CHAPTER IV

REPRODUCTION
INTRODUCTION

Reproduction is one of the fundamental activity of the organism. The aquatic environment permits the organisms to adapt and reproduce their species successfully. In recent years this environment is being threatened by a number of pollutants which may alter the reproductive capacity of the animals. Thus the main function of reproduction is to replace population losses due to death and emigration (Warren, 1971).

The hydrocarbons are known to cause considerable physiological changes in an organism. The assimilation of hydrocarbons within the organism induces a sequence of biological effects. The effects of hydrocarbon on the gonads is deleterious and affects the whole population.

The available literature reveals that very less work has been carried out on the histological changes in gonads of crustaceans exposed to toxicants. Nagabhushanam et al. (1983) noticed the histopathological lesions in the gonads of freshwater prawn, Macrobrachium lamerrii exposed
observed the effect of benzene on changes in gonadal structures of marine crab, *Scylla serrata*.

The present investigation was undertaken with a view to observe the histological changes in the gonads of the freshwater prawn, *Macrobrachium lamerrii* after exposure to benzene.
MATERIAL AND METHODS

The freshwater prawns, *Macrobrachium lamertii* were collected from Kham river near Aurangabad and acclimatized to laboratory conditions for 3-4 days in aquaria with aeration. During acclimatization, water was changed daily and they were fed with algae and starved one day before the experiment.

Histopathological studies

Intermolt (stage C), laboratory acclimatized mature prawns were selected for this study. The mature females were divided into nine groups, the first group served as base control and remaining eight groups were exposed separately to 1.1, 1.02, 0.95, and 0.89 ppm of benzene for 24, 48, 72 and 96 hours and 0.1 (1/10 of 48 hrs. LC50) ppm of benzene for 7, 14, 21 and 30 days respectively. Mature males were exposed to 1.09, 1.0, 0.95 and 0.90 ppm of benzene for 24, 48, 72 and 96 hours and 0.1 ppm (1/10 of 48 hrs. LC50) for 7, 14, 21 and 30 days respectively. The water media was changed daily and requisite amount of benzene was
added. The prawns were not fed during the course of experiment but those exposed to chronic treatment were fed twice a week. At the end of exposure period the experimental and control prawns were sacrificed. The tissues i.e. ovaries and testes were quickly excised and fixed in Bouin's fixative. The fixed tissues were dehydrated in graded series of alcohol, embedded in paraffin wax and sections were cut at 6-7 μ and stained with haematoxylin and counter stained by eosin (Harris, 1900).

Recovery studies on pre-exposed prawns in normal water:

Healthy, intermolt (stage C), laboratory acclimatized mature prawns were exposed to 0.1 ppm of benzene for a period of 30 days. After 30 days of exposure, the same prawns were transferred to normal tap water and after 7, 14, 21 and 30 days, 4-5 prawns were sacrificed and their gonads were quickly excised and fixed in Bouin's fluid. The serial sections were cut at 6-7 μ and stained with haematoxylin and counter stained by eosin (Harris, 1900).
OBSERVATIONS AND RESULTS

Effect of lethal and sublethal concentration of benzene on the ovary of the freshwater prawn, *Macrobrachium lamierii*:

Control ovary:

The ovary of *M. lamierii* (Fig. 1) is covered with an outer epithelial membrane followed by connective tissue and an inner germinative epithelium. In the early stages of development the germinative zone or zone of proliferation is distinguished by the presence of compact mass of oogonial cells which undergo meiotic division and give rise to primary oocytes (previtellogenic oocytes). The primary oocyte has large round nucleus with 2-3 nucleoli and these grow in size and mature into secondary oocytes or ova (vitellogenic oocytes). Each vitellogenic oocyte is covered with a thin layer of follicle cells. The mature oocytes or vitellogenic oocytes are completely filled with yolk globules and granules. The nutritive cells are present in close proximity of oocytes and supply the nutritive material to the developing oocytes. The degenerating
oocytes are almost of the same size as the vitellogenic oocytes and the appearance of vacuoles is distinctive. The degenerating ova are surrounded by nutritive phagocytes which increase in their size with the increase in vacuolisation. In fully mature ovary all the above described stages of developing oocytes as well as follicular cells and phagocytes can be seen.

