Chapter 1

INTRODUCTION

Life arose in the earth over four billion years ago in the form of chemotrophic unicellular organisms in the coacervates (Lehninger et al., 2008). The evolution was then lead to the appearance of phototrophic cyanobacteria over 1.3 billion years ago. As a consequence, cyanobacteria introduced O$_2$ to the atmosphere which was released as a byproduct of photosynthesis (Harman, 1956, Haugaard, 1968). Consequently the force of evolution played its crucial role to introduce and evolve the aerobes in the O$_2$ rich environment. Aerobes utilized O$_2$ for the oxidation of carbon and hydrogen rich molecules to culminate energy. On the other hand O$_2$ got reduced in the process. But the aerobes paid the price for it when incomplete reduction of O$_2$ has consequenced to produce oxygen derived free radicals commonly known as reactive oxygen species (ROS) (Balentine, 1982, Petkaw, 1982). ROS usually have unpaired electron and, therefore, can potentially oxidize all the biomolecules present in their vicinity (Halliwell and Gutteridge, 2001). The phenomenon has came to be known scientifically in middle of the $20^{th}$ century when Gerschman and his colleagues proposed the “free radical theory of oxygen toxicity” describing the toxic effects of elevated oxygen levels on aerobes (Gerschman et. al., 1956). Then the detailed mechanisms of oxygen toxicity have been established with the involvement of electron leakage in respiratory chain to produce superoxide anions and its derived free radicals as ROS (Gutteridge and Halliwell, 2000). However, to neutralize or lower down the effect of ROS, all most all aerobes are equipped with the protection system called as antioxidant defence system which efficiently controls the level of ROS in cells (Sies, 1993 and 1997). When ROS generation exceeds the antioxidant capacity of the cell, then damages occur especially to lipids, proteins and nucleic acids leading to a stress condition in animals commonly called as “oxidative stress” (Hagen, 2003). Since much study on the oxidative stress physiology of vertebrates has been performed therefore, currently a growing interest has been noticed on the oxidative stress physiology of invertebrates.

1.1 Reactive oxygen species and oxidative stress

1.1.1 Reactive oxygen species

Reactive oxygen species (ROS) is a collective term used for oxygen-derived free radicals i.e. superoxide anion radical (O$_2^-$), hydroxyl radical (OH$^*$), hydroperoxyl radical (HO$_2^*$) and peroxy radicals (ROO$^*$) (Fridovich, 1983). Some derivatives of oxygen are reactive in nature but do not contain unpaired electrons such as hydrogen peroxide (H$_2$O$_2$), lipid peroxides (ROOH), singlet oxygen (¹O$_2$) and hypochlorous acid (HOCl) are also come
under the category of ROS (Halliwell and Cross, 1994). All these chemical entities are highly oxidant in nature and can attack to damage the biomolecules efficiently. Ground state diatomic oxygen (O₂), despite have two unpaired electrons, is sparingly reactive due to parallel spin of its unpaired electrons (Turrens, 2003).

1.1.1.1 Sources of ROS

1.1.1.1.1 Mitochondria

Mitochondria are the primary source of ROS production. Under normal physiological conditions, more than 90% of oxygen consumed by the cells is utilized by mitochondria where it undergoes tetra-electronic reduction to water with the help of several redox centres present in the electron transport chain (ETC). These redox centres are localized in the inner mitochondrial membrane. However, about 1-3% of the total oxygen consumed by mitochondria is converted to ROS (Halliwell and Gutteridge 2001, Turrens, 2003). The superoxide anion radical, the precursor of the other ROS, is formed by the univalent

Fig.1.1 Proton movement and electron transport in mitochondria: the involvement of various redox centers.

reduction of triplet–state molecular oxygen (\(^{3}\text{O}_2\)) due to electron leakage in ETC. The electron leakage is mediated enzymatically by enzymes particularly by complex I and III of ETC (fig. 1.1) (Andreyev et. al., 2005). Subsequently, further incomplete reduction of \(\text{O}_2^{*}\) by second and third electron reduction lead to production of hydroxyl radical (\(\text{OH}^{*}\)) or hydrogen peroxide molecule (\(\text{H}_2\text{O}_2\)), respectively. Therefore, the role of mitochondrial complex enzymes is vital in the ROS generation. The process of \(\text{O}_2^{*}\) generation is also non-enzymatically mediated by redox reactive compounds of the mitochondria such as the semi-
ubiquinone compound of ETC. The superoxide radical formed then under goes spontaneous
dismutation or by the enzymatic dismutation into hydrogen peroxide (Fridovich, 1975, 1995)
in mitochondria. In biological tissues, superoxide radical can also be converted non-
enzymatically into the non-radical species singlet oxygen ($^{1}\text{O}_2$) and hydrogen peroxide
(Steinbeck et al., 1993). Despite an anticipation of a positive relation between $\text{O}_2$ uptake and
ROS production, hypoxic state of organisms also accompanied with elevated ROS
production. The mechanism is mainly explained due to back flow and leakage of electrons in
respiratory chain (Turrens, 2003). Due to the above fact, it is believed that mitochondria act
as the main hub for ROS generation. However, other sources are also there which serve as
the centre for ROS generation.

**1.1.1.1.2 Other sources of ROS**

Other than mitochondria, ROS can also be formed *in vivo* by various chemicals and
enzymatic reactions as shown in diagram 1.1.

