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Arsenic Toxicity on Respiratory Physiology and Organic Reserves of Gills of *Mystus vittatus* (Bloch)

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Abstract

The effect of heavy metal, arsenic on changes in total oxygen consumption at different sublethal concentration and different time intervals of 10, 20 and 30 days was studied in a fresh water teleostean cat fish, *Mystus vittatus* (Bloch). The average oxygen consumption by this fish in normal water was 0.614 ml/g/hr in control. The glycogen, protein, triglyceride, acid and alkaline phosphatases content were decreased 21.49–61.78%, 11.56–47.22%, 14.76–65.05%, 7.98–54.35% and 16.67–38.71%, respectively in arsenic exposed fish. A significant decreased in oxygen consumption and organic reserves of gills were recorded at every time and every concentration of arsenic trioxide as compared to control fishes. The effect was more pronounced as the concentration of arsenic trioxide and duration of exposure increased.

Keywords: Arsenic; Gill; Glycogen; *Mystus vittatus*; Oxygen consumption; Protein; Triglyceride.

Introduction

Fishes are exclusively aquatic animals. A number of workers have studied the effects of different toxicants on various species of fishes including Prakash and Verma, (2018), Srivastava Prakash, (2019) and Kumar et al., (2019). The arsenic is a widespread environmental contaminant, which enters the aquatic ecosystem from natural and anthropogenic sources. The drinking water containing more than 10 µg/L of arsenic is harmful to the body and chronic exposure to arsenic contaminated water and food causes cancer (WHO, 2001). Arsenic is the first metalloid to be identified as a human carcinogen and most cases of chronic arsenic sis are associated with continual intake of arsenic-contaminated water (Ananth et al., 2014).

The most frequently used arsenic compound is arsenic trioxide. It is used in the synthesis of inorganic agrochemicals like phosphate fertilizers and pesticides and various organic compounds. Fish is the major source of arsenic exposure and humans who consume arsenic exposed fish may be threatened by arsenic toxicity. Fish tissues, skin, liver, muscles, kidney, gill, brain, gastrointestinal tract and blood are commonly involved in arsenic poisoning (ATSDR, 2006; Prakash and Verma, 2019a, 2020a, 2020b and 2020c; Verma and Prakash, 2019a, 2019b and 2020). Hence, the utility of fish in assessing contaminations in water has gained prominence in recent years (Ananth et al., 2014). The present work is an endeavour to study the effect of sublethal concentration of arsenic trioxide on total oxygen uptake by fish and changes in organic reserves of gills in a freshwater catfish, *Mystus vittatus* (Bloch).

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Materials and Methods

The healthy Mystus vittatus ranging from 7.0–8.0 cm in length and weighting 8.0–9.0g were collected from ponds in and around Balrampur (U.P.) and washed with 1% solution of KMnO4 for five minute and then transferred to the plastic jar containing 50L dechlorinated tap water for acclimatization. Fishes were acclimated to laboratory conditions for 15 days at room temperature. The LC50 values of arsenic trioxide for 24, 48, 72 and 96 hours were 4.71, 4.16, 3.68 and 3.20 ppm, respectively (Prakash and Verma, 2019b). Based on 96 LC50, fish were exposed to sublethal concentrations (10%, 20% and 30%) for treated and control period of 10, 20 and 30 days. A control group was maintained in an identical environment. The fishes were regularly fed with commercial food and the medium was changed daily to remove faeces and food remnants. The total oxygen consumed by fish were measured by the method of Ray and Kumar (2013) and Sharma and Kumar (2013).The rate of oxygen uptake per gram weight of fish per hour was calculated and the values were expressed as ml O_2/g/hour.

The fishes were sacrificed from both experimental and control groups on 10th, 20thand 30th days of exposure periods. The gills were homogenized in 0.25 M sucrose solution and centrifuged at 1000x g for 10 minutes. The supernatants were filtered and the filtrates were used for analysis of glycogen, protein and triglyceride by standard method of David (1992). The data in this paper is presented with mean ± mean standard error and the statistical significance of difference between control and experimental group was calculated by student's t-test.

Results and Discussion

Oxygen uptake of *Mystus vittatus* exposed to sublethal concentrations of arsenic trioxide is given in the Table 1. In the present study, oxygen uptake rate decreased from 16.50% to 67.15% in fish, Mystus vittatus exposed to 10–30% sublethal concentration of arsenic trioxide for a period of 10, 20 and 30 days. In the present study, the oxygen consumption was gradually decreasing with increasing concentration of arsenic and exposure periods. Minimum (-16.50%) and maximum (-67.15%) decline over control in the rate of respiration was noticed in 10% and 30% sublethal concentration on 10 days and 30 days of exposure, respectively (Table 1).

In the present study, it has been found in *Mystus vittatus* that short and long term exposure of arsenic trioxide decreased the total oxygen consumption to a significant level as compared to control. This finding corroborates the finding of Sornaraj et al., (1995) in *Channa punctatus* after the exposure of heavy metals, Ray and Kumar (2013) in *Clarias batrachus* after the exposure of parathion and sevin and Roy and Kumari (2016) in *Channa punctatus* after the exposure of agrochemicals. The decrease in the rate of oxygen consumption in *Mystus vittatus* after arsenic trioxide exposure may be due to coagulation "Film anoxia" in which mucous is lost from gills, as a result of which oxygen from surroundings media is adversely affected. The decrease in oxygen consumption can also result from the disintegration of the respiratory epithelium. Branchial lesion together with coagulation film anoxia is likely to result in serious respiratory distress and related hypoxia (Roy and Kumari, 2016). Decrease in oxygen uptake by gills can result in oxygen debt but also loses its effective mechanisms for 'histoxic anoxia' in which gill tissue not only suffers from oxygen debt but also loses its effective mechanism for removing carbon dioxide from blood. Anoxia or hypoxia increases carbohydrate consumption and thereby induces a sort of respiratory stress on organisms even at a sublethal level resulting in additional expenditure of energy (Verma and Prakash, 2019a).

Table 1: Changes in the oxygen uptake of *Mystus vittatus* at different sublethal concentrations of arsenic (O_2ml/g/hr)(N=6)

Experimental group	Exposure Periods (Days)		
	10	20	30
Control	0.618±0.55	0.609±0.64	0.615±0.56
10% As2O3	0.516±0.43(-16.50%)`	0.425±0.49(-30.21%)	0.363±0.65*(-40.98%)
20% As2O3	0.469±0.51(-24.11%)	0.377±0.56(-38.09%)	0.278±0.55*(-54.80%)
30% As2O3	0.315±0.49*(-49.03%)	0.267±0.67**(-56.15%)	0.202±0.39**(-67.15%)

*Significant at P< 0.05 ; ** significant at P< 0.01.

Table 2: Alterations in organic reserves of gills in arsenic induced *Mystus vittatus*

Experimental Group	Experimental Duration		
	10 Days	**20 Days**	**30 Days**
Glycogen (mg/g)			
Control	2.28±0.25	2.27±0.43	2.25±0.29
10%	1.79±0.43	1.62±0.32	1.50±0.33
	(-21.49%)	(-28.63%)	(-33.33%)
20%	1.59±0.41	1.29±0.34*	1.15±0.37*
	(-30.26%)	(-43.17%)	(-48.89%)
30%	1.25±0.32	1.18±0.34*	0.86±0.21**
	(-45.18%)	(-48.02%)	(-61.78%)
Protein (mg/g)			
Control	17.39±0.21	17.34±0.23	17.32±0.31
10%	15.38±0.33	14.76±0.31	13.43±0.32
	(-11.56%)`	(-14.88%)	(-22.46%)
20%	13.45±0.28	12.58±0.35	11.21±0.42*
	(-22.65%)	(-27.45%)	(-35.28%)
30%	11.54±0.31	10.34±0.21*	9.14±0.24**
	(-33.64%)	(-40.37%)	(-47.22%)
Triglycerides (mg/g)			
Control	4.54±0.23	4.58±0.33	4.55±0.21
10%	3.87±0.22	3.17±0.22	2.76±0.31*
	(-14.76%)	(-30.78%)	(-39.34%)
20%	3.12±0.23	2.66±0.31*	2.25±0.28*
	(-31.28%)	(-41.92%)	(-50.55%)
30%	2.93±0.25	2.27±0.34*	1.59±0.29**
	(-35.46%)	(-50.44%)	(-65.05%)
Alkaline phosphatase (µg Oleic acid mg/hr)			
Control	4.02±0.34	4.05±0.32	4.03±0.12
10%	3.35±0.31	3.10±0.24	2.82±0.48*
	(-16.67%)	(-23.46%)	(-30.02%)
20%	3.22±0.33	3.01±0.26	2.70±0.39*
	(-19.90)	(-25.68%)	(-33.00%)
30%	3.12±0.18	2.90±0.28**	2.47±0.26**
	(-22.39%)	(-28.40%)	(-38.71%)
Acid phosphatase (µg Oleic acid mg/hr)			
Control	1.88±0.46	1.82±0.29	1.84±0.34
10%	1.73±0.23	1.52±0.42	1.22±0.42*
	(-7.98%)	(-16.48%)	(-33.70%)
20%	1.57±0.52	1.32±0.24	1.01±0.47**
	(-16.49%)	(-27.47%)	(-45.11%)
30%	1.42±0.45	1.12±0.21*	0.84±0.32**
	(-24.47%)	(-38.46%)	(-54.35%)

*Significant at P< 0.05 ; ** significant at P< 0.01.

In the present investigation, arsenic exposed fish, *Mystus vittatus* showed a significant decrease in glycogen, protein triglyceride, acid and alkaline phosphatases contents of gill at all sublethal concentrations as compared to control. The glycogen, protein, triglyceride, acid and alkaline phosphatases content were decreased 21.49–61.78%, 11.56–47.22%, 14.76–65.05%, 7.98–54.35% and 16.67–38.71%, respectively in arsenic exposed fish (Table 2). The percentage of alteration in gills was directly proportional to the concentration of arsenic trioxide and duration of exposure. During experimental periods fishes showed various behavioural changes like increase in surface activity, opercular beating and mucous secretion over body (Prakash and Verma, 2019a). The increased activity demands extra energy and thereby a depletion of all the three components of the fish.

In the present study, during stress condition, the available glycogen were quickly exhausted to

meet increased energy demand and to maintain the uninterrupted and increasing energy requirement, the protein and triglyceride breakdown commenced to supply necessary precursor to carry on carbohydrate metabolism by TCA pathway, to release the much needed energy (Prakash and Verma, 2019b and 2020a; Verma and Prakash, 2019a and 2020). The carbohydrate resource was also used by the fish to produce protective coating around the body in the form of mucous.

Enzyme, acid and alkaline phosphatases are known as "inducible enzymes" and their activity goes up in the presence of any toxicant to counteract the toxic effect of toxicant (Leland, 1983). According to Parthasarathi and Karuppa (1998), alkaline phosphatase is capable to inactivate the phosphorylase enzymes involved in glycogen synthesis. Thus, any alteration in this enzyme affects the carbohydrate metabolism. Acid phosphatase is a lysomal enzyme that hydrolyses the easter linkage of phosphate esters and helps in autolysis of the cell after its death. Thus the increased activities of acid and alkaline phosphatases observed in the liver of test fishes exposed to sugar factory effluents can be attributed to the destruction of the cell membrane and lysosomes which intern leads to hepatic damage.

Thus, depletion in glycogen, protein and triglyceride content in liver may be due to the inhibition of enzymes as well as breakdown of stored glycogen, protein and triglyceride content to meet additional energy requirements under stress conditions.

Conclusion

In conclusion, this study showed that arsenic trioxide altered the oxygen consumption rate and would bring deleterious changes in the physiology of gills of freshwater catfish *Mystus vittatus* by damaging the gill epithelium leading to the loss of mucous secretion.

