Impacts of Raw and Processed Water on Fish

(Histopathological and Histochemical analysis on ovary of *Clarias batrachus)*

BY

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A THESIS FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE MASTER'S DEGREE OF SCIENCE

CENTRAL DEPARTMENT OF ZOOLOGY – FISH AND FISHERIES

INSTITUTE OF SCIENCE AND TECHNOLOGY

TRIBHUVAN UNIVERSITY

KATHMANDU, NEPAL

2010

CONTENTS

ABBREVIATION

Atretic oocyte	:AO
Atretic Follicles	:AF
Cytoplasm	:C
Fusion of oocyte	:FO
Germinal epithelium	:GE
Nuclear Membrane	:NM
Nucleus	:N
Ovigerous lamella	:OL
Primary oocyte	:pO
Theca	:TH
Tunica Albuginea	:T
United Nation	:UN
Vacuoles	:V
Water Treatment Plant	:WTP
World Health Organisation	:WHO
Yolk Globules	:YG
Yolk Vesicles	:YV
Zona Granulosa	:ZG
Zona Radiata	:ZR

INTRODUCTION

Water is a ubiquitous chemical substance that is composed of hydrogen and oxygen and is vital for all known forms of life. From a biological standpoint, water has many distinct properties that are critical for the proliferation of life that set it apart from other substances. It carries out this role by allowing organic compounds to react in ways that ultimately allow replication. All known forms of life depend on water.

Water is vital both as a solvent in which many of the body's solutes dissolve and as an essential part of many metabolic processes within the body. Metabolism is the sum total of anabolism and catabolism. In anabolism, water is removed from molecules (through energy requiring enzymatic chemical reactions) in order to grow larger molecules (e.g. starches, triglycerides and proteins for storage of fuels and information). In catabolism, water is used to break bonds in order to generate smaller molecules (e.g. glucose, fatty acids and amino acids to be used for fuels for energy use or other purposes). Water is thus essential and central to these metabolic processes. Therefore, without water, these metabolic processes would cease to exist, leaving us to muse about what processes would be in its place, such as gas absorption, dust collection, etc.

An original recommendation for water intake in 1945 by the Food and Nutrition Board of the National Research Council read: "An ordinary standard for diverse persons is 1 milliliter for each calorie of food. Most of this quantity is contained in prepared foods." The latest dietary reference intake report by the United States National Research Council in general recommended (including food sources). 2.7 liters of water total for women and 3.7 liters for men. Specifically, pregnant and breastfeeding women need additional fluids to stay hydrated.

Water fit for human consumption is called drinking water or potable water. Water that is not potable can be made potable by filtration or distillation (heating it until it becomes water vapor, and then capturing the vapor without any of the impurities it leaves behind), or by other methods (chemical or heat treatment that kills bacteria). Sometimes the term safe water is applied to

potable water of a lower quality threshold (i.e., it is used effectively for nutrition in humans that have weak access to water cleaning processes, and does more good than harm). Water that is not fit for drinking but is not harmful for humans when used for swimming or bathing is called by various names other than potable or drinking water, and is sometimes called safe water.

Water treatment describes those process used to make water more acceptable for a desired end-use. During the purification process undesirable chemicals and biological contaminants are removed from the raw water. In Kathmandu, the KUKL (Kathamndu Upathyaka Khanipani Limited) has undertaken the responsibilities Kathmandu Valley drinking water management since 1st falgun 2065 B.S. (February 13, 2008). The standard for drinking water quality is typically set by the Government of Nepal or by International Standards. However, in Kathmandu it is found to be satisfactory according to Nepal drinking water quality standard in some aquifers but ammonia was high in Jwagal plant.

Ground water from the depth and confined aquifers is usually microbial safe and chemically stable in the absence of direct contamination; however, shallow or unconfined aquifers can be subject to contamination from discharge or seepage associated with agricultural practice (e.g., pathogens, nitrates and pesticides), on-site sanitation and sewerage (pathogens and nitrates) and industrial wastes. Hazards and hazardous events that can have impact on catchments and that should be taken into consideration as a part of hazard assessment.

Great attempts have been made to understand the general toxicology. Due to alarming increase in population special emphasis is being given to the histopathological evidences of damage tissue or changes occurring in animals exposed to toxic chemicals. Such studies have also offered opportunity to locate the effects of pollutants on various organs and organ system of animals, the reproductive system being one of them. So far, only few a few attempts have been made to study the effect of water parameters on the reproductive organs of fishes (Sangalanga 1974; Saxena and Gorg 1978; Kapur et. al. 1978; Benoit, 1975 and Pandey and Shukla, 1980). The present paper records

the effect of raw and processed water on ovary of the fish (*Clarias batrachus*) during pre spawning phase.

Attempt has been made to study the histopathological changes induced by non-lethal exposure of phosphamidon recovery in *Labeo rohita* figerlings, Medda et al (1992).

In mid 70's toxicologist took considerable interest to study the consequences of insecticides on the blood of fishes. Blood is the primary target of pesticidal action (Kennedy et al., 1970) and since circulatory system is intimately related to the respiratory system, any change in one will affect the other. The changes in fish blood exposed to varying degrees of environmental stressors/pesticides have been recorded in the publication of Mishra and Srivastava (1984), Singh et al (1991),Kumar and Banerjee (1919) and others.

Tiwary (1977) in *C. batrachus*, Towheed (1982) in male *Amphipnous cuchia* and Singh (1988) in female *Mastacembulus cuchia* with stimulated gonadal maturation on thyroxin administration. Singh and Singh (1990) have reported the effect of thiourea on the serum levels of thyroid hormones during the prespawning and spawning phases of the fresh water, catfish, *Clarias batrachus*.

