VOLUME 11 ISSUE 2 2025

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 11, No. 2, 878-905 (2025)

International Journal of Zoological Investigations

Contents available at Journals Home Page: www.ijzi.net

Editor-in-Chief: Prof. Ajai Kumar Srivastav

Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Assessment of the Environmental Impact of Acrylamide on Liver and Reproductive Health in Freshwater Fish *Anabas testudineus*

Shehma Mamunni Musliyarakath, Kathirakkottil Maneesha, Mashirin Reeha and Chitra Kumari Chidambaran*

Endocrinology and Toxicology Laboratory, Department of Zoology, University of Calicut, Malappuram, Kerala 673 635, India

**Corresponding Author*

Received: 10th February, 2025; **Accepted:** 22nd June, 2025; **Published online:** 12th November, 2025

https://doi.org/10.33745/ijzi.2025.v11i02.086

Abstract: Acrylamide is an organic compound formed during high-temperature cooking of certain foods and poses ecological consequences through its potential to contaminate water sources and harm aquatic life when released into the environment. The objectives of this study are to investigate the sublethal effects of acrylamide on hepatic biotransformation processes, and reproductive health in a freshwater fish *Anabas testudineus*. Fish exposed to sublethal levels of acrylamide, i.e., 13.2 µg L^{-1} for 4 d changes the specific growth rate, length-weight relationship, and condition factor thereby illustrating negative allometric growth pattern. Decline in the absolute and relative weights of liver and gonadal tissues suggests detrimental structural damage. Alterations on the activities of various enzymes within liver subcellular compartments, including ethoxyresorufin-O-deethylase, UDP-glucuronosyl-transferase, glutathione S-transferase, gamma-glutamyl transpeptidase, and flavin monooxygenase indicated limited detoxification potential against acrylamide. Sublethal exposure to acrylamide resulted in reduced gonadal tissue weights, poor sperm quality, compromised oocyte growth, and reduced fecundity rate along with alterations in gonadal steroidogenic enzyme activities indicating disruptions in steroidogenic pathways and impaired reproductive health of the fish. Liver tissue lesions such as vacuolization and aggregation of melanomacrophages along with germ cell loss, seminiferous tubule thickening, and ovarian structural deformities demonstrates toxic impacts of acrylamide. The study concludes that acrylamide exposure has significant multifaceted impacts on *Anabas testudineus*. The findings emphasize potential ecological consequences of acrylamide exposure, including implications for population dynamics and sustainability in natural environments.

Keywords: Condition factor, Detoxification, Sperm quality, Gonadal steroidogenesis, Histology, Liver lesions, Liver enzymes, Biomarkers, Acrylamide

Citation: Shehma Mamunni Musliyarakath, Kathirakkottil Maneesha, Mashirin Reeha and Chitra Kumari Chidambaran: Assessment of the environmental impact of acrylamide on liver and reproductive health in freshwater fish *Anabas testudineus.* Intern. J. Zool. Invest. 11(2): 878-905, 2025.

https://doi.org/10.33745/ijzi.2025.v11i02.086

Introduction

Acrylamide gained significant public attention when its presence was detected in certain cooked foods in the early 2000s. The global production of acrylamide largely depends on the demand for its various applications, such as it functions predominantly as a fundamental precursor for the synthesis of polyacrylamide, a polymer of paramount importance in diverse sectors encompassing water treatment, mineral processing, petroleum extraction, and pulp and paper fabrication, along with various minor-scale applications (Taeymans *et al*., 2004). In the global context, China emerges as the principal contributor to both the production and consumption of acrylamide, commanding a 50% share of the global production capacity. Subsequently, North America and Western Europe follow suit as significant participants in this production landscape (Zhang *et al*., 2005). It is noteworthy that Japan distinguishes itself as the foremost exporter of acrylamide, annually dispatching approximately 54,000 tonnes of this compound to international markets (Yoshida *et al*., 2002). Studies in animals have shown that acrylamide exposure have toxic effects on the nervous system and can increase the risk of certain types of cancer.

The Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) in 2002 jointly stipulated fundamental threshold limit values, no-observed-adverse-effect levels, and tolerable daily intake levels for acrylamide, set at 0.5 mg/kg body weight/day. Notably, cigarette smoking emerges as another substantive source of acrylamide exposure. Acrylamide undergo enzymatic or non-enzymatic processes in the body to form reactive intermediates, such as glycidamide. These intermediates can covalently bind to cellular biomolecules, particularly proteins and DNA. This covalent binding forms adducts, which are abnormal molecular structures that can interfere with normal cellular processes (LoPachin and Gavin, 2012). Besides humans and other terrestrial animals, the impact of acrylamide on the aquatic organisms is very concerning. Once acrylamide enters aquatic ecosystems, it has several implications for aquatic organisms and the ecosystem as a whole (Naiel *et al*., 2023). Due to its solubility in water, acrylamide easily mix into the water column and be taken up by aquatic organisms through direct exposure or ingestion of contaminated water or sediments (Tepe and Cebi, 2017). This lead to bioaccumulation in aquatic organisms, potentially affecting their health and behavior. The persistence of acrylamide in aquatic environments contribute to long-term contamination and pose risks to aquatic life (Xu *et al*., 2020).

The presence of acrylamide in aquatic ecosystems is a concerning environmental issue that arises primarily from anthropogenic activities and industrial processes. Acrylamide, a chemical compound with diverse industrial applications, can find its way into aquatic environments through various pathways, leading to potential ecological and human health risks (Tepe and Cebi, 2017). In aquatic ecosystems, fish assume the role of sentinel species, commonly employed for the assessment of connections between exposure and deleterious outcomes associated with various compounds. The selection of fish as preeminent subjects in toxicological research stems from multifaceted considerations, encompassing parameters such as size, age, and condition factor (Neetha *et al*., 2022). In the current investigation, the experimental focus centers on *Anabas testudineus* (Anabantidae) as a designated model organism. These attributes encompass the widespread species distribution within local natural habitats, consistent year-round availability, ease of laboratory maintenance, non-invasive qualities, and its economic significance (Priyatha and Chitra, 2022). The choice of test model is predicated upon the intention to scrutinize the repercussions of acrylamide toxicity through the exploration of detoxification pathways and reproductive physiology.

The biotransformation phenomenon primarily

transpires through a two-phase process operating within the liver, categorized as phase I and phase II pathways. Phase I detoxification entails the involvement of enzymes recognized as functionalization enzymes, serving to modify the chemical structure of the toxicant. Subsequently, in phase II pathways, conjugation enzymes partake in the attachment of water-soluble molecules to the modified toxicant, culminating in the initiation of the elimination process (Ziegter, 1988). Reproduction is a critical process for the survival and sustainability of fish populations. Any impairment in reproductive success can have cascading effects on population dynamics, genetic diversity, and overall health of the ecosystem. A comprehensive assessment of crucial reproductive endpoints, encompassing gonadosomatic index, sperm indices, oocyte measurements, fecundity rate, gonadal steroidogenic enzymes, and gonadal histology, was employed to assess the reproductive status of the fish. Thus the study underscores the importance of addressing and mitigating the risks associated with environmental contaminants like acrylamide.

Materials and Methods

Collection and maintenance of test animal

The adult freshwater cichlid fish, *Anabas testudineus*, weighing 28 ± 1.5 g and length 12 ± 1.5 cm was collected from the Alankritha Fish farm, Chathamangalam, Kozhikode District, Kerala, India. To minimize disturbance, the fish were transported in a well-aerated polythene bag and subsequently acclimatized for a period of 15 days prior to the commencement of the experiments. During this acclimatization period, the fish were housed in a 180 L capacity cement tank, which provided them with a constant supply of dechlorinated tap water and a well-functioning lighting system, maintaining a 12 h light and 12 h dark cycle. Throughout the acclimatization and experimental period, the fish were fed standard commercial fish pellets once a day, while their health status was continuously monitored.

Preliminary tests

The physico-chemical features of the tap water were analyzed as per American Public Health Association guidelines (APHA, 1998). The standardized physico-chemical features of water, including water temperature (28 ± 2°C), pH (7.0 ± 0.5), dissolved oxygen (8.64 ± 0.6 mg L^{-1}), and other water parameters including water hardness, concentrations of particulate matter, total organic carbon, unionized ammonia, nitrate, chlorine, and other metallic impurities, and chemical oxygen demand were estimated according to the standard protocols as prescribed in APHA (1998) guidelines to ensure the dilution in test medium never influence the test results. The chemical analysis ensured the water quality for the survival and growth of fish without any sign of stress, and care was taken not to exceed the limits specified in OECD guideline 203 throughout the experimental duration (OECD, 1992).

Chemicals

Acrylamide of 99.9% purity was purchased from Himedia Research Laboratories, Mumbai, India. All the other chemicals were incurred from local commercial sources.

Experimental design

The selection of test concentration of the toxicant (acrylamide) was based on the median lethal concentration or LC_{50}-96 h determined by probit analysis in *Anabas testudineus*, which was 132 µg L^{-1} concentration (Ligina *et al*., 2022). In the present study, one-tenth of the median lethal concentration of acrylamide was selected as a sublethal concentration i.e., 13.2 µg L^{-1}. After the acclimatization period, fish were randomly categorized into separate aquarium glass tanks (40 L; 30 cm width × 60 cm length × 30 cm depth), and the experiment on *A. testudineus* was conducted during its spawning phase in July and August. Acrylamide exposure was conducted under static conditions for the test duration, without renewal of the exposure solution during this period. From the acrylamide stock solution (1 mg/ml), 528 µl was added to each 40 L

experimental tank and thoroughly mixed to achieve a uniform sublethal concentration of 13.2 µg L^{-1}.

For the biochemical analysis in liver tissue, fish (N = 160) were used regardless of sex. Each experimental group consisted of 10 fish in replicates, totaling 20 fish per group, and the experiment was maintained for 1, 2, 3, and 4 days along with the respective control group for each acrylamide treatment. For the analysis of reproductive parameters (N = 160), each tank contained a total of 20 fish, with 10 males and 10 females per group. During the analysis of sperm indices and testicular steroidogenic enzyme activities, only the male fish were used, while the remaining 10 female fish were utilized for assessing female reproductive parameters, including oocyte measurement, fecundity, and steroidogenic enzyme activities. Each of these groups also maintained 20 control fish, with 10 males and 10 females per group.

Sample collection and tissue processing

At the end of every treatment period, fish were caught very carefully by using a small dip net, one at a time with the least disturbance. The body weights of the fish and liver weights, from the control and treatment groups, were recorded to evaluate specific growth rate, length-weight relationship, condition factor, and hepatosomatic index. A portion of liver was cleaned of debris and used for biochemical analysis, while the remaining portion was preserved in 10% buffered formalin for histological analysis.

Gonad tissues (ovary and testes) were excised carefully, weighed, and recorded to assess gonadosomatic index. Sperm collected from the control and treatment groups were used for the evaluation of sperm indices, and also the size of oocytes, and fecundity rate was measured and recorded as per the standard protocol. A part of gonadal tissues, both ovary and testes, were preserved in the 10% buffered formalin for histological analysis.

Specific growth rate, length-weight relationship and condition factor

After every treatment period, the length and weight of the fish captured from each group were recorded. The specific growth rate (SGR) of the fish was calculated by the formula as follows:

Log (Initial - Final body weight)/ No. of exposure days x 100.

The length-weight relationships of the fish after log transformation were determined by linear regression analysis as per the cube law proposed by Le Cren (1951).

$$W = aL^b$$

Where, W=Weight of fish (g), L is observed total length (cm), 'a' is the regression intercept and 'b' is the regression slope. The logarithmic transformation of the above formula is:

$$\text{Log } W = \log a + b \log L.$$

Then Fulton's condition factor (K) was calculated according to Htun-Han's (1978) equation as per the formula given below:

$$K = W \times 100 / L3$$

Where, W=weight of fish (g), L=Length of fish (cm).

Absolute and relative weights of liver and gonadal tissues

Liver and gonads (testes and ovary) collected from both control and treatment groups were weighed accurately to calculate the absolute and its respective relative weights. The relative weight of liver/ gonadal tissues or hepatosomatic index/ gonadosomatic index (HSI or GSI) was measured using the standard formula and expressed in percentage (King, 1995; Sulistyo *et al*., 2000).

HSI or GSI = (Tissue weights (g)/ Fish weight (g)) × 100.

Processing of liver tissue

Liver subcellular fractions such as cytosolic, mitochondrial, and microsomal fractions were prepared as described by Graham (2015). The liver tissues collected from both male and female fish, regardless of sex differences, were weighed, and chopped finely. Then a 20% (w/v)

homogenates were prepared in ice-cold 0.25 M sucrose with a Teflon homogenizer on crushed ice for a minute. Homogenates were centrifuged at 500 g for 15 min at 4 °C, the pellet contains nuclei, and the supernatant contained mitochondria, cytoplasm, and microsomal fractions. The supernatants obtained were centrifuged at 12,000 g for 20 min at 4 °C to obtain the mitochondrial pellets, which were resuspended in 0.25 M sucrose to get the mitochondrial fractions. The obtained supernatant was further centrifuged at 100,000 g for 60 min at 4 °C in Beckman Ultracentrifuge to obtain microsomal pellets and cytosolic fractions as the supernatant. The microsomal pellets were resuspended in 0.25 M sucrose to get the microsomal fractions. The obtained cytosolic and microsomal fractions were stored at -80 ºC until used for the biochemical analysis. Protein was estimated by the method of Lowry *et al.* (1951) in the subcellular fractions with bovine serum albumin as the standard.

Biochemical analysis in liver tissue

Assay of 7-ethoxyresorufin O-deethylase (*EROD*)

EROD activity was determined in the subcellular fractions of the liver tissue as described by Fernandes *et al*. (2002). Hepatic subcellular fractions obtained from the differential centrifugation were incubated with the reaction mixture containing 100 mM phosphate buffer, pH 7.4, 0.25 mM NADPH, and 4.15 µM 7-ethoxyresorufin. The reaction was stopped by adding ice-cold acetone, and absorbance was read at 537 nm against the blank. The standard curve was plotted using 7-ethoxyresorufin at different concentrations, and the activity is expressed as the amount of resorufin generated minute^{-1} mg protein^{-1}.