Acknowledgments

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Biochemical Activity of Superoxide Dismutase Enzyme in Liver of *Labeo rohita* in River Gomti

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Abstract

Most of aquatic animals are dependent on molecular oxygen. Yet all living forms are prone to oxygen toxicity. This oxygen toxicity has been attributed to reactive oxygen metabolites including the Superoxide radicals(O_2), Hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH-). These highly reactive substances can directly or indirectly cause substantial damage to living cells. The enzyme superoxide dismutase is essential for the survival of all respiring and metabolically active cells. Only respiring and metabolically active cells can generate reactive oxygen metabolites and they are controled at physiological concentration by a repertoire of cellular antioxidant defenses such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Glucose-6-Phosphate dehydrogenase (G-6-PDH) and Glutathione-S-transferase (GST).

Keywords: CAT; GPx; G-6-PDH; GST; OH; ROMs.

Introduction

The concept of oxygen as a requirement for aerobic life is mentioned inancient Indian civilization. For example, the Sanskrit word "PRAN VAYU" meaning the "the gas of life" appears frequently in the "VEDAS". For centuries, considered critical to life itself, oxygen remains and will always remain, one of the essential elements. The Biochemical Scientists have envisaged much interest on the role of oxygen derived free radicals in various diseases. "Reactive Oxygen Species (ROS) or Free Radicals" have been implicated in over hundred diseases from arthritis and haemorrhagic shock to AIDS (Southern 1988, Halliwell and Gutteridge 1989). The ROS is also related with plant defence system (Deepmala, 2019). The wide range of diseases implied increased formation of free radicals leading to cell and tissue injury in most, if not all human disease(Halliwell and Gutteridge; 1984 and 1989). The metabolism of oxygen generates reactive intermediates i.e. free radicals (Otto and Moon, 1995). A radical might donate its unpaired electrons to another molecule. It might take an electron from another molecule in order to pair or it might simply join to that molecule.

Superoxide is the best known free radical of all oxygen derived species (Fridovich, 1978) because it is the best intermediate in the sequential univalent reduction of oxygen that leads to formation of H_2O. (Florence, 1990; Harris, 1992; Winkle et al., 2007). The hazardous effects of reactive oxygen species are quite well-known, however their detoxification, which is one of the prerequisite of aerobic life, cannot over sighted for this reason. The nature has equipped the biosystem with multiple defenses. (Otto and Moon, 1996; Winkle et al., 2008; Mishra et al., 2008). The antioxidant systems responsible for cellular protection against oxidative stress are

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diversified as the free radicals themselves (Heffner and Repine, 1989). The Superoxide Dismutase (SOD) catalyzes the dismutation between two moles of superoxide anion to yield one mole of oxidize product (oxygen) and one mole of reduced product (Klug et al., 1972; Babich et al., 1993). This is ananalogoue to the dismutation of hydrogenperoxide to oxygen and water catalyzed by catalase (Mishra et al., 2008; Singh et al., 2009; Mishra et al., 2018; Dar et al., 2019; Abhijith et al., 2012). In rat, mice and fish, the Mn-SOD is localized in mitochondria whereas the Cu-Zn-SOD in cytoplasm. Various studies have been carried out to study the limnological condition and heavy metal pollution in the fresh water bodies and its impact on fish physiology (Prakash et al., 2015a, 2015b, 2015c; Srivastava and Prakash, 2018; Prakash and Verma, 2019a, 2019b, 2019c, 2020a, 2020b, 2020c; Prakash, 2020a, 2020b; Verma and Prakash, 2018, 2019a, 2019b, 2020a,) but no study on activity of superoxide dismutase was done.

The experimental fish *Labeo rohita* is most common fresh water non air breathing edible fish. It has antioxidant defense system which utilizes the enzymatic and nonenzymatic mechanisms. It can be expected that fish antioxidant defense mechanisms depend on oxygen consumptions. This antioxidant defense mechanism of fish will be detected by the Cu-Zn-SOD and Mn-SOD activities in liver (metabolic tissue) of *Labeo rohita.*

Materials and Methods

The fish *Labeo rohita* was collected from different sites of Gomti river at district Jaunpur, U.P. India and stocked in earthen container and acclimatized in laboratory conditions. The physico–chemical parameters of water such as temperature, pH, alkalinity and DO were analyzed by following standard methods.

After acclimatization fishes were sacrificed by decapitation and liver tissues were taken out then homogenized and centrifuged. The clear supernatant was taken for biochemical studies. The protein and superoxide dismutase were estimated bythe method of Lowry et. al. (1954) and Mc Cord and Frodovich, (1969), respectively. The superoxide anion were generated in a system comprised of NADH and PMS. The superoxide anion reduce the nitro blue tetazolium (NBT) forming a blue formazan measured at 560nm optical density. For the assay the tissue homogenate were diluted 1:4 for liver tissues. To find out the amounts of Cu-Zn -SOD and Mn-SOD in tissues, 2mm KCN solution was added to the mixture to inhibit CU, Zn SOD, Manganese SOD remain unaffected. (Fridovich, 1974; Nandi and Chatteree 1988; Crapo et al., 1978; Mishra et al. 2008). The unit of enzyme activity was defined as the amount of enzyme required to inhibit the optical density at 560nm of NBT reduction by 50% in one minute under assay condition. The data in this paper have been presented with mean ± mean standard error and the statistical significance of difference between control and experimental group was calculated by ANOVA.

Results and Discussion

Table 1: Physico- chemical parameters of water samples collected from different sites of Gomti river.

Sites	Temp. 0C	PH	Alkalinity mg/lit	DO mg/lit
Kalichabad	24.0±1.35	7.3	194±16.3	11.9±1.83
Katghara	24.2±1.89	7.4	190±17.8	11.3±1.07
Gularghat	24.2±1.18	7.5	199±13.8	7.90±0.99
Hanuman ghat	23.8±1.45	7.1	170±16.8	11.2±0.93
Achalaghat	23.8±0.21	7.0	169±13.6	11.5±0.54
Surajghat	24.1±1.20	7.2	171±13.9	11.9±1.96

Table 2: Total Cu-Zn SOD and Mn-SOD activities (unit mg-1 protein) in liver of *Labeo rohita* collected from different experimental sites. (Activity expressed as mean ±SD of 5 observations).

Experimental sites of River Gomti in Jaunpur	Total SOD	Cu-Zn SOD	Mn-SOD
Kalichabad	9.0±0.381	6.6±0.158	2.3±0.223
Katghara	8.±0.165	6.1±0.007	2.1±0.158
Gularghat	7.2±0.370	5.6±0.212	1.4±0.158
Hanuman ghat	7.1±0.444	5.8±0.244	1.3±0.200
Achalaghat	8.6±0.527	6.2±0.254	2.2±0.273
Surajghat	9.1±0.316	6.8±0.158	2.3±0.158

Table 2a: ANOVA of Cu/Zn SOD of data Table 2.

	S.S.	df	MS	F	P
Total	6.0	28			
Between experimental sites	5.1	5	1.04	27.7	<0.001
Error	0.9	23	0.0373		

Table 2b: ANOVA of Mn-SOD of data Table 2

	S.S.	df	MS	F	P
Total	5.70	28			
Between experimental sites	5.6	5	1.14	21.43	<0.001
Error	1.4	23	0.0539		

The physico- chemical parameters of the water

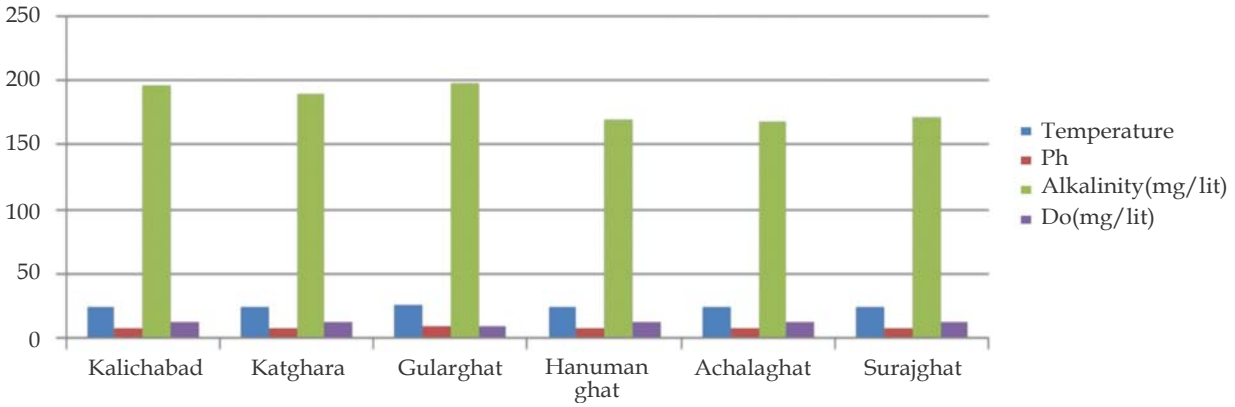

Fig. 1: Physico- chemical parameters of different sites of Gomtiriver at Jaunpur

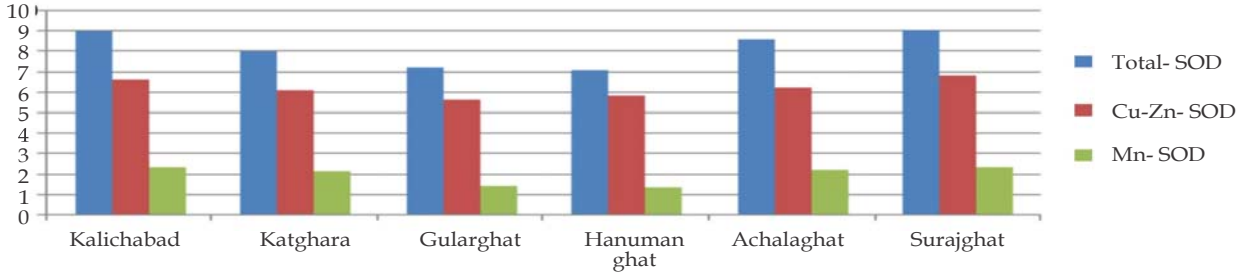

Fig. 2: Cu-Zn SOD &Mn-SOD activities (unit mg-1 protein) in liver of *Labeo rohita* collected from different sites