The present investigation has been done with the aim to study the effect of raw and processed water correlation with the histological and structural changes in the ovary of an air breathing teleost, *C. batrachus*. More emphasis has being given to the study of spawning phase of the present study.

AIMS AND OBJECTIVES OF THE STUDY

1. GENERAL OBJECTIVES:-
    i. Effect of different water parameters from WTP on fish.
    ii. Study for improved progeny and developed immunology.

2. SPECIFIC OBJECTIVES:-
    i. Effects of raw and processed water on the reproductive organ (ovary) of *Clarias batrachus.*
    ii. Hitopathological and Histochemical analysis of ovary of *Clarias batrachus.*

Study Area:

The study in impacts of raw and processed water on fish was carried out at Jawgal, near Bagmati River, Kathmandu (Nepal). The WTP is located at UN Park at Jawgal, which has been distributing water since last many years in Kupandol area.

The research work was carried out from the date 10.07.2009 to 10.08.2009 under the supervision Dr. Archana Prasad (thesis supervisor), Central Department of Zoology, Tribhuvan University, Kirtipur, Nepal. Daily Observation was kept under notice with the help of record book.

DESCRIPTION OF THE SPECIMEN

Fish: *Clarias batrachus*
Local Name: “Mungri” or “Magur”
Classifaction : Leo.S.Berg (1947)

Phylum: Chordata
Division: Gnathostomata
Super Class: Pisces
Class: Osteicthyes
Sub Class: Actinopterygii
Order: Osterophyss
Sub Order: Siluroidea
Family: Claridae
Genus: *Clarias*
species: *batrachus*

Short description

An air breathing teleost *Clarias batrachus* is an annual breeder completing its gonadal cycle in four phases: Pre-spawning (April-June); Spawning (July-September); Post-spawning (October-December); and Resting (January-March). Usually, females with well developed ovaries were found in July- August. The maximum length of the *Clarias batrachus* may be 47.0 cm and the weight 1,190g. They are potamodromous and lives in freshwater; brackish water; at the depth of 1 to several meters of range. They are found in tropical places at 29 °N – 7 °S at the temperature of 10 °C – 28 °C. Dorsal spines (total): 0; Dorsal soft *rays* (total): 60; Anal spines: 0; Anal soft rays: 47-58.Body compressed posterior, Upper jaw a little projecting. Spine of pectoral fin is rough on its outer edge and serrated on its inner edge. Occipital process more or less triangular, its length about two times in its width; distance between dorsal and occipital process 4-5 times in distance from tip of snout to end of occipital process. Genital papilla in males is elongated and pointed (Ref. 52012, www.fishbase.com).

LITERATURE REVIEW

The earliest reference on the use of chemical can be traced back to as early as 1000 B.C. when Homer spoke of sulphur for fumigants and other forms of pest controls. Romans used hellebore for the control of rats, mice and insects (Shepard, 1951). Chinese employed arsenic sulphide to control insects on garden plants (900 A.D). Nowadays, a number of arsenic and inorganic derivatives are in extensive use of pest investigation.

Great attempts have been made to study the general toxicology. Duodoroff et al., (1953) studied the effects of acute toxicity of some organic insectides to fishes. Adlung (1957) attempted to study the toxic effects of endosulfan to fresh water fish and found that toxicity varies with temperature. Haynes et, al. (1959) briefly evaluated the effects of sevin to goldfish. Ludemann newmann (1960) reported the acute toxicity of some insecticides to a common carp, *Cyprinus carpio.* They also studied the mechanism of acute toxicity of some newly formulated insecticides. Mathur (1960) evaluated the lethal dose for a number of insecticides. Lewallen and Wilder (1962) studied the toxicity of some organophosphorus and carbamate group of pesticides on *Salmo gairdeneri.*

Many authors have detailed the formation of atretic oocytes (preovulatory corpora atretica) in the teleostean ovary (Ball, 1960; Lofts and Bern, 1972; Browning, 1973; Erickson et. al., 1985). However, atretic oocytes have generally been considered to be the degenerating oocytes which are lost subsequently from the ovary other than ovulation (Erickson et. al., 1985). Regarding the functional significance of atretic follicles in teleost, it has been described the site of ovarian steroid biosynthesis because of its glandular appearance (Hoar, 1969; Browning, 1973). Beach (1959) however believed its intimate association with the removal of the yolk. This was also supported by Sathyanesan (1962). Further it has been suggested by Munkittrick and Leatherland (1984) that the process of atresia is under the influence of gonadotropin.

Clarias batrachus has been nominated as among 100 of the “World’s Worst invaders” (Dr. Mathew Baber, Postdoctoral Research Associate, Department of Natural Resources, University of New Hamsphire, Durham, USA).

Verma. (1996) reported that fishes have been armed with various powerful natural hormonal defense systems for their protection against environmental toxicant and pathogens like interstitial nutritive cells and rosette cells functioning both as phagocytic, mainly peroxisomes and nutritive.

According to Khanna (1985), in *Clarias batrachus*, the Ovaries are paired elongated sac like structures lying in the body cavity, ventral to the swim bladder. They are flattened ovoid bodies, each measuring about 4-6cm in length, about 2 cm in width and 1cm in thickness. They are attached to the body walls by the means of the mesovarium. The anterior end is free but their caudal ends may become united into one. The hinder end of each ovary is continued posterior into a short oviduct. The oviducts fuse and open to the exterior by a separate genital aperture or by a common urinogenital opening.