**Effect of lethal concentration of benzene on ovarian development of prawn, *M. lamertii***:

On exposure to 1.1 ppm of benzene for 24 hours destruction of epithelial layer and evidence of degeneration of oocytes was seen. No other conspicuous changes have been observed (Fig. 2). 48 hours exposure to 1.02 ppm of benzene shows degenerated oocytes. Disorganisation of nucleus was also observed (Fig. 3). 72 hours exposure to 0.95 ppm of benzene shows shrinkage within the oocytes. However, disappearance of nuclei and nucleoli was noticed (Fig. 4). Loss in epidermal layer and disintegrated nuclei and nucleoli was
Effect of benzene on the ovary of freshwater prawn, *Macrobrachium lamerrii*.

Fig. 1 : T.S. of control ovary of freshwater prawn, *M. lamerrii* showing various developmental stages of oocytes. Haematoxylin - Eosin X 100.

Fig. 2 : T.S. of ovary of freshwater prawn, *M. lamerrii* exposed to 1.1 ppm of benzene for 24 hours. Haematoxylin-Eosin X 100. Note: Distruption of epithelial layer and degeneration of oocytes.

Fig. 3 : T.S. of ovary of freshwater prawn, *M. lamerrii* exposed to 1.02 ppm of benzene for 48 hours. Haematoxylin-Eosin X 100. Note: Degeneration of oocytes and disorganisation of nucleus.

DO - Degenerating oocytes
FC - Follicular cells
N - Nucleus
NU - Nucleous
PVO - Previtellogenic oocytes
VO - Vitellogenic oocytes
observed at 96 hrs. exposure to 0.89 ppm of benzene. Vacuolisation at the periphery of oocytes was noticed (Fig. 5).

**Effect of sublethal concentration (0.1 ppm) of benzene on ovarian development of prawn *Macrobrachium lamerrii* :**

After 7 days exposure, vacuolisation towards the periphery of oocytes, disorganisation in nuclear material and yolk globules were observed in the oocytes (Fig. 6). 14 days exposure caused shrinkage in ooplasmic material, vacuolisation in nucleus and oocytes are found to be scattered or loosely arranged (Fig. 7). After 21 days of exposure, increased number of degenerating oocytes and disappearance of nucleus was found (Fig. 8). At the end of 30 days of exposure degeneration and maximum damage to ovarian structure was observed (Fig. 9).
Fig. 4: T.S. of ovary of freshwater prawn,  
*M. lamerrii* exposed to 0.95 ppm of  
benzene for 72 hours.  
Haematoxylin-Eosin X 100.  
Note: Shrinkage within oocytes.

Fig. 5: T.S. of ovary of freshwater prawn,  
*M. lamerrii* exposed to 0.89 ppm of  
benzene for 96 hours.  
Haematoxylin-Eosin X 100.  
Note: Vacuolisation at the periphery of  
oocytes.

Fig. 6: T.S. of ovary of freshwater prawn,  
*M. lamerrii* exposed to 0.1 ppm of  
benzene for 7 days.  
Haematoxylin-Eosin X 100.  
Note: Oocyte showing vacuolisation  
and disorganisation in nuclear material.

DO - Degenerating oocytes  
V - Vacuoles  
YG - Yolk globules
Fig. 7: T.S. of ovary of freshwater prawn, *M. lamerrii* exposed to 0.1 ppm of benzene for 14 days. 
Haematoxylin-Eosin X 100. 
Note: Oocytes are loosely arranged.

Fig. 8: T.S. of ovary of freshwater prawn, *M. lamerrii* exposed to 0.1 ppm of benzene for 21 days. 
Haematoxylin-Eosin X 100. 
Note: Increased the number of degenerating oocytes.

Fig. 9: T.S. of ovary of freshwater prawn, *M. lamerrii* exposed to 0.1 ppm of benzene for 30 days. 
Haematoxylin-eosin X 100. 
Note: Degeneration and maximum damage to ovarian tissue.

DO - Degenerating oocytes  
N - Nucleus.
Recovery studies on pre-exposed female prawns in normal water:

The freshwater prawns, *M. lamertii* which had been previously exposed to benzene were maintained in the normal water for detection of persistent effects. After 7, 14, 21 and 30 days the histological changes which occurred were as follows:

After 7 days, the ovary seems to be slightly recovered, but ovarian areas showed vacuolisation and degenerating oocytes (Fig. 10). After 14 days, there is no sign of vacuolisation and development of follicular cells was clear (Fig. 11). Nuclear envelope is prominent, epithelial layers are found to be intact after 21 days (Fig. 12). After 30 days, ovarian picture is almost as normal as control ovary; yolk globules and their follicular arrangement becomes normal (Fig. 13).