Assay of UDP-glucuronosyltransferase (*UGT*)

The assay of UDP-glucuronosyltransferase activity was determined in the hepatic subcellular fractions as described by Zhivkov (1970). The hepatic subcellular fractions were pretreated with 0.2% Triton X-100 on ice and the reaction was initiated by the addition of 81 µM *p*-nitrophenol in a shaking water bath for 30 min at 30 ºC. The reaction was stopped by the addition of 0.2 M ice-cold trichloroacetic acid, centrifuged at 800 *g* for 10 min, and alkalinized with 10 N potassium hydroxide. The absorbance was measured at 405 nm against the blank. The enzyme activity is expressed as the amount of *p*-nitrophenol generated minute^{-1} mg protein^{-1}.

Assay of glutathione S-transferase (*GST*)

Glutathione S-transferase activity was assayed in the hepatic subcellular fractions by the method described by Habig *et al*. (1974). Briefly, the reaction mixture contained potassium phosphate buffer (80 mM; pH 7.4), 1-chloro-2,4-dinitro-benzene (1mM), and reduced glutathione (1 mM) were incubated with the hepatic fractions at room temperature. The change in absorbance was recorded at 340 nm, and the enzyme activity is expressed as µM 1-chloro-2,4-dinitrobenzene conjugate formed minute^{-1} mg protein^{-1}.

Assay of gamma-glutamyl transpeptidase (*GGT*)

Gamma-glutamyl transpeptidase (GGT) was assayed in the hepatic subcellular fractions according to the method as described by Christiansen *et al.* (1998). The tissue samples were mixed with sodium hydroxide (60 mM, pH 7.9), glycylglycine (150 mM), and L-γ-glutamyl-3-carboxy-4-nitroanilide (6 mM). The change in absorbance was measured at 410 nm for 5 min using Shimadzu UV-Visible spectrophotometer and the enzyme activity is expressed as nmol *p*-nitroaniline liberated/ min/ mg protein.

Assay of flavin-containing monooxygenase (*FMO*)

Flavin-containing monooxygenase enzyme was assayed in the hepatic subcellular fractions as described by Rose (2002). Briefly, the reaction mixture consists of 2× tricine/ KOH buffer, pH 8.5 (500 µl), 2 mM dithiothreitol (DTT; 10 µl), 1 mM 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB; 60 µl), 10 mM NADPH (10 µl), and sample, which was made up to 1 ml total volume and incubated for 1 min, then absorbance was recorded for 2 min at 412 nm. Then 100 mM methimazole was added, mixed, and incubated for 1 min, and absorbance

was recorded for 2 min at 412 nm. The change in the absorbance was calculated, and expressed as nmol of nitro-5-thiobenzoate (TNB) oxidized/ min/ mg protein.

Assay of aspartate aminotransferase

The activity of aspartate aminotransferase was assayed in the hepatic subcellular fractions by the method described by Reitman and Frankel (1957). The reaction mixture containing aspartate (0.1 M) and 2-oxoglutarate (2 mM) dissolved in phosphate buffer (0.1 M; pH 7.4) was vortexed and incubated at 37 °C for 1 h. After incubation, 250 µl of 2,4-dinitrophenyl hydrazine was added and incubated at room temperature for 20 min. Finally, to stop the reaction, sodium hydroxide (0.1 N) was added, mixed, and incubated at room temperature for 10 min. The absorbance was read at 510 nm against the blank. A standard calibration was prepared by using different concentrations of sodium pyruvate. The results are expressed as µM glutamate formed mg protein^{-1}.

Assay of alanine aminotransferase

The activity of alanine aminotransferase was assayed in the hepatic subcellular fractions by the method described by Reitman and Frankel (1957). The reaction mixture containing DL-α-alanine (0.2 M) and 2-oxoglutarate (2 mM) dissolved in phosphate buffer (0.1 M; pH 7.4) was vortexed and incubated at 37 °C for 1 h. After incubation, 250 µl of 2,4-dinitrophenyl hydrazine was added and incubated at room temperature for 20 min. Finally, to stop the reaction, sodium hydroxide (0.4 N) was added, mixed, and incubated at room temperature for 10 min. The absorbance was read at 510 nm against the blank. A standard calibration curve was prepared by using different concentrations of sodium pyruvate. The results are expressed as µM pyruvate formed mg protein^{-1}.

Determination of sperm indices

Male fish were gently held without stress, and the catheters were inserted into the urogenital tract for the collection of uncontaminated milt. The collected sperm were transferred to a small Petri plate and processed for assessing sperm viability, sperm motility, and sperm count.

Sperm viability

Sperm suspension (20 µl) was mixed with an equal volume of 0.05% Eosin-Y. After a minute of incubation at room temperature, slides were observed under light microscope at 100X magnification for live and dead sperm and morphological abnormalities were also noted. Viable sperm remained colourless while non-viable sperm stained in Eosin appeared pink (Wyrobek *et al*., 1983). Sperm were counted in both control and treatment groups and viability is expressed in percentage (Eliasson, 1977).

Sperm motility

Collected sperm was diluted with diluent (1:50) and placed on a Neubauer-type haemocytometer prepositioned on the microscope stage at 40X magnification. Sperm swimming activity was recorded for 2 min, and counted for motile and non-motile sperm. First non-motile sperm was counted followed by motile sperm and the motility is expressed as a percentage of motile sperm of the total sperm counted.

Sperm count

Prior to determination of spermatozoa concentration, sperm was diluted 100 times with an immobilizing solution, Tris-Sucrose solution (10: 100 mM at pH 8.5). A droplet of diluted sperm suspension was placed on a counting chamber of the hemocytometer (depth 0.1 mm) with a coverslip. Sperms were counted by using a microscope at 40X magnification and sperm concentration was calculated according to method as described by Caille *et al*. (2006). Spermatozoa concentration is expressed as 10^9 spermatozoa/ ml.

Determination of oocyte diameter

Prepared histology slides were placed on the microscope stage and observed under low magnification to locate the ovarian follicles containing oocytes. Then switched to a higher magnification objective (40x or 100x) to measure

the diameters of individual oocytes using Image J software (v. 1.49b, National Institute of Health, http://imagej.nih.gov/), which measured the diameter of all oocytes present in the image (Kennedy, 2018). The well-preserved, round oocytes were chosen for measurement, and those observed as oblique or irregularly shaped are avoided. After measuring 20 oocytes from each fish, the average diameter was calculated by adding the individual oocyte diameters and dividing by the number of oocytes measured.

Rate of fish fecundity

The fecundity was estimated by the gravimetric method using the ovaries from spawning stage. Briefly, after 48 h of preservation in modified Gilson's fluid, the eggs were completely liberated, washed thoroughly, and spread on the blotting paper to air dry, and counted using the sub-sample by the following equation (Grimes and Huntsman, 1980):

$$F = nG/g$$

Where, F = fecundity; n = number of eggs in the sub-sample; G = total weight of the ovaries; g = weight of the sub-sample.

Assay of gonadal steroidogenic enzymes

The activities of 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD) were measured in the gonads according to Bergmeyer (1974) with minor modifications. A part of gonadal tissues were cleaned of debris, and homogenate (1% w/v) was prepared in 5 mM potassium phosphate buffer containing 1 mM EDTA and 20% spectroscopic grade glycerol and supernatant was collected after centrifugation at 10,000*g* for 10 min at 4 ºC.

For the assay of 3β-HSD, an aliquot of the supernatant was mixed with pyrophosphate buffer (100 mM), NAD (0.5 mM), and dehydroisoandrosterone (0.1 mM), and absorbance was read immediately at 340 nm for 5 min at 30 s interval in a spectrophotometer against the blank. The units are expressed in µmol of NAD reduced/ min/ mg protein.

The assay mixture of 17β-HSD contained pyrophosphate buffer (100 mM), NADPH (0.5 mM), and 1,4-androstenedine-3,17-dione (0.8 mM) was read at 340 nm immediately after the addition of sample at 30 s interval for 5 min in a spectrophotometer against the blank. The enzyme activity is expressed as µmol of NADP formed/ min/ mg protein.

Histopathological analysis

Liver and gonadal tissues fixed in 10% neutral buffered formalin for 24 h were dehydrated in a series of ascending grades of alcohol, then cleared in xylene until they became translucent. The tissues were embedded in molten paraffin wax for an hour for the complete impregnation to make the tissue blocks. Sections were made using rotary microtome at 4 to 6 µm thickness, which was double stained with hematoxylin and eosin, and finally mounted in DPX mountant (Roberts and Smail 2001). Triplicate slides of tissues were examined under the light microscope at 40X magnifications. Photomicrographs were taken using a Canon shot camera fitted to the Leica CTR6 LED Trinocular Research Microscope.

Statistical analyses

Statistical analyses were performed using International Business Machine Corporation-Statistical Package for the Social Sciences (IBM-SPSS) software, version 24.0. Normal distribution and homogeneity of variance were ensured before conducting the analysis. Statistical analysis was performed using a one-way analysis of variance (ANOVA). For the comparison of equal variances, Duncan's Multiple Range Test was done as a post-hoc test. Differences were considered significant at $P<0.05$ against the control groups, which were denoted as asterisks (*) in the Figures. Data are represented as Mean ± Standard Deviation using 20 fish per group for biochemical analysis, while 10 fish per group were used for reproductive parameters.

Table 1: Specific growth rate, length-weight relationship and condition factor of the fish, *Anabas testudineus* after acrylamide exposure for 4 days (n = 20, i.e., 10/ group)

	Experiment groups	Specific growth rate	Logarithmic equation (Log W = log a + b log L)	Correlation coefficient 'r'	Coefficient of determination 'r²'	Condition factor 'K'
Male	Control	0.7052	Log W = log 9.222 + 1.616 log L	0.933	0.8717	2.396
	1 d	1.0312	Log W = log 8.635 + 0.954 log L	0.795	0.6335	1.685
	2 d	1.5452	Log W = log 7.979 + 0.068 log L	0.529	0.0028	1.452
	3 d	0.6418	Log W = log 9.727 + 0.506 log L	0.280	0.0786	1.328
	4 d	0.5282	Log W = log 8.173 + 0.458 log L	0.293	0.0862	1.150
Female	Control	0.6816	Log W = log 7.916 + 1.709 log L	0.362	0.1314	2.362
	1 d	0.3073	Log W = log 6.321 + 0.177 log L	0.795	0.0065	2.171
	2 d	0.2198	Log W = log 8.646 + 0.972 log L	0.187	0.1836	1.686
	3 d	0.1690	Log W = log 15.77 + 0.327 log L	0.717	0.0515	1.630
	4 d	0.9447	Log W = log 9.945 + 0.582 log L	0.498	0.2487	1.403

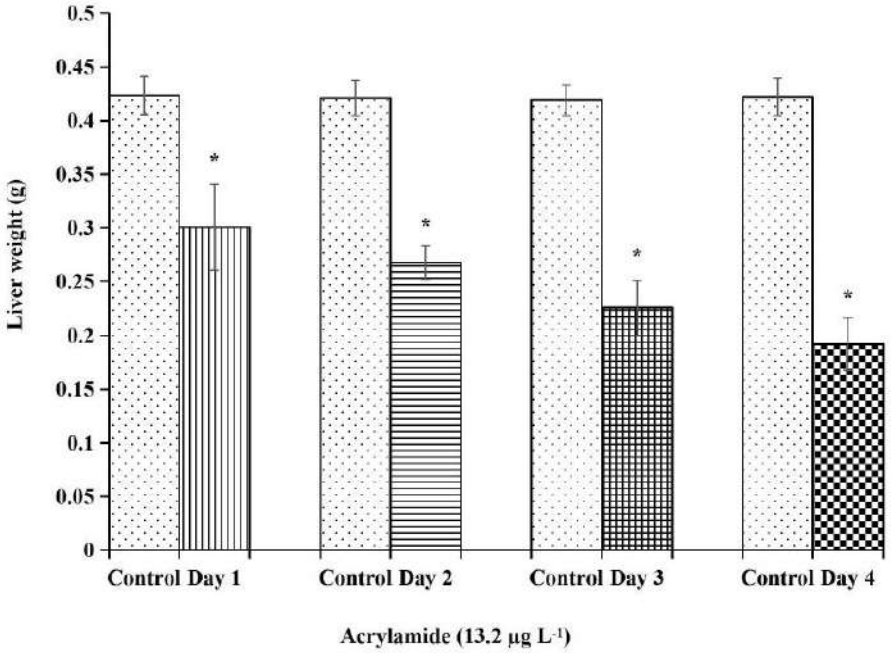

Fig. 1: Effect of acrylamide on the weight of liver tissues in the fish, *Anabas testudineus* (Mean ± SD; n = 20 fish/ group). Asterisks (*) denotes significance at P<0.05 against control group.

