water. These lowered levels of oxygen consumption in higher salinities must have been due to significant reduction in osmotic gradients resulting in slight hyper-osmoticity in 50% sea water and more or less isosmoticity in 100% sea water at the end of acclimation period. Thus, the crabs exposed to higher salinities become osmo-conformers and depend mainly on evasive mechanisms of osmoregulation, whereas those exposed to lower salinities resort to hyper-regulation and seem to invoke mainly compensatory methods of osmoregulation. The osmoregulatory picture seen in this crab falls into type I of Lockwood's classification viz., hyper-osmoregulation of body fluids in lower salinities and iso-osmoticity in higher salinities.

Ionic composition of tissues

Intracellular Na^+ and Cl^- ion composition of the muscle and hepatopancreas was found to follow in general the trend

of changes in the blood during acclimation to 50% and 100% sea water and during the reverse acclimation to fresh water but the amplitude of change in tissues was too low compared to that of blood. The actual concentrations were several times lower in tissues than in blood. It looked as though there was no ionic parity between blood and tissues inspite of a tremendous increase in the concentration of Na^+ and Cl^- ions in hepatopancreas of especially 100% sea water acclimated crabs and a moderate increase of these ions in the muscle. Even in fresh water crabs large gradients exist in the ion concentrations between blood and tissues. But optimum cell function requires an iso-osmotic composition of tissue fluids with that of blood, and this has been called as the isosmotic regulation of the intracellular fluid (Florkin, 1962). Therefore, this ionic disparity between blood and tissues necessarily implies the existence of other important and major intracellular osmotic effectors. It is a known fact that relatively high K^+ concentration in tissues, especially muscle, plays a significant role in adding to the inorganic osmotic effector profile of the tissues. But, this aspect did not form part of this dissertation. However, it is a well known fact that the intracellular osmotic concentration is greatly augmented by organic osmotic effectors which are known to be mainly the free amino acids (Bricteux-Gregoire and coworkers, 1962; Florkin and coworkers, 1964;Schoffeniels and Gilles, 1970,

1972; Vincent marique and Gilles, 1970a, 1970b; Gerard and Gilles, 1972a, 1972b; Hoffmann and Hendil, 1976; Tan and Choong, 1981). The concentration differences between the blood and the tissues, with respect to Na^+ and Cl^- ions, observed in the present work, indicate that these ions participate only partially in the intracellular isosmotic regulation. The organic osmotic effectors, particularly, the free amino acids are the complementary isosmotic regulation components and their modulations during the acclimation of crabs will be discussed presently. Before proceeding to this aspect, mention of tissue specific differences in ion composition is needed. The hepatopancreas behaved differently from the muscle. When exposed to higher salinities, the hepatopancreas showed not only excessive dehydration but also excessive uptake of Na^+ and Cl^- ions, whereas the muscle showed moderate uptake of ions in both salinities. In 100% sea water the hepatopancreas was the most affected tissue with a heavy impregnation of inorganic ions, particularly chloride, which might have become injurious to this tissue. This might be the cause for the loss of appetite in crabs after 3 days of exposure to 100% sea water and consequent lethargy and also atrophy of this tissue reported by Venkata Reddy (1976). The muscle maintained a relatively low inorganic ion composition in

100% sea water and this conservative trend of the muscle might probably account for the survival of nearly 50% of the crabs in this medium. However, even this moderate increase in the inorganic ion composition of the muscle coupled with considerable dehydration, seems to have caused lethargy and failure of escape reactions which were evident in early stages of exposure to 100% sea water.