Effect of lethal and sublethal concentration of benzene on testis of the prawn, *Macrobrachium lamertii*

Control testis:

Testis of *M. lamertii* (Fig. 14a) consists of several testicular tubules which are closely packed
Fig. 10: T.S. of ovary of freshwater prawn, *M. lamerrii* after 7 days in normal water.  
Haematoxylin-Eosin X 150.  
Note: Slightly recovered, but ovarian area shows vacuolisation and degenerating oocytes.

Fig. 11: T.S. of ovary of freshwater prawn, *M. lamerrii* after 14 days in normal water.  
Haematoxylin-Eosin X 150.  
Note: Developing follicular cells area clear.

Fig. 12: T.S. of ovary of freshwater prawn, *M. lamerrii* after 21 days in normal water.  
Haematoxylin-eosin X 200.  
Note: Nuclear envelope is prominent, and epithelial layers are found to be intact.

Fig. 13: T.S. of ovary of freshwater prawn, *M. lamerrii* after 80 days in normal water.  
Haematoxylin eosin X 250.  
Note: Normal structure of ovary as control.

DO – Degenerating oocytes,  
FC – Follicular cells  
N – Nucleus  
NU – Nucleolus  
PVO – Previtellogenic oocytes  
V – Vacuoles  
VO – Vitellogenic oocytes
together. The tubules are continuous and lead directly into the vas deferens. Each tubule has an outer layer of connective tissue and inner layer of germinal epithelium with a central lumen. In the initial stages of development, the germinal cells give rise to spermatogonia and occur in clusters around the germinal ridge. The spermatogonia are identified by their size, nucleus and position of nucleolus. The ensuring stage consist of a rapid proliferation of spermatogenic elements by division of spermatogonia into primary and then secondary spermatocytes and spermatids. Spermatids undergo morphological changes during maturation and finally crescent shaped sperms are produced. All progressive spermatogenic stages are visible in the lobules. In regressive phase the lumen decreases in size and the testis is reduced to a network of connective tissue with distinct germ cells lining the walls.

Effect of lethal concentration of benzene on testis development of the prawn, Macrobrachium lamerrii.

On exposure to 1.09 ppm of benzene for 24 hours, there is a rearrangement of testicular tubules and degeneration of spermatogenic elements (Fig. 15).
Effect of benzene on the testis of freshwater prawn, *Macrobrachium lamereii*.

Fig. 14a: T.S. of control testis of freshwater prawn, *M. lamereii* showing different developmental stages of spermatogenesis in testicular follicles. Haematoxylin-eosin X 280.

Fig. 14b: T.S. of testis showing spermatozoa. Haematoxylin-Eosin X 250.

SG - Spermatogonia  
SPC - Spermatocytes  
SPT - Spermatids  
SZ - Spermatozoa  
TF - Testicular follicle
After 48 hours to 10 ppm of benzene the testis showed ruptured testicular follicle and scattered spermatogenic mass (Fig. 16). After 72 hours exposure to 0.95 ppm of benzene the testis disintegrated tissue with reduced spermatogenic material (Fig. 17).

**Effect of 0.90 ppm of benzene on the prawns**

At the end of 96 hours exposure to 0.90 ppm benzene there was extensive damage to testicular tissue. In some lobules, spermatogenic mass was clumped together at one side of the lumen (Fig. 18).

**Effect of sub-lethal concentration (0.1 ppm) of benzene on testis development of prawn**

*Macrobrachium lamerrii*

After 7 days of exposure to benzene there was less damage to testicular tissue. No conspicuous changes have been observed (Fig. 19). 14 days exposure showed irregular arrangement of testicular tubules and necrosis of tubules (Fig. 20). After 21 days of exposure vacuolisation, degeneration and
Fig. 15 : T.S. of testis of freshwater prawn, 
M. lamerrii exposed to 1.09 ppm of 
benzene for 24 hours. 
Haematoxylin-eosin X 100. 
Note : Dearrangement of testicular tubules.