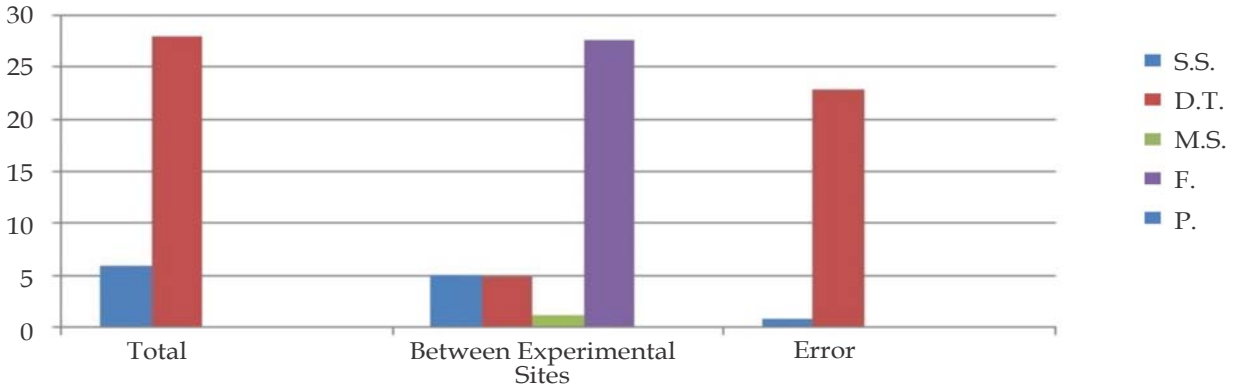

Fig 3: ANOVA of Cu/Zn SOD of data

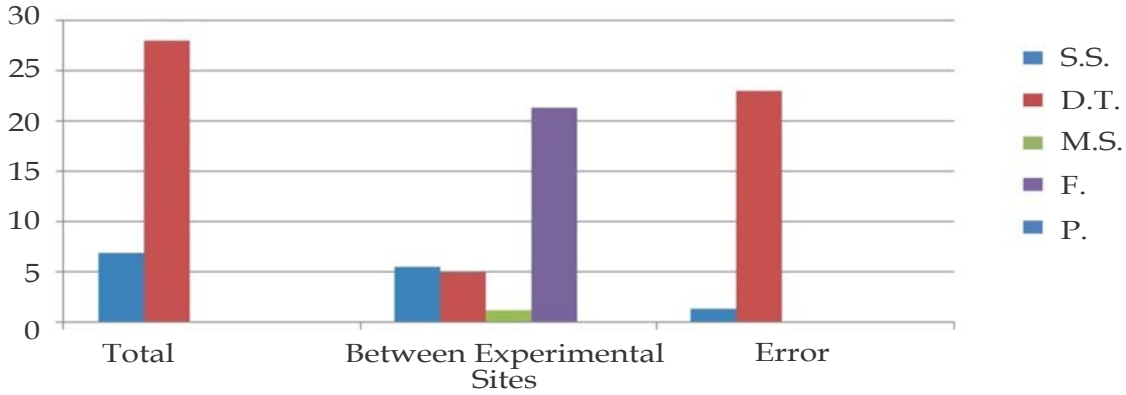

Fig 4: ANOVA of Mn-SOD of data

samples collected from six different sites of Gomti river at Jaunpur. In these experimental sites temperature was almost identical (23.8-24.020C). There was a nominal difference in pH and alkalinity in all the sites but dissolve oxygen show variation in different sites (Table 1).

The liver plays very important role in metabolism. The Superoxide dismutase, Cu-Zn-SOD Mn-SOD activities in liver of *Labeo rohita* are presented in table and Graph 2, Table 2a and Table 2b, respectively. The highest SOD activities was seen in fish collected from experimental site Kalichabad and lowest SOD activities observed in fishes collected from Hanuman ghat (Table and Fig. 2). The highest Cu-Zn-SOD was found in fishes collected from Surajghat site and lowest in Gularghat site (Table and Fig. 2). Similarly the Mn-SOD activity was observed highest and lowest in fishes collected from Kalichabad and Hanuman ghat respectively (Table and Fig. 2). The ANOVA results indicate that SOD activities of liver in *Labeo rohita* collected from different experimental sites were significantly with each other (Table and Fig. 1- 4).

The result shows that the liver of this more active non air breathing fish, *Labeo rohita* possess an enhanced antioxidant defense system while less active fishes have poor antioxidant defense system. More active fish are metabolically active tissue higher O_2 consumption cause oxidative stress which is inhibited by SOD. SOD forms the primary line of defense against oxidative stress.

Conclusion

The present study reveals the role of dissolved oxygen in the activity of superoxide dismutase enzyme in fresh water non air breathing fish *Labeo rohita*. High DO contents produces oxyanion that cause high oxidative activity of SOD. The present study clearly indicated that the physico-chemical parameters affect the SOD, Cu-Zn-SOD and Mn-SOD activities resulting the severe damage of cellular compounds and also conformational changes in nucleic acid, protein, lipids and carbohydrates. The fishes facing such severe problem of reactive oxygen species also produce the ADS (Antioxidant Defense System) to protect themselves.

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Thyroid Hormone Induced changes in Collagen Metabolism of *Duttaphrynus Melanostictus* in Relation to Age

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Abstract

The present study investigated the correlation between thyroid hormones (i.e. thyroxine (T4) & triiodothyronine (T3)) and total collagen content in tissues of Duttaphrynus melanostictus. Administration of both T4 and T3 (0.5 µg/gm) separately for seven consecutive days led to changes in the total collagen content of dorsal skin, ventral skin and muscle of both young and adult common Indian toads. Thyroxine is important for both collagen synthesis and matrix metabolism (Yen, 2001). Duttaphrynus melanostictus were collected from Berhampur University campus and acclimated at laboratory conditions for 2/3 days. Levels of collagen were estimated following the method of Neuman and Logan (1950), as modified by Leach (1960). T4 treatment appeared to stimulate the deposition of collagen as evident from values of total collagen of dorsal and ventral skin. On the other hand, T4 treatment inhibits the increase in total collagen in the muscle region. On the contrary, T3 treatment decelerated the collagen deposition in dorsal skin and muscle tissue. A tissue-specific action of T3 administration in common Indian toad was shown in ventral skin where the total collagen content increased though differing to some extent in a degree of response. There is an age-related response to collagen metabolism which is tissue specific. The total collagen content declined during maturity whereas showing an acceleration in the post-maturity period.

Keywords: T4; T3; Collagen; Duttaphrynus melanostictus.

Introduction

Collagen may be a fibrous structural macromolecule gift within the animate thing matrix and animal tissue of animals (Ramshaw et al., 2009). It is the sole most plentiful protein in the set of all animals. It is missing in plants and unicellular life forms where polysaccharides and cellulose takes up its job. In the invertebrates, collagen is found in the body walls and cuticles. Particularly in mammals, collagen contains 25-30% of the macromolecule substance of the complete body (Muller & Werner, 2003) and represents well over 70% of the dry weight of human skin (Rycker et al., 1984). It is found in the corneas, bones, blood vessels, cartilage, dentin of teeth, etc. It is found as elongated fibrils in fibrous tissues such as the skin, tendons and ligaments. It comprises 1-2% of muscle tissue wherever it's an important element of the endomysium. Collagen is created largely by the embryonic cell of animal tissue and conjointly by sort of alternative animal tissue cells (Kadler et al., 2007; Silvipriya et al., 2015).

Thyroid hormones are responsible for the early development of vertebrates, especially in amphibian metamorphosis (White and Nicoll, 1982). Thyroxine is arguably the most important hormone in anuran development and affects development through exogenous and endogenous means (Storz, 2003). Amphibian metamorphosis is a highly synchronized mechanism in which essentially all tadpole tissues are transformed (Storz, 2003)

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and understanding the role of the environment in triggering ontogenetic polyphenism is important in understanding the coordinated evolution of the physiological systems involved in metamorphosis (Storz, 2003, Denver 1997).

Thyroxine is important for both collagen synthesis and matrix metabolism (Yen, 2001). Hypothyroidism is the cause for accumulation of glycosaminoglycans (GAGs) in the extracellular matrix, which may, in turn, predispose to tendon calcification (Oliva et al., 2013). Impacts of select Na and Ca- channel blockers on collagen synthesis and deposition were estimated in cultured human dermal fibroblasts and aortic smooth muscle cells by immunoassay. Channel blockers tested demonstrated inhibitory effects on collagen type I deposition to the ECM by fibroblasts, each to a different degree. Ascorbic acid significantly increased collagen I ECM deposition. (Ivanov et al., 2016). Thyroid hormones have been reported to stimulate collagen and GAG production, but reported outcomes, including which specific collagen types are affected, are variable throughout the literature. The ability of thyroxine (T4) to preferentially stimulate collagen production, as compared with GAG, in articular chondrocyte derived scaffold-free engineered cartilage (Whitney et al., 2017). Collagen formation was found to be variable but generally slower than increase in the weight of the thyroid (Harkness, et al., 1953).

Materials and Methods

For the present study, the common Indian toads of both sexes were collected and reared.

Collection and Maintenance: They were acclimated in the laboratory condition at room temperature for 3–4 days in wire-netted plastic cages (75*40*35 cm) size containing a moist sand bed. They were forced-fed with goat liver (composition mg/g wet wt: 110±41 protein, 84±16 lipid, 2.3±1.1 glycogen) every day, and water was provided ad libitum. All collected animals were used within five to seven days of collection. The estimation of various biochemical parameters were completed with all the batches of animals of various sizes irrespective of their sexes.

Treatment: After laboratory acclimation, animals of mixed sexes of different age groups were divided into control and treated groups. The control and treated group contain both young and adult group animals. Each group consists of five animals. There were two treatments of T4 and T3 separately. The treated group of toads were injected intramuscularly with thyroxine (T4) and T3, Na-salts (Fluka A.G.) at a dose of 0.5 µg/gm dissolved in 0.65% NaCl solution, pH 8.3 in separate batches; while the control animals received an equal value of 0.65% NaCl solution, pH 8.3. This injection schedule continued for seven days at a fixed time. The animals were sacrificed on the eighth day for the estimation of biochemical parameters.

Tissue Processing and Statistical analysis: Following the method of Neuman and Logan (1950) as modified by Leach (1960), dorsal skin and ventral skin tissues of both control & treated group animals were processed for the extraction and estimation of collagen fractions. Using correlation, the statistical significance of the data was evaluated.

Results

Following results show the correlation between body weight and total collagen content of Duttaphrynus melanostictus in relation to age with the administration of T4 & T3 (0.5 µg/gm).

I. T4 treatment

A. Dorsal skin

There was a significant positive correlation between the body weight and the total collagen content of dorsal skin of controls ($r= 0.859$, $P< 0.01$). Initially it increased upto maturation period with increasing body weight. However, the T4 treated ($r =0.510$; P, NS) animals exhibited a very similar trend as compared to control animals. There was an increase in total collagen of T4 treated animals as compared to controls before the onset of maturity while it declined during the postmaturity period (Table 1 & 2, Fig. 1).

B. Ventral skin

The total collagen content of the control animals ($r=0.577$; P, NS) declined at the young age. With the onset of maturity, it remained constant and with the increase in age, it shows higher elevation during postmaturity period. However, T4 treated ($r=0.020$; P, NS) animals, it exhibited biphasic characteristics. It decreased up to the onset of maturity period. Then it increased with increasing body weight subsequently (Table 1 & 2, Fig. 2).

C. Muscle

The body weight and total collagen content of muscle showed a significant positive correlation in

controls (r =0.720; P< 0.02) while it is insignificant in treated animals (r =0.547; P, NS) (Table 1 & 2, Fig. 3).

II. T3 treatment

A. Dorsal skin

There was a significant positive correlation between the body weight and total collagen content of dorsal skin of controls (r =0.859; P< 0.01). The total collagen content increased after the onset of maturity with increase in age. The treated (r =0.892; P< 0.001) animals also showed a significant positive correlation. There was a decrease in total collagen content in treated animals as compared to controls (Table 1 & 3, Fig. 4).

B. Ventral skin

The total collagen content of control animals (r =0.577; P, NS) declined in young age. With the onset of maturity, it remained constant and with increase in age. There was a significant positive correlation between the body weight and total collagen content in T3 treated animals (r = 0.715; P< 0.02) (Table 1 & 3, Fig. 5).

C. Muscle

There was a significant positive correlation between the body weight and total collagen content of muscle of controls (r=0.720; P< 0.02). The total collagen content increases during the post maturity period. The T3 treated animals also showing the significant positive correlation (r =0.785; P< 0.01) between the body weight and total collagen content (Table 1 & 3, Fig. 6).

In vivo effects of thyroxine (T4 & T3) (0.5 µg/gm), i.e. Treated-I & Treated-II on total collagen characteristics in dorsal skin, ventral skin & muscle tissues of Duttaphrynus melanostictus.