Organic osmotic effectors

That "the intracellular fluid is isosmotic or only slighty hyper-osmotic to the extracellular fluid" is well known (Conway and Mc Cormak, 1953; Appelboom and coworkers, 1958; Maffly and Leaf, 1959; Shoffeniels, 1967; Florkin and Schoffeniels, 1969). It was explained earlier that the intracellular fluid has however, a lower inorganic ion concentration than the extracellular fluid and that the cell's osmotic deficit is compensated by organic substances, particularly by the free amino acids. Extensive studies have been made in recent times on the role of free amino acids as the intracellular osmotic effectors (Robertson, 1965; Gilles and Schoffeniels, 1969; Lange, 1963; 1968; Schoffeniels and Gilles, 1970, 1972; Gerard and Gilles, 1972a,b; Gilles, 1972c, 1973a,b. 1974a, Venkatachari and Keshavan, 1973; Venkatachari, 1974; Gilles and Gerard, 1974; Gerard, 1975; Venkata Reddy, 1976; Tan and Choong, 1981). The investigations

made in this direction in the crab Oziotelphusa (Paratelphusa) senex senex are found to be in agreement with earlier findings. It was observed in the present work that the contribution of free amino acids to blood osmalarity was negligible (1.0% to 1.6%) whereas the same was very high in the cells of muscle and hepatopancreas (55% to 70%). It was also noted that the elevation or depression in the free amino acid levels of tissues was in line with the increase or decrease of inorganic ions. These inorganic ions, particularly Cl^-, seemed to trigger the process of mobilization of amino acids inside the cells as suggested by the previous investigators (Chaplin, Hyggins and Munday, 1965; Gilles, 1969, 1974c; Gilles, Hague and Kearney, 1971). It is known that oxygen consumption under salinity stress is some how influenced by the chloride gradient between the blood and external medium (Flemister and Flemister, 1951; Pampathi Rao and Madanmohan Rao, 1963; Ramamurthi, 1965). A striking correlation between oxygen consumption and chloride gradient was demonstrated in this dissertation (Chapter II). Penetration of more Cl^- into the cells in higher salinities has been shown to suppress the metabolic rate. This in turn might be correlated to decreased catabolism of intracellular amino acids with a consequent increase in the free amino acid content of the cells under

salinity stress. In the reverse acclimation to fresh water, the chloride ion content of cells fell steeply. It was shown that the oxygen consumption also increased steeply in the reverse acclimation to fresh water. This elevation in the metabolic rate in fresh water may be correlated to increased catabolism of amino acids resulting in a fall in the free amino acid content of cells in line with chloride. Such results are in accordance with the previous reports (Siebers and coworkers, 1972; Siebers, 1972; Chaplin, Huggins and Munday, 1970; Lee and Pyung, 1970; Gilles, 1974a, 1975). During acclimation to higher salinities, there was a decrease in the uptake of oxygen by the crabs and increase in the intracellular amino acid content and in the reverse acclimation to fresh water, the metabolic rate increased and the amino acid content of cells decreased. This implies a corresponding decrease in CO_2 and NH_3 production under hyper-osmotic stress conditions and increase in the production of CO_2 and NH_3 under hypo-osmotic stress conditions (Siebers, 1972; Chaplin, Huggins and Munday, 1970; Gerard and Gilles, 1972a; Mangum and coworkers, 1976; Vincent-Marique and Gilles, 1970a). The crab is known to be an osmo-conformer in higher salinities and hyper-osmotic regulator in lower salinities. As such during the hyper-osmotic regulation in lower salinities, the metabolic products of amino acids, viz., CO_2 and NH_3 which are known to increase are supposed

to act as counter ions for the active uptake of Na^+ and Cl^-. NH_4^+ or H^+ ions are supposed to act as counter ions for the active Na^+ uptake and HCO_3^- ion has been suggested as the counter ion for Cl^- uptake (Krogh, 1938, Shaw, 1960; Maetz, and Garcia Romeu, 1964; Kerstetter, Kirschner and Rafuse, 1970; Maetz, 1956, 1973).