**Diagram 1.1** Major other pathways of ROS production.

$\text{O}_2^\cdot\cdot$ radicals are generated at several extra-mitochondrial sites in the cytoplasm. Enzymatic dismutation of $\text{O}_2^\cdot\cdot$ produces $\text{H}_2\text{O}_2$. Microsomal cytochrome P-450 enzymes constitute a potential source of $\text{O}_2^\cdot\cdot$ (Goeptar et al., 1995). Proteolytic conversion of xanthine dehydrogenase to xanthine oxidase produces oxidants (Yokoyama et al., 1990). Peroxisomal $\beta$-oxidation of fatty acids also generates $\text{H}_2\text{O}_2$ as a by-product. Deamination of dopamine by monoamine oxidase generates $\text{H}_2\text{O}_2$ (Fahn and Cohen, 1992).

Non-enzymatic production of ROS also occurs *in vivo*. $\cdot\text{OH}$ is produced by the
reduction of $\text{H}_2\text{O}_2$ by $\text{O}_2^\cdot\cdot$ in a two-step process catalyzed by transition metals in the classical Fenton reaction as follows.

$\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \cdot\text{OH} + \text{OH}^- + \text{Fe}^{3+}$ \hspace{5pt} (**Fenton reaction**)  
$\text{Fe}^{3+} + \text{O}_2\cdot\cdot \rightarrow \text{Fe}^{2+} + \text{O}_2$  
$\text{O}_2\cdot\cdot + \text{H}_2\text{O}_2 \rightarrow \cdot\text{OH} + \text{OH}^- + \text{O}_2$ \hspace{5pt} (**Haber-Weiss reaction**)  


Production of $O_2^{•−}$ is mediated by numerous redox cycling compounds such as reduced coenzymes, prosthetic groups or xenobiotics or herbicide like paraquat, anticancer agent like adriamycin etc. Spontaneous dismutation of $O_2^{•−}$ also yields $H_2O_2$. In addition, $O_2^{•−}$ may react with nitric oxide (NO$^•$) producing peroxynitrite (Beckman and Koppenol, 1996). Finally, oxidants are also generated as a response of phagocytic cells to pathogens (Moslen, 1994).

1.1.2 Biological significance of ROS and oxidative stress

1.1.2.1 Harmful role: oxidative stress

1.1.2.1.1 On proteins

ROS attack all the major macromolecules (proteins, lipids and nucleic acids) present in their vicinity. Deleterious modifications of membrane proteins are very common by ROS attack. In general, proteins are susceptible for modification of amino acids, oxidation of -SH groups, reduction of disulfides, altered enzyme activity, protein-protein cross-linking, peptide bond cleavage, loss of metals in metalloproteins, altered antigenicity and increased proteolytic susceptibility due to ROS attack (Stadtman, 1992). Further, sulphur containing amino acids and thiol groups particularly are very susceptible sites for protein oxidation. Besides, many amino acids undergo irreversible modifications, such as His, Lys, Pro, Arg and Ser by forming carbonyl groups upon oxidation by ROS (Levine \textit{et al.}, 1990; Stadtman, 1990) (diagram 1.2).

1.1.2.1.2 On lipids

ROS can deform all types of lipids by reacting with them. Particularly, polyunsaturated fatty acids (PUFA) are very sensitive to free radical attack. The oxidation reaction of PUFA by ROS once initiated, can be propagated in a chain-like fashion. Radical-mediated elimination of hydrogen atom from β-carbon of lipid molecule forms a carbon-centered conjugated diene lipid radical. It further reacts with oxygen to form a peroxyl radical (lipid hydroperoxide) capable of initiating further oxidation. Ultimately, it leads to production of low molecular weight alkanes, alkenes, hydroxy or epoxy derivatives, ketones or polyhydroperoxides like toxic products (Storey, 1996). As a consequence, structural and functional basis of the lipoprotein membrane are deformed leading to several pathophysiological disorders (Richter, 1987).

1.1.2.1.3 On nucleic acids

Attack by ROS can cause strand breaks and base modifications, leading to point mutations in DNA (Sies, 1993). Recently, unwinding of DNA molecules possible by ROS attack has been used as a marker for DNA damage (Shugart, 1988 a and b).
Diagram 1.2 Oxidative stress, ROS production and their clearance by antioxidant defence system. NO- NADP(H) oxidase, XO- xanthine oxidase, NA- nucleic acids.

If the ROS mediated damage is severe and prolonged then animals are pushed to several physiological disorders. In such conditions, animals are referred as in “oxidative stress” condition (Halliwell and Gutteridge, 2001) (diagram 1.2).

1.1.2.2 Beneficial role: as necessary evil

Below surplus concentrations, ROS play an important role as regulatory mediators in many physiological processes such as monitoring of oxygen tension in the control of ventilation, redox regulation of cell adhesion, amplification of immune responses and various signal transduction pathways (Droge, 2002). The beneficial roles of \( \text{O}_2^- \) include regulation of vascular function, cell division, inflammation, apoptosis and bactericidal activity of neutrophils. \( \text{H}_2\text{O}_2 \) acts as an important component of leukocyte-mediated defense against
bacteria. Phagocytic cells generate ROS to kill the engulfed microorganisms (Vrba and Modriansky, 2002). Traditional Chinese medical treatment includes killing of cancer cells by treating the local area with insect products that generates ROS (Mitsuhashi, 1997). On the other hand, aerobes have evolved the protection system to defend against the toxic effect of ROS. The protection system is commonly known as antioxidant defence system.

**1.2 Strategies to counterbalance ROS: antioxidant defence system (ADS)**

Aerobes have their own defence strategies to remove surplus ROS by means of various small antioxidant molecules and a battery of enzymes collectively known as antioxidant defence system (ADS). Functionally, an antioxidant is defined as any substance that when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate (Halliwell and Gutteridge, 2001) (diagram 1.2).