CONTROL

Sl No.	Initial Body wt. (gm)	Final body wt. (gm)	Dorsal skin (mg/gm)	Ventral skin (mg/gm)	Muscle (mg/ gm)
1	15	14	252.539	207.388	200.426
2	19	22	271.646	224.605	223.720
3	24	20	280.459	199.430	213.151
4	27	25	264.581	182.522	252.646
5	28	28	251.650	246.677	206.890
6	45	46	269.993	202.762	234.741
7	50	52	281.227	205.200	298.822
8	55	59	323.457	206.368	343.448
9	65	67	350.384	255.129	397.294
10	69	72	394.821	318.510	245.186

Table-1

TREATED-I

Sl No.	Initial Body wt. (gm)	Final body wt. (gm)	Dorsal skin (mg/gm)	Ventral skin (mg/gm)	Muscle (mg/ gm)
1	13	25	286.094	425.008	72.400
2	27	21	319.150	442.367	56.208
3	31	29	302.445	368.189	81.741
4	33	30	335.767	205.399	119.501
5	34	31	322.177	210.869	135.195
6	41	36	171.865	123.139	33.062
7	54	50	240.896	158.649	70.590
8	62	54	289.811	345.646	158.538
9	69	61	473.129	366.816	111.592
10	81	74	441.314	373.994	373.994

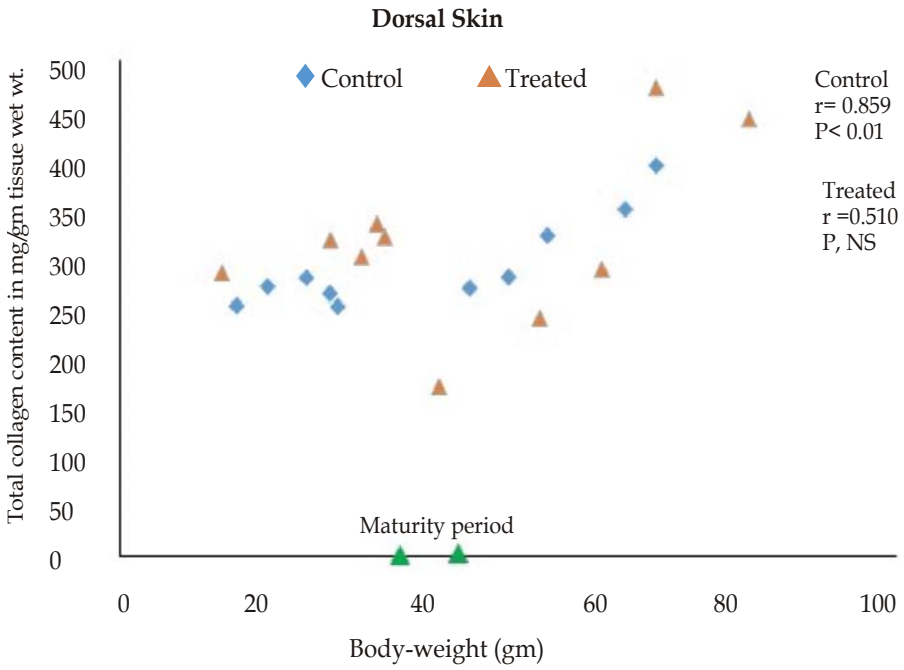

Fig. 1: Correlation of total collagen content in dorsal skin of control (r =0.859398395; P< 0.001) and T4 (r =0.509743145; P, NS) treated toads, Duttaphrynus melanostictus through different ages. Values are µg/g tissue wet weight. Dose - low dose (0.5 µg/gm).

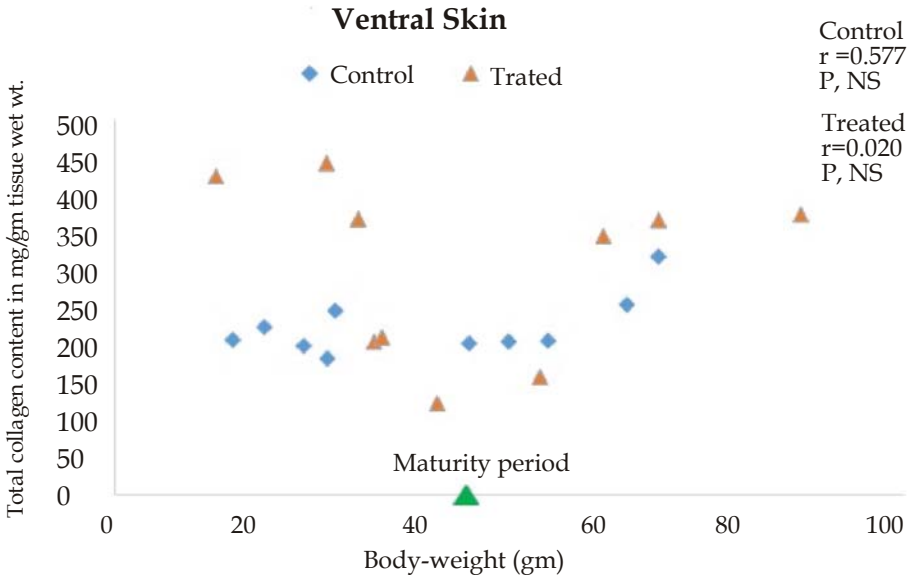

Fig. 2: Correlation of total collagen content in Ventral skin of control (r =0.576702986; P, NS) and T4 (r =0.020572113; P, NS) treated toads, Duttaphrynus melanostictus through different ages. Values are µg/g tissue wet weight. Dose - low dose (0.5 µg/gm).

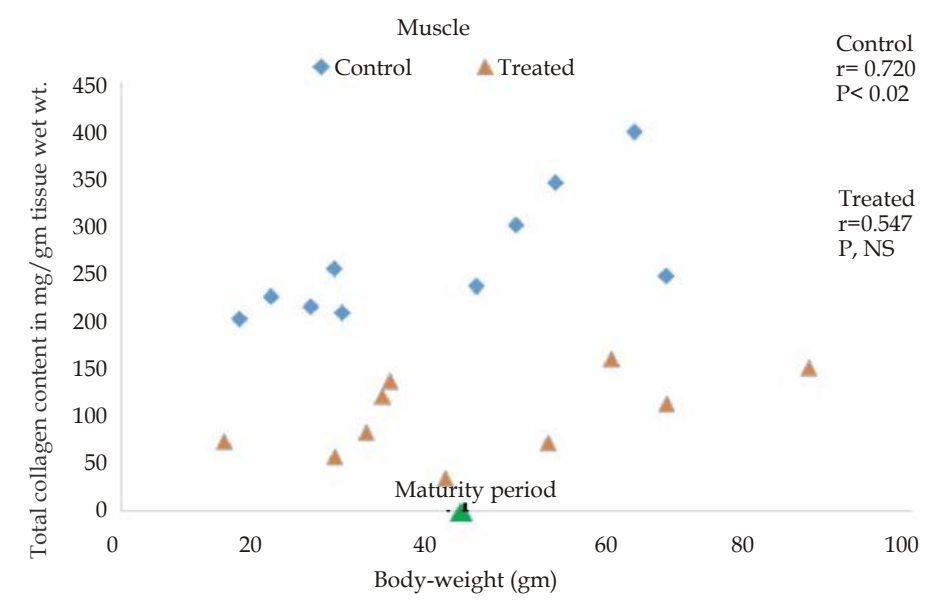

Fig. 3: Correlation of total collagen content in Ventral skin of control (r =0.576702986; P, NS) and T3 (r =0.71509395; P< 0.02) treated toads, Duttaphrynus melanostictus through different ages. Values are µg/g tissue wet weight. Dose - low dose (0.5 µg/gm).

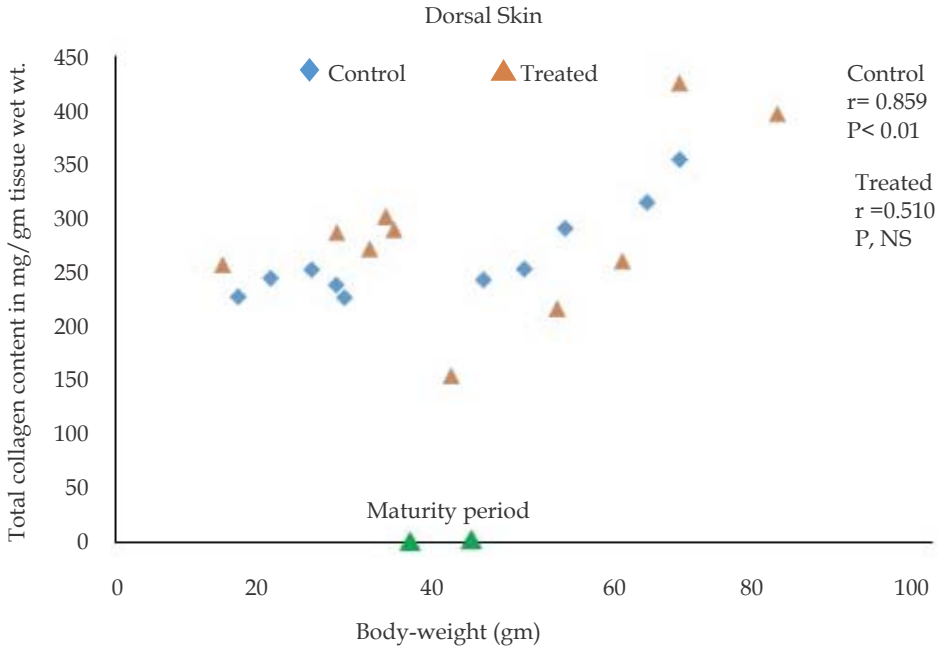

Fig. 4: Correlation of total collagen content in Dorsal skin of control (r =0.859398395; P< 0.01) and T3 (r =0.892517292; P< 0.001) treated toads, Duttaphrynus melanostictus through different ages. Values are µg / g tissue wet weight. Dose - low dose (0.5 µg / gm).

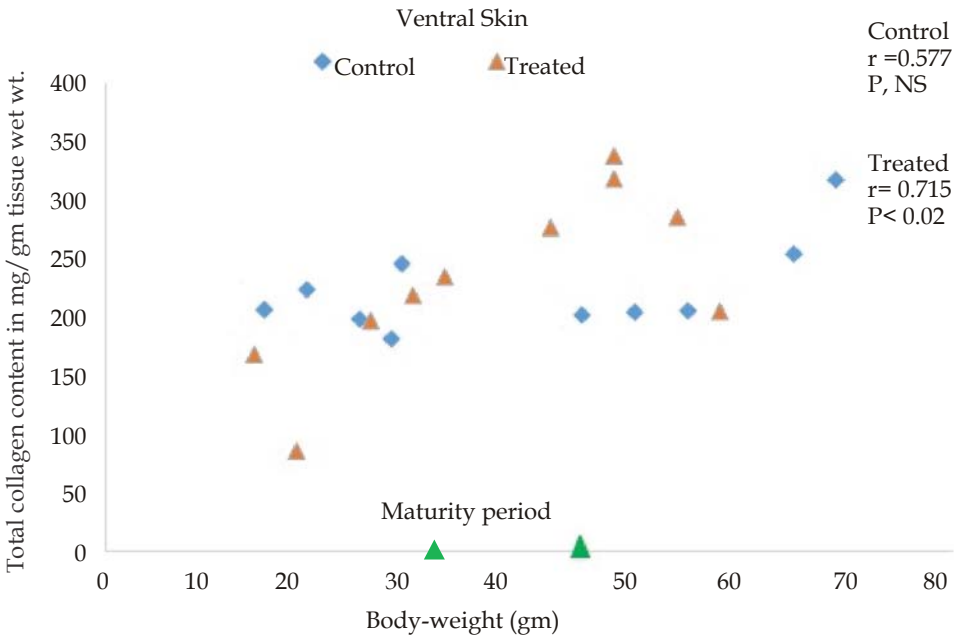

Fig. 5: Correlation of total collagen content in Ventral skin of control (r =0.576702986; P, NS) and T3 (r =0.71509395; P< 0.02) treated toads, Duttaphrynus melanostictus through different ages. Values are µg/g tissue wet weight. Dose - low dose (0.5 µg/gm).