Another important mechanism to account for the modulations in amino acid content of cells under conditions of osmotic stress is the influx of amino acids from the blood into the cells in higher salinities and the efflux of these amino acids from the tissues into the blood in lower salinities (Jeffries, 1966; Clark, 1968; Binns, 1969; Vincent-Marique and Gilles, 1970a,b; Gerard and Gilles, 1972a). In the present study the blood ninhydrin positive substances increased significantly in the early stages of acclimation of the crabs to 50% and 100% sea water with a later decline to normal levels in 50% sea water and to lower levels in 100% sea water. Such initial increase in free amino acid content could be due to "increased degradation" or "decreased synthesis of blood serum peptidic material" (Siebers and coworkers, 1972; Siebers, 1972; Gilles, 1977; Tan and Choong, 1981). The free amino acids thus available in the blood may be actively transported into the cells. The decrease in free amino acid content of blood seen in the later stages of acclimation to 50% sea water and 100% sea

water is suggestive of such influx of amino acids from the blood into the intracellular fluid. During the reverse acclimation to fresh water from 50% sea water and 100% sea water, a highly significant increase in blood amino acid levels was observed in the early stages with a later decline. This observation lends support to the view that during hypo-osmotic shocks the amino acids leakout of cells into the blood in the early stages and later get converted to blood serum peptides and proteins. It may further be argued that the amino acids leaking out of cells into the blood during hypo-osmotic stresses may be catabolized in the gills and the energy derived thus may be used up for the active transport of Na^+ and Cl^-.

Enzymes

The free amino acid content of blood and tissues is subjected to alterations in hyper- and hypo-osmotic stress conditions. Hence, the patterns of key enzymes of amino-acid metabolism viz: glutamate dehydrogenase (GDH) and aminotransferases (AAT and AlAT) have been studied during the acclimation of the crabs to 50% and 100% sea water and reverse acclimation to fresh water. GDH activity forms the marker of amino-acid oxidations in cells and it catalyzes the oxidative deamination of glutamic acid leading to the formation of an important

TCA cycle intermediary product, α - ketoglutaric acid. Aspartate aminotransferase (AAT) activity indicates the formation of another important TCA cycle intermediary product viz: oxaloacetic acid from aspartic acid and alanine amino-transferase (AlAT) activity marks the formation of pyruvate from alanine. Such conversion of amino acids to carbohydrates (gluconeogenesis) may be required under stress conditions when the animals normally suffer from hypo-glycemia.

The GDH enzyme activity in the muscle showed an initial elevation with a later reversal to control level in 50% sea water, suggesting the operation of some homeostatic mechanisms. Such mechanisms were reflected in the attainment of steady state in oxygen consumption of the whole animal, compensation for water loss in this tissue, slight hyper-osmotic regulation of the body fluids and a moderate regulation of inorganic and organic effectors in the muscle. In the hepatopancreas, the enzyme activity was inhibited, perhaps resulting in the decreased amino acid catabolism. In 100% sea water, the GDH activity in both tissues was highly elevated in the early periods of accli-mation with a later suppression over the control level suggest-ing failure of compensatory mechanisms in 100% sea water. Upon reversal to fresh water the GDH activity in tissues returns to control values relatively quickly. In general, it may be said that the enzyme activity in tissues parallels the oxygen con-sumption of whole animals.

The AlAT and AAT enzyme activities were elevated in the early stages of acclimation to 50% sea water. In later stages the enzyme activities declined to normal levels in the muscle but were suppressed far below the normal in hepatopancreas. However, in 100% sea water the activity levels of both AlAT and AAT were highly elevated in both the tissues throughout the period of acclimation suggesting the operation of gluconeogenic pathways. The enzyme activities generally returned to control levels before 7 days of reversal to fresh water.