**1.2.1 Enzymatic antioxidants and supporting enzymes of ADS**

Antioxidant enzymes (AOEs) include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Other two enzymes, namely glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PD) help in increasing the efficiency of the enzymatic antioxidant defence system. In addition, detoxicant enzymes such as glutathione S-transferases (GSTs) metabolize the toxic electrophiles and xenobiotics (Hayes and Strange, 1995).

**1.2.1.1 Superoxide dismutases (SOD)**

SOD enzymes (EC 1.15.1.1) were discovered by McCord and Fridovich in 1969. Three different kinds of SODs have been characterized in aerobes depending on the prosthetic group they carry: copper-zinc SOD (Cu/Zn SOD), manganese SOD (MnSOD) and extra cellular SOD (EC-SOD) (Fridovich, 1995). CuZn SOD, a cytosolic protein with 32 kDa homodimer containing both Cu (II) and Zn (II) at its active sites. Copper is essential for its catalytic activity, and zinc gives stability to the protein structure (Fridovich, 1975). Mn SOD, is a 50 kDa protein (in crabs, Bunker et al., unpublished result) with manganese (III) at its active site. It is mainly localized in the cytosol of crustaceans and mitochondria of vertebrates. EC-SOD immunologically is a distinct form of SOD other than cytosolic Cu/Zn SOD. It is a 135 kDa secretory tetrameric glycoprotein containing copper and zinc (Marklund, 1982). It is predominantly found in extracellular or interstitial spaces and has
affinity for heparan sulfate and proteoglycans located on the endothelial and other cell surfaces (Abrahamsson et al., 1992). Apart from this, Fe, Co and Ni containing SOD have also been identified in invertebrates and bacteria, respectively. However, in crustaceans, cytosolic Cu/Zn SOD is replaced by Mn SOD (Brouwer et al., 2003). Additionally they may contain Fe SOD. SOD catalyzes dismutation of two \( \text{O}_2^- \) molecules into \( \text{H}_2\text{O}_2 \) and \( \text{O}_2 \) (reaction 1).

1.2.1.2 Catalase (CAT)

CAT (EC 1.11.1.6, 225-250 kDa) is a tetramer located primarily in the peroxisomes. Catalase contains a heme in its active site responsible for its catalytic activity. It principally functions in detoxifying \( \text{H}_2\text{O}_2 \) to oxygen and water (reaction 2).

1.2.1.3 Glutathione peroxidases (GPx)

GPx (EC 1.11.1.9) were discovered by Mills in 1957. It is a 85 kDa enzyme contains four protein subunits, each containing one atom of selenium (Se) at its active site as selenocysteine. It catalyses the removal of \( \text{H}_2\text{O}_2 \) and organic hydroperoxides coupled with the oxidation of reduced glutathione (reaction 3).

1.2.1.4 Glutathione reductase (GR)

GR (EC 1.6.4.2) is a dimer of 44 kDa enzyme which plays a major role in recycling glutathione. It catalyses the reduction of oxidized glutathione (GSSG) back into GSH with the help of NADPH (Halliwell and Gutteridge, 2001) (reaction 4).

1.2.1.5 Glucose-6-phosphate dehydrogenase (G6PD)

G6PD (EC 1.1.1.49) is a 110 kDa enzyme catalyzes the first step of the pentose phosphate pathway. It mainly provides NADPH, required for the action of GR (reaction 5).

1.2.1.6 Glutathione S-transferases (GST)

GST (EC 2.5.1.18) form a family of enzymes those detoxify the xenobiotics which are more prevalent in marine dwellers. The family of enzymes catalyzes conjugation of GSH with hydrophobic compounds bearing an electrophilic center (reaction 6).

AOEs act in a coordinate manner to help living tissues in defending them from oxidative damage as outlined in reactions from 1 to 6.
All though abundant work has been performed on the structural and functional aspects of the above enzymes in other animals, not much information is available on the above enzymes in marine invertebrates in general, and mud crabs in particular.

1.2.2 Non-enzymatic antioxidants

Non-enzymatic antioxidant molecules are known to directly remove ROS in the cellular compartment. Glutathione, ascorbic acid, uric acid, α-tocopherol, carotenoids, flavonoids and ubiquinol are the important non-enzymatic small antioxidant molecules. (Irshad and Chaudhuri, 2002). Ascorbate helps in neutralizing $O_2^*$ and $^*$OH radicals. It assists in recycling the tocopherol radical, inhibits lipid peroxidation and protects the polyunsaturated fatty acids present in the cell membranes. Besides these, transferrin and ceruloplasmin also act as scavengers of ROS (Gutteridge and Quinlan, 1993). β-carotene can efficiently scavenges singlet oxygen. The role of tocopherol in removing ROS is remarkable. Ubiquinone exercises its main function in mitochondria as a part of the electron transport chain. Carotenoids are potent quenchers of singlet oxygen. Uric acid acts as an endogenous radical scavenger and antioxidant. It can scavenge rapidly singlet oxygen, peroxyl radical (ROO') and $^*$OH radical (Halliwell and Gutteridge, 2001).