Fig. 16 : T.S. of testis of freshwater prawn, 
M. lamerrii exposed to 1.0 ppm of 
benzene for 48 hours. 
Haematoxylin-Eosin X 100. 
Note : Ruptured testicular follicle 
and scattered spermatogenic mass.

Fig. 17 : T.S. of testis of freshwater 
prawn, M. lamerrii exposed to 0.95 ppm 
of benzene for 72 hours. 
Haematoxylin-eosin X 100. 
Note : Disintegrated tissue with 
reduced spermatogenic material.

L  = Lumen 
SC = Spermatocytes 
SG = Spermatogonia 
SZ = Spermatozoa 
TF = Testicular follicle
Fig. 18: T.S. of testis of freshwater prawn, 
*Melania lamerrii* exposed to 0.90 ppm of 
benzene for 96 hours. 
Haematoxylin-eosin X 100. 
Note: Extensive damage of tissue.

Fig. 19: T.S. of testis of freshwater prawn, 
*M. lamerrii* exposed to 0.1 ppm of 
benzene for 7 days. 
Haematoxylin-eosin X 100. 
Note: Loosely arrangement and 
deformation of testicular tubules.

Fig. 20: T.S. of testis of freshwater prawn, 
*M. lamerrii* exposed to 0.1 ppm of 
benzene for 14 days. 
Haematoxylin-eosin X 100. 
Note: Irregular arrangement and 
necrosis of testicular tubule.

DTF - Deformed testicular follicle 
PZ - Proliferating zone 
SC - Spermatocytes 
ST - Seminiferous tubules 
SZ - Proliferating zone 
TF - Testicular follicle
more damage of the testicular tissue was noticed (Fig. 21). At the end of 30 days of exposure to benzene an extensive damage to testicular tissue was observed such as degeneration and disintegration of tubules with spermatogenic material (Fig. 22).

**Recovery studies on pre-exposed male prawns in normal water**

After 7 days, there is no sign of recovery. Degenerated tissue was noticed (Fig. 23). After 21 days, testis seems to be recovered. Testicular tubules are intact and filled with spermatophores. Proliferating zone is prominent (Fig. 24). After 30 days, testicular picture is almost as normal as control. There is appearance of proliferating zone and additional spermatogonial cells in the testicular tubules (Fig. 25).
Fig. 21: T.S. of testis of freshwater prawn, M. lamellipex exposed to 0.1 ppm of benzene for 21 days.
Haematoxylin-Eosin X 100.
Note: Vacuolisation, degeneration and more damage of the tissue.

Fig. 22: T.S. of testis of freshwater prawn, M. lamellipex exposed to 0.1 ppm of benzene for 30 days.
Haematoxylin-eosin X 100.
Note: Damaged tissue.

Fig. 23: T.S. of testis of freshwater prawn, M. lamellipex after 7 days in normal water.
Haematoxylin-eosin X 100.
Note: No sign of recovery degenerated tissue was noticed.

DF - Degenerating follicle
DTF - Deformed testicular follicle
ST - Seminiferous tubules
V - Vacuoles
Fig. 24: T.S. of testis of freshwater prawn, *M. lamertii* after 21 days in normal water. Haematoxylin-eosin X 200. Note: Testicular tubules are intact and filled with spermatophores.

Fig. 25: T.S. of testis of freshwater prawn, *M. lamertii* after 30 days in normal water. Haematoxylin-eosin X 200. Note: Testicular picture is almost as normal.

SC  Spermatocytes
SG  Spermatogonia
SZ  Spermatozoa
TF  Testicular follicle
DISCUSSION

Benzene exposure induced significant alterations in the gonads of *M. lamertii*.

The alterations observed in the ovary of the prawn after benzene stress are such as shrinkage of ooplasm, degeneration of tissue, alteration in normal cell shape, vacuolisation, disappearance of nucleus and nucleoli.

After acute and chronic treatment, increase in exposure period leads to increase in the damage to the tissue. The observed cellular deformities following the benzene exposure may be due to severe dislocation of the metabolic mechanism. The disappearance of nucleus and nucleolus results in the decline of the reproductive activity. The observed ooplasmic vacuolisation may probably be due to the sudden alteration in membrane permeability leading to the active transport of benzene molecules. Such structural damage is caused by the alteration of intercellular ionic composition. Dixon and Léduc (1981) stated that cytolysis is
brought about by an increased cytoplasmic viscosity followed by swelling of the cytoplasmic membrane and it occurs along with karyolysis. Sastry and Miller (1981) reported destabilisation of intracellular lysosome which results in the release of hydrolases into the cytoplasm in response to the toxic stress and thus resulting in autolytic cellular damages. A similar mechanism might have resulted in the present study.