GDH/TAT ratio

The ratio between the activities of glutamate dehydrogenase and total aminotransferases (GDH/TAT) revealed interesting correlations with the oxygen consumption of the whole animals. This ratio indicates the extent of mobilization of amino acids into oxidative reactions over that of mobilization into the formation of amphibolic substances (gluconeogenesis). On transfer of the crabs from fresh water to 50% sea water this ratio showed a drastic decrease within 1 hour of exposure in both the tissues studied viz: the hepatopancreas and muscle suggesting decreased oxidative deamination activities. Similarly, the oxygen consumption of the animal was also highly suppressed within the first hour of transfer to 50% sea water. Later periods of exposure resulted in a gradual increase in the

GDH/TAT ratio which was reflected in the gradual attainment of a steady state in oxygen consumption of whole animals during acclimation to 50% sea water. It is interesting to note here that the muscle showed full compensation for water loss and the hepatopancreas partial compensation and the body fluid showed hyper-ionic regulation in the later periods of acclimation to 50% sea water. Further, the influx of free amino acids from the blood into the cells is known to be an active process (Vincent-Marique and Gilles, 1970a,b; Gerard and Gilles, 1972a; Gilles, 1977, 1979). Thus, there appears to be some relationship between the attainment of a steady state in oxygen consumption of the whole animals, an increased GDH/TAT ratio in cells, after an initial drop, and the operation of several compensatory mechanisms in later periods of acclimation to 50% sea water. During the reverse acclimation to fresh water from 50% sea water the GDH/TAT ratio increased in the early phase with a concomitant elevation in the whole animal oxygen consumption suggesting increased oxydative deamination of amino acids and their consequent decreased levels in cells.

In the early phase of exposure of the animal to 100% sea water the response was quite contrary to what it was in 50% sea water. The GDH/TAT ratio was elevated over the normal with a subsequent decline. Similarly the oxygen consumption showed an initial increase over the normal with a later suppression. Since 100% sea water exerts extreme

stress conditions on the animal, being highly hyper-osmotic and resulting in large and abrupt osmotic and ionic gradients between blood and ambient medium, the oxidative metabolism of amino acids might be elevated initially with a later decline consequent on reduction of osmotic gradients. Hence the GDH/TAT ratio directly represents the cellular oxidations which become reflected as oxygen consumption of whole animals. The decline in GDH/TAT ratio in the later periods indicates a general decrease in the oxidative metabolism of amino acids leading to their increased levels in cells. The increased levels of amino acids in cells may also be due to their influx from blood. Thus the main organic osmotic effectors, viz., the free amino acids increase in cells under hyper osmotic stress conditions. However, part of the amino acids thus mobilized seems to participate in gluconeogenic activities in view of the higher levels of aminotransferases maintained in the cells during acclimation to 100% sea water. The crabs were observed to starve after 3 days of exposure to 100% sea water. This suggests the possible onset of hypoglycemic conditions, and in order to avoid death due to hypoglycemia, the amino acid metabolism seems to shift in the direction of gluconeogenesis.

In the reverse acclimation to fresh water from 100% sea water, the GDH/TAT ratio showed a general increase

corresponding to the increase in the oxygen consumption of the animals suggesting increased oxidation of amino acids under hypo-osmotic conditions. This picture fits well into the general trends reported (Chaplin, Huggins and Munday, 1970; Siebers, 1972; Gilles and Jobsis, 1972; Gilles, 1973b; 1974a). Thus, amino acid metabolism seems to have a direct influence on the oxygen consumption of the animal under changing salinities.

GDH/FAA, AAT/FAA and AlAT/FAA ratios

The ratios like GDH/FAA, AAT/FAA and AlAT/FAA represent the relative mobilization of free amino acids (FAA) into the activities of glutamate dehydrogenase (GDH) and aspartate (AAT) and alanine aminotransferases (AlAT). In 50% sea water the mobilization of amino acids into the activities of glutamate dehydrogenase and aminotransferases was evident in the initial stages of exposure in the muscle tissue but in the hepatopancreas the amino acids were mobilized preferentially into AAT activity with a suppression in GDH and AlAT activities. In the later stages of acclimation to 50% sea water suppression of mobilization of amino acids into catabolic activities was evident probably leading to the elevation in amino acid content of the cells which are essential for the regulation of ionic influxes from the hyper-ionic body fluids.