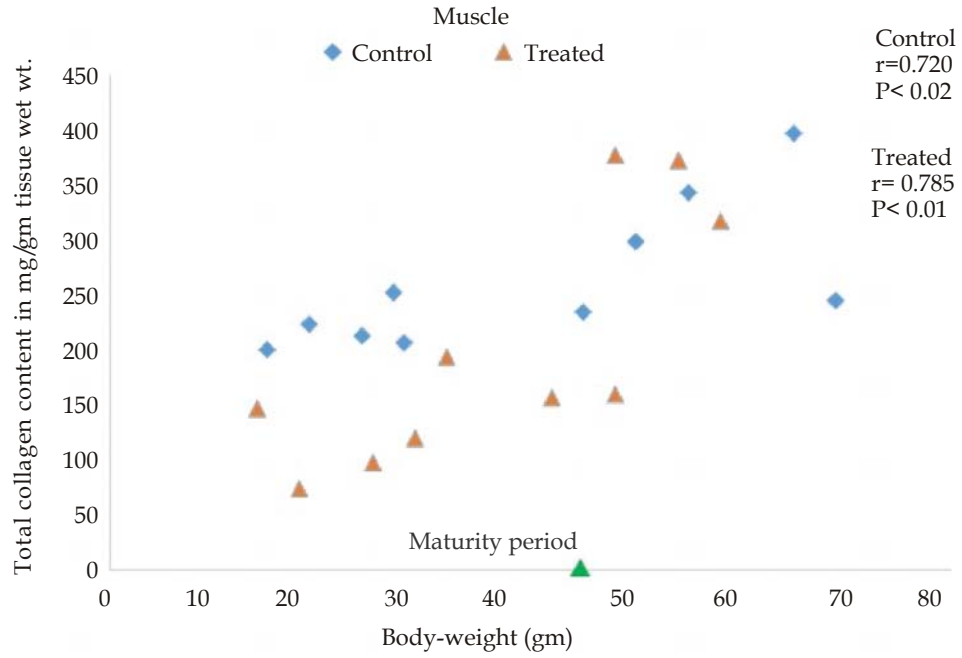

Fig. 6: Correlation of total collagen content in Muscle of control (r =0.720144006; P< 0.02) and T3 (r =0.785391931; P< 0.01) treated toads, Duttaphrynus melanostictus through different ages. Values are µg/g tissue wet weight. Dose - low dose (0.5 µg/gm).

Discussion

This have been reported to influence the metabolism of collagen. Hyperthyroidism potentially can be caused by an increased catabolism of both soluble and insoluble collagen. Hypothyroidism appears to be accompanied by lower rates of collagen catabolism (Kivirikko et al., 1965). The rate of collagen synthesis is declined both in hyperthyroidism and in hypothyroidism (Kivirikko et al., 1967).

One of the well-known parameters to accesses ageing in vertebrates is the changes associated with the characteristics of connective tissue protein collagen (Sinex, 1968). With advancing age, the number of intra and intermolecular cross-link ages increase in collagen molecule. This eventually leads to a derangement in physiological functions of various tissues. Cross-linked collagen in extracellular space may not effectively permit the transport of nutrients and oxygen to tissues. Vital organs like heart and kidney may not function effectively. The contraction mechanism of skeletal muscle may be impaired due to such cross-linkages. The deposition of calcium salts in collagen-containing matrix of bone may be disturbed. Thus, the ageing of collagen might affect the ageing of the organism as a whole. Such changes in the characteristics of collagen during ageing provide excellent support to the "cross-linking theory" of ageing (Panigrahy and Patnaik, 1973). A decrease in solubility (Verzar, 1964; Hall, 1976) and in soluble/ insoluble collagen ratio (Sinex, 1968; Walford et al., 1969) is the consequence of increased number of cross-links in collagen molecule (Mishra, 1987).

Several workers have thoroughly reviewed the hormonal control of mammalian collagen metabolism and its implications for growth and aging (Everitt and Burgess, 1976). Since the collagen characteristics undergo considerable modification during development, growth and aging, it is necessary to verify the involvement of hormones in such processes.

Higher-dose thyroxine is known to reduce the production of collagen in mammalian tissues. Hyperthyroidism appears to increase the catabolism of collagen (Kivirikko et al., 1963, 1967). Fink (1967) also stated that thyroxine causes bone collagen degradation. The total collagen content in the tendon decreases after treatment with thyroxine and increases in lizards treated with thiourea suggest that the hormone induces collagen degradation in garden lizards. Induced anabolic actions on collagen have also been reported of thyroxine (Drozdz et al., 1979).

Total content of collagen indicates approximately the balance between the synthesized and degraded amounts. There was a positive correlation between body weight and total collagen content in relation to age. The total collagen content is increased both in dorsal and ventral skin by the T4 treatment showing the deposition of collagen in youngs while adult toads showing degradation of collagen in dorsal skin and muscle tissue. T^3 treatment resulting in collagen degradation at both young and adults. In a majority of cases the quantity of collagen has been shown to increase with advancing age. In skeletal muscle (Schaub, 1963) and cardiac muscle (Schaub, 1964/5) of rat, the collagen content increases with age. A similar pattern was observed in the skeletal muscle of garden lizard (Haseeb and Patnaik, 1978). On the other hand, the total collagen content in the bone of garden lizard Calotes versicolor increased till sexual maturity and remained constant there after (Panigrahy and Patnaik, 1973) which supports our results. Increased collagen degradation is observed in hyperthyroidism (Kivirikko et al., 1963, 1967). Hyperthyroidism also stated by Fink in 1967 leads to increased degradation of bone collagen (Brahma and Pattnaik, 1982). T4 treatment showing collagen degradation reduces the total collagen content in both dorsal and ventral skin. Retardation of growth by decreasing synthesis of collagen followed by collagen catabolism (Pattnaik and Mishra, 2018).

Conclusion

An analysis of the graphs indicates an age-related response that is dependent on dose and specific tissue. There are several earlier reports that there has been an age-dependent response of these two parameters to thyroid hormones (Andia, 1984; Choudhury, 1992; Pattnaik et al., 2015; Mohanty, 2018). Such a dependency may result possibly due to the simultaneous effect of other hormones such as growth hormone and pituitary secretions, gonadal and adrenocortical hormones, nutritional status and other environmental factors. The correlation results indicated an increase in collagen in response to T4 and a decrease in the same in T3 with tissue-specific action.

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Effect of Hydroponic Maize fodder as a Substitute for Green Grass during Summer on Milk Yield in Indigenious Breed and Cross Breed Cows of Ganjam District

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Abstract

Milk yield in the dairy cattle depend on quality management of feed and fodder. To get better milk production, proper utilizations of nutrients is highly essential for the dairy animals. Usually farmers feed their animals with natural resources like roughage, rice bran, wheat bran etc. without considering balance quantity of feed. During summer season the availability of Green Grass is very less which is essential for the milking animals. The study is carried out to find out the effect of Hydroponic Maize Fodder (HMF) as a substitute of Green fodder during summer season. 20 lactating cows were selected for this Study (10 cows from Indigenous breed and 10 cows from Cross Breed). Both Breeds of cows again divided into two groups, five cows in each group from. The treated cows of each group were fed concentrate feed along with 10 kg Hydroponic Maize Green Fodder where as the control group cows fed with concentrated feed without HMF. The feeding trail was tested for 3 months.After the trial period milk yield were recorded for 7 consecutive days & found that the milk yield was significantly higher in the animal fed with HMF in both treated groups as compare to control groups. The use of HMF during summer season can enhance and maintain dairy farming productivity and improve farmer's income. Hydroponic Maize Fodder can be a substitute for green grass during summer season on milk yield in indigenous breed and cross breed cows of Ganjam district.

Keywords: HMF, Cows, Milk.

Introduction

The method of Hydroponic Fodder production was introduced during 1800s (Kerr et al., 2014). Sprouted grains were fed as a feed by the dairy farmers of Europe to their cows during the winter to improve the fertility and maintain the milk production. To fulfil the green fodder demand, the most important alternatives is hydroponic fodder as an extra supplement to the meagre pasture. The hydroponics word is discovered from a couple of Greek words: 'hydro' which means water and the word 'ponos' which means labour. Hydroponic fodder can be produced in low cost both in sophisticated, large automated commercial systems where the ambient environment is suitable for fodder production. In these days, hydroponics can also be developed in the harsh climates like poor soil area, urban area and also in the desert where the traditional cost for agriculture is very high. Developing of hydroponic is a pleasure, soil free, free from fertilizer, chemical, pesticides, herbicides. Developing of grains will result to increase the amount of quality and quantity of protein, minerals, vitamin and sugars. The most important benefit of nutrition delivered by the sprouted hydroponic fodder is to enhance the performance and general health of dairy cattle with minimizing the cost of the feed. Therefore, the study is intended at "Economic value and Nutritional improvement

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of hydroponically sprouted maize fodder". (Life Sciences International Research Journal, 2015)

Advantages of Hydroponic fodder

The hydroponic system reduces the wastage of water as it is directly applied to the roots of the plant and is frequently used for number of times (FAO, 2015). Hydroponics is a growing plants method in the base of water solution, free from soil and produce quick and nutrients rich fodder from Maize, Ragi, Bajra, barley, oats, Cowpea and Horse gram, etc. (Bakshi et al., 2017). The hydroponic system need very less time and space than conventional systems. The root of hydroponic fodder's plant are usually very smaller as compare to the traditional fodder as a result more numbers of plants grows in lesser space. Approximately 600 to 1000 kg green maize fodder in a day can be produced within 7–8 days of growth cycle in 45–50 m^2 area compared to the traditional farming (Naik and Singh, 2013; Rachel Jemimah et al., 2015). Hydroponic fodder contains rich source of vitamin C, vitamin A, vitamin E, riboflavin, biotin, niacin, thiamine, free folic acid, anti-oxidants such as B-carotene (Cuddeford, 1989; Finney, 1982; Naik et al., 2015) and minerals (Chung et al., 1989; Bhise et al., 1988; Fazaeli et al., 2012).

Objective of the study

To know the effect of hydroponic maize fodder as a substitute for green grass during summer on milk yield in indigenous breed and cross breed cows of Ganjam district.

Materials and Methods

The study has conducted to find out the effect of Hydroponic maize fodder as a substitute of Green fodder during summer season. 20 lactating cows were selected for this Study (10 cows from Indigenous breed and 10 cows from Cross Breed). Both Breeds of cows again divided into two groups, 5 cows in each group from (Indigenous Breed & Cross Breed). The treated cows from each group were fed concentrate feed along with the supplementation of 10 kg Hydroponic Maize Green Fodder where as the control group of cows were fed with concentrated feed without hydroponic maize fodder. The feeding trail is tested for 3 months (March, April, and May). During the trial period milk yield were recorded & found that the milk yield was significantly higher in the animal fed with Hydroponic Maize Fodder both in Indigenous breed & crossbreed cows group as compare to the control group cows. After feeding for a time of three months the samples of the milk were collected for 7 consecutive days. The milk samples from all groups were collected & measured by measuring cylinder and recorded daily. The data has been statistically analyzed by student's t-test (Microsoft excel 2007).

Results and Discussion

The contents of nutrients in the concentrate mixture are according to the BIS specifications of compounded Dairy animal feed (Table 1). Hydroponic Fodder looks just similar to mat of 20–30cm tallness containing seeds of sprouted implanted inside the white roots with green sprouts (Naik et al., 2011a, Naik et al., 2013b). The DM content (on fresh basis) of the hydroponics maize fodder was slightly lower than the concentrate mixture. According to Naik and Singh (2013) harvests of 5 to 6 folds on the basis of fresh (1 kg seed produces 5–6 kg HMF) and content of DM is 11 to 14 percent are familiar for HMF; even though, occasionally the content of DM is up to 18.3 percent is observed in this study. The hydroponics maize fodder had high in EE, CP, NFE and lower CF, TA and AIA %. Earlier, Naik et al., (2012(b)) has confirmed higher EE, NFE and CP and CF is lower, TA and AIA % in the maize hydroponics fodder as compare to conservative maize fodder.