1.2.2.1 Glutathione (GSH)

GSH (L-$\gamma$-glutamyl-L-cysteinylglycine) plays its crucial role in maintaining the redox status of the cell due to its thiol group (Sies, 1999). It is a tri-peptide containing glutamate, cysteine and glycine (fig. 1.2A). Reduced glutathione is a substrate of antioxidant enzymes such as Se-dependent GPx and GSTs. Upon utilization by GPx, GSH forms a dimer by disulphide bridge to produce GSSG (fig. 1.2B). The ratio of GSH/GSSG is therefore an important indicator of cellular redox status. GSH serves several vital functions such as
detoxifying electrophiles, maintaining the essential thiol status of proteins by preventing the oxidation of -SH groups or by reducing disulfide bonds induced by oxidant stress, scavenging ROS, serving as an electron donor for certain antioxidant enzymes and detoxification processes by formation of conjugates with harmful endogenous and xenobiotic compounds and modulating many critical cell processes (DeLeve and Kaplowitz, 1991; Lu, 1999). Its deficiency is known to contribute to oxidative stress and is supposed to play a key role in the pathogenesis of many diseases (Wu et al., 2004).

**Figure 1.2** Structure of GSH (A) and GSSH (B).

![Structure of GSH and GSSH](image)

1.2.2.2 *Ascorbic acid (AA)*

Chemically ascorbic acid is a carbohydrate (fig. 1.3) and is required for the biosynthesis of collagen, for the biosynthesis of steroid and peptide hormones. It also helps to prevent or reduce the oxidation of biomolecules (Luck *et al.*, 1995). It is an important antioxidant molecule which can directly remove ROS upon reacting with them. Not much study has been performed on the role of ascorbic acid in combating OS in marine invertebrates. Interestingly, invertebrates have the power of synthesizing ascorbic acid which the higher vertebrates do not have.

**Figure 1.3** Structure of ascorbic acid.

![Structure of ascorbic acid](image)

1.2.2.3 *Vitamin E*

It is a lipid soluble vitamin present in membranes having a hydroxyl group that reacts with unpaired electrons and reduces peroxyl radical (Nordberg and Arner, 2001). It is a scavenger of singlet oxygen, superoxide radical and lipid peroxyl radicals and thus protects against lipid peroxidation (Miller *et al.*, 2005). Its lipid soluble nature allows it to concentrate
within the phospholipid bilayer of cell membrane and within blood lipoprotein. Vitamin E is itself converted to a weakly reactive radical which interferes in the chain reaction of peroxidation (Miller et al., 2005) (fig. 1.4)

**Figure 1.4** Structure of vitamin E.

1.3 Mitochondrial complex enzymes and oxidative stress

Mitochondria are the hub for oxidative stress. This is because of the production of the major quantity of ROS due to leakage of electrons during oxidative phosphorylation. Oxidative phosphorylation is a process catalyzed by the respiratory chain enzymes of the inner mitochondrial membrane that carry out electron transfer from different universal electron acceptors to O₂ (fig. 1.1). The process is accompanied by H⁺ pumping into the intermembrane space via complex I, III and IV and then back to the matrix via complex V (ATPase). The free energy available in the last step coupled with ATP synthesis by the F₆F₁-ATP synthase complex (Navarro and Boveris, 2007). Eventually, electrons get leaked particularly at complex I and III which then reduces oxygen to O₂⁻. It indicates that the process of oxidative phosphorylation includes both the energetic machinery to culminate with ATP production and generation of ROS in the mitochondria. Therefore, study of the functional level of complex enzymes is of major importance in oxidative stress research. The fact is well studied in vertebrates. However, with regard to the work done on the above aspects of mitochondria, very few articles are available showing the change in respiratory enzymes and its consequences in response to pollutants and seasonal variations in marine invertebrates in general, and mud crabs in particular (Kong et al., 2008, Vijayavel et al., 2005). This indicates the presence of a lacuna in the understanding the functional level of the respiratory enzymes of mud crabs with respect to altered environmental conditions.

1.4 A general review on the experimental species

1.4.1 Systematic

The genus *Scylla* has Phylum-Arthropoda, Sub phylum- Mandibulata, Class-Crustacea, Order-Decapoda, Family-Portunidae, Genus-*Scylla*, Species-*serrata*,

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tranquebarica, oceanica and paramamosain. Commonly mud crabs are called as mangrove crab, Indo-West Pacific salmon crab, serrated swimming crab, edible mud crab etc.

In India, only two species namely, S. serrata and S. tranquebarica occur. Gopikrishna and Shekhar (2003) had shown differences between two Indian Scylla species collected from Pulicat lake of Chennai by analyzing restriction digestion products of 16S and 12S rRNA. Mohanty et al. (2006) distinguished the above two species of Scylla collected from Chilika lagoon of Odisha on the basis of morphological parameters. Recently, Imai et al. (2004) classified Scylla sp. collected from various parts of the globe by using molecular markers like ITS-1 and 16S rDNA.

1.4.2 Morphology

The body of Scylla serrata is broad, transverse and somewhat convex at its carapace with even surface. The carapace is formed with fused tergites and serrated anteriorly with six teeth and anterolaterally, with nine teeth on each side. Crabs have a pair of each antennae, antennules and stalked eyes in the anterior side. The body is divisible into three parts namely cephalic, thoracic and abdominal regions. Mouth is positioned at mid anterocephalic region with well distinct mouth parts like mandible and maxillae etc. The podal appendages constitute of a pair of strong chelifeds anteroventrally with movable fingers called dactylus, three pairs of walking legs mid ventrally and posteroventrally a pair of appendages with pedal like modification. Chelipeds in mud crabs act as its hand and are used for walking and burrowing along with the middle three pairs of legs. The last pair of pedal like legs are used for swimming (Fig. 1.5). Thorax of the mud crabs is divided into six segments. An abdominal flap covers the thorax ventrally. The mature males have the triangular abdominal flap where as it is more flattened at the base as well as at the middle in case of females. Abdominal flap in both the sexes has soft and flattened structures called pleopods, which have fine cilial divisions throughout the edges. Male mud crabs have only two pairs of abdominal pleopods where as females have four pairs. Biologically, the broad abdominal flap with more numbers of pleopods in case of females helps them in bearing eggs till their hatching (fig. 1.6).