In 100% sea water, GDH/FAA, AlAT/FAA and AAT/FAA ratios showed a gradual decreasing trend over the control level throughout the course of acclimation except in the initial periods. Such a decrease in the ratios suggest a relative decrease in the catabolic activities of amino acids and their consequent accumulation inside the cells as organic osmotic effectors. Lowered oxygen consumption of whole animals throughout the acclimation period except for 48 hours in the early stages (Chapter I) and decreased NH_3 production in the same crab acclimated to higher salinities (Krishna moorthy and Srihari, 1973) lend support to this view. However, in the initial stages, these ratios were elevated over the normal level suggesting the mobilization of amino acids into oxidative as well as transamination processes. This is understandable in view of the large osmotic gradient faced by the animal in the initial stages of exposure to 100% sea water when the animals resort to some homeostatic mechanisms for maintaining a constant internal osmotic and ionic levels. However, such homeostatic mechanisms seem to break down in later stages of acclimation to 100% sea water. As the osmotic gradients become gradually reduced and as the animals become osmoconformers, the energy required for osmotic work is minimised and this explains why the GDH/FAA, AlAT/FAA and AAT/FAA ratios become decreased resulting in the conservation of free amino acids inside

the cells. However, the activity levels of AlAT and AAT remained at higher levels over the normal suggesting the mobilization of some amino acids into gluconeogenic activities leading to the formation of carbohydrates. Thus, the tissues under salinity stress (100% sea water) showed two types of responses one being the maintenance of higher amino acid content for electrostatic equilibrium to check the ionic fluxes and the other being the maintenance of carbohydrate levels through gluconeogenesis to prevent hypoglycemic conditions.

During the reverse acclimation of the crabs to fresh water from 100% sea water, the GDH/FAA, AlAT/FAA and AAT/FAA ratios in tissues increased immediately after transfer indicating increased mobilization of amino acids into oxidative deamination and transamination activities leading to the depletion of intracellular amino acid content concomitant with the decrease in inorganic ions. However, the extent of depletion of amino acids due to efflux and the extent of depletion due to increased catabolism cannot be visualised in view of the fact that both these events occurr simultaneously.

Relation between Cl^- and GDH activity

There appears to be a specific relationship between the intracellular Na^+ and Cl^- ions and activity levels of oxidative enzymes of amino acid catabolism.

This aspect has been studied particularly with respect to intracellular Cl^- ions and GDH activity. As the animals were transferred to higher salinities the intracellular Cl^- ions increased gradually and the activity levels of GDH were generally decreased. In the reverse acclimation to fresh water, the chloride ions appeared to leakout of the cells and concomitantly the GDH activity was elevated. Thus, the chloride ion seems to suppress the activity of GDH enzyme in particular and all oxidative enzymes of amino acid catabolism in general. But this suppression seems to occur only when the intracellular chloride content reaches a threshold level, for in the initial stages of transfer to 100% sea water, when the intracellular chloride concentration was comparatively lower, the GDH, activity was elevated to significantly higher levels in view of the large osmotic gradient between the body fluid and ambient medium. Suppression of the enzyme activity followed only after the intracellular chloride ion concentration reached a threshold level. Thus variations in Na^+ and Cl^-, particularly Cl^-, concentration inside the cells seem to exert some influence on the enzyme activities of amino acid oxidative metabolism in animals adapting to different salinities. This generalisation is quite in agreement with seversal earlier reports (Snoke, 1956;

Schoffeniels and Gilles, 1963; Chaplin, Huggins and Munday, 1965; Gilles, 1969, 1974c; Gilles, Hogue and Kearney, 1971).