Generally, both the ovaries are of equal size, but occasionally they are unequal also. They are thin, flaccid and translucent when immature, but on maturity, they become enlarged and lobulated, while the ripe ova are seen bulging out.

With the respect of advancing time the smooth of the ovary gets disrupted and the ripe ova bulges out adhering the granular appearances visible through the transparent ovarian wall. The colour changes in different phases of its annual reproductive cycle like, it was yellowish in early stage of development but it changed to reddish brown as the degree of vascularisation increased in the latter stage of development.

Histologically, the ovary is made up of an outer wall with developing oocytes and encloses a cavity. The outermost layer consisting of connective tissue is peritoneum, which is so thin that it is difficult to different from adjoining layer. The middle layer is called tunica albuginea which is made up of connective tissue, muscle fibers and blood capillaries. During the pre spawning season, tunica albuginea becomes thickened and same gets thin during the

spawning time. The germinal epithelium, which is the inner most layer consists of a single layer of cubodial cells which possess scanty cytoplasm and a large nucleus with nucleolus.

The development of a new crop of oogonia takes place in ovigerous lamella which is the part of germinal epithelium. The earlier stage of the oocytes is mainly found in the proximity of the ovarian wall while advance ones lay near the lumen cavity

The wall of the ovary is fairly thick during the non-breeding season but become highly vascular during the spawning period. The germ cells or oogonia are found in clusters. As the oogonia increase in size, there is an increase in the quality of Ooplasm. The developing eggs are known as Oocyte. Considering the nuclear and cytoplasmic changes he distinguished oocytes into different growing stages before they attain their maturity.

STAGE 1. (S I)

These oocytes are more or less spherical in shape and frequently found to be in clusters along the margin of ovigerous lamella. They are provided with a large distinct nucleus containing nucleoli and numerous chromatin threads. These oocytes are found to be in variable numbers thought the year.

STAGE 2. (S II)

There is the further increase in the size of the oocytes, number of nucleoli and basophilia of the cytoplasm. Several small nucleoli of various sizes are seen along the pheriphery of the nuclear membrane. Many oocytes at this stage, process a yolk nucleus, lying near to the nuclear membrane of the cytoplasm. Later, the yolk nucleus moves towards the pheriphery of the oocyte.

STAGE 3. (S III)

This is still large in size, and is distinguished by the appearance of the thin layer of follicular cells around the cytoplasm. A few nucleoli pass out of the nuclear membrane, and are seen in the cytoplasm of the oocyte.

STAGE 4. (S IV)

There is further increase in the size of the oocyte and a large number of small, clear vacoules called the yolk vesicles appear in the pheriphery of the ooplasm. The vesicles appear empty in the early stage and are not stainable. Many oocytes shows an undulated nuclear membrane, and the nucleoli enter into the pockets of the nuclear membrane, and pass out to the ooplasm.

STAGE 5. (S V)

As the oocyte grow further, the yolk vesicles increase in number and fill the entire ooplasm. A vitelline membrane of zona radiata is also clearly visible, between the ooplasm and follicular layer of zona granulosa. Nucleolar extrusion continues at this stage.

STAGE 6. (S VI)

This is characterized by appearance in the form of minute granules in the extravesicular ooplasm. They appear first in the pheripherial region and accumulate there in large numbers. Then yolk proceeds centripetally, till the whole ooplasm becomes impregnated with them. The yolk granules fuse to form larger globules, and the oocyte is of considerable size. A thin layer of fibroblast, known as theca is also distinguishable outside the follicular layer.

STAGE 7. (VII)

There is a heavy deposition of yolk globules which are fairly large in size. The yolk vesicles also fuse to become large. The nucleus migrates gradually towards the pheriphery. Some yolk vesicles push towards the pheriphery of the egg and form cortical alveoli.

However, these oocytes are of various shapes depending upon the stresses imposed on them and are found in a large numbers thought the year.

The nucleus contains about 2-8 nucleoli together with some fragmented chromatin materials. The cytoplasm is widespread and stains immensely with eosin while the nuclear materials with haematoxylin. A thin wrinkled layer covering the oocyte is sometimes indistinct due to densely stained cytoplasm.

METHODS AND METHODOLOGY

Adult fish *Clarias batrachus* weighing 250 +- 5g and 30 cm in length were purchased from the local supplier in the month of early July, 2009 and were acclimatized in a clean aquarium containing tap water for 15 days.

To observe the effects from different condition provided to them, the fishes were kept on an aquarium (usually 7 fishes in each aquarium) at different condition i.e. raw water, processed water and tap water. All the fishes were fed with same protein diet i.e liver of chicken (200g/fish). The water of the aquarium was changed every day after 24 hours at the evening before feeding. The aquarium was kept clean by changing the water every 24 hours by netting the fishes on to the other aquarium provided with same water quality that is to be examined. The experiment was conducted for 30 days. Before the dissection of the fishes, they were kept unfed for 24 hours.

The sample tissues were collected from each provided conditions and preserved in fixative to undergo the further observation. Meanwhile, investigation in their behavior pattern of the remaining fishes were also observed and written in a record book to get the best output of the study.

1. Analysis and instrumentation

The water quality parameters like dissolved oxygen (DO), carbondioxide (CO2), temperature, ph - value, iron, total ammonia – N, nitrate – N, nitrite – N, and organic ammonia were analyzed at Water Analysis Laboratory, Balkhu, Kathmandu. The methods of analysis, equipments used for individual parameters have been described as follows.