The observed alteration in the ovarian structure of Macrobrachium lamereii after exposure to benzene collaborates the earlier reports of several investigators. Gyananath (1982) reported changes in cell shape, degeneration of oocytes in the ovary of M. lamereii after dimecron exposure. Bodkhe (1983) observed alterations in the histological structure of gonads like pycnosis of nutritive cells and nucleus due to shrinkage of oocytes and vacuolisation in the ooplasm of the oocyte of the crab, Barytelphusa cunicularis after exposure to carbamate. Victor and Sarojini (1986) while studying the effect of organophosphorous insecticide,
dimecron on the histological structure of the ovary of freshwater prawn, *Caridina rajadhari* observed alterations in the histological picture of the ovary. They observed ooplasmic vacuolisation, atresia of the mature oocytes, inhibition of oocyte development, nuclear pycnosis and hypertrophy of haemocytes. Sarojini *et al.* (1986) reported that fenitrothion toxicity causes damage by disintegration of nucleolus which is followed by vacuolisation in nucleus, in the ovarian tissue of freshwater prawn, *M. lamertii*. Gangshettiwar (1986) observed the effect of phenol on *M. lamertii* and noted rupturing of oocyte membrane, changes in cell shape, vacuolisation and degeneration of nucleoli and nucleus of oocytes. Jaiswal (1986) also noted similar changes in ovarian picture of *M. kistnensis* exposed to naphthalene.

The general changes observed in the testis of *Macrobrachium lamertii* during lethal and sub-lethal exposure to benzene were rupturing of testicular follicle layers, deformed tubules, reduced spermatogenic mass, vacuolisation and degeneration of tissue. Abnormal changes in testis
might be due to damage to androgen secreting cells during benzene exposure which would eventually lead to testicular damage. However, decline in spermatozoa numbers might have resulted from reduction in circulating testosterone level. In some tubules reduced spermatogenic material was noticed indicating inhibition of spermatogenesis. Bodkhe (1983) reported irregular arrangement of spermatozoa in the testicular tubules of the crab, Barytelphusa cunicularis when exposed to sevimol. SambasivaRao (1984) found inhibition of spermatogenesis, fibrosis of lobules, fatty necrosis and total disintegration of reproductive element in testicular lobules of the crab, Scylla serrata after exposure to organophosphate and organochlorine pesticides. Deshpande (1985) reported thickening of lobular wall, impairment of lobules, reduced spermatogenic mass, affected interstitial cells, vacuolisation and degeneration of the testis of M. kistnensis after pesticidal treatment. Sarojini et al. (1986) found highly affected germinal epithelium in testis of M. lamerrii on chronic exposure to fenirotrothion. Gangshettiwar (1986)
showed thickening and rupturing of testicular tubules, deformation of tubules affecting proliferating zone, reduced spermatogenic mass, vacuolisation and degeneration of tissues of prawn, *M. lameltri* after exposure to phenol.

Jaiswal (1986) observed the effect of naphthalene on testis of *M. kistnensis* and noticed changes like dearrangement of follicles, degeneration, necrosis and rupture of testicular wall, reduction in spermatogenic mass and damage to tissue. These results coincides with the results obtained in the present investigation and it clearly indicates that the benzene has deleterious effects on the gonads of *M. lameltri*. The pollutants have different affinities to different biochemical components. Hydrocarbons which are lipophilic in nature, generally are deposited in lipid rich tissues (Gesamp, 1977). The gonads have lipid in greater quantities, so naturally benzene might migrate to the gonads and this fact is indirectly responsible for the destruction of gonads.
On the other hand gonadal changes after benzene treatment might be due to alteration in gonadotropic hormone as they are regulated by the varied titre of gonadotropic hormone (Hoar, 1965).

In the present investigation, the pre-exposed prawns were found to recover after they are kept in normal water for 30 days. This recovery may be due to either metabolism of the accumulated benzene or its release from the body of prawn. Brown (1976) stated that certain organisms possess mechanism to detoxify pollutants. Once the pollutants enters the body, the organisms tries to metabolize it or it will excrete.