The foregoing account shows that only the salient aspects of metabolic response to salinity stress in the field crab have been investigated and the author does not profess to have covered all the ground. He is also aware of several dificiencies in his work which could not be made good in view of several limiting factors while carrying out this project. For instance, a suitable technique adaptable in our laboratory conditions for determining the ECF volume of tissues has to be developed in order to calculate the precise concentration of intracellular ions and other osmotic effectors. It would also be interesting if the fate of individual amino acids were followed in the blood and tissues including the gills of the crabs. Studies on the time course of proteolytic enzyme activity and protein content in the blood may throw more light on changes in the equilibrium between blood serum peptidic materials and amino acids in animals adapting to different salinities. A similar evaluation of the changes in protein content and proteolytic enzyme activity in the tissues along with a detailed investigation in the patterns of nitrogen excretion would further substantiate the point stressed in this dissertation that

modulations in the intracellular amino-acid catabolism play an important role in the tissues of animals adapting to heterosmotic media rather than changes in the equilibrium between proteins and amino acids. It would be worthwhile to study further the time course of glucose metabolism in order to understand the extent of hypoglycemia under salinity stress and to substantiate the proposed shift in metabolism in the direction of gluconeogenic pathways, particularly in higher salinities.

An attempt has been made to correlate the GDH activity of the tissues in general to oxygen consumption of the whole animal. However, tissue specific differences in GDH activity were observed which could be further elaborated if data on tissue respiration were available. The author is aware of such lacunae in the present work and therefore, proposes to extend future studies in such directions.

SUMMARY

1. The fresh water field crab Oziotelphusa (Paratelphusa) senex senex (FABRICIUS) was selected as the experimental animal as it survives direct transfer to 50% and 100% sea water. Some aspects of metabolic adjustments to salinity stress have been investigated earlier in this crab but the time course of metabolic changes is completely lacking. No attempt has so far been made to understand the patterns of reverse acclimation from sea water to fresh water in this crab in particular and in crustaceans in general. Hence, the present investigation was aimed at understanding the temporal sequence of metabolic adjustments to salinity stress.

2. Crabs were abruptly transferred to 50% and 100% sea water and the time course of metabolic response was investigated. crabs acclimated to 50% and 100% sea water were suddenly transferred to fresh water (reverse acclimation) and the time course of metabolic adjustments was studied.

3. Time course of changes in 1) Oxygen consumption of whole animals, 2) water content in tissues, 3) Na^+ and Cl^- ions in the blood and tissues, 4) total free

amino acids in the blood and tissues and 5) activity levels of some enzymes of amino acid metabolism viz: glutamate dehydrogenase (GDH), aspartate aminotransferase (AAT) and alanine aminotransferase (AlAT) in the tissues has been followed in all the salinities. The tissues selected were the muscle and hepatopancreas.

4. A new technique, "the continuous flow method" has been devised and used for the study of the time course of oxygen consumption in crabs.

5. Using the new set up, the oxygen consumption of whole animals was studied in relation to sex, size and diurnal rhythms.

6. The unit oxygen consumption was found to be greater in males than in females. Smaller crabs showed greater metabolic rate than the larger ones and the weight regression curves followed the usual pattern, 'b' values conforming nearly to the surface rule (i.e., 2/3 value) in the normal crabs, whereas they became gradually depressed in crabs acclimated to sea water, the depression being greater in 100% sea water than in 50% sea water. During their reverse acclimation from sea water to fresh water, the value of the slope gradually returned nearly to the 2/3 level.

7. With respect to diurnal variations, both the sexes showed two significantly high peaks of oxygen consumption, one at 10 A.M. and the other at 8 P.M. in summer whereas in winter, the males showed two maxima one at 2 P.M. and the other at 12 midnight but the females showed only one maximum value at 2 A.M. but these maximal values in winter were non-significant. Changes in oxygen consumption in relation to sex, size and diurnal rhythms were briefly discussed.

8. The metabolic rate of the crabs decreased significantly immediately after transfer to 50% sea water and a steady state was attained after 10 days of transfer.

9. Transfer to 100% sea water resulted in a significant elevation of oxygen consumption over the normal level for 48 hours initially and later depression followed. No steady state was attained. until 288 hours.

10. There was no compensation in oxygen consumption of the animals in both the higher salinities.

11. During the reverse acclimation from sea water to fresh water, the oxygen consumption of the crabs increased to normal levels by about 72 hours. These results indicate that metabolic adjustments in the crabs are quicker in lower salinities than in higher salinities.