Table I: Analysis and Instrumentation

Sl. No	Parameters	Methods of analysis	Instruments used
1.	pH – value	Electrometric	pH – meter
2.	Temperature	Thermometric	Thermometer
3.	Total NH	With standard AgNO	Colorimetrics
4.	Organic ammonia	Nesslerization	HACH kit
5.	Nitrite	TKN – method	Glass wares
6.	Nitrate	Brocine sulphate	HACH kit
7.	Inorganic ammonia	Brocine sulphate	Glass wares
8.	Total Iron	Brocine sulphate	HACH kit
9.	D.O	Modified winkler method	Glass wares
10.	Carbondioxide	Tritrimetric with standard alkali	Glass wares

2. METHODS FOR HISTOLOGICAL STUDY:

A. Fixation and Microtomy

Before undergoing fixation and microtomy objects to be studied under microscope needs certain preparation like fixative block preparation, sectioning, staining and mounting etc.

FIXATIVE: A fixative is a chemical which kills tissue with a minimum changes in volume, structure and chemical makeup. It hardens the tissue with post mortem changes in shape, size and also renders some substances insoluble.

Bouin's fluid (alcoholic): The fixative was prepared by taking, following chemicals together in a clean dry tube.

(Saturated) Picric Acid (1.4% aq.)	75ml
Commercial Formalin	25ml
Glacial Acetic Acid	05ml

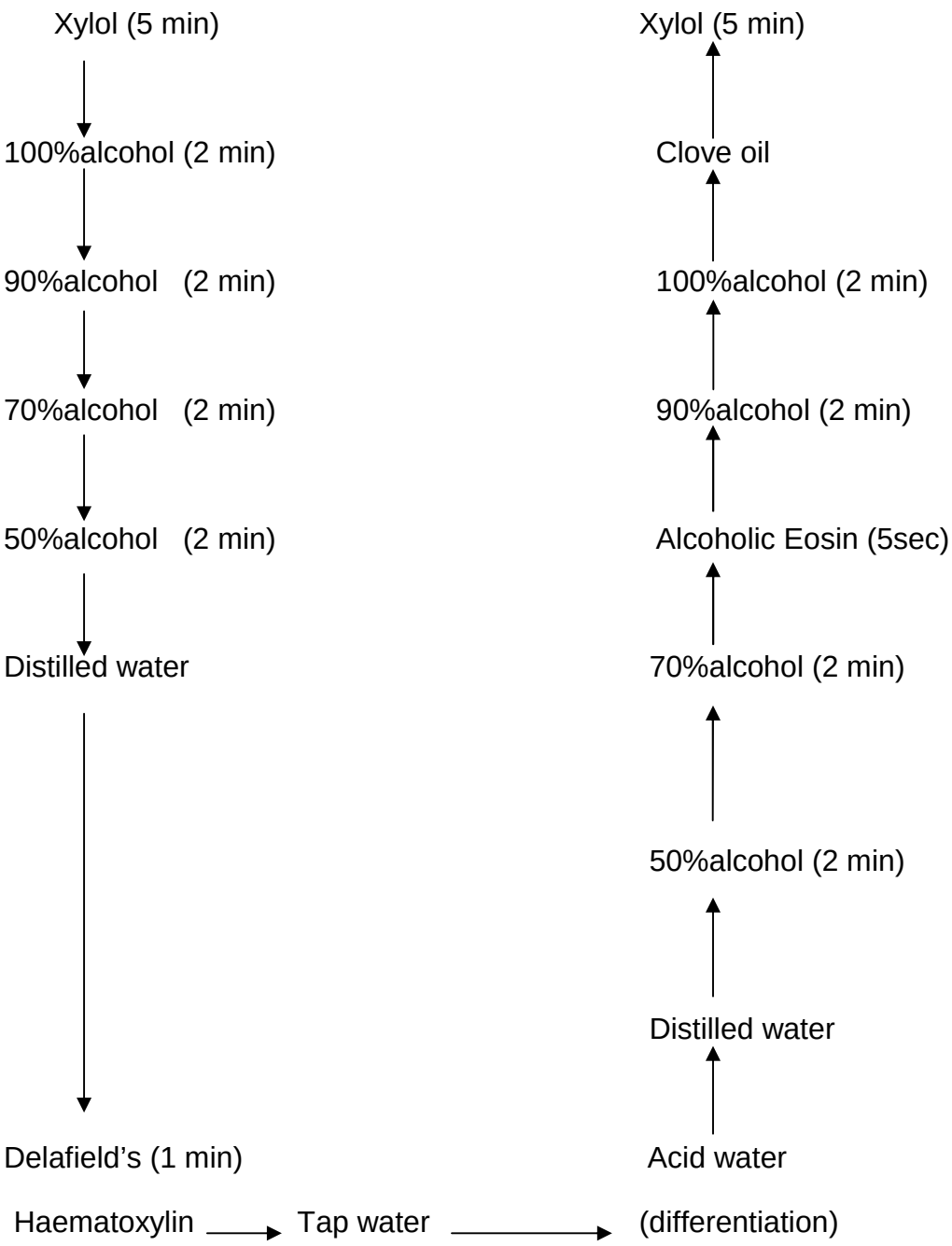
After 24 hours of fixation in the above fixative, the tissues were washed under running water for next 24 hours. Then tissue were dehydrated in 50% alcohol (3 changes at one hour interval each) followed by 70% alcohol. Thereafter, tissues were further dehydrated (90% and absolute alcohol) and cleared in Xylene and finally embedded in paraffin wax (M.P. 58 - 60°C). The block ready for sectioning was finally adjusted on the microtome machine and the tissues were sectioned at the thickness of 5 micron.