Results

Specific growth rate, length-weight relationship, and condition factor

Anabas testudineus subjected to acrylamide exposure at a sublethal concentration of 13.2 µg L^{-1} over a 4-day period exhibited noteworthy alterations in their specific growth rate, as well as their condition factor. Moreover, a decline was observed in the length-weight relationship of bothmale and female fish, a comparison being made against their corresponding control groups (Table 1). The specific growth rate of fish exposed to acrylamide demonstrated a distinct variation when contrasted with the control groups (Fig. 1). The length-weight relationship, in conjunction with the condition factor (K), displayed a negative allometric growth pattern in acrylamide-exposed

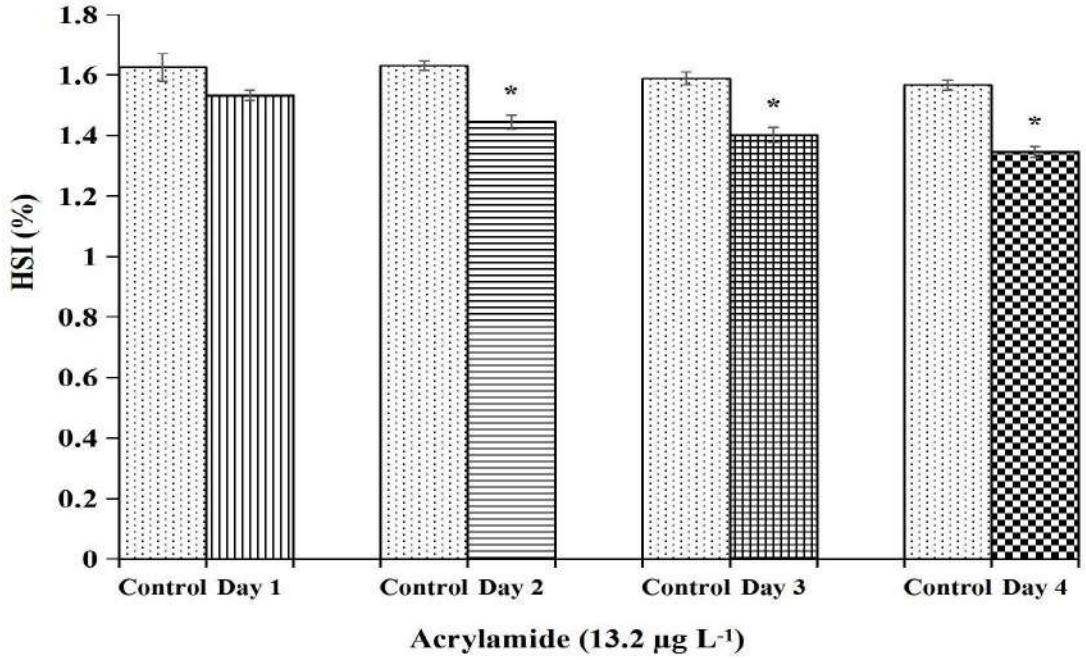

Fig. 2: Effect of acrylamide on the hepatosomatic index of the fish, *Anabas testudineus* (Mean ± SD; n = 20 fish/ group). Asterisks (*) denotes significance at P<0.05 against control group.

fish. This was manifested in a significant (P<0.05) reduction of the condition factor or K value, wherein the value declined from 2.396 to 1.150 in male fish, and decreased from 2.362 to 1.403 in female fish, corresponding to an extended exposure duration (Table 1).

Absolute and relative weights of liver and gonadal tissues

The sublethal exposure of fish to acrylamide resulted in a statistically significant (P<0.05) decline in both the absolute and relative weights, as indicated by the hepatosomatic index of liver tissues (Fig. 2). This reduction followed a time-dependent pattern in comparison to the respective control groups (Table 2). Fish exposed to sublethal concentration of acrylamide showed a significant (P<0.05) reduction in the absolute and relative weights of testis and ovary, which was duration-dependent when compared to the corresponding control groups (Table 2).

Hepatic biotransformation enzymes

The analysis of phase I and phase II enzymes involved in hepatic biotransformation in cytosolic, mitochondrial, and microsomal fractions showed minimal variation in the control groups for days 1, 2, 3, and 4. Therefore, the control values from day 4 were used as representative values for comparison with all treatment groups in each subcellular fraction. Hence separate control values for each exposure duration were not presented.

Exposure of fish to acrylamide at a sublethal concentration elicited a statistically significant (P<0.05) reduction in the activity of ethoxy-resorufin-O-deethylase (EROD), while UDP-glucuronosyl transferase (UGT) activity increased in all subcellular compartments of hepatocytes (Figs. 3, 4). Acrylamide exposure decreased the activity of glutathione S-transferase(GST) (Fig. 5) whereas elevated the gamma-glutamyl trans-peptidase (GGT) activity in hepatic subcellular fractions (Fig. 6). The activity of FMO enzyme was found to decline in all subcellular fractions of liver tissues (Fig. 7). Liver biomarker enzymes, alanine (ALT) and aspartate aminotransferase (AST), displayed a significant (P<0.05) increase across all subcellular fractions of liver tissues, following a

Table 2: Effect of acrylamide on the absolute and relative weights of gonads in the fish, *Anabas testudineus* (Mean ± SD; n = 20, i.e., 10/ group; Asterisks (*) represent significance at P<0.05 against control group)

Gonadal weights	Experiment groups	Absolute weight (g)	Relative weight (GSI in %)
Testis	Control	0.236 ± 0.034	0.879 ± 0.052
	Day 1	0.158 ± 0.021*	0.745 ± 0.072*
	Control	0.233 ± 0.022	0.865 ± 0.047
	Day 2	0.139 ± 0.016*	0.701 ± 0.053*
	Control	0.231 ± 0.028	0.873 ± 0.049
	Day 3	0.118 ± 0.013*	0.688 ± 0.038*
	Control	0.239 ± 0.029	0.861 ± 0.049
	Day 4	0.102 ± 0.011*	0.636 ± 0.045*
Ovary	Control	3.640 ± 0.086	12.96 ± 0.91
	Day 1	2.487 ± 0.254*	09.57 ± 0.80*
	Control	3.611 ± 0.075	11.99 ± 0.89
	Day 2	1.587 ± 0.167*	07.02 ± 0.45*
	Control	3.682 ± 0.081	13.01 ± 0.88
	Day 3	1.351 ± 0.071*	06.22 ± 0.36*
	Control	3.594 ± 0.077	12.68 ± 0.89
	Day 4	1.177 ± 0.040*	06.44 ± 0.28*

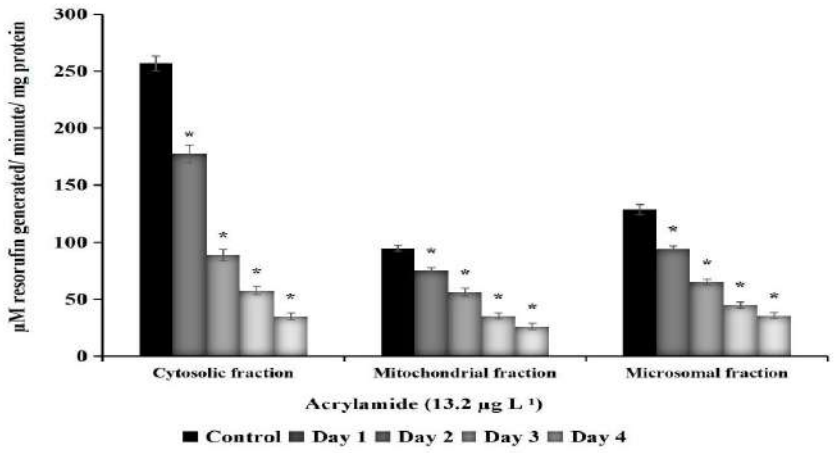

Fig. 3: Effect of acrylamide on the activity of ethoxyresorufin-O-deethylase (EROD) enzyme in subcellular fractions of liver tissues in the fish, *Anabas testudineus* (Mean ± SD; n = 20 fish/ group). Asterisks (*) denotes significance at P<0.05 against control group.

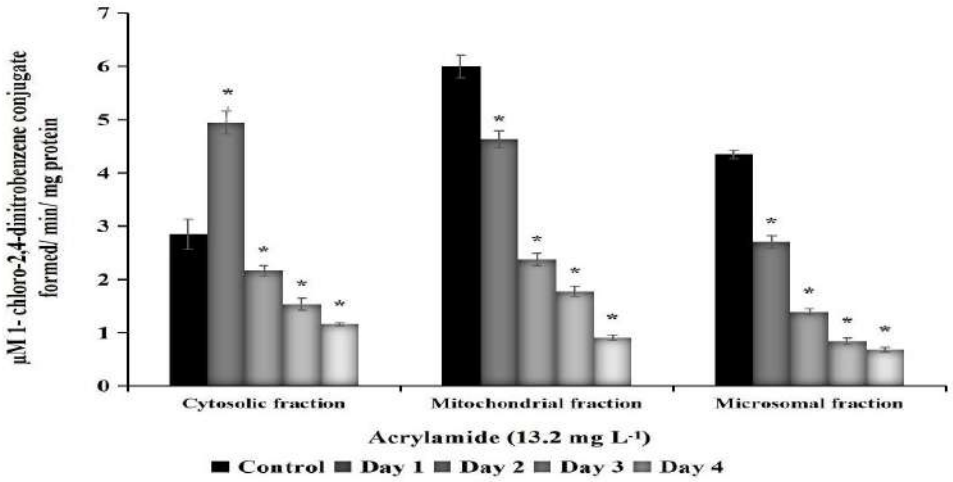

Fig. 4: Effect of acrylamide on the activity of glutathione-S-transferase enzyme in subcellular fractions of liver tissues in the fish, *Anabas testudineus* (Mean ± SD; n = 20 fish/ group). Asterisks (*) denotes significance at P<0.05 against control group.

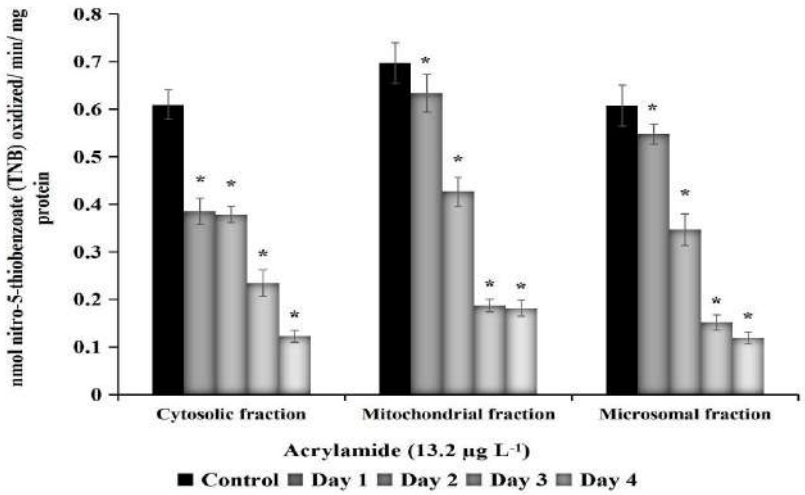

Fig. 5: Effect of acrylamide on the activity of flavin-containing monooxygenase enzyme in subcellular fractions of liver tissues in the fish, *Anabas testudineus* (Mean ± SD; n = 20 fish/ group). Asterisks (*) denotes significance at P<0.05 against control group.

time-dependent trend in comparison to the respective control groups (Figs. 8, 9).

Reproductive parameters

The exposure of fish to acrylamide led to a significant (P<0.05) decrease in sperm viability over the course of treatment from day 1 to day 4 than the control group, where the sperm viability dropped from 21.8% to 29.89% in a time-dependent manner (Fig. 10). The fish exhibited a gradual decline in sperm motility after 2 days of exposure to acrylamide. The reduction in sperm motility continued over time, culminating in a 10% decrease by the end of the 4-day acrylamide exposure period (Fig. 11). A significant (P<0.05) decrease of 2 to 3 million sperm count was observed as a consequence of acrylamide exposure, and this reduction progressed in a time-dependent manner (Fig. 12). Following exposure

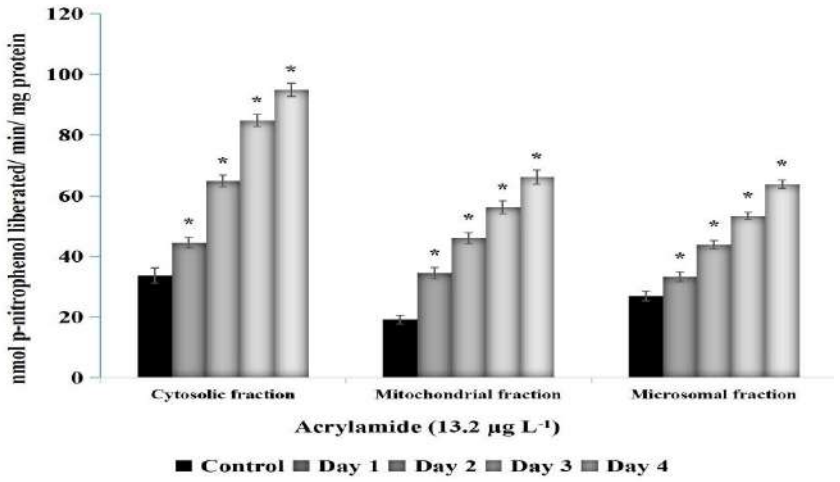

Fig. 6: Effect of acrylamide on the activity of UDP-glucuronosyl transferase (UGT) enzyme in subcellular fractions of liver tissues in the fish, *Anabas testudineus* (Mean ± SD; n = 20 fish/ group). Asterisks (*) denotes significance at P<0.05 against control group.

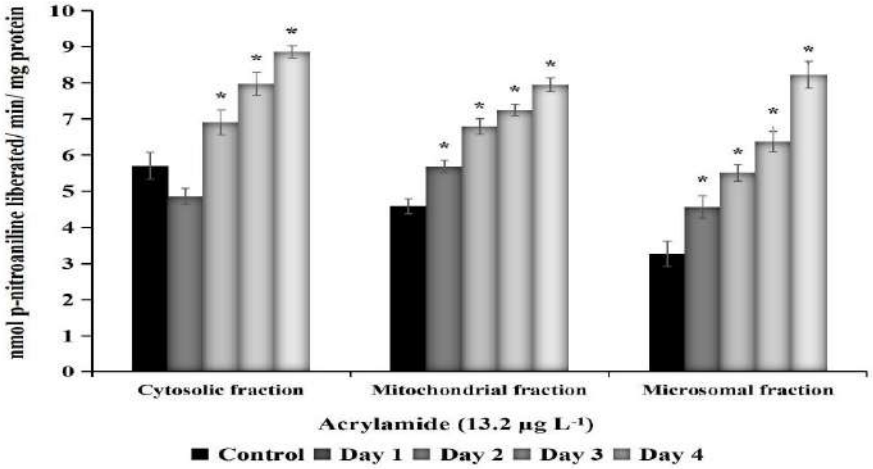

Fig. 7: Effect of acrylamide on the activity of gamma-glutamyl transpeptidase enzyme in subcellular fractions of liver tissues in the fish, *Anabas testudineus* (Mean ± SD; n = 20 fish/ group). Asterisks (*) denotes significance at P<0.05 against control group.

to acrylamide, there was an observable and statistically significant (P<0.05) reduction in the diameter of fish oocytes. This reduction became notably pronounced after 2 days of exposure, and continued to regress significantly by the 4-day acrylamide treatment (Fig. 13). Similarly, the fecundity rate of the fish underwent a substantial decline, also exhibiting statistical significance (P<0.05), as evident across the treatment period, with the fecundity rate decreasing from 10.09% on day 1 to 55.95% on day 4 of acrylamide exposure (Fig. 14). In the current investigation, exposure to acrylamide yielded notable reductions in the activities of testicular and ovarian steroidogenic enzymes (Fig. 15), following a progressive temporal pattern.