According Naik et al., 2015, Hydroponics fodder needs very little amount of water to grow. These plants grow without soil and only the tap water can be used. The plants grow within very short duration (approx. 7 days). In our country, grain maize must be preferred for hydroponics fodder production. Hydroponics maize fodder feeding can enhance the nutrients digestibility of the ration and that will leads to increase the production of milk (8-13 per cent). In some circumstances, where the conservative green fodder can't be able to grown-up effectively, hydroponics maize fodder can be harvest by the dairy farmers to feed their livestock with low cost strategies during the scarcity of green grass. "The hydroponics fodder's nutrient contents are higher with few particular non-leguminous fodders but similar to leguminous fodders." As developed grains (hydroponics maize fodder) are very rich in enzyme and it is commonly in the nature of alkaline, so that hydroponics fodder's feeding improves the productivity of the livestock by increasing a strong immune system due to nullification conditions of the acid. On the other hand, it helps to the anti-nutritional elimination factors like grains phytic acid. Hydroponics maize

fodders are very good foundation of chlorophyll and also it contains a factor of grass juice, which increases the performance of the dairy animal." (Naik et al., 2015).

Indigenous Breed Cows

In the case of Indigenous breed cows, the animals (Treated Group) feed with Hydroponic Maize fodder and concentrated feed shows little higher milk yield as compare to the animals(Control group) fed with concentrate feed and paddy straw without Hydroponic maize fodder. Whichis significantly high (P< 0.01) result on avg. milk production per day. Addition of Hydroponic Maize fodder along with concentrate improved the milk yield and Health status of indigenous breed cows. (Fig. 1 & Table 2)

Cross Breed Cows

The animals (Treated Group) feed with Hydroponic Maize fodder with concentrated feed shows higher milk yield as compare to the animals (Control group) fed only concentrate feed without Hydroponic maize fodder. Which was also found highly significant (P< 0.5) result on avg. milk production per day. The animal fed with Hydroponic Maize fodder not only enhanced the milk production but also developed their health, body growth and positive effects on mortality, conception rate and abortion. (Fig. 2 & Table 2)

Table 1: Composition of Chemical (on % DM basis) of fodder and feeds

Parameters	Concentrate mixture	Hydroponics maize fodder
Dry matter (on fresh basis)	92.4	18.3
Crude protein	21.68	13.3
Ether extract	4.83	3.27
Crude fibber	8.39	6.37
Nitrogen free extract	58.27	75.32
Total ash	6.83	1.75
Acid insoluble ash	1.16	0.57

Table 2: Effect of Hydroponic Maize Fodder with nutritional supplement on milk yield of Indigenous Breed and Cross Breed dairy cows. Value of milk yield is kg/day (Mean ± SEM), Numbers in parentheses indicate number of animals used, significant at different Level.

Effect of Hydroponic Fodder	Indigenous breed cows	Cross breed cows
Control	4.5 ± 0.19 (5)	10.3 ± 0.31 (5)
P	P < 0.01	P < 0.5
Treated	6.0 ± 0.09 (5)	13.4 ± 0.35 (5)

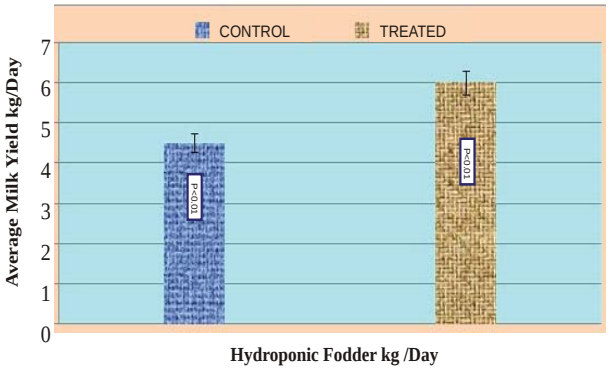


Fig. 1: Effect of Hydroponic Maize Fodder with nutritional supplement on milk yield ofIndigenous Breed Dairy Cows. Value of milk is kg/day. Columns represent the mean values and vertical Bars SEM

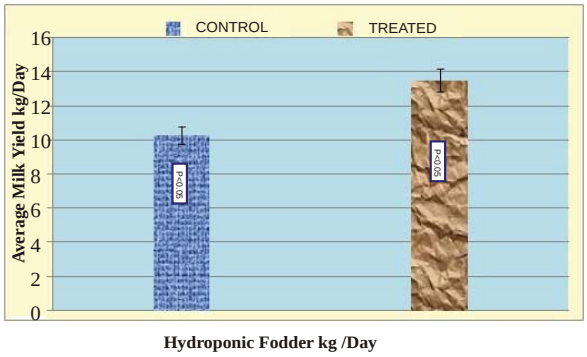
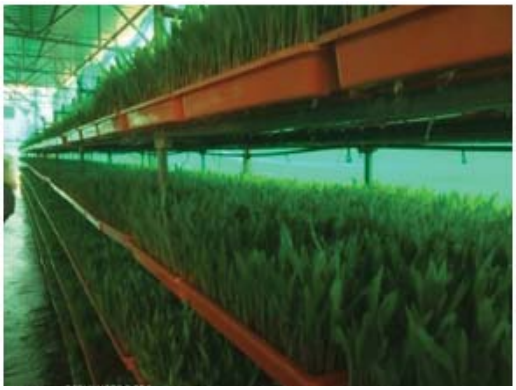


Fig. 2: Effect of Hydroponic Maize Fodder with nutritional supplement milk yield of Cross Breed Dairy Cows. Value of milk is kg/day. Columns represent the mean values and vertical Bars SEM

Pic 1 . Process of hydroponic maize fodder



Pic 2 hydroponic maize fodder

Pic 3 hydroponic maize fodder

Pic 4. Feeding of Hydroponic maize fodder

Conclusion

In the developed countries where there is scarcity of quality fodder and feed, the hydroponic fodder production is less competitive than traditional production of fodder when compared on per kg dry matter basis. (Bakshi et al., 2017). Paddy straw was used as a main source of roughage and chemical composition was similar to other roughages like jowar straw and wheat straw. Hydroponic fodder is highly relished, palatable and digestible by the animals. If the milk yield of a dairy cows is about 5 k/g per day, than 1 k/g mixture of concentrate can be substituted by 10 k/g per day green fodder maize without disturbing the daily milk production providing that the majority of the livestock should be satisfied by ad lib roughage such as jowar straw. (Naik et al., 2012). The procedure of hydroponically developing green fodder allowed regulating the conditions of the climate for optimal growth with definite output per day. It has proved that Green fodder production through Hydroponics technology could be a real alternative source to overcome the fodder deficiency.

Maize is the best source of fodder producing under hydroponics, the system of hydroponics can be prepared using low cost material, no soil media and no nutrients are added for hydroponics production. But, in rural areas, where the timer facility is not used, it was difficult to get the uniform growth of fodder (Kammar et al., 2019).

It is accomplished that the effect of hydroponic fodder can be a substitute for green grass during summer on indigenous breed and cross breed cows of Ganjam district.

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Variations in Temperature and Trends in Tarai Region of Uttrakhand under Changing Climate

Ravi Kiran

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Abstract

The present investigation was carried out using the long term (1981-2015) meteorological data recorded at Agrometeorological Observatory situated at NEB CRC, GBPUA& T, Pantnagar Uttarakhand. The analysis revealed that there is a decreasing trend of maximum temperature at the rate of 0.007°C per year over the years. However minimum temperature shows an increasing trend at the rate of 0.031°C per year. Average temperature range shows an increasing trend at the rate of 0.025°C per year. Maximum temperature anomaly shows a decreasing trend. Minimum temperature anomaly has an increasing trend. Temperature range anomaly showed a decreasing trend. The decadal analysis of temperature shows similar trend. The average monthly maximum temperature found to be at peak in the month of May and lowest in July. The lowest average monthly maximum and minimum temperatures were found in January. Average temperature range was highest in April and lowest in August.

Key words: Maximum; Minimum; Range; Temperature; Trend; Variabilty; Pantnagar; Climate Change; Anomaly.

Introduction

The Indian Himalayan region is one of the most sensitive regions of the country in terms of climate change. Uttarakhand state is highly vulnerable to frequent climatic catastrophic events. It has wide geographical variations. It spreads between 28°43′ to 31°27′ N latitudes and 77°34′ to 81°02′ E longitudes, in the northern part of India with a total geographical area of 5.33 M ha. The 92.57% area is mountainous and 7.43% is occupied by plains.

Since last few decades abrupt changes in temperature has been noticed by researchers. IPCC has stated that the climate change is mainly attributed to anthropogenic activities. Current global mean warming is 0.65 to 1.06°C above preindustrial time over the period of 1880-2012. Climatic extremes and abrupt weather phenomena are becoming an unexpected crisis in human life and human activity, now a days (Jewson and Caballero, 2003; Meze-Hausken et al., 2009).

The trend analysis of temperature recorded for several years provides information about temperature variability. A deep understanding of the influence of changing climate on agricultural production is must to cope up with changes in temperature. Keeping in view the above facts an analysis was made on long term data of temperature recorded at GBPUA and T, Pantnagar.

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Data and Methodology

The present analysis was made using the temperature data from 1981 to 2015 recorded at NEBCRC, GBPUA&T, Pantnagar which is situated in Udham Singh Nagar district (290 N Latitude, 79°

E Longitude and 243.8 m abobe mean sea level). This area lies in Tarai belt of India, located in the foothills of Himalaya with annual rainfall of about 1400 mm. The monthly meteorological data of maximum (Tmax, °C) and minimum (Tmin, °C) temperature were collected from the Agrometeorological Observatory situated at Normen E. Borlague Crop Research Centre situated at Pantnagar and verified for errors. Temperature range was calculated by subtracting T max from Tmin on annual basis.

Further the data were processed at decadal and annual scales and various statistical analyses were made to draw any final conclusion. The magnitude of the trends of increase and decreasing were derived and tested by the Mann-Kendall (Mann, 1945) test and slope of regression line using the least square method. Its range, mean, coefficient of variation (CV) were calculated. Trends and variations were determined by the relationship between the two variables, temperature and time. The magnitudes of the trends of increasing or decreasing temperatures were derived and slope of the regression line using the least square method.

Results and Discussion

Variations in Maximum and Minimum Temperature: The results of the analysis on long period average of maximum temperature on annual basis is presented in Fig. 1. It show that maximum temperature decreases at the rate of 0.007°C per year. The results of the analysis of minimum temperature on annual basis, presented in Fig. 2. It depicts that minimum temperature increases at the rate of 0.031°C per year. The average temperature range on annual basis depicts positive trend at the rate of 0.025°C per year (Fig. 3). The average monthly maximum temperature found to be at peak in the month of May and lowest in July (Fig. 4). The lowest average monthly maximum and minimum temperatures were found in January (Fig. 5). Average temperature range was highest in April and lowest in August (Fig. 6).

Trends of temperature anomalies: Average maximum Temperature Averagemaximum Temperature Anomaly at Pantnagar for 1981-2015 show a showed a decrease of 0.007 per year (Fig. 7). On the other hand Average minimum Temperature Anomaly (°C) at Pantnagar for 1981-2015 show an increase of 0.025 per year (Fig. 8). Average Temperature Range Anomaly (°C) showed a decrease of 0.031 per year (Fig. 9). The rise in minimum temperature anomalies shows that it would have significant inpact on agricultural production in the region.