Staining: Sections of tissue are usually stained with two dyes to bring contrast between different histological structures. This makes detailed study easier. Staining with two dyes is known as double staining. The most common double staining practiced in a class work is with haematoxylin and eosin.

The general staining, paired with haematoxylin and eosin.

Stain	Common use	Nucleus	Cytoplasm	Red blood cell (RBC)	Collagen fibers	Specifically stains
Haematoxylin	General staining when paired with eosin	Blue	-	-	-	Nucleic acids—blue ER—blue
Eosin	General staining when paired with haematoxylin	-	Pink	Orange / red	Pink	Elastic fibers—pink Reticular fibers—pink

B. Staining Procedure:


Xylol (5 min)
100%alcohol (2 min)
90%alcohol (2 min)
70%alcohol (2 min)
50%alcohol (2 min)
Distilled water
Delafield's (1 min)
Haematoxylin
Tap water
Acid water
Distilled water
50%alcohol (2 min)
70%alcohol (2 min)
Alcoholic Eosin (5sec)
90%alcohol (2 min)
100%alcohol (2 min)
Clove oil
Xylol (5 min)
(differentiation)

Permanent slide preparation:

The slide with the paraffin ribbon containing sections of tissue is dipped in Xylol for about 5 minutes. The paraffin dissolves. The slide is transferred to absolute alcohol.

Haematoxylin is in aqueous medium. Presuming eosin in 70% alcohol the procedure to be followed is given below.

Time in each grade of alcohol is about two minutes with two changes in absolute alcohol. Time required for staining and differentiation is determined by trial. A small amount of DPX is put on the slide, depending upon the size of the cover slip to be used. The amount should be just enough to form a very thin film on the slide. The cover slip is put on it. If the air bubbles are locked between the cover slip and the slide, it may be removed by leaving the slide overnight on a hot plate.

OBSERVATION

Report on physical, chemical and bacteriological analysis of water

Water Analysis Laboratory, Balkhu, Kathmandu.

Source of Sample: Deepboring (Raw water)

Location of Sample: Jawgal, Lalitpur

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Table I. Raw Water

S.No.	Parameters	Unit	Results
1	Total Dissolved Solids	mg/l	576
2	Total Alkalinity	mg/l	690
3	Total Hardness	mg/l	356
4	Bicarbonates	mg/l	690
5	Ammonium	mg/l	94
6	Calcium	mg/l	80
7	Free carbondioxide	mg/l	144
8	Chloride	mg/l	5.88
9	Nitrite	mg/l	1.8
10	Nitrate	mg/l	22
11	Dissolved Oxygen	mg/l	0

Here concentration of ammonium is very high and the dissolved oxygen is nil. The amount of nitrate and nitrite found in this water is less.

Report on physical, chemical and bacteriological analysis of water

Water Analysis Laboratory, Balkhu, Kathmandu.

Source of Sample: Deepboring (Processed water)

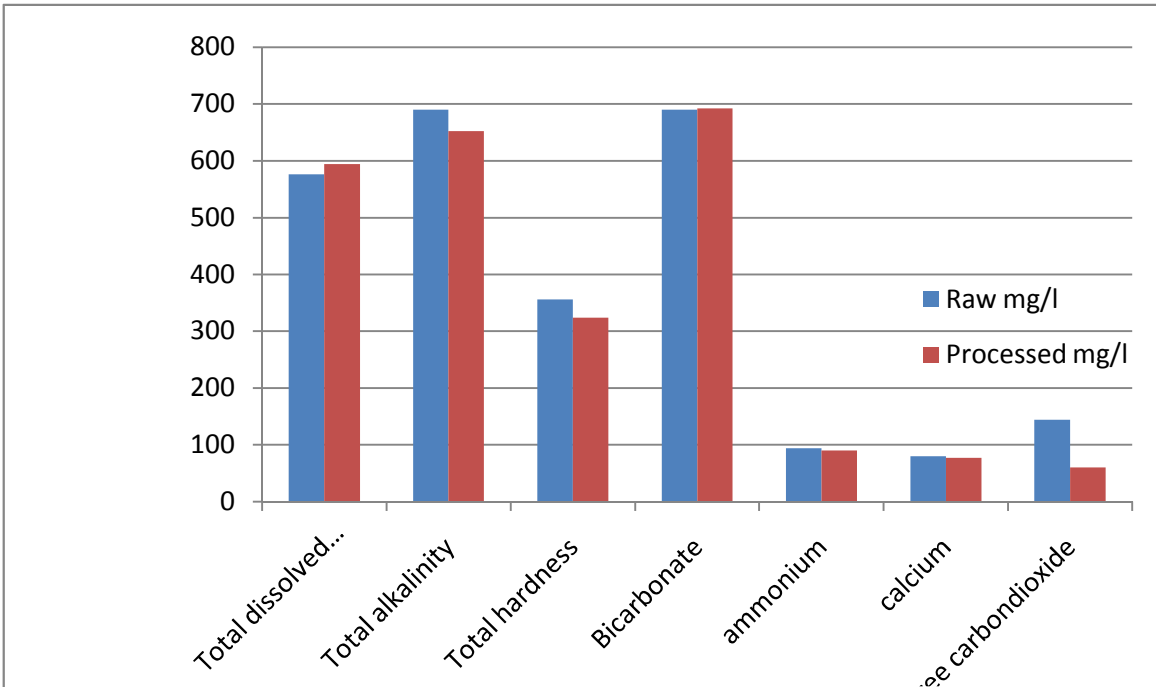
Location of Sample: Jawgal, Lalitpur.