Histology of liver tissues

Anabas testudineus, subjected to acrylamide exposure at a concentration of 13.2 µg L^{-1}, exhibited conspicuous histological alterations within liver tissues (Fig. 16). The histological

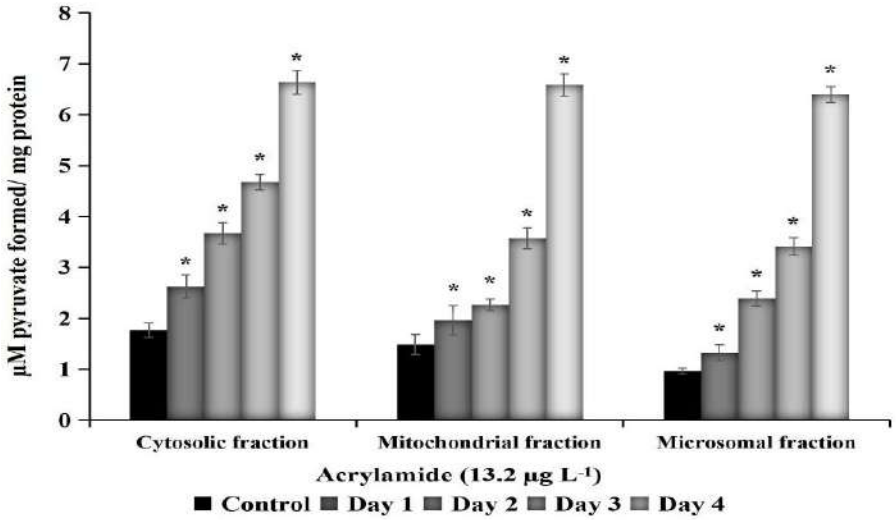

Fig. 8: Effect of acrylamide on the activity of alanine aminotransferse enzyme in subcellular fractions of liver tissues in the fish, *Anabas testudineus* (Mean ± SD; n = 20 fish/ group). Asterisks (*) denotes significance at P<0.05 against control group.

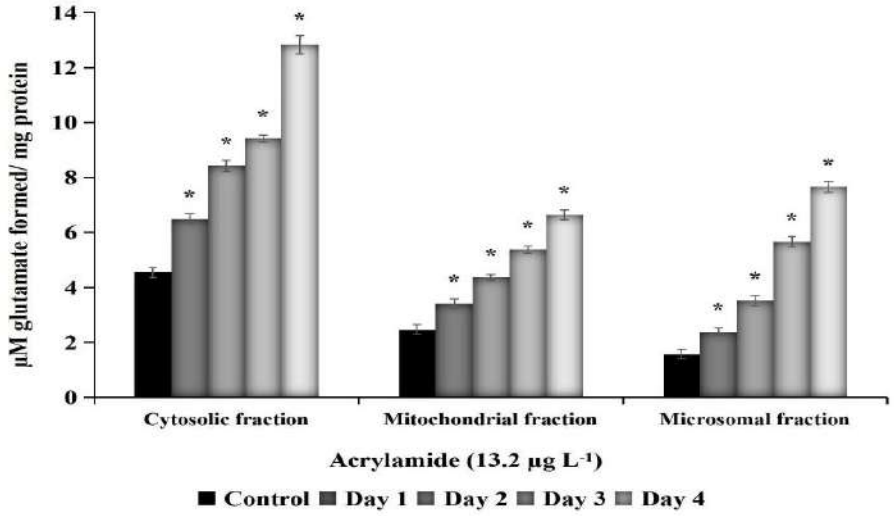

Fig. 9: Effect of acrylamide on the activity of aspartate aminotransferase enzyme in subcellular fractions of liver tissues in the fish, *Anabas testudineus* (Mean ± SD; n = 20 fish/ group). Asterisks (*) denotes significance at P<0.05 against control group.

architecture of the control liver displayed normalcy, characterized by homogeneous cytoplasm and prominent nuclei (Fig. 16a). Upon sublethal exposure to acrylamide for a single day, the histological manifestations included the aggregation of melanomacrophages and infiltration of erythrocytes (Fig. 16b). Furthermore, following a 2-day exposure to acrylamide, hepatocytes exhibited signs of degeneration (Fig. 16c). On day 3 of acrylamide exposure, vacuolization became a notable event (Fig. 16d). Subsequently, a 4-day exposure to acrylamide resulted in the maximal manifestation of melanomacrophage aggregation when compared to the other exposure groups (Fig. 16e).

Histology of gonadal tissues

Anabas testudineus exposed to acrylamide

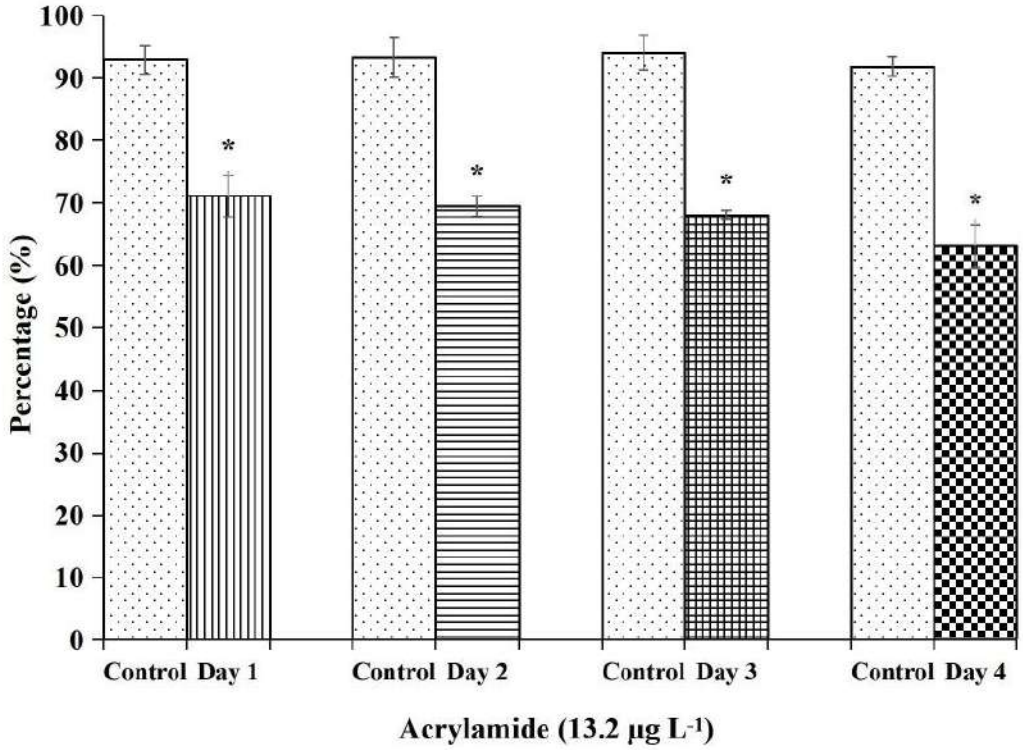

Fig. 10: Effect of acrylamide on the sperm viability of the fish, *Anabas testudineus* (Mean ± SD; n = 10 fish/ group). Asterisks (*) denotes significance at P<0.05 against control group.

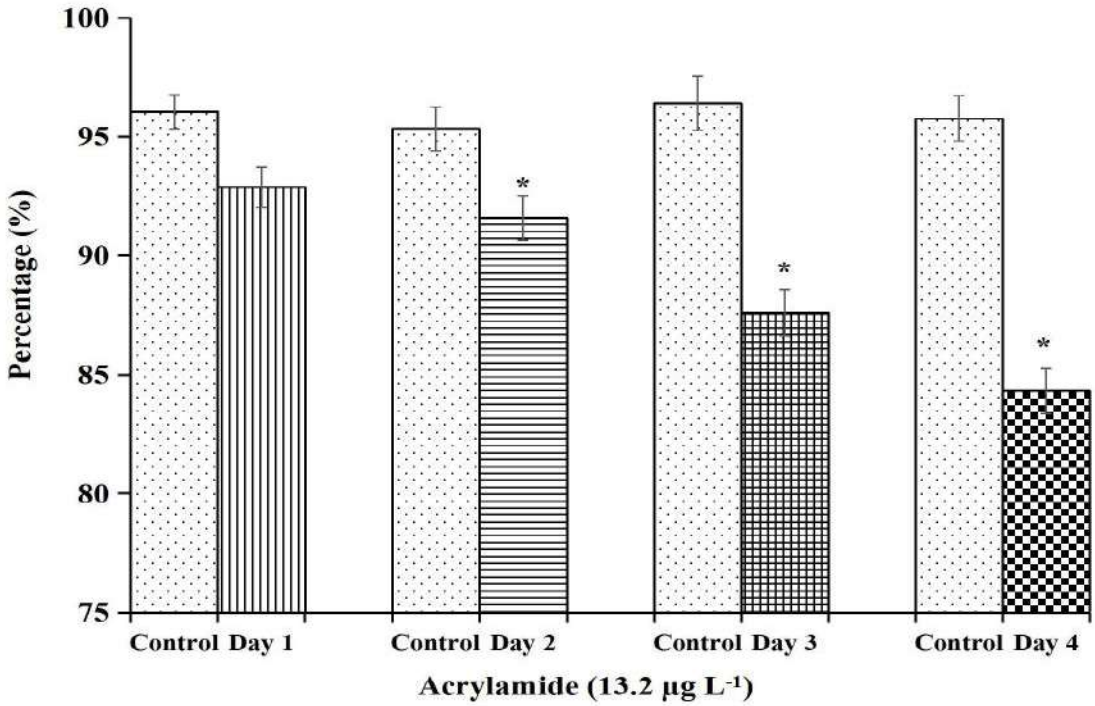

Fig. 11: Effect of acrylamide on the sperm motility of the fish, *Anabas testudineus* (Mean ± SD; n = 10 fish/ group). Asterisks (*) denotes significance at P<0.05 against control group.

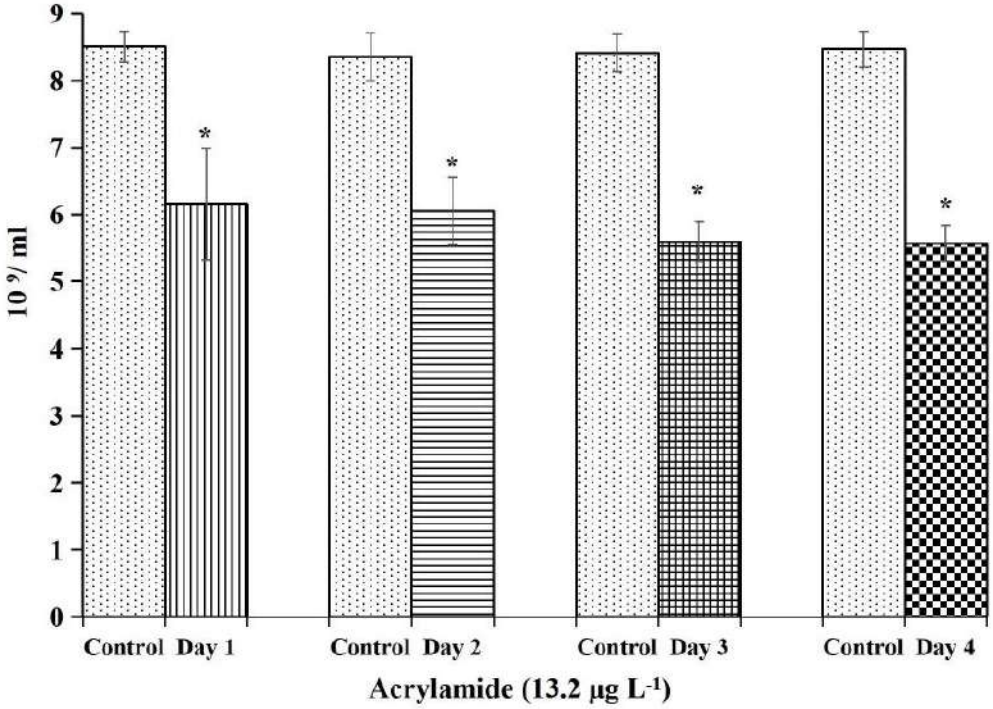

Fig. 12: Effect of acrylamide on the sperm count of the fish, *Anabas testudineus* (Mean ± SD; n = 10 fish/ group). Asterisks (*) denotes significance at P<0.05 against control group.