12. The oxygen consumption of whole animals adapting to different salinities seems to be governed by the osmotic and ionic gradients between the body fluids and the ambient medium of which the chloride ion gradient seems to be important.

13. The crabs exposed to 50% sea water showed normal feeding and locomotor activities. But the crabs transferred to 100% sea water were observed to show escape reactions in the early stages but became lethargic after 3 days. They were also observed to starve after 3 days. Hence, starvation stress appears to be super-imposed over salinity stress in 100% sea water.

14. There was significant decrease in the water content of both tissues in both higher salinities, the maximum dehydration being around 72 hours in the muscle in both salinities and around 72 and 168 hours in the hepatopancreas of 50% and 100% sea water acclimated crabs respectively.

15. The muscle showed full compensation for water loss and the hepatopancreas only partially in 50% sea water whereas both the tissues failed to show compensation in 100% sea water.

16. On reversal to fresh water, the tissues that had lost water regained normal levels of water content relatively quickly indicating that regulation of tissue water content and consequent cell volume regulation is relatively quicker in lower salinities rather than in higher salinities.

17. The Na^+ and Cl^- ion concentrations of the blood showed a gradual increase in the crabs acclimating to 50% and 100% sea water and upon reversal to fresh water, the ions seemed to leak out of the blood.

18. The nearly iso-ionic condition of blood observed in the early phase of acclimation to 50% sea water increased to slightly hyper-ionic levels in the later periods, suggesting that the crabs are capable of limited hyper-ionic and consequently hyper-osmotic regulation in 50% sea water.

19. The crabs began with large ionic gradients between their body fluids and external medium, with respect to Na^+ and Cl^-, immediately after their transfer to 100% sea water, but these gradients were gradually diminished.

20. With respect to the combined effect of both Na^+ and Cl^-, the crab's body fluid became nearly iso-ionic

to the medium at the end of the acclimation period in 100% sea water.

21. When returned to fresh water, the ionic composition of the crab's body fluid was restored to normal level but this was highly hyper-ionic to the external medium.

22. Therefore, the crab was found to be a good hyper-regulator in fresh water, slight hyper-regulator in 50% sea water and a conformer in 100% sea water with regard to the major inorganic osmotic effectors viz: Na^+ and Cl^-. From this, one can visualise "the anisosmotic regulation of the ECF".

23. It was rather interesting to draw correlation between the time course of oxygen consumption of the animals acclimating to different salinities and that of ionic gradients between the body fluids and ambient media. Larger the ionic gradients, higher was the oxygen consumption, smaller the ionic gradients, lower the oxygen consumption, with respect to both Na^+ and Cl^-. The correlation between the Cl^- gradient and oxygen uptake was particularly striking in 100% sea water. The probable reasons for this similarity were discussed.

24. The intracellular ion composition was found to be increasing upon acclimation of the animals to higher salinities and decreasing during their reversal to fresh water, with some tissue specific differences.

25. The hepatopancreas was letting in these inorganic ions liberally whereas the muscle was "conservative", especially in 100% sea water.

26. The hepatopancreas seemed to be a poor regulator of its inorganic and organic osmotic effectors as also its water content whereas the muscle showed better powers of osmoregulation.

27. The intracellular ionic composition was found to be always hypo-ionic to the ECF suggesting that other inorganic osmotic effectors particularly K^+ and organic osmotic effectors such as the free amino acids play a complimentary role in the isosmotic regulation of the intracellular fluid.

28. The free amino acid (TNPS) levels of the blood of crabs acclimated to higher salinities showed an initial elevation with later decline to nearly normal levels in 50% sea water. However, in 100% sea water, higher levels were maintained.

29. On reversal to fresh water from 50% and 100% sea water highly significant elevation in the blood amino acid levels was found and they were maintained upto 7 days with a later drop to normal levels. These modulations in blood amino acid levels were probably due to their free influx and efflux into and outside the cells under hyper- and hypo-osmotic stress conditions respectively. Changes in equilibrium between serum peptidic materials and free amino acids might be involved.