Table II. Processed Water

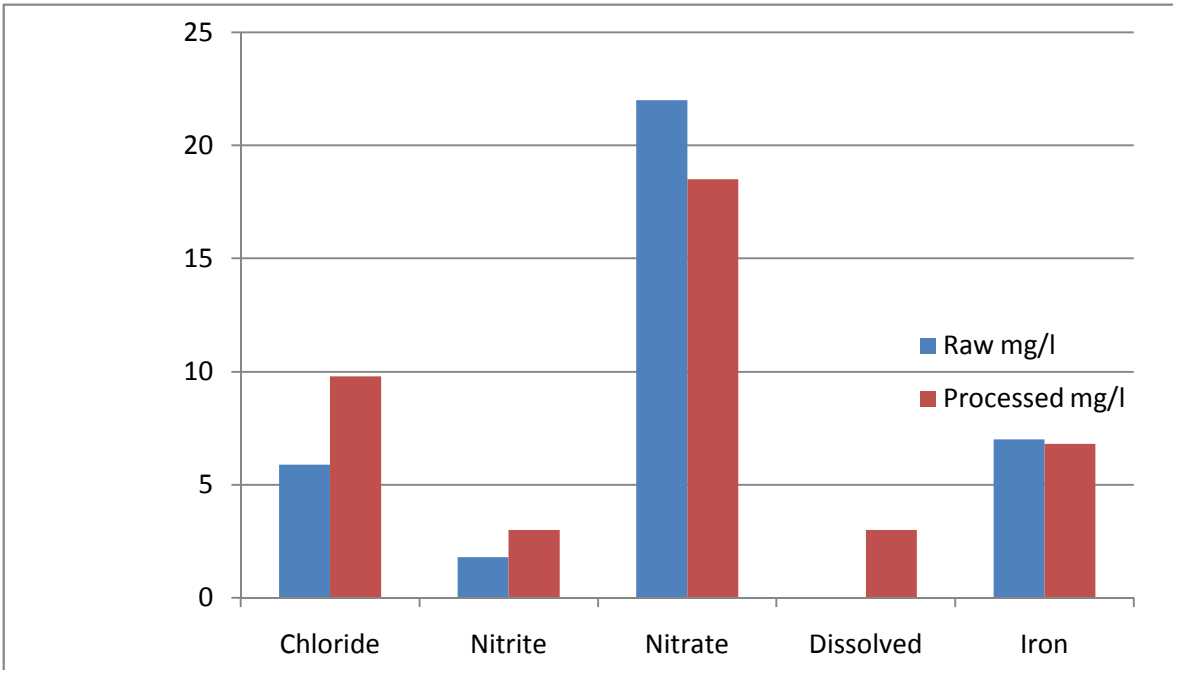
S.No.	Parameters	Unit	Results
1	Total Dissolved Solids	mg/l	594
2	Total Alkalinity	mg/l	652
3	Total Hardness	mg/l	324
4	Bicarbonates	mg/l	692
5	Ammonium	mg/l	90
6	Calcium	mg/l	77
7	Free carbondioxide	mg/l	60
8	Chloride	mg/l	9.8
9	Nitrite	mg/l	3
10	Nitrate	mg/l	18.5
11	Dissolved Oxygen	mg/l	3

Here the concentration of the ammonium is comparatively low than raw water but the dissolved oxygen is found to be rising. The amount of nitrate and nitrite in this water is very high.

Text fig.I



Text fig.II



1.Tap water

The fishes were kept in aquarium provided with tap water and feed (boiled liver of chicken) at a normal temperature of 26^{o}C. For the first day the behavior pattern of the fish was highly aggressive. This may be due to the environmental fluctuation also, but later they all came to normal as they adjusted themselves to that environment.

As we know the specimen fish was highly carnivorous and detritus feeder in nature, the fish fed voraciously on the provide meal to them. The stockings density of the fish in the aquarium was 7 fish in one aquarium of size 50x50x125 cm^{3} at the volume of 40 liters of water.

Thereafter, we noticed no changes in the mortality and abnormal behavioral changes in the fishes.

When the animals were sacrificed after 7 days, histomorphological study of the ovary reveals no any significant changes on these fishes. All the oocyte was in normal condition. There was no appearance of the cloudy mass over the oocyte, no vacoulisation, neither clumping of the oocytes. The germinal epithelium was thin and tunica albuginea was thick as seen in (plate I, fig-1).

After 10 days the remaining fishes provided under same condition showed marked significant changes like the colour were changing from greenish to pale yellowish. Some of the undergoing atresia can be observed (plate I, fig-2). The size of the some oocytes were relatively large those of the previous observation.

At the last day of dissection, that is on 14th day the ovary was seen more enlarged and somewhat yellowish in colour. The yolk can be seen at 10x under the microscope. The nucleus was located near the pheriphery (plate I, fig-3 and fig-4).

2.Raw water

The fishes kept under this condition were found to be very interesting as they showed varied changes in their behavior and morphology. There were high rate of mortality due to the enormous growth of bacteria on their body during the time of experimentation. Even small wounds in their body would make fatal as the raw water contained bacteria and impropotionate water quality parameters. According to the reports of the water analysis, turbidity, alkalinity, total iron, and manganese are found to be higher than the limits recommended by WHO.