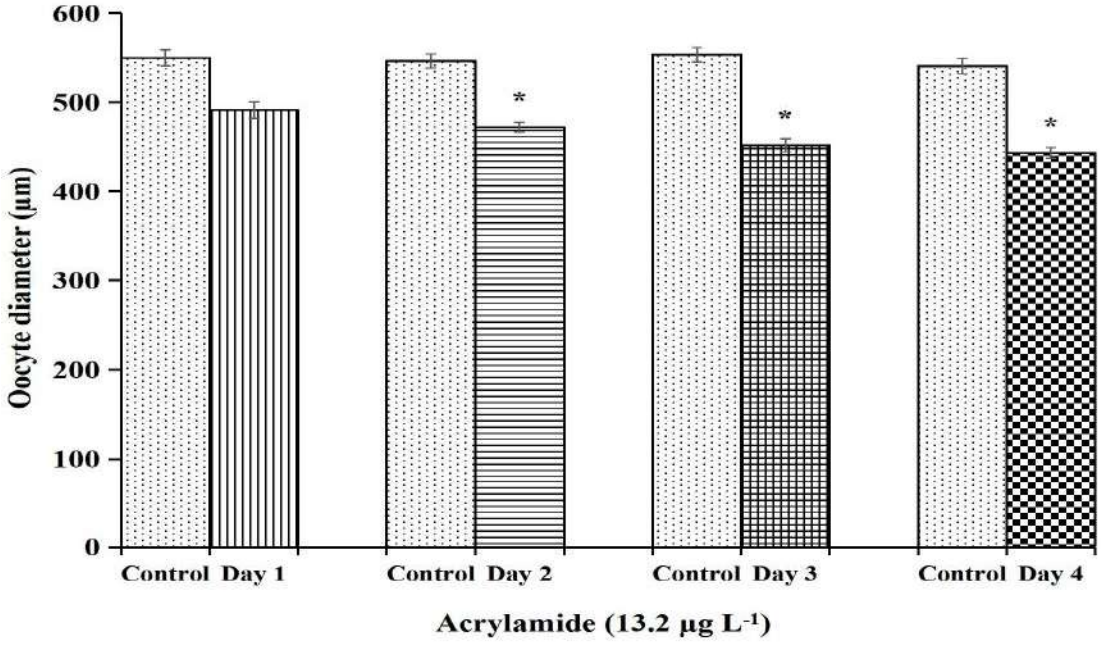

Fig. 13: Effect of acrylamide on the oocyte diameter in the fish, *Anabas testudineus* (Mean ± SD; n = 10 fish/ group). Asterisks (*) denotes significance at P<0.05 against control group.

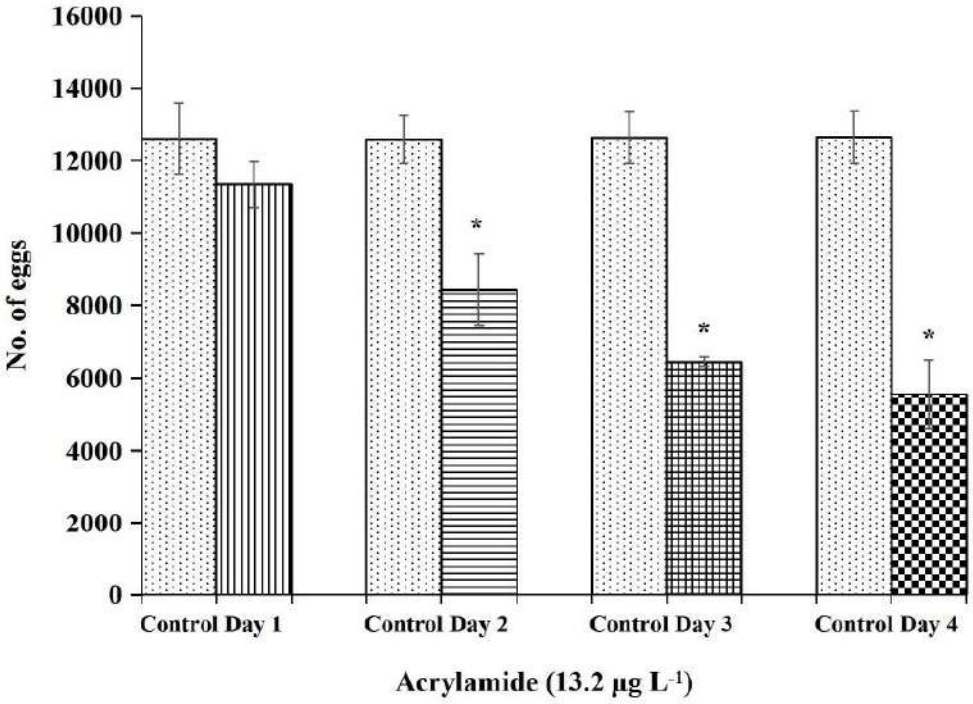

Fig. 14: Effect of acrylamide on the rate of fecundity in the female fish, *Anabas testudineus* (Mean ± SD; n = 10 fish/ group). Asterisks (*) denotes significance at P<0.05 against control group.

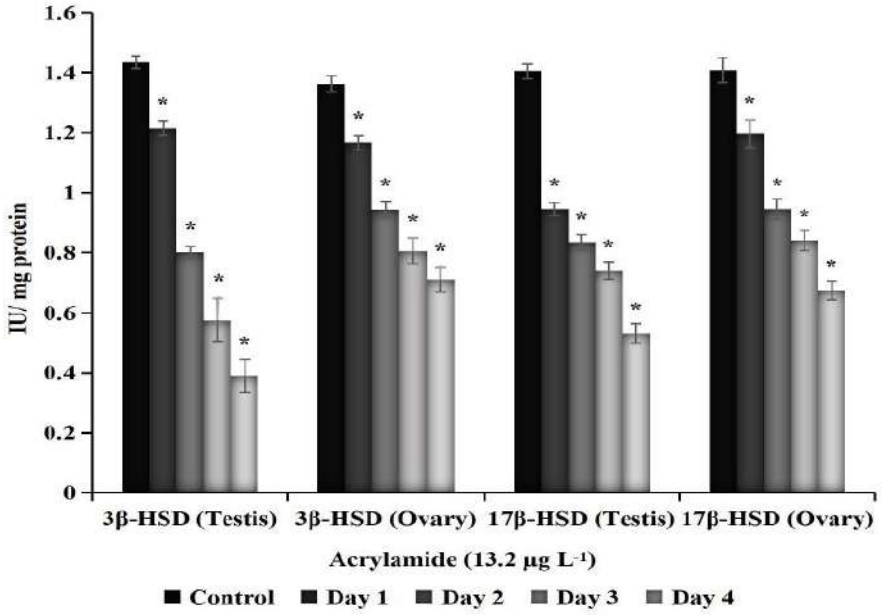

Fig. 15: Effect of acrylamide on the activities of gonadal steroidogenic enzymes in the fish, *Anabas testudineus* (Mean ± SD; n = 10 fish/ group/sex). Asterisks (*) denotes significance at P<0.05 against control group.

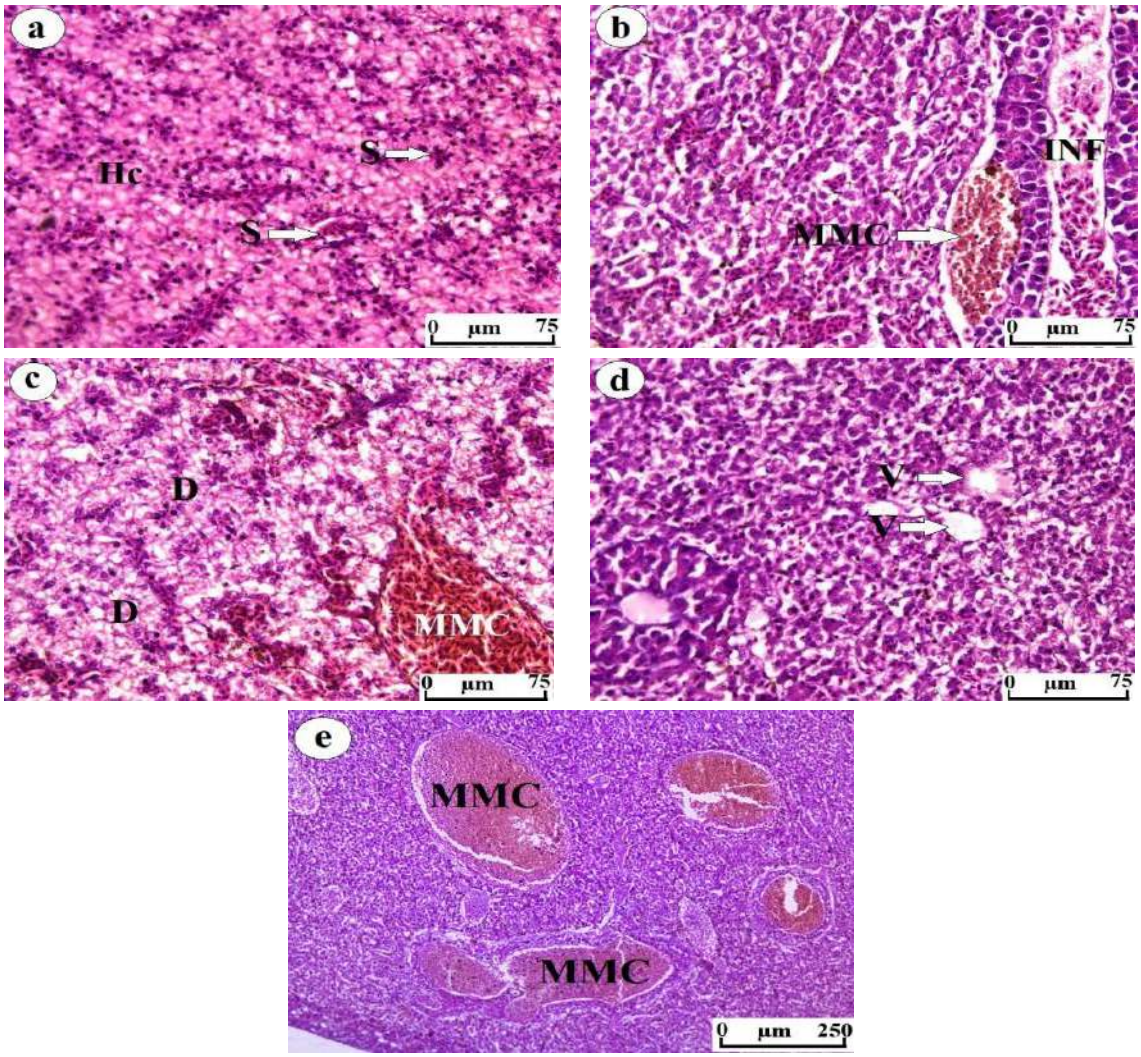

Fig. 16: Photomicrographs showing liver of *Anabas testudineus* exposed to acrylamide. **a -** Control (Hc - Hepatocytes, S – Sinusoids); **b -** Acrylamide-exposed group (13.2 µg L^{-1}) for 1 day (MMC - melanomacrophage aggregation, INF - erythrocyte infiltration); **c -** Acrylamide-exposed group for 2 day (MMC - melanomacrophage aggregation, D - degenerated hepatocytes); **d -** Acrylamide-exposed group for 3 day (V – Vacuolization); **e -** Acrylamide-exposed group for 4 day (MMC - melanomacrophage aggregation).

exhibited pronounced histological aberrations within the testicular tissue, as evidenced in Figure 17. The histological integrity of the control testes appeared unremarkable, featuring organized stages of spermatogenesis orderly arranged within compact seminiferous tubules (Fig. 17a). Sublethal exposure to acrylamide for one day caused severe consequences, including the depletion of spermatozoa (Fig. 17b). On day 2 of acrylamide exposure, the testicular architecture became distorted, accompanied by a notable loss of germ cells (Fig. 17c). Prolonged exposure, extending to 3 days, exacerbated these effects, leading to the loss of germ cells, thickening of seminiferous tubule epithelium, and the emergence of interstitial fibrosis (Fig. 17d). Exposure to acrylamide for 4 days showed severe histological lesions including a substantial loss of germ cells, thickened seminiferous tubule epithelium, and interstitial fibrosis. Remarkably, day 4 group exhibited more severe lesions in comparison to other exposure durations (Fig.17e).