1.4.3 Geographic distribution

Scylla serrata is widely distributed in tropical and subtropical continents. They are distributed in Indo-Pacific regions, South Africa, Indonesia, Philippines, Taiwan, Japan, China, East and West coast of Australia, Western Samoa, Salmon Island, Fiji, New
Caledonia, Malaysia and Singapore (Oshiro, 1991, Roy and Das, 2000; Roy and Bhadra, 2005).

Fig. 1.5 *S. serrata* ventral view (A) showing abdominal flap and different body parts, dorsal view (B) showing carapace and legs.

1.4.4 Ecology

Since mud crabs prefer brackish water habitat, they migrate from high salinity to estuarine or brackish water bodies during their post larval or juvenile stages (Srinivasagam *et al*.
al., 2000). Tagging, telemetric and ultrasonic transmitter studies and laboratory experiments have clearly demonstrated the anadromous behavior of mud crabs in relation to habitat selection (Hyland et al., 1984; Oshiro, 1991; Webley et al., 2009). Muddy bottoms of mangrove forest with fluctuating salinity are preferred by S. serrata (Motoh, 1979; Vay, 2001), which is established by tagging experiments (Hyland et al., 1984). Oshiro (1991) reported that S. serrata prefers marshy mangroves at Okinawa. A recent study showed that habitat selection differs with respect to life cycle stages in Scylla sp. Usually, megalopa larvae of Scylla sp. prefer a structurally complex habitat rich with refuge and food. But crablets of S. serrata strongly prefer sea grass habitat (Webley et al., 2009). The nature of substratum for benthic life in estuaries in Indian coast for Scylla sp. varies from sandy to sandy muddy or totally muddy with wide fluctuating hydro biological factors. The environmental factors for Scylla in India may vary with a wide range of temperature (13 °C - 40 °C) and from a low dissolved oxygen to over saturated dissolved oxygen content (4-10 mg/l, table 1.1).

**Table 1.1** Hydrobiological fluctuation in the biggest estuary of India i.e. Chilika lagoon where Scylla species are available (Mohapatra et al., 2007a; Panigrahi et al., 2007).

<table>
<thead>
<tr>
<th>Factors</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkalinity (mg/l)</td>
<td>70 – 118.2</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>1.7 – 32.73</td>
</tr>
<tr>
<td>Dissolved Oxygen (mg/l)</td>
<td>3.58 -9.98</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>18.4 – 30.5</td>
</tr>
</tbody>
</table>

1.4.5 **Composition and nutritional values of crab meat**

Investigations have revealed that crab meat is a rich source of proteins with low fats (table 1.2). Therefore, it is recommended to patients with atherosclerosis symptoms (Gangal and Magar, 1967; Zafar et al., 2004). Better growth of rats was noticed when crab proteins

**Table 1.2** Biochemical compositions of crab meat. Units are calculated in approximate % wet wt. for all the parameters (Zafar et al., 2004).

<table>
<thead>
<tr>
<th>Composition</th>
<th>Male</th>
<th>Female</th>
</tr>
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<tbody>
<tr>
<td>Protein</td>
<td>17.69</td>
<td>19.39</td>
</tr>
<tr>
<td>Fat</td>
<td>0.59</td>
<td>0.61</td>
</tr>
<tr>
<td>Moisture</td>
<td>85</td>
<td>79</td>
</tr>
<tr>
<td>Ash</td>
<td>2.2</td>
<td>2.5</td>
</tr>
</tbody>
</table>
are replaced with vegetable proteins (Gangal and Magar, 1967). Presence of essential elements and free amino acids have also been identified in crab meat (Chiou and Huang, 2003; Mohapatra et al., 2007b and 2009).

1.4.6 Reproduction and life cycle

1.4.6.1 Breeding environment

It is reported that though the mud crabs are marine in origin, they are euryhaline and during post larval or juvenile stages migrate to brackish water bodies where they grow and attain adulthood (Naidu, 1955; Hyland et al., 1984; Oshiro, 1991; Srinivasagam et al., 2000; Webley et al., 2009). It indicates that the physical factors of marine and estuarine environment such as osmotic pressure, temperature, dissolved oxygen and other hydrobiological factors profoundly influence life cycle of mud crabs, particularly during larval and early adulthood stages. Recent studies have demonstrated that both ecological condition and pathophysiological status of mud crabs influence the life cycle and growth of Scylla sp. both in natural as well as in cultured conditions (Hamumante et al., 1979; Babu and Manjulatha, 1993; Mwaluma, 2002; Manjulatha, 2003; Davis et al., 2005; Weng et al., 2007; Webley et al., 2009).