The dead fishes had a slimy and gelatinous secretion over their body. The fish look whitish in colour due to the invasion of bacteria on their body. Rigormortos occurred very quickly after the cessation of fish life. The off smelling of the fish was noticed few hours later.

Histological studies of the ovary of *Clarias batrachus* after 7 days event

in the raw showed atretic follicles with some its portion with hypertrophic condition of zona granulosa and zona radiata shows deviation (plate II, fig-1).

After 10 days, vacuoles were clearly seen with irregular shape of follicles (plate II, fig2). Prominent channels and vesicles were formed in the cytoplasm and the lipid level seems to be less as the staining was very light (plate II, fig3).

Then, after 14 days the yolk mass was negligible and cytoplasmic shrinkage was very prominent making large gaps in the ooplasm. The cell boundary seems as they all was merging into one another (plate II, fig-4).

3. Processed water

To achieve the objective or purpose of the study, the fishes that are to be experimented were kept in an aquarium provided with processed water supplied with the standard water quality parameters chart from KUKL. When the fish were first kept in this condition for the first time, they showed aggressive behavior; this may be due to environmental fluctuation, but later they all came to normal as they adjusted themselves to that environment.

Since, we know that *Clarias batrachus* is a one of the very highly resistive fish even though they were dying at intervals of time during the research work due to vulnerable growth of the bacteria over their body. The bodies of the dead fishes look silvery white in colour and the whole texture was covered with slimy jelly mass like secretion.

Histologically studies of the ovary of these fishes showed few marked significant, as there was change in the structural pattern of the cell organells in the ovary.

After 7 days of, the yolk globules were seen bit elongated in the ooplasm and the yolk vesicles were reduced in number (plate III, fig-1). However, the zona granulosa and zona radiata was more organized than in case of raw water ovary (plate III, fig-2).

Ooplasmic shrinkage occurred after 10 days and at some places few nucleus undergoing abnormal mitosis were seen (plate III, fig-3).nuclear membrane was ruptured at some place of the nucleus (plate III, fig-4).

After 14 days of the event, some changes were observed in the cell organells of the ovary. The clumping of the cells were seen though hypertropism of the zona granulosa was not seen (plate III, fig-5 and fig-6).

PLATE I

Microphotographs of section of ovary during spawning phase stained with haemotoxylin and eosin. (10x)

After 7 days

Fig1.Thick ovarian wall consisting of thin outer peritoneum (P), thick middle layer of Tunica Albuginea (T) and innermost Germinal Epithellium (G).

Note: presence of large numbers of Oocytes. The nucleus is located towards the center of the oocyte.

After 10 days

Fig2.Different stages of the oocytes can be seen from the GE towards the center of ovary.

After 14 days

Fig3. Yolk of the ovary.

Fig4. Presence of large numbers of oocytes.

PLATE II

Microphotographs of section of ovary during spawning phase stained with haemotoxylin and eosin. (10x)

After 7 days

Fig1. Atretic follicles (AF) were seen. A portion of AF with hypertropic condition of ZG, ZR shows deviation.

Note: Finger like projection in the Ooplasm can be seen.

After 10 days

Fig2. Vacuoles can be seen in the Ooplasm.

Note: The follicles are of irregular shape.

Fig3. Prominent Vesicles and cannels can be seen in the cytoplasm.

Note: the lipid level seems to be less as the staining was light.

After 14 days

Fig4. The AF with hypertrophic ZG was clearly seen. The yolk mass was negligible.

Note: The cytoplasm shrinkage was very prominent making large gaps in the Ooplasm. The cells boundary was not seem as they all was merging into one another (clumping).

PLATE III

Microphotographs of section of ovary during spawning phase Stained with haemotoxylin and eosin. (10x)

After 7 days

Fig1. Yolk Globules (YG) can be seen along the periphery. These are bit elongated in the ooplasm.

Fig2. Yolk Vesicles (YV) at the periphery is reduced in numbers and is thicker. Zona granulosa (ZG) and zona radiata are more organized than in raw water.

After 10 days

Fig3. Nucleus (N) formation though Nuclear Membrane (NM) was ruptured at same place.

Note: Few N undergoing abnormal mitosis

Fig4. In the Yolk mass, the Yolk Vesicles (YV) was seen more in numbers along the ZG. Ooplasmic shrinkage was not that prominent but few channel like structure can be seen.

After 14 days

Fig5. Almost regular boundary of ZG, ZR seems to be more organized.

Note: Hyper tropism of ZG was not seen.

Fig6. A round structure can be seen in nucleoli which did not take stain. The clumping of the cells was seen. (40x)

DISCUSSION

The Histomorphological study revealed that the structural abnormalities in both vitellogenic and maturing oocytes followed by atresia. These include: clumping of cytoplasm, vacuolation, disorganisation and liquefication of yolk – granules and ooplasm and also shrinkage in nuclear material in vitellogenic follicles after the treatment of the fishes into raw and processed water. The same result was also found by Sahai (1988); Patwardhan and Gaikward (1990);Dutta et al (1993) Guraya (1993) after 24 hours exposure to the sublethal concentration of malathion.

Dutta et.al.(1994) reported follicular atresia, clumping of cytoplasm, increased nucleoli number, shrunken nuclear material and fusion of the oocytes after the treatment of malathion in *Heteropneustes fossilis*. They also observed the reduction in estrogen level.

Baronia et. al. (1993) while, studying long term malathion administration on ovary of albino rats, observed enlarged germinal epithelium, vacoulisation, irregular and damaged ovum, pycnotic nuclei and large cystic follicles. However, in our case the evidence i.e vacuole formation was seen in the ovary of raw water fishes to overcome the stress (plate II, fig-2 and fig-3).