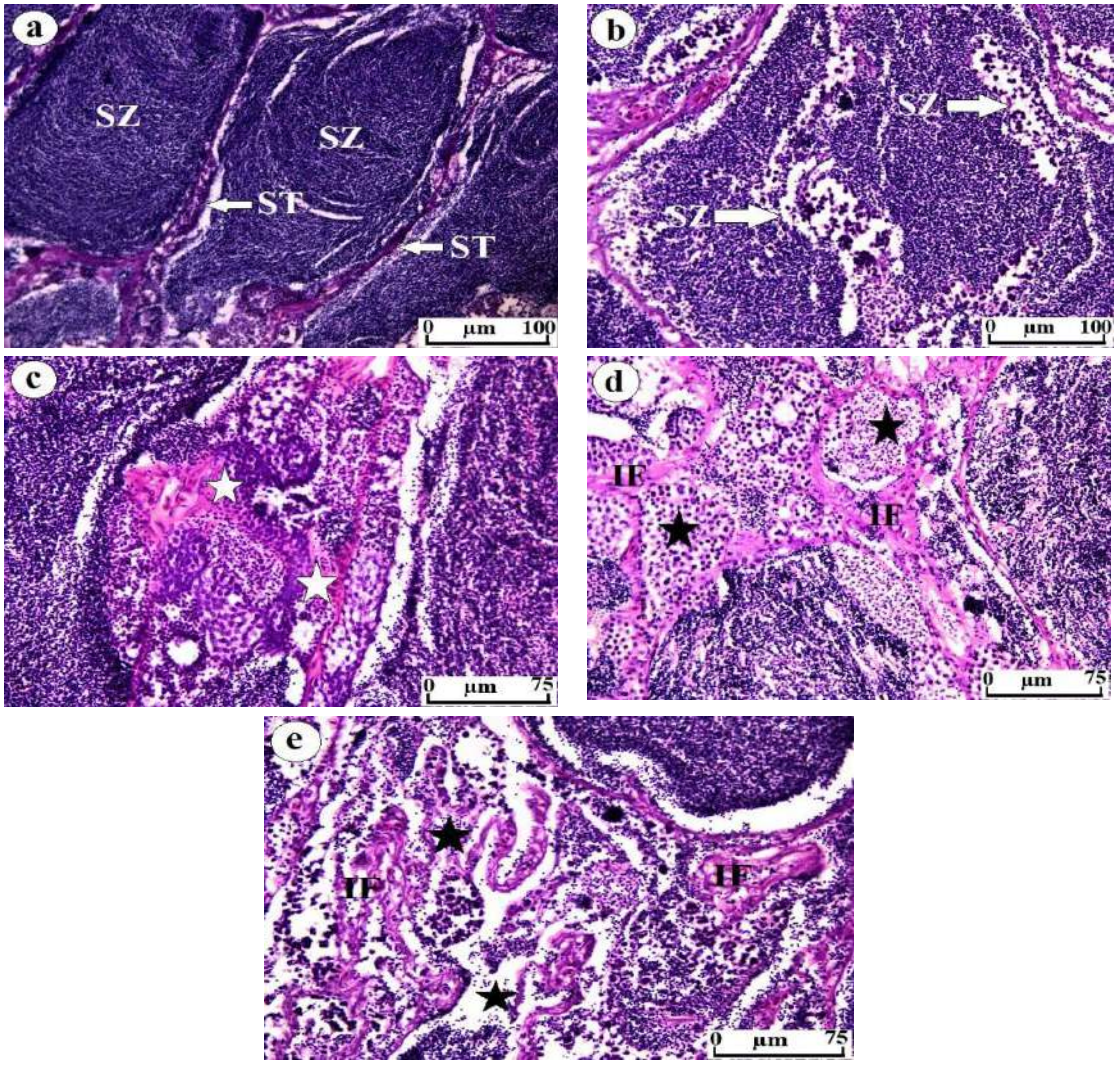

Fig. 17: Photomicrographs showing testis of *Anabas testudineus.* **a -** Control (ST - Seminiferous tubule, SZ – Spermatozoa); **b -** Acrylamide-exposed group (13.2 µg L^{-1}) for 1 day (Arrow - loss of spermatozoa); **c -** Acrylamide-exposed group for 2 day (Asterisks (*) - Germ cell loss); **d -** Acrylamide-exposed group for 3 day (Asterisks (*) - Germ cell loss, IF - Interstitial fibrosis); **e -** Acrylamide-exposed group for 4 day (Asterisks (*) - Germ cell loss, IF - Interstitial fibrosis).

The ovaries of control groups revealed mature oocytes characterized by centrally positioned prominent oil vacuoles, alongside the presence of loosely arranged spherical eggs (Fig. 18a). Exposure of fish to sublethal concentrations of acrylamide elicited noteworthy histological alterations in ovarian tissue. Specifically, within the exposure duration of 1 to 2 days, instances of atretic follicles and epithelial blebbing were more prominent (Figs. 18b, c). Over a 3-day acrylamide exposure period, ovarian tissue displayed signs of atretic follicles and oocyte degeneration (Fig. 18d). In 4-day acrylamide exposure group there was a remarkable oocyte degeneration alongwith epithelial blebbing within the follicular region (Fig. 18e).

Discussion

Acrylamide is one of the noteworthy organic pollutants exhibiting profound neurotoxic consequences, and it functions as a monomeric unit in the synthesis of polyacrylamide, thereby warranting focused attention on the consequential implications in aquatic life (Tareke *et al*., 2002).

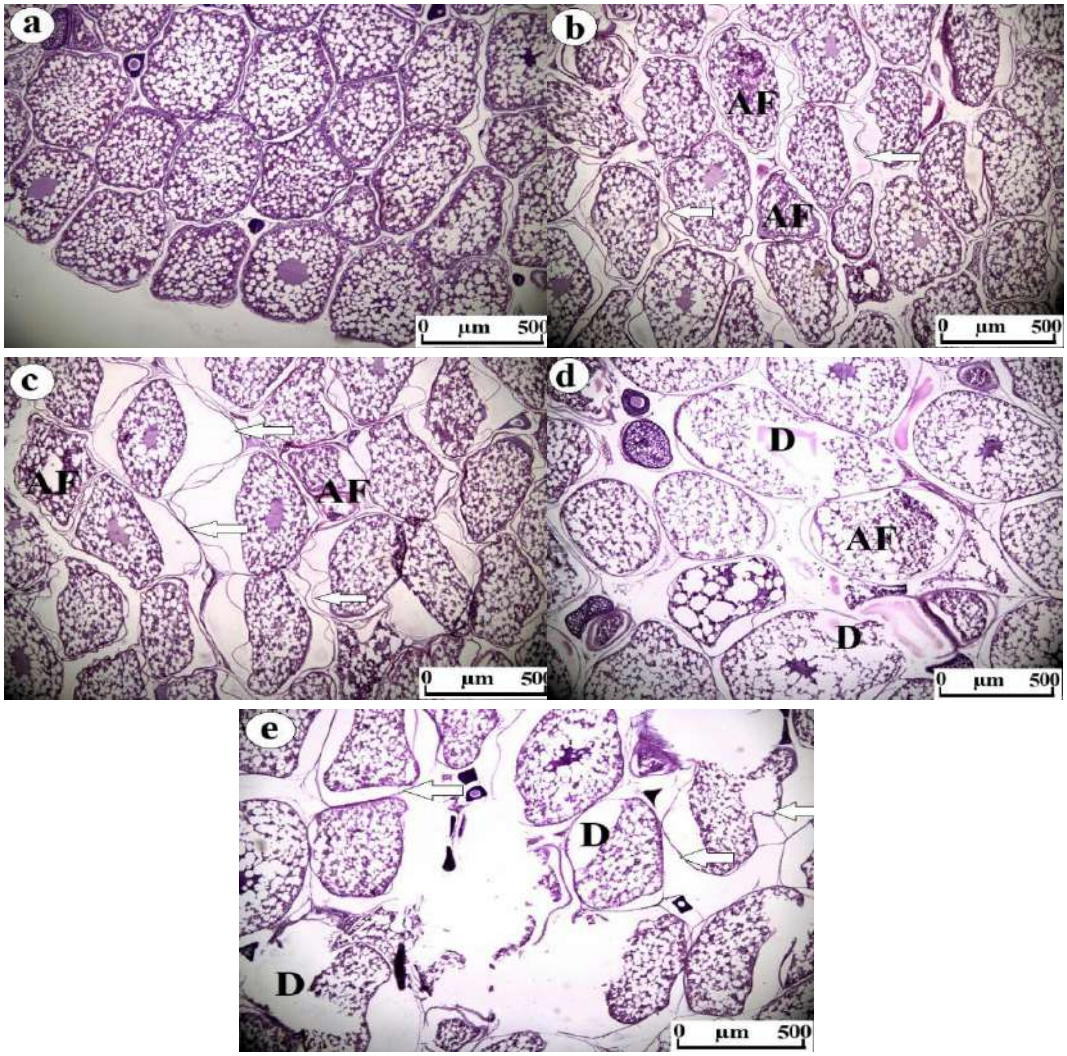

Fig. 18: Photomicrographs showing ovary of *Anabas testudineus.* **a -** Control; **b and c-** Acrylamide-exposed group (13.2 µg L^{-1}) for 1 and 2 day (AF - Atretic follicle, Arrow - Blebbing of follicular epithelium); **d -** Acrylamide-exposed group for 3 day (AF - Atretic follicle, D - Oocyte degeneration); **e -** Acrylamide-exposed group for 4 day (D - Oocyte degeneration, Arrow - Blebbing of follicular epithelium).

The current investigation assessed the hepatic biotransformation processes and reproductive impairment on the freshwater fish *Anabas testudineus* after acrylamide exposure. According to our previous findings (Ligina *et al*., 2022), the 96 h median lethal concentration (LC_{50}) for acrylamide was determined to be 132 µg L^{-1}. Fish subjected to a sublethal concentration of 13.2 µg L^{-1} for varying durations such as 1, 2, 3, and 4 days exhibited a series of stress responses within the fish.

The study of specific growth rate, length-weight relationship, and condition factor in fish provides valuable insights into the overall health, growth dynamics, and physiological condition of fish populations in aquatic environments. These parameters are fundamental tools used by ecologists, and environmental researchers to assess the well-being of fish species and the health of their habitats. *Anabas testudineus* subjected to acrylamide exposure at a sublethal concentration of 13.2 µg L^{-1} over a 4-day period exhibited noteworthy alterations in their specific growth rate, as well as their condition factor. Moreover, a

decline was observed in the length-weight relationship of both male and female fish, a comparison being made against their corresponding control groups. The specific growth rate of fish exposed to acrylamide demonstrated a distinct variation when compared with the control groups. Specific growth rate is a measure used to understand how fast an individual fish is growing relative to its initial size (Elliott and Hurley, 1995). It provides essential information about the growth performance and efficiency of fish populations in response to various environmental factors, such as food availability, temperature, and water quality (Bolger and Connoly, 1989). In the present study, the variation in the specific growth rate of fish demonstrated poor water quality and induction of stress due to the exposure of acrylamide.

The length-weight relationship is a mathematical expression that describes the relationship between the length and weight of individual fish. It is an important tool for estimating the overall body condition and health of fish (Froese, 2006). It is also used to estimate the size of fish from their weight, which is valuable in population assessments, stock monitoring, and evaluating the health of fish populations. The length-weight relationship, in conjunction with the condition factor (K), displayed a negative allometric growth pattern in acrylamide-exposed fish. This was manifested in a significant ($P<0.05$) reduction of the condition factor or K value, wherein the value declined from 2.396 to 1.150 in male fish, and decreased from 2.362 to 1.403 in female fish. Alteration in the length-weight relationship after acrylamide exposure indicated shifts in the energy allocation in the fish *Anabas testudineus* due to the toxicant exposure.

The condition factor provides information about the energy reserves of the fish, nutritional status, and overall physiological condition (Schloesser and Fabrizio, 2017). Alterations in the condition factor indicated the exposure to acrylamide affected the fish health status. Similarly, in another study, exposure to triclosan at sublethal concentration has been shown to cause negative allometric growth in the fish *Anabas testudineus* (Neetha *et al*., 2022).

The assessment of acrylamide-induced gonadal toxicity in *Anabas testudineus* was carried out through the analysis of gonad absolute weights (testis and ovary) as well as the determination of gonadosomatic index (GSI), representing relative gonad weights. The observed statistically significant decline in both the absolute and relative weights of liver tissues, as indicated by the hepatosomatic index (HSI), following sublethal exposure to acrylamide suggested the potential impact of this compound on the physiological health of the fish. These findings reveal that acrylamide exposure induced alterations in the liver, a vital organ responsible for detoxification, metabolism, and maintaining homeostasis. The reduction in liver weight and HSI is indicative of a compromised metabolic state in the fish exposed to acrylamide, and this could be associated to various factors, such as altered enzymatic activities, disrupted cellular homeostasis, or impaired metabolic pathways (van den Berghe, 1991). It is worth noting that the liver played a crucial role in allocating more resources towards detoxifying acrylamide leading to alterations in liver morphology and functions.

The observed decline in both absolute and relative testicular weights consequent to acrylamide exposure could be attributed to a diminished count of spermatogonia, spermato-cytes, or spermatozoa, a deduction substantiated by the histological observations. Similarly, the female fish subjected to acrylamide displayed a reduction in both absolute and relative ovarian weights, which serves as an indicative marker of ovarian toxicity. In another study, *Oreochromis niloticus* exposed to palm oil mill effluent exhibited alterations in reproductive performance, as underscored by the perturbation in gonado-somatic index illustrating potential impacts of effluent contamination within the aquatic milieu (Zulfahmi *et al*., 2018).

The liver, a principal organ responsible for detoxification, is particularly susceptible to

aquatic pollutants, rendering it a prime target tissue. Various classes of hepatocellular pathology serve as robust biomarkers of toxic insults and reflect the biological endpoints influenced by pollutants. The present study employed biomarker enzymes within hepatic subcellular fractions encompassing cytosolic, mitochondrial, and microsomal compartments to elucidate bio-transformation processes in *Anabas testudineus*.

Ethoxyresorufin-O-deethylase (EROD) is one of the key enzymes in the phase I reactions of biotransformation pathway that respond rapidly to various xenobiotics. In fish, EROD is a specific biomarker enzyme used to measure the activity of the cytochrome P450-dependent monooxygenase induction by the exposure to xenobiotic chemicals (Stegeman *et al*., 1997). Usually, cytochrome P450 catalyses the oxidation, reduction and hydrolysis reactions of phase I system. As a result, the enzyme introduces functional group to the toxic substrate, thereby making it more polar and allowing the toxin to be easily excreted or further biotransformed into other water soluble products (Anzenbacher and Anzenbacherova, 2001). Reduction in the activities of EROD indicated low detoxification potential of hepatocytes of the fish against acrylamide exposure.

In the phase II reactions of biotransformation pathway, the metabolites formed in phase I reactions bind to endogenous water-soluble compounds through a variety of enzymes. The microsomal UDP-glucuronosyltransferase enzyme catalyses the conversion of hydrophobic xeno-biotic substrates to more hydrophilic UDP glucuronic acid. The glucuronide conjugates and other endogenous compounds formed as a result of glucuronidation are polar, water-soluble conjugates that are excreted from the body in bile or urine depending on the size of metabolite (Asifa and Chitra, 2017). Acrylamide exposure elevated the activity of UGT indicating an adaptive mechanism of the fish to reduce the potential adverse effects of acrylamide.

GST is a family of enzymes that play a crucial role in the biotransformation and detoxification of various endogenous and exogenous compounds within organisms. GST catalyze the conjugation of glutathione with electrophilic compounds, making them more water-soluble and facilitating their elimination from the body (Vaish *et al*., 2020). GST is the most important enzyme of phase II reactions, which catalyses the conjugation of xenobiotics having electrophilic functional groups to glutathione (Riol *et al*., 2001). A time-dependent reduction in GST activity suggested the failure of biotransformation system to detoxify or eliminate the toxicant.