1.4.6.2 Coupling

Copulation in mud crabs Scylla serrata takes around 2-3 days. In the first step, the male climbs dorsally over the female and clasps and carries her with his cheliped for a period of 3 to 4 days until the female under goes molting (Fig.1.6 C). The male then helps the female to shed her shell and then again climbs her ventrally for true mating. The copulation continues for 7 to 12 hours or even for days after which the transfer of non-motile spermatozoa passed into the female seminal receptacle. The act of sperm transfer lasts for 5 to 7 hours (Joel and SanjeevaRaj, 1982; Bhavanishankar and Subramoniam, 1998). The male can show polygamy with one to two days interval. Similarily, one female also can copulate with two males and receives the sperm from the both. In such case, spermatozoa deposited by the second crab are only utilized by the female for fertilization (Srinivasagam et al., 2000). The sperm in female seminal receptacle can survive up to 9 to 12 months with repetitive extrusion of eggs in 2 to 3 batches under laboratory conditions (Chen, 1976; Srinivasagam et al., 2000). Fertilization is internal in Scylla and females bearing about 1 to 5 millions of eggs (Fig. 1.6 E) migrate to the
Fig. 1.6 Reproduction in the crab *S. serrata*. A- male mud crab (a- triangular abdominal flap), B- female mud crab (a- flattened abdominal flap), C- Coupling in mud crab (a- male mud crab climbs over female, b- female mud crab), D- After false coupling, female crab molts after which true coupling takes place (a-male helps in molting of females and protect the soft female crab from predation and autobalism, b- molted female crab, c- old exoskeleton of molted female), E- Ovigerous female (a-millions of eggs in the abdomen of female), F- hatching of eggs into first larval form i.e. zoea (a-tail of zoea which helps in movement), G- megalopa, the second larval stage of mud crab (a-pre-developed cheleped, b- rudimentary tail which develop into abdominal flap), H- first instar crab (crablets), I- adult crab.

the shoreline in order to hatch (Srinivasagam *et al.*, 2000; Hill, 1974 and 1996). Hatching of eggs needs one to two hours in laboratory conditions which is influenced by temperature (Hill, 1974). No information is available about its hatching in natural conditions. However, the spawning in *S. serrata* is highly influenced by environmental factors (Hill, 1996). The eggs are hatched after fertilization and undergo development through only two larval stages i.e. zoea and megalopa (Fig. 1.6 F and 1.6 G). The zoea-I can also emerge from the abdomen of the female and undergo metamorphosis to the first instar crab (Oshiro, 1991).
1.4.6.3 Growth and maturation

Reports about the growth of mud crabs in nature are scanty. However, it is species specific (Vay, 2001). The larval development of mud crabs is found to be affected by their diet. The growth of adult mud crabs is reported to be enhanced by supplementing essential fatty acids in their diets (Suprayudi et al., 2004). Age is also an important factor contributing to the growth of mud crabs in nature. It is reported that the size of *S. tranquebarica* is about 8 to 12 cm and 14 to 15 cm in first and second years, respectively (Oshiro, 1991). Age at which mud crabs attain reproductive maturity is species and sex specific and is influenced by geographical location (Vay, 2001). In general, carapace width is used as an index of maturity in mud crabs. In Australia, mud crabs attain physiological maturity at carapace width from 9 to 11 cm (Heasman et al., 1983 and 1985; Knuckey, 1996) where as in South Africa the value is 9.2 cm (Robertson and Krugar, 1994). Also similar observations were made for mud crabs of Chilika lagoon of India where it was reported to be 12.1 cm and 7.9 cm of carapace width for females of *S. tranquebarica* and *S. serrata*, respectively (Mohanty et al., 2006).

1.4.6.4 Natural breeding and induced breeding

Several reports support the perennial breeding activity of mud crabs. In Rasimi River of Kenya, spawning in *S. serrata* is reported to be throughout the year with a peak after June (Dorcu, 2002). In South Africa, a similar report was furnished by Davis et al. (2004) for *S. serrata* with a peak in late winter or in early spring. In Kabira Bay of Ishigaki Island, the high and low peak of spawning in *S. serrata* was observed from May to June and from October to November, respectively (Oshiro, 1991). In Chilika lagoon of India, *S. serrata* juveniles were observed throughout the year and also in case of *S. tranquebarica*, the juveniles were observed throughout the year with a peak in May to September (Mohanty et al., 2006). However, the breeding activity in *S. serrata* and *S. tranquebarica* was reported to occur from August to November and from March to June in the lagoon (Mohanty et al., 2006). Induced breeding in crabs has opened a new avenue in aquaculture. In India, Central Institute of Brackish water Aquaculture (CIBA), Chennai has taken initiative to breed the crabs in laboratory condition in order to avail crab seeds on commercial basis. In CIBA, it was achieved by unilateral eye stalk ablation (ESA) method.

Unilateral or bilateral ESA in mud crabs stops the source of hyperglycemic hormone (HGH), responsible for metabolic regulation as well as stops the secretion of molt inhibiting
hormone (MIH) in the female crabs. This facilitates molting followed by copulation and subsequent breeding. ESA in female mud crabs increases food intake, faster growth of ovary irrespective of their developmental stage or season. As a result, crabs gain weight significantly and their oocyte size increases with advancement of extrusion of eggs. Consequently, a single female can produce millions of eggs in laboratory (~4 millions in case of S. tranquebarica, ~1.3 millions in case of S. serrata).

1.4.6.5 Survivability

Literature regarding survivability of Scylla sp. after their hatching in nature is very scanty. However, Hill (1975) reported that percentage of mortality of S. serrata during hatching is about 41-60% in nature. On the other hand, Srinivasagam et al. (2000) reported that hatching of about 90% of the berried crabs constitutes pre-zoeal stages under laboratory condition. The zoea larvae are positive phototactic in nature hence most of the times they are predated by surface feeders. However, once they achieve the adulthood, they can defend themselves from predators with their strong chelepeds.