Standard deviation and coefficient of variation: Thestandard deviation and coefficient of variation is shown in Table 1. The standard deviation in case of maximum temperature was found lowest in November (0.79) and highest in June (2.1). In case of minimum temperature was found lowest in August (0.449) and highest in February (1.24). The standard deviation in case of temperature range was found in August lowest (0.73) and highest in May (2.48).

TThe coefficient of variation in case of maximum temperature was found in August (2.4%) and highest in January (9.07%). The coefficient of variation in case of minimum temperature was found in August (1.78%) and highest in June (15.31%) and in case of temperature range it was found in November (8.82%) and highest in January

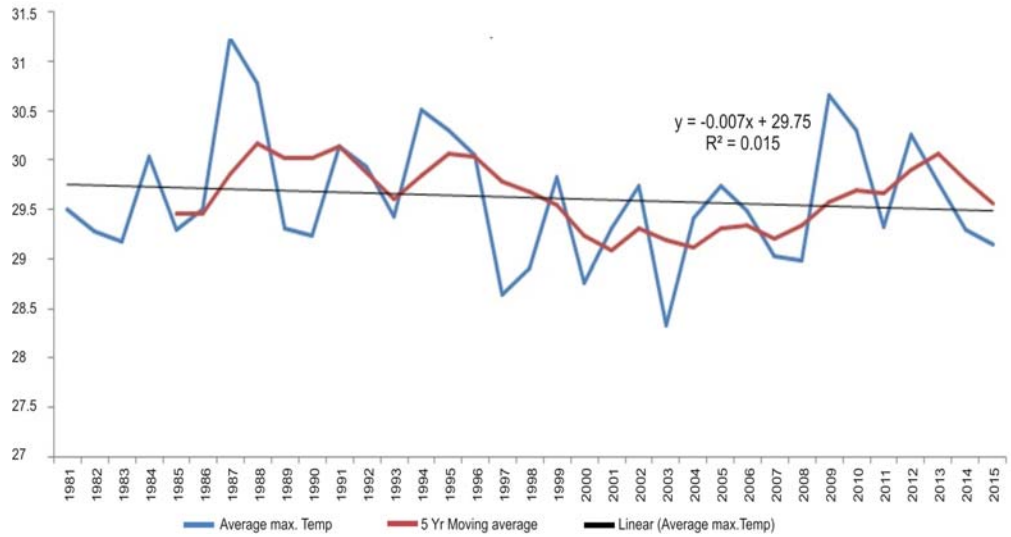

Fig. 1: Average maximum temperature (°C) at Pantnager for 1981–2015

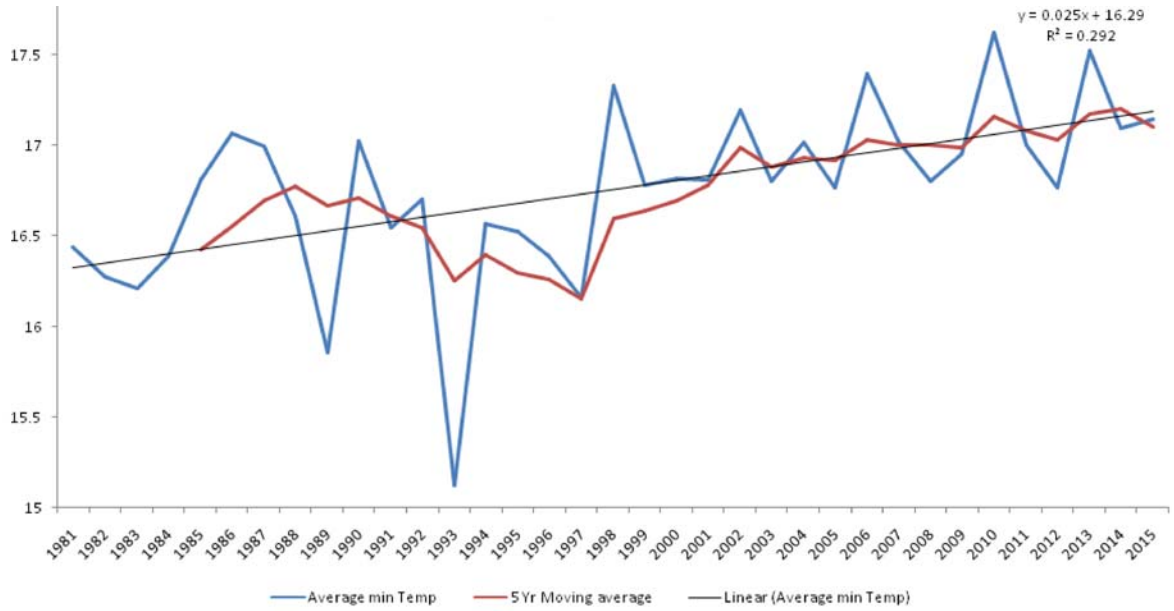

Fig. 2: Average minimum temperature (°C) at Pantnager for 1981–2015

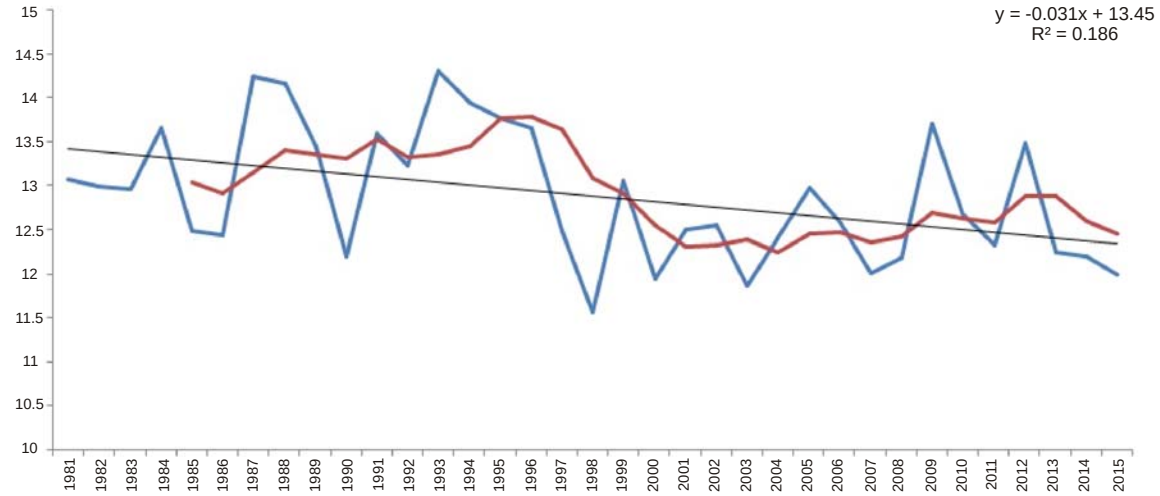

Fig. 3: Average temperature range (°C) at Pantnager for 1981–2015

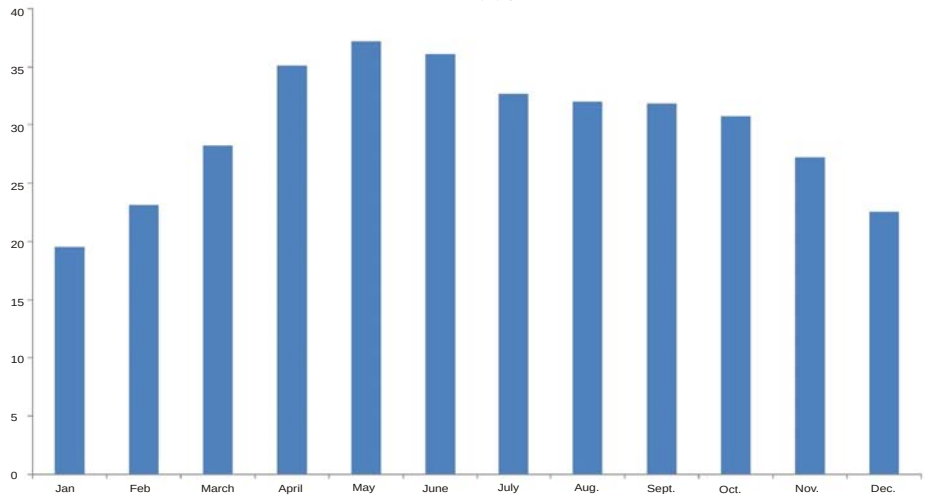

Fig. 4: Average maximum temperature (°C) at Pantnager for 1981–2015

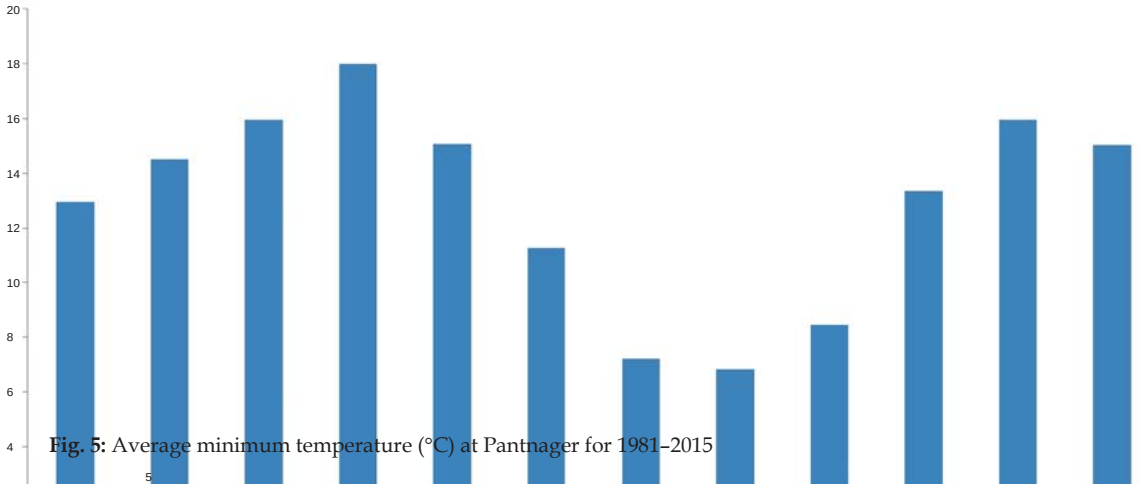

Fig. 5: Average minimum temperature (°C) at Pantnager for 1981–2015

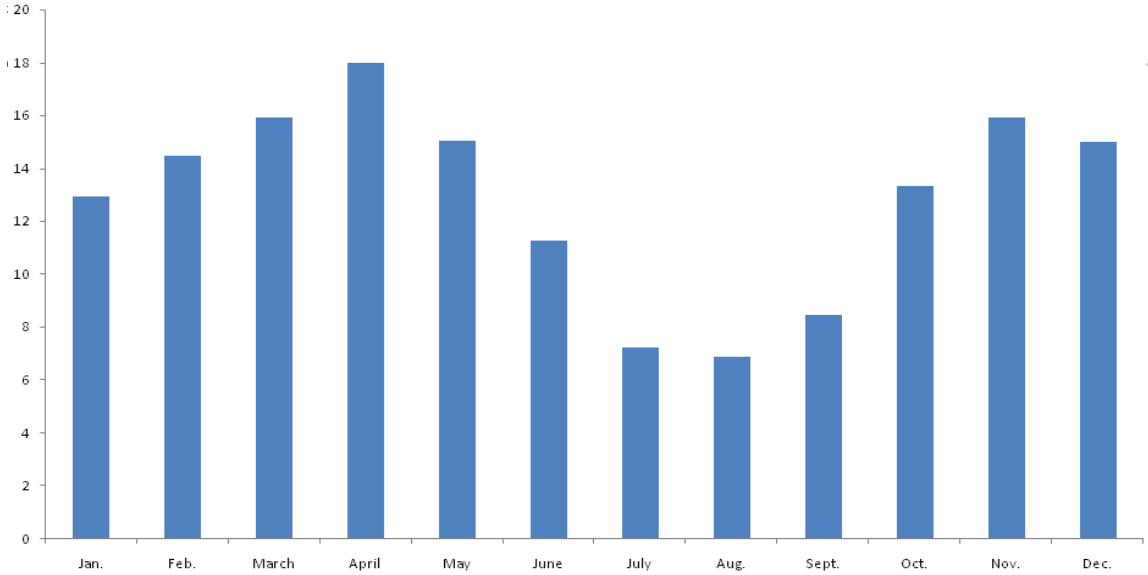

Fig. 6: Average temperature range (°C) at Pantnager for 1981–2015

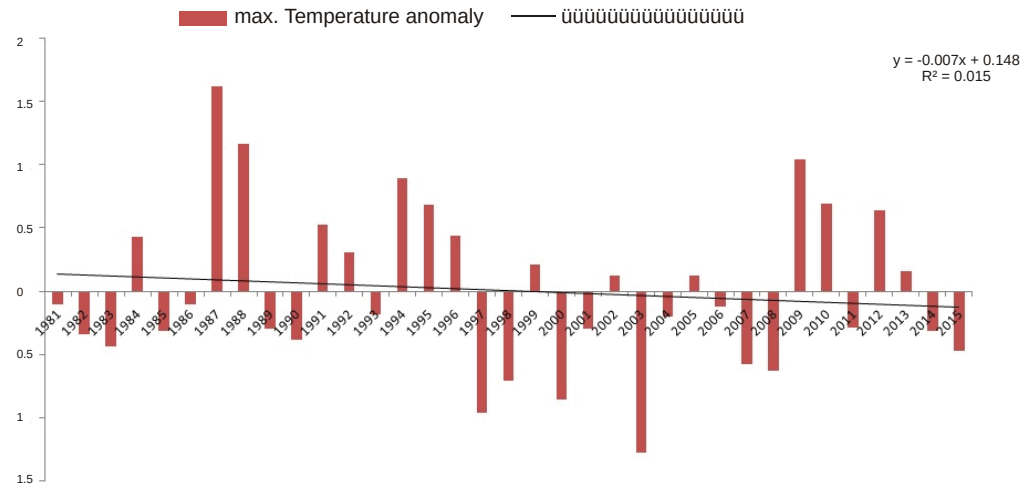

Fig. 7: Average maximum temperature Anomaly(°C) at Pantnager for 1981–2015

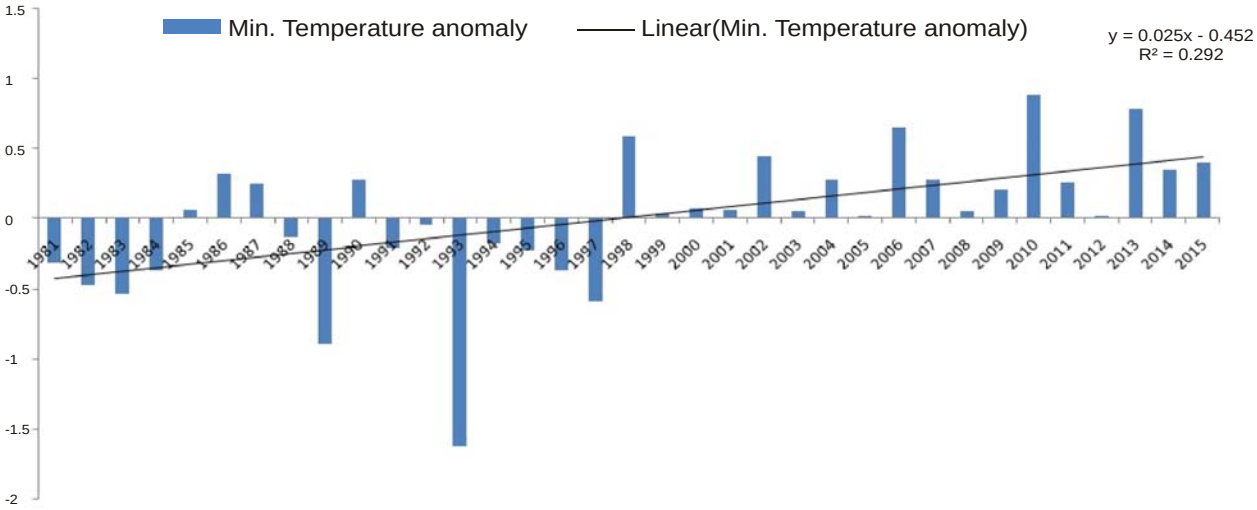

Fig. 8: Average minimum temperature Anomaly(°C) at Pantnager for 1981–2015

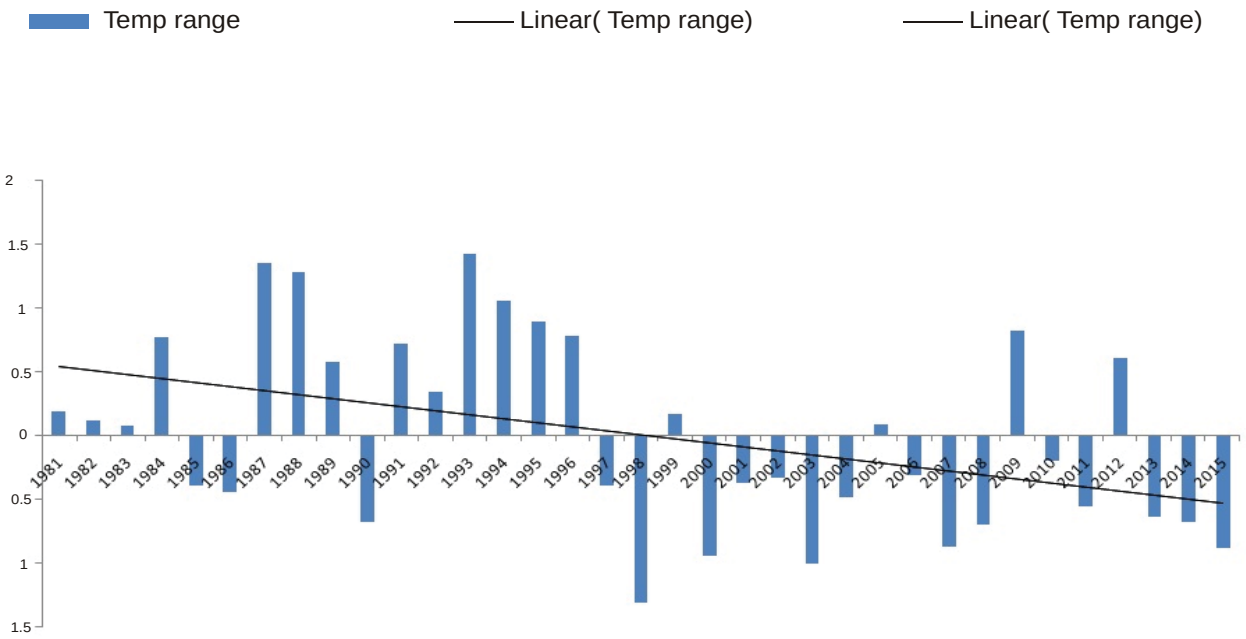

Fig. 9: Average temperature range Anomaly (°C) at Pantnager for 1981–2015

Table 1: Standard deviation and coefficient of variation (%) for Maximum, minimum and range of temperature for 1981–2015 at Pantnagar.

Month	T_{max}		T_{min}		T_{min}	
	Standard Deviation	CV(%)	Standard Deviation	CV(%)	Standard Deviation	CV(%)
Jan.	1.766699	9.070629	1.003624	15.31248	2.146144	16.60735
Feb.	1.394226	6.028153	1.245078	14.39159	1.617021	11.16947
March	1.948872	6.908093	0.917312	7.456093	1.804956	11.34581
April	1.846327	5.270347	1.113925	6.517544	1.712804	9.538288
May	1.664826	4.484985	1.811091	8.203466	2.489541	16.54966
June	2.196915	6.084675	0.70096	2.819953	2.218289	19.72063
July	1.03048	3.155289	0.564526	2.219795	0.875416	12.12487
Aug.	0.785934	2.460339	0.448815	1.787479	0.736035	10.76815
Sept.	0.843413	2.654944	0.62475	2.676935	1.006202	11.9368
Oct.	1.036453	3.371676	1.086703	6.235184	1.446857	10.86928
Nov.	0.791679	2.910892	1.005506	8.907291	1.403854	8.824515
Dec.	1.225842	5.455115	1.009753	13.50195	1.811408	12.0818

Table 2: Variation of Maximum, minimum and range of temperature for 1981-2015 at Pantnagar.

Time	*T max*	Tmin	T range
1981-1990	21.0-39.8	5.2-25.8	6.1-21.2
1991-2000	17.9-40.0	4.9-26.7	5.1-21.3
2001-2010	17.9-39.1	6.2-26.3	5.6-20.3
2011-2015	20.5-40.1	6.8-26.3	6.3-18.1

(16.60%)..