GGT plays a multifaceted role in the biotransformation of xenobiotics, encompassing the degradation of glutathione conjugates, modulation of cellular glutathione levels, and exerting influence over the activation or deactivation of specific compounds (Hendrich and Pitot, 1987). GGT is predominantly localized within the liver, with its expression more evident in biliary epithelium, and its elevated level shows potential perturbations in the cellular physiology and metabolic processes of *Anabas testudineus*.

FMOs are the most important enzymes of phase 1 reactions that plays a major role in the detoxification machinery, contributing to the modification and inactivation of a wide range of xenobiotics and endogenous compounds. FMOs are primarily associated with oxidative reactions that introduce oxygen into substrates, leading to their modification and often rendering them more water-soluble and less toxic, thereby facilitating their elimination from the body. The catalytic activity of FMO is quite different from the CYP450 enzymes as it forms less toxic metabolites than the CYP enzymes (Motika *et al*., 2007). In the present study, the activity of FMO enzyme declined in all subcellular fractions of liver tissues, and this could be due to the accumulation of substrates or inability of the hepatic tissues to eliminate acrylamide during the exposure period.

ALT and AST are enzymes involved in transaminase reactions, which facilitate the interconversion of amino acids and α-keto acids.

They play crucial roles in amino acid metabolism, and are often used as marker enzymes of liver functions (Gella *et al*., 1985). ALT catalyzes the conversion of alanine and α-ketoglutarate to pyruvate and glutamate. This reaction is important for the energy balance of hepatocytes, and facilitates the transport of ammonia from peripheral tissues to the liver for detoxification. AST is another aminotransferase enzyme that catalyzes the conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate. The elevated activities of ALT and AST in the subcellular compartments of liver tissues indicate liver tissue damage due to acrylamide exposure. Similar increase in alanine and aspartate aminotransferase enzymes has been reported after chlorpyrifos exposure in the plasma of *Cyprinus carpio* (Banaee *et al*., 2011), liver of *Clarias batrachus* (Narra *et al*., 2011), *Oreochromis niloticus* (Majumder and Kaviraj, 2019), and *Pseudetroplus maculatus* (Raibeemol and Chitra, 2018).

The quality of sperm was evaluated by several sperm indices, such as sperm viability, sperm motility and sperm concentration. Fish sperm remain quiescent in the genital tract and in the seminal plasma and they become transiently motile or metabolically active only after the release into the surrounding water (Alavi and Cosson, 2006). Sperm viability is a crucial parameter reflecting the proportion of viable and functional sperm cells within a population where viable sperm remained colourless and non-viable sperm stained in eosin appeared pink (Wyrobek *et al*., 1983; Asifa and Chitra, 2019). A reduction in sperm viability suggests compromised cellular integrity, impaired physiological function, or increased susceptibility to stressors, which collectively can hinder successful fertilization processes. The time-dependent nature of this decline further underscores the progressive and cumulative impact of acrylamide on sperm viability. It has been reported that acrylamide induce oxidative stress and disrupt cellular processes (Ligina *et al*., 2022), which can lead to cellular damage. Such oxidative stress can detrimentally affect sperm membranes and DNA integrity, consequently reducing sperm viability.

Sperm motility is a pivotal factor in the success of fertilization, as it enables sperm cells to navigate through the female reproductive tract and ultimately reach the ova. Several factors that affect sperm motility includes pH, temperature, ions and osmolality that facilitate in the activation of axonemal movement (Alavi and Cosson, 2006; Zhou *et al*., 2015). A reduction in sperm motility severely compromise the ability of sperm to reach and fertilize eggs, and this could be possibly due to disturbances in the cellular machinery responsible for propulsion thereby fails to provide cellular energy production, membrane integrity, or biochemical signaling pathways essential for coordinated movement (Pereira *et al*., 2017). The present study suggests the potential impact of acrylamide to disrupt vital cellular processes essential for proper sperm functionality, which can ultimately hinder fertilization rates and reproductive outcomes in the fish *Anabas testudineus*.

Sperm count is a fundamental indicator of the quantity of available sperm for fertilization (Bertolla, 2020). A reduction in sperm count, as seen in this study, can significantly compromise the chances of successful fertilization. Sperm count reduction is often indicative of disruptions in the spermatogenesis process through which sperm cells are produced in the testes (Zhang *et al*., 2019). Factors that interfere with spermatogenesis can lead to decreased sperm production, contributing to lower sperm counts, which was evident in the histological analysis.

Oocyte diameter is a crucial metric reflecting the developmental stage and quality of oocytes within the ovary (Krisher, 2004). A reduction in oocyte diameter observed in the current study signifies compromised oocyte growth and maturation, which can subsequently impact the quality and potential of eggs for successful fertilization. The more pronounced reduction in oocyte diameter after 2 days of exposure suggests a rapid and sensitive response of oocytes to

acrylamide-induced stress. Oocytes are highly sensitive to environmental perturbations, and their diameter can serve as an early indicator of adverse effects on reproductive processes (Maskey *et al*., 2019). The continued and significant reduction in oocyte diameter over the 4-day treatment period implies a sustained and cumulative impact of acrylamide exposure on oocyte development. This observation highlights the potential of acrylamide to impair the reproductive success of *Anabas testudineus* by compromising the quality and potential of oocytes for fertilization. Fecundity rate is a pivotal measure representing the reproductive potential of female fish, reflecting the number of eggs produced over a specific period (Alam and Pathak, 2010). The observed decline in fecundity rate indicated impaired egg production and poor egg quality that affects the reproductive capacity, thereby affecting population dynamics and sustainability.

The enzymatic activities of 3β-hydroxy steroid dehydrogenase and 17β-hydroxy steroid dehydrogenase plays critical roles as oxidoreductase enzymes within the framework of steroid hormone biosynthesis (Labrie *et al*., 1992). Both enzymatic entities make contributions to the gonadal steroidogenic pathways (Simard *et al*., 2005). This phenomenon serves as indicative of antagonistic effect of acrylamide on the gonadal steroidogenic processes that could stem from its ability to interfere with hormonal signaling or directly affect enzyme activities (Yildizbayrak and Erkan, 2018; Sajla *et al*., 2019). This disruption could subsequently lead to reduced synthesis of sex steroids, impacting gametogenesis, oocyte maturation, and overall reproductive functions. This observation clearly indicates the potential role of acrylamide to disrupt normal reproductive hormone synthesis and, consequently, to hinder reproductive success in the fish *Anabas testudineus*.

The hepatotoxic effects of acrylamide was documented by the histological alterations observed in the liver tissues of *Anabas testudineus*. *Anabas testudineus*, subjected to acrylamide exposure at a concentration of 13.2 µg L^{-1}, exhibited conspicuous histological alterations within liver tissues. The aggregation of melanomacrophages and erythrocyte infiltration in the liver tissues suggests an initial inflammatory response. This could be indicative of the attempt of liver tissue to cope with the introduced stressor. Such inflammatory responses are common reactions to xenobiotic exposure and could be a sign of early immune activation in response to acrylamide-induced stress. The study observed hepatocyte degeneration indicating the progression of liver tissue damage. Degeneration of hepatocytes could signify impaired liver function and potential disruption of detoxification mechanisms due to acrylamide exposure.

The appearance of vacuoles in hepatocytes suggests potential disruptions in cellular homeostasis. Vacuoles can be indicative of altered lipid metabolism, cellular stress, or other underlying cellular dysfunctions (Camargo and Martinez, 2007). Vacuolization of hepatocytes indicate an imbalance between the rate of synthesis of metabolic substances in the parenchymal cells and the rate of release into the systemic circulation (Gingerich, 1982). Vacuole formation is a cellular defensive mechanism of hepatocytes against toxic substances that prevent by interfering with the biological activities of hepatocytes (Mollendroff, 1973).

The observed conspicuous aggregation of melanomacrophages suggests a potential manifestation of its toxic effects. This phenomenon could be indicative of an intensified inflammatory response as the exposure duration progresses. Melanomacrophagic centers (MMCs) are characterized as nodular accumulations of macrophages that possess the capacity to synthesize melanin, hemosiderin, and lipofuscin. The augmentation in both the size and abundance of MMCs is typically elicited in response to environmental stressors. These MMC alterations are recognized as dependable indicators of water quality and the presence of chemical

contamination, thereby serving as biomarkers for such conditions (Agius and Roberts, 2003).

Anabas testudineus exposed to acrylamide exhibited pronounced histological aberrations within the testicular tissue. Sublethal exposure to acrylamide caused depletion of spermatozoa, distorted testicular architecture, thickening of seminiferous tubule epithelium, and the emergence of interstitial fibrosis reflecting the detrimental impact of the toxicant on spermatogenesis and testicular health. The effects are associated with combination of factors, including direct cellular toxicity, disruption of hormonal regulation, compromised blood supply, mitochondrial dysfunction, and inflammatory responses. These mechanisms collectively contribute to the impaired testicular functions and reproductive health in the fish *Anabas testudineus*. A histology study conducted in adult albino rat model showed that acrylamide treatment caused testicular damage in the form of decreased seminiferous tubules diameters and epithelial height, degeneration of tubules, and arrested spermatogenesis (Hasanin *et al*., 2018).

The histopathological assessment of the ovarian tissue revealed notable leisons after acrylamide exposure, characterized by atretic follicles, epithelial blebbing, and pronounced oocyte degeneration. These observed effects are likely attributable to the direct impact of acrylamide on ovarian function, potentially through its modulation of follicular development and the induction of follicular atresia (Aldawood *et al*., 2020). Atretic follicles refer to ovarian follicles that have undergone a process of degeneration or regression, leading to their reduced size, disrupted structure, and loss of normal cellular components (Tilly *et al*., 1991). The formation of atretic follicles can arise from a complex interplay of factors involving hormonal imbalances, oxidative stress, nutrient deficiencies, endocrine disruptors, inflammation, apoptotic signaling, and genetic influences (Zhou *et al*., 2019). Follicular epithelial blebbing, also known as membrane blebs, refers to a cellular phenomenon characterized by the formation of small, bubble-like protrusions or bulges on the surface of epithelial cells lining the ovarian follicles that emerge as a result of changes in cell membrane dynamics and cytoskeletal rearrangements (Gabaeva, 1977). These blebs are often observed in various cellular contexts, including during processes like cell migration, apoptosis, and cellular stress responses. Oocyte degeneration refers to the process of structural and functional deterioration of oocytes, which are the immature eggs within ovarian follicles (Samarin *et al*., 2019). During oocyte degeneration, the oocytes undergo changes in their cellular morphology, internal structures, and biochemical composition. These changes can include alterations in nuclear structure, cytoplasmic components, and organelles. Oocyte degeneration can occur due to a variety of factors that disrupt normal oocyte development and maturation, leading to their dysfunction and eventual loss (Hatirnaz *et al*., 2022).

Conclusion

In conclusion, the present study sheds light on the impact of acrylamide exposure on hepatic biotransformation enzymes and tissue biomarkers in the fish *Anabas testudineus*, highlighting the potential disruption of crucial metabolic pathways. A comprehensive assessment of crucial reproductive endpoints, encompassing gonado-somatic index, sperm indices, oocyte measurements, fecundity rate, gonadal steroidogenic enzymes, and gonadal histology, was employed to assess the reproductive status, which stated reproductive impairment in the fish, *Anabas testudineus*. The overall findings highlight that sublethal impacts of acrylamide in the aquatic environment can be used as a bioindicator to monitor environmental health conditions. Further research in this area will provide deeper insights into the mechanisms underlying these alterations, aiding in the development of effective strategies for the conservation and protection of aquatic ecosystems and the organisms that inhabit them.

Ethical Statement

The care and use of the fish strictly adhered to the guidelines and regulations set forth by the Animal Welfare Board of India and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) under the Ministry of Environment, Forest, and Climate Change, Government of India.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

The authors sincerely acknowledge the Central Sophisticated Instrumentation Facility (CSIF), University of Calicut, Malappuram district, and the Department of Science and Technology- Fund for the Improvement of Science and Technology Infrastructure (DST-FIST; TPN No.: 106238, File No. SR/FST/LS-I/2024/1382) for providing the necessary facilities and equipment to complete the work.

Conflict of Interest

The authors declare no conflicts of interest.

References

Agius C and Roberts RJ. (2003) Melano-macrophage centres and their role in fish pathology. J Fish Dis. 26(9): 499-509.

Alam M and Pathak JK. (2010) Assessment of fecundity and gonadosomatic index of commercially important fish, *Labeo rohita* from Ramganga River. Int J Pharma Bio Sci. 1: 1-6.

Alavi SMH and Cosson J. (2006) Sperm motility in fishes. (II). Effects of ions and osmolality: a review. Cell Biol Int. 30(1): 1-14.

Aldawood N, Alrezaki A, Alanazi S, Amor N, Alwasel S, Sirotkin A and Harrath AH. (2020) Acrylamide impairs ovarian function by promoting apoptosis and affecting reproductive hormone release, steroidogenesis and autophagy-related genes: An *in vivo* study. Ecotoxicol Environ Saf. 197:110595.

Anzenbacher P and Anzenbacherova E. (2001) Cytochromes P450 and metabolism xenobiotics. Cell Molec Life Sci. 58(5): 737-747.

APHA. (1998) American Public Health Association, American Water Works Association, and Water Environment Federation. Standard methods for the examination of water and wastewater. Washington, D.C: APHA-AWWA-WEF. https://www.worldcat.org/title/standard-methods-for-the-examination-of-water-wastewater/oclc/779509419).

Asifa KP and Chitra KC. (2017) Hepatic biotransformation of chlordecone and induction of hepatotoxicity in the cichlid fish, *Pseudetroplus maculatus* (Bloch, 1795). Int J Appl Res. 3: 521-526.

Asifa KP and Chitra KC. (2019) Effect of chlordecone on the reproductive potential of the cichlid fish, *Pseudetroplus maculatus* (Bloch, 1795). J Aquat Res Mar Sci. 2(2): 163-177.