30. The amino acid concentrations showed a gradual increase in both tissues of crabs acclimated to higher salinities with some tissue specific differences. This increase was generally in proportion to the increase in the inorganic ions, particularly chloride.

31. The intracellular free amino acid content showed positive regression on the Cl^- ion concentrations, suggesting some role for Cl^- ions in the modulation of amino acid content of cells subjected to salinity stress.

32. It looks as though the free amino acid content increased under higher salinity stress in order to exclude

the entry of excessive inorganic ions into the cells and to protect them from "ionic injury".

33. In the hepatopancreas of 100% sea water exposed crabs, however, the increase in the free amino acid content did not keep pace with that of chloride.

34. The extreme dehydration observed in the hepatopancreas of 100% sea water exposed crabs coupled with excessive influx of Na^+ and Cl^- ions and a relatively lesser role played by the free amino acids might have been the causative factors for the 'ionic injury' to this tissue leading to its 'salogenous atrophy' and concomitant lethargy and starvation observed in the crabs during their sojourn in 100% sea water. This may also account for their greater mortalities in this medium.

35. Considerable dehydration and consequent shrinkage of the muscle, coupled with a moderate influx of Na^+ and Cl^- ions may probably account for the lethargy and loss of 'escape reactions' in the crabs exposed to 100% sea water.

36. The increased mobilization of amino acids in tissues subjected to higher salinity stress was attributed partly to their decreased mobilization into catabolic

activities and partly to their influx from the ECF, consequent on the increase in the inorgonic ion composition. The general decreasing trends observed in oxygen consumption, CO_2 and NH_3 production of the crabs acclimating to higher salinities point to the first possibility and the decreasing trends found in the blood amino acid content in the later stages of acclimation point to the second possibility.

37. In the reverse acclimation process to fresh water, the amino acid levels in tissues fell steeply and this was in line with the decrease in chloride content. The decrease in amino acid content in tissues under hypo-osmotic stress conditions could probably be due to increased catabolism of amino acids in cells and also to their efflux into blood. Increased oxygen consumption, Co_2 and NH_3 production in animals acclimating to lower salinities strengthen the former view and increased levels of free amino acids seen in the blood of animals under hypo-osmotic stress conditions lend support to the latter view.

38. Glutamate dehydrogenase activity showed differential trend in the muscle and hepatopancreas of 50% sea water acclimated crabs. The muscle recorded an initial

rise in GDH activity upto 72 hours of exposure to 50% sea water with a later reversal to the control level whereas the enzyme activity was inhibited in the hepatopancreas throughout the period of exposure.

39. In 100% sea water, the GDH activity was highly elevated in the early periods of acclimation in both tissues. However, in later stages, the GDH activity was suppressed over the normal level.

40. The AlAT and AAT activities were elevated in both the tissues in the early periods of acclimation to 50% sea water but in later stages, the enzyme activities declined to normal levels in the muscle whereas in the hepatopancreas they were depressed below the control values.

41. In 100% sea water, the AlAT and AAT activities were highly elevated in both tissues throughout the period of acclimation.

42. During the reverse acclimation, the GDH activity in tissues returned to the control values relatively quickly, but the AlAT and AAT activities showed differential behaviour in the time course of attaining the normal levels.

43. Modulations in enzyme activities were correlated to the probable modulations in amino acid catabolism, both in the direction of oxidative deaminations as well as transaminations involving gluconeogenic pathways. The implications underlying changes in GDH/TAT, GDH/FAA, AlAT/FAA and AAT/FAA ratios were discussed.

44. Patterns in the time course of GDH activity in tissues could be related to changes in intracellular NaCl content, particularly the Cl^-. An inverse relationship was found to exist between Cl^- concentration and GDH activity suggesting that Cl^- plays an inhibitory role on the expression of GDH activity.

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