1.4.7 Ecophysiology

1.4.7.1 Effects of Environmental factors on physiology of marine invertebrates

Several environmental factors of aquatic ecosystem change with season and thereby, modulate physiology of aquatic organisms including that of invertebrates (Di-Giulio et al., 1989; Winston, 1991; Winston and de-Guilio, 1991). In general, it has been demonstrated that spring and summer mostly have major impacts on lysosomal enzymes, oxidative stress biomarkers, peroxisomal enzyme activities in mussels. This suggests that temperature, reproductive cycle and food availability can affect the biology of invertebrate species (Bocchetti and Regoli, 2006). Verlecar et al. (2008a) reported that magnitude of oxidative damages in tissues of a mollusc in Arabian Sea (Perna viridis) varies with season. It indicates, ecological fluctuations are not only prevalent in fully or partially restricted water bodies like lakes and lagoons but also occur in large water bodies like riverine systems or oceanic bodies. In saline water bodies, aquatic organisms usually exposed to various insults in their environment are basically as a result of the fluctuations in salinity, temperature, dissolved oxygen content, alkalinity, pollutants, UV radiations etc. (Lesser et al., 2006).

Salinity is one of the distinguishable abiotic variables of estuarine ecosystem that fluctuates widely during the year and thereby, plays a significant role in the physiology of inhabiting invertebrate species (Schmidt-Nielson, 1997). It is observed that survival rate of
larvae of *Metapenaeus monoceros* (Fabricius), a crustacean species, and their metamorphosis to post larval stages is maximum high salinity (40 ppt) (Kumlu *et al*., 2001). On the other hand, it is noticed that survival of halibut larvae (*Hippoglossus hippoglossus*) is better at medium range salinity (15-20 ppt) and is influenced by temperature too (Opstad and Rust, 2004; Doroudi *et al*., 2006). Cook *et al*. (2005) demonstrated that the eggs of Lingcod *Ophiodon elongatus* survive and undergo normal hatching at 20-30 ppt salinity and their survivability and hatching decrease with increasing salinity. Similarly, lower salinity has been demonstrated to have an adverse effect on shrimp *Litopenaeus schmitti* as it causes immune-suppression in its hemolymph (Lamela *et al*., 2005). Kinsey and Lee, (2003) have shown that acute salinity stress influences the activity of enzyme arginine kinase in euryhaline crabs. Further, it was demonstrated that above effect of salinity stress is nullified by sufficient acclimation time (Holt and Kinsey, 2002). Salinity stress may be controlled by the crabs in vivo due to their integrated osmoregularity capacity. Salinity was also reported to alter lysosomal membrane function, sequestration and detoxification of xenobiotics, immune-response and antioxidant enzyme status in marine and estuarine animals (Regoli, 1998, 2000). The mechanism for the above biochemical processes may be attributed to redox status of the cells (Regoli, 1998, 2000).

Several authors have reported about the effect of temperature on the physiology of marine invertebrates (Lesser *et al*., 2006; Manduzio *et al*., 2005). It has been observed that increase in temperature not only augments mitochondrial respiration in vitro but also causes a decrease in its coupling behavior in intertidal mud clam *Mya arenaria* (Abel *et al*., 2002).

### 1.4.7.2 Effects of Environmental factors on physiology of mud crabs

#### 1.4.7.2.1 Effect of salinity

Mud crabs *S. serrata*, an euryhaline species, generally inhabits intertidal zones and estuaries throughout the Indo-Pacific region (Chen and Chia, 1996a) including Chilika lagoon of India (19º 28' and 19º 54' N and 85º 05' and 85º 38' E). Several aspects of *S. serrata* are reported to be modulated by environmental salinity and/or temperature (Hill, 1974; Chen and Chia, 1996a, 1996b; Hai *et al*., 1998; Ruscoe *et al*., 2004). A seasonal variation in fat and protein content of abdominal muscle of the species were attributed to the changing salinity of the water (Zafar *et al*., 2004). It is also reported that larval survival, growth and development of *Scylla serrata* are considerably influenced by salinity (Hill, 1974; Hamasaki, 2003). There are reports those describe about the manifestation of
physiological and biochemical variations especially that of O₂ consumption of euryhaline crabs at altered salinity conditions (Findley et al., 1978; Chen and Chia, 1996b; Robles et al., 2002). It is reported that the excretion in *S. serrata* shifts from ammonotelism to ureotelism with increased salinity (Chen and Chia, 1996a and 1996b). Ruscoe et al. (2004) proposed that limiting the rotifer supply as staple food to *Scylla zoea* larvae at salinity 10 to 35 ppt and temperature 30°C enhances a maximal survibility and its production. It has been demonstrated that *S. serrata* experiences oxidative stress in response to cold stress (Kong et al., 2005 and 2007). Also it was noticed that mitochondrial count in its different tissues considerably change by cold stress (Wang et al., 2007). Similarly, healthy adult crabs without any hormonal or pharmacological disturbances can withstand thermal shock up to 40°C (Hamumante et al., 1979).

1.4.7.2.2 Effect of seasons

Although several studies have clearly demonstrated strong correlation between the antioxidant defence system and seasons in relation to changing environmental conditions in molluscs (Bocchetti and Regoli, 2006; Filho et al., 2001; Manduzio et al., 2004; Power and Sheehan, 1996; Verlecar et al., 2008a and 2008b; Viarengo et al., 1991), cephalopods (Zielinski, and Portner, 2000) and other euryhaline crabs (Kucharski and Da Silva, 1991, Valle et al., 2009), not much information is available on arthropods in general and mud crabs in particular. However, Kong et al. (2008) reported that seasonal factors like high temperature with a prevalent low salinity during summer in tropical climate can enhance both protein and lipid oxidation in tissues of mud crabs.