Banaee M, Sureda A, Mirvaghefi AR and Ahmadi K. (2011) Effects of diazinon on biochemical parameters of blood in rainbow trout (*Oncorhynchus mykiss*). Pesticide Biochem Physiol. 99: 1-6.

Bergmeyer MU. (1974) Steroid dehydrogenase. In: Methods of enzymatic analysis, (eds.) Bergmeyer H.U. and Gawehn K., 2nd edn., Academic Press, New York. pp 476-477.

Bertolla RP. (2020) Sperm biology and male reproductive health. Sci Rep. 10: 21879.

Bolger T and Connoly PL. (1989) The selection of suitable indices for the measurement and analysis of fish condition. J Fish Biol. 34: 171-182.

Caille N, Rodina M, Kocour M, Gela D, Flajshans M and Linhart O. (2006) Quantity, motility and fertility of tench *Tinca tinca* (L.) sperm in relation to LHRH analogue and carp pituitary treatments. Aquacult Int. 14: 75-87.

Camargo MMP and Martinez CBR. (2007) Histopathology of gills, Kidney and liver of a Neotropical fish caged in an urban Stream. Neotrop Ichthyol. 5(3): 327-336.

Christiansen T, Korsgaard B and Jespersen A.(1998) Effects of nonylphenol and 17beta-oestradiol on vitellogenin synthesis, testicular structure and cytology in male eelpout *Zoarces viviparous*. J Exp Biol. 200(2): 179-192.

Eliasson R and Treichl L. (1977) Supravital staining of human spermatozoa. Fertil Steril. 22(2): 134-137.

Elliott JM and Hurley MA. (1995) The functional relationship between body size and growth rate in fish. Funct Ecol. 9(4): 625-627.

Fernandes D, Potrykus J, Morsiani C, Raldua D, Lavado R and Porte C. (2002) The combined use of chemical

and biochemical markers to assess water quality in two lowstream rivers (north east Spain). Environ Res. 90(2):169-178.

Froese R. (2006) Cube law, condition factor and weight-length relationships: History, meta-analysis and recommendations. J Appl Ichthyol. 22(4): 241-253.

Gabaeva NS. (1977) Characteristics of the follicular epithelium in the fishes with demersal role. Concerning the classification of unilaminate monomorphous follicular epithelium of the vertebrates. Arkh Anat Gistol Embriol. 73(9): 48-55.

Gella FJ, Olivella T, Cruz Pastor M, Arenas J, Moreno R, Durban R and Gomez JA. (1985) A simple procedure for the routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate. Clin Chim Acta. 153(3): 241-247.

Gingerich WH. (1982) Hepatic toxicology of fishes, In: Aquatic Toxicology, (ed.) Weber L.J., Raven Press, New York. pp. 55-105.

Graham JM. (2015) Fractionation of subcellular organelles. Curr Protocols Cell Biol. 69(1): 1-3.

Grimes CB and Huntsman GR. (1980) Reproductive biology of vermilion snapper, *Rhomboplites aurorubens* from North Carolina and South Carolia. Fish Bull. 78: 137-146.

Habig WH, Pabst MJ and Jakoby WB. (1974) Glutathione S-transferases the first enzymatic step in mercapturic acid formation. J Biol Chem. 249(22): 7130-7139.

Hasanin NA, Sayed NM, Ghoneim FM and Al-Sherief SA. (2018) Histological and ultrastructure study of the testes of acrylamide exposed adult male albino rat and evaluation of the possible protective effect of vitamin E intake. J Microsc Ultrastruct. 6(1): 23-34.

Hatirnaz S, Hatırnaz ES, Ellibes Kaya A, Hatırnaz K, Soyer Calıskan C, Sezer O, Dokuzeylul Gungor N, Demirel C, Baltaci V, Tan S and Dahan M. (2022) Oocyte maturation abnormalities - A systematic review of the evidence and mechanisms in a rare but difficult to manage fertility pheneomina. Turk J Obstet Gynecol. 19(1): 60-80.

Hendrich S and Pitot HC. (1987) Enzymes of glutathione metabolism as biochemical markers during hepatocarcinogenesis. Cancer Metastasis Rev. 6(2): 155-178.

Htun-Han M. (1978) The reproductive biology of the dab *Limanda limanada* (L.) in the North Sea: Gonadosomatic index, hepatosomatic index and condition factor. J Fish Biol. 13(1):351-377.

Kennedy J. (2018) Oocyte size distribution reveals ovary development strategy, number and relative size of egg batches in lumpfish (*Cyclopterus lumpus*). Polar Biol. 41: 1091-1103.

King M. (1995) Fisheries biology assessment and management. Fishing News Books Blackwell Science Ltd., London, p. 341.

Krisher RL. (2004) The effect of oocyte quality on development. J Anim Sci. 82: E14-23.

Labrie F, Simard J, Luu-The V, Belanger A and Pelletier G. (1992) Structure, function and tissue-specific gene expression of 3β-hydroxysteroid dehydrogenase/5-ene-4-ene isomerase enzymes in classical and peripheral intracrine steroidogenic tissues. J Steroid Biochem Mol Biol. 43(8): 805-826.

Le Cren ED. (1951) The length-weight relationships and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). J Anim Ecol. 20(2): 201-219.

Ligina V, Martin R, Aiswarya MV, Mashirin KR and Chitra KC. (2022) Acute and sublethal effects of acrylamide on the freshwater fish, *Anabas testudineus* (Bloch, 1792). Environ Sci Pollut Res. 29: 90835-90851.

LoPachin RM and Gavin T. (2012) Molecular mechanism of acrylamide neurotoxicity: lessons learned from organic chemistry. Environ Health Perspect. 120(12): 1650-1657.

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. (1951) Protein measurement with the Folin phenol reagent. J Biol Chem. 193: 265-275.

Majumder R and Kaviraj A. (2019) Acute and sublethal effects of organophosphate insecticide chlorpyrifos on freshwater fish *Oreochromis niloticus*. Drug Chem Toxicol. 42(5): 487-495.

Maskey E, Crotty H, Wooten T and Khan IA. (2019) Disruption of oocyte maturation by selected environmental chemicals in zebrafish. Toxicol *In Vitro* 54: 123-129.

Mollendroff T. (1973) Cytology and cell physiology. 3rd edn., Academic press, New York.

Motika MS, Zhang J and Cashman JR. (2007) Flavin-containing monooxygenase 3 and human disease. Expert Opin Drug Metab Toxicol. 3: 831-845.

Naiel MAE, Negm SS, Ghazanfar S, Farid A and Shukry M. (2023) Acrylamide toxicity in aquatic animals and its mitigation approaches: An updated overview. Environ Sci Pollut Res Int. 30(53): 113297-113312.

Narra MR, Begum G, Rajender K and Venkateswara Rao J. (2011) Sub-lethal effect of chlorpyrifos on protein metabolism of the food fish *Clarias batrachus* and monitoring of recovery. Toxicol Environ Chem. 93(8): 1650-1658.

Neetha R, Nikhil J and Chitra KC. (2022) A comparative

study on the sensitivity of selected freshwater fishes against triclosan exposure. Drug Chem Toxicol. 46(6): 1116-1129.

OECD. (1992) Guidelines for the testing of chemicals 203.Fish, acute toxicity test. OECD, Paris.

Pereira R, Sa R, Barros A and Sousa M. (2017) Major regulatory mechanisms involved in sperm motility. Asian J Androl. 19(1): 5-14.

Priyatha CV and Chitra KC. (2022) Evaluation of the reproductive cycle and gonadal development in the climbing perch, *Anabas testudineus* (Bloch 1792) in captivity. J Fish. 10(1): 101206.

Raibeemol KP and Chitra KC. (2018) Hematological and biochemical changes in the freshwater fish, *Pseudetroplus maculatus* exposed to sublethal concentrations of chlorpyrifos. Res Rev J Life Sci. 8(3): 108-116.

Reitman S and Frankel S. (1957) Colorimetric methods for aspartate and alanine aminotransferase. Am J Clin Pathol. 28: 55-60.

Riol MJM, Valinas MCN, Fernandez MAG and Lopez MP. (2001) Glutathione S-transferases from rainbow trout liver and freshly isolated hepatocytes: Purification and characterization. Comp Biochem Physiol C Toxicol Pharmacol. 128(2): 227-235.

Roberts RJ and Smail DA. (2001) Laboratory methods. In: Fish Pathology, (ed.) Roberts R.J., 3rd edn., Harcourt Publishers Limited, pp. 380-390.

Rose RL. (2002) Measurements of flavin-containing monooxygenase (FMO) activities. Curr Protoc Toxicol. doi: 10.1002/0471140856.tx0409s13.

Sajla K, Raibeemol KP and Chitra KC. (2019) Induction of ovarian toxicity in the freshwater fish, *Pseudetroplus maculatus* (Bloch, 1795) after sublethal exposure of dibutyl phthalate. Int J Sci Res Biol Sci. 6(5): 26-38.

Samarin AM, Samarin AM, Ostbye TKK, Ruyter B, Sampels S, Burkina V, Blecha M and Policar T. (2019) The possible involvement of oxidative stress in the oocyte ageing process in goldfish *Carassius auratus* (Linnaeus, 1758). Sci Rep. 9: 10469.

Schloesser RW and Fabrizio MC. (2017) Condition indices as surrogates of energy density and lipid content in juveniles of three fish species. Trans Am Fish Soc. 146(5): 1058-1069.

Simard J, Ricketts ML, Gingras S, Soucy P, Feltus FA and Melner MH. (2005) Molecular biology of the 3beta-hydroxysteroid dehydrogenase/ delta5 - delta4 isomerase gene family. Endocr Rev. 26(4): 525-582.

Stegeman JJ, Woodin BR, Singh H, Oleksiak MF and Celander M. (1997) Cytochromes P450 (CYP) in tropical fishes: catalytic activities, expression of multiple CYP proteins and high levels of microsomal P450 in liver of fishes from Bermuda. Comp Biochem Physiol Part C Pharmacol Toxicol Endocrinol. 116(1): 61-75.

Sulistyo I, Fontaine P, Rincarh J, Gardeur JN, Migaud H, Capdeville B and Kestemont P. (2000) Reproductive cycle and plasma level of steroid in male Eurasian Perch (*Perca fluviatilis*). Aquat Living Resour. 13: 99-106.

Taeymans D, Wood J, Ashby P, Blank I, Studer A, Stadler RH, Gonde P, Van Eijck P, Lalljie S, Lingnert H, Lindblom M, Matissek R, Muller D, Tallmadge D, O'Brien J, Thompson S, Silvani D and Whitmore T. (2004) A review of acrylamide: an industry perspective on research, analysis, formation, and control. Crit Rev Food Sci Nutr. 44(5): 323-347.

Tareke E, Rydberg P, Karlsson P, Eriksson S and Tornqvist M. (2002) Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J Agric Food Chem. 50(17): 4998-5006.

Tepe Y and Cebi A. (2017) Acrylamide in environmental water: A review on sources, exposure, and public health risks. Expos Health 11: 3-12.

Tilly JL, Kowalski KI, Johnson AL and Hsueh AJ. (1991) Involvement of apoptosis in ovarian follicular atresia and postovulatory regression. Endocrinology 129(5): 2799-2801.

Vaish S, Gupta D, Mehrotra R, Mehrotra S and Basantani MK. (2020) Glutathione S-transferase: A versatile protein family. 3 Biotech. 10(7): 321.

van den Berghe G. (1991) The role of the liver in metabolic homeostasis: Implications for inborn errors of metabolism. J Inherit Metab Dis. 14(4): 407-420.

Wyrobek AJ, Gordon LA, Burkhart JG, Francis MW, Kapp RW Jr, Letz G, Malling HV, Topham JC and Whorton MD. (1983) An evaluation of the mouse sperm morphology test and other sperm tests in nonhuman mammals. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res. 115(1):1-72. https://doi.org/10.1016/0165-1110(83)90014-3.

Xu W, Tan L, Zhao T, Zhu X and Wang J. (2020) Toxicity assessments of acrylamide in aquatic environment using two algae *Nitzschia closterium* and *Scenedesmus quadricauda*. Environ Sci Pollut Res Int. 27(16): 20545-20553.

Yildizbayrak N and Erkan M. (2018) Acrylamide disrupts the steroidogenic pathway in Leydig cells: Possible mechanism of action. Toxicol Environ Chem. 100(2): 235-246.

Yoshida M, Ono H, Kameyama M, Chuda Y, Yada H, Kobayashi H and Ishizaka M. (2002) Determination of acrylamide in processed foodstuffs in Japan. Nippon Shokuhin Kagaku Kogaku Kaishi. 49(12): 822-825.

Zhang Y, Zhang G and Zhang Y. (2005) Occurrence and analytical methods of acrylamide in heat-treated foods. J Chromatogr A. 1075(1-2): 1-21.

Zhang YW, Liu J, Ma KH and Tan Y. (2019) Effects of autophagy on spermatogenesis and sperm motility. Zhonghua Nan Ke Xue. 25(1): 72-76.

Zhivkov V. (1970) Measurement of uridine diphosphate glucuronic acid concentrations and synthesis in animal tissues. Biochem J. 120(3): 505-508.

Zhou J, Chen L, Li J, Li H, Hong Z, Xie M, Chen S and Yao B. (2015) The semen pH affects sperm motility and capacitation. PLoS One 10(7): e0132974.

Zhou J, Peng X and Mei S. (2019) Autophagy in ovarian follicular development and atresia. Int J Biol Sci. 15(4): 726-737.

Ziegter DM. (1988) Detoxication: oxidation and reduction. In: The liver: biology and pathobiology, (eds.) Arias I.M., Jakoby W.B., Popper H., Schacter D. and Shafritz D.A., Raven Press, New York, pp. 363-374.

Zulfahmi I, Muliari M, Akmal Y and Batubara AS. (2018) Reproductive performance and gonad histopathology of female Nile tilapia (*Oreochromis niloticus* Linnaeus 1758) exposed to palm oil mill effluent. Egypt J Aquat Res. 44(4): 327-332.