The effects of three sublethal concentration of the electroplating industry have been reported by Gupta and Saxena (1991) on the ovaries of the *Heteropneustes fossilis*. It showed significant higher percentage of the atretic oocyte. The numbers of the nucleoli in these atretic oocytes were significantly reduced form the control value. Similar results have been observed in the present study. This suggested that amplification or transcription or both, of ribosomal genes were effected even by the improportionate water quality parameters.

According to Khanna (1985), the main function of the follicular atresia is to limit the number of oocyte that would undergo vitellogenesis and become mature for ovulation. The same case was observed in plate II, fig-3, fig-4 and

plate III, fig-2, fig-3). Atretic pre-vitellogenesis follicles, possibly give origin to the interstitial cells of the ovary, and may secrete steroid hormones required for the growth and maturation of the normal follicles. Histochemical and in-vitro studies have failed to demonstrate any enzyme activity in these atretic follicles. Further studies may finally decide whether the atretic follicles of the teleost are associated with degeneration and absorption of the yolk only or they have some other function also.

Mani and Saxena (1985) who studied the effect of sublethal concentration of fenitrothion and carbofuran on the oocytes of the *Channa punctatus* causing more atretic oocytes (both in young and mature) has convincingly favored the observation of Gupta and Saxena.

Singh and Singh (1980) expressed that pesticides produce low level of serum and blood gonadotropin in the fish due to accumulation or direct toxic action on the ovary. Jagdeesh and Sahai (1987) observed degenerative gonadotrophs in the pituitary of *Heteropneustes fossilis* after exposure to malathion, which lead to the secretion of lesser amounts of gonadotropin changing the reproductive physiology of fish. The cyclic change in the serum T3 probably, explains the biphasic nature of thyroid hormone action, a higher potency of T3 than T4 and the probable prohormone nature of T4 as documented by De.et.al.(1989) in *Heteropneustes fossilis*.

In the present investigation oocyte swelling, formation of the cloudy mass over the follicles, loosening of inter-cellular attachment are incessant degenerative changes in the ovary of the raw water fishes was observed (plate II, fig-2, fig-3, and fig-4). Unceasing furrows, cleft and pits occur according to the functional demand under physiological and pathological condition of the ovary. On contrary, the histology of the ovary of the processed water fishes were seem to be normal as seen in the normal fishes.

According to Matty (1985) the teleost pituitary contains and releases a thyrotropic hormone (TSH) which regulates thyroid function. He stated that the teleost thyroid could be stimulated by mammalian growth hormones and thyrotropic hormone. Fish pituitary contains variable amount of TSH which

cause thyroid involvement in growth and reproduction on other metabolic activities.

Further, he stated that the pituitary of some fishes have a TSH content that may be 10-12 times as great as that found inmammals.

G. M. Sinha and P. C Pal have reported the variation in the lipid level of the ovary of *Clarias batrachus* during annual ovarian cycle. Maximum lipid level has detected in the organs like liver, body muscle and ovary during maturation phase. Further, the stored nutrient may serve as the source of energy to be utilized in metabolism, ovarian maturation and spawning activity. However, they observed decrease in the content during spawning phase onwards and minimum in the periods between resting and spawning phase. Meanwhile, in our case we found the same result but little earlier to spawning phase on the ovary of raw water after 14 days (plate II, fig-3) while the others remain constant.

From the above account it is evident that the impact of raw water has more effect on reproductive organ i.e. ovary of *Clarias batrachus* rather than by processed water. On contrary, the processed water has negative impact on the ovary since the abnormal changes on the histology of the ovary had occurred. The clumping of the cell was observed at the later stage of maturation i.e after 14 days of treatment in processed water (plate III, fig-5 and fig-6). But these changes were marked less significant than those with of raw water impacts on ovary of *Clarias batrachus*.

SUGGESTIONS

1. Understanding the reason for variation in the raw water quality is important, as it will influence the requirement for treatment.
2. Identification and implementation of control measures should be based on the multiple-barrier principle. The strength of this approach is that a failure of one barrier may be compensated by effective operation of the remaining barriers during water treatment process.
3. Awareness should be emphasized by the renounced organization like WHO, UNICIEF, NGOs, Governments, etc., on the impact of raw and processed water on human health as well as on aquatic flora and fauna.
4. Ritual bathing in the polluted rivers like Bagmati is still practiced today. Many of the worshippers are unaware of the water quality parameters and its effect on health. They must be made precise consciousness about the water parameters and its effect on their reproductive systems.
5. I request to grant more encouragement at sub cellular and molecular studies as water being necessity for being life affects the metabolism of life.
6. If I get the opportunity and facilities, I would like to continue this work at sub cellular and endocrinology level.

SUMMARY

Histomorphological study of the ovary of tap water fishes showed no any significant changes. All the oocyte was in normal condition. There was no appearance of the cloudy mass over the oocyte, no vacoulisation, neither clumping of the oocytes. The germinal epithelium was thin and tunica albuginea was thick at the early stage spawning phase and some oocytes had undergone atresia late spawning phase.

In case of raw water fishes there was enormous growth of bacteria over the body of the fishes. The histomorphological study of the ovary revels that the fishes may have come upon the stress due to high level in the ammonia, nitrate, nitrite and low DO.

On contrary, little significant changes were observed in the ovary of these fishes since the level of chlorine was high and not suitable for the sustenance of the fishes.

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