1.4.7.2.3 Effect of pollutants

Pollutant like naphthalene, a fumigant, is shown to induce oxidative stress in *S. tranquebarica* in nature (Vijayavel et al., 2006). It also causes reproductive dysfunction by altering vitellogenesis in *S. serrata* (Vijayavel and Balasubramanian, 2008). It has been reported that the retention of the pharmacological compounds like enrofloxacin and ciprofloxacin are high in hepatopancreas of *S. serrata* but their rate of elimination is slow (Fang et al., 2007). *S. serrata* has been shown to protect itself from cadmium toxicity by enhancing the level of stress resistance metal protein (Rao et al., 2005). Considering the crab *S. serrata*, Oosterom et al., (2010) opined that GST enzyme can be taken as an important biomarker for pollution stress in the aquatic environment.
1.4.8 History of work on crabs and *Scylla* sp. in India

Literature survey reveals that scientific research on crabs in India started in mid-twentieth century and it was basically related to morphological description, and taxonomical aspects of the species. Chhapgar (1957) described about the various types of crabs present in mangrove habitats of Bombay regions. Krishnamurthy and Jeyasaleelam (1981) reported the presence of around 20 species of crabs in Pichavaram. The first systematic report on crabs of India was documented by Das in 1985 at Andaman Nicobar islands. Chakraborty et al. (1986), Anonymous (1987a and 1987b) and Mandal and Nandi (1989) reported the presence of more than 55 species of crabs under 30 genera in Sundarbans. Das and DevRoy (1989) described 31 species of crabs from Andaman Nicobar islands. Srinivasgam et al. (2000) and Kathirvel et al. (1981 and 2004) reported the presence of only two species i.e., *S. serrata* and *S. tranquebarica* in Indian water bodies. Work on growth of the crab *Scylla* sp. was initiated by Babu and Manjulatha (1993) which was related to eye stalk ablation, sexual maturity and hatchery management. Proteolytic activity analysis of seminal plasma of *Scylla* was reported in detail by Jayasankar and Subramanian (1997). In 1998, Bhavanishankar and Subramoniam have developed a procedure for cryopreservation of spermatozoa of crab (*S. serrata*). Rao et al. (2005) studied the physiological response of metallothionein in *S. serrata* to cadmium exposure. In 2004-2005, Mohapatra et al. reported the food and feeding habits of *S. serrata* and *S. tranquebarica* in Chilika lagoon of Orissa by considering the smaller sized *Scylla* sp. as *S. serrata*. Vijayavel et al. (2004 and 2006) studied the toxicological aspects of naphthalene on the oxidative metabolism of mud crabs both *S. serrata* and *S. tranquebarica*. Mohanty et al. (2006) described the occurrence of the above two species of *Scylla* in Chilika lagoon of Odisha. Recently, Mohapatra et al. (2010) deduced a relationship among carapace width, body weight and gonado-somatic index for the mud crab *S. serrata*.

1.4.9 Aquaculture importance of the species

The mud crab (*Scylla serrata*) is an important commercial species. The average annual landing of mud crabs is about 24,000 tones in Indo-Pacific zones (Sivasubramaniam and Angell, 1992; Anon, 2005). Recently much attention is being paid to consider it as a potential candidate for aquaculture (MaheshRaj, 1992; Kathirvel et al., 2004; Ruscoe et al., 2004; Mohanty et al., 2006; Webley et al., 2009). The mud crabs are cosmopolitan in distribution and have exploited every aquatic niche thereby considerably contributing to the food web in its habitat. The species is an excellent research model to understand the
molecular and physiological basis of stress in aquatic animals due to its high adaptability to
stress tolerance (Hill, 1974 and 1976; Chen and Chia, 1996a; Hai et al., 1998; Hamasaki, 2003). In addition, mud crabs have a direct role in maintaining marine as well as brackish water ecology. Mud crabs in particular act as burrowers; as a result they help in aeration, mixing and nutrient flow in the soil. Being burrower mud crabs enhance aeration and nutrient flow in the soil (Montague, 1980 and 1982; Bertness, 1985). Consequently, soil fertility is augmented that helps in facilitating the survival of other plants and soil dwelling animals. In addition, bioturbation structures created by crabs trap sediments and mangrove seeds (Chaudhury and Choudhury, 1994) and significantly contribute to the conservation of mangrove plants. One of the important roles of crabs particularly in mangrove environment is the production of millions of meroplanktonic larvae, which serve as potential food source for a large number of planktophagus organisms including rich number of edible fishes. Crabs stabilize the complex food web in the mangrove ecosystem. Despite its high commercial value, information on its biochemistry and physiology especially in relation to free radical mediated metabolism is scanty and needs attention for its use in aquaculture.

Finally, it can be concluded that crustaceans like crabs may become susceptible to various stresses when they cross over the threshold value of several environmental factors (Manduzio et al., 2005; Lesser, 2006). Therefore, S. serrata are rightly known for its wide range of environment stress tolerance and attenuation of physiological homeostasis particularly with respect to changing salinity (Hill, 1974; Hai et al., 1998) and temperature (Hill, 1974; Chen and Chia, 1996a and 1996b; Hamasaki, 2003). Different mechanisms on how these marine invertebrates adapt either at physical or physiological level to avoid the environmental stress are being explored by various workers (Weihrauch et al., 2004; Lesser et al., 2006; Abele et al., 2007). As evidenced from the above, studies made in past on mud crabs were basically addressed to their characterization, distribution and taxonomy. However, not much work has been done on the physiology particularly on oxygen metabolism of mud crabs. Oxygen metabolism is an important aspect of aerobes. A fluctuation in oxygen metabolism due to changes in hydrobiological parameters may have adverse effect on the metabolism of aquatic organisms by pushing them to stress condition known as “Oxidative stress”. Although there are some information available on oxidative stress and antioxidant defence system in invertebrates particularly in molluscs and insects, not much information is available on crabs.