Decadal Trend: The decadal analysis oftemperature is presented in Table 2. The lower limit of Tmax was 17.9-21.0 and the higher limit was 39.1-40.1. The lower limit of Tmin was 4.9-6.8 and the higher limit was 25.8-26.3. The lower limit of Trange was 5.1-6.3 and the higher limit was 18.1-21.3. Murthy et al., 2004 also found similar results at Ranichauri in the mid Himalayan region.

The annual trend of temperature has shown the decreasing trend for all the 13 districts of Uttarakhand for maximum whereas in case of minimum temperature the trend showed (Tripathi et al., 2014) 99.9% significant increasing trend for all the districts (Yadav et al., 2014).An increasing trend is reported in surface temperature over the period of 1900-82 from 73 stations of India (Hingane et al., 1985) (Arora et al., 2005).

Conclusions

A decreasing trend has been found of maximum temperature at the rate of 0.007°C per year over the years. However minimum temperature shows an increasing trend at the rate of 0.031°C per year. Average temperature range depicts increasing trend at the rate of 0.025°C per year. Maximum temperature and temperature range anomaly shows a decreasing trend. Minimum temperature anomaly has an increasing trend. The increasing trend of minimum temperature is of great concern in respect of the production and productivity of major crops in this region.

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Impact of Climate Change on Fisheries and Aquaculture

Avijit Bakshi[1], Doli Halder[2], Ashis Kumar Panigrahi[3]

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Abstract

Pollution is, undoubtedly, the most alarming issue of concern among all environmental hazards. The rate and degree of pollution is gradually increasing with the explosion in population of the world. Due to some anthropogenic activities and malpractices of some scientific methodologist gradual changes in climate turned the global situation into a critical position affecting different aspects of agriculture, fisheries and others. Impact of climate change is conspicuously observing in the field of aquaculture posing adverse impact on its productivity and variety. Objective of the study was to prepare a review concerning about the impact of climate changes on fisheries and aquaculture sector. This review can be useful to find out a possible way for relieving the constrain through constructing some solution through various scientific activities. In present generation, the altered climatic condition shows a great impact on Ocean temperature, variation in water level, impact of sea level rise. For this climate change, there is a great impact on inland water. It is altering distribution and productivity of marine and freshwater species, and changing food webs. This making the concerns for viable of aquatic ecosystems for fisheries and aquaculture. Besides, it can also hamper our future scenarios. If these harmful effects are randomly proceeds, the future generation will also undergo a dangerous condition. Global warming is the main fact of these climate changes. Therefore, at first the main point is to reduce global emission of GHGs, which is the main anthropogenic factor. Fisheries and aquaculture require specific adaptation and mitigation actions for responding to the chances for and threats to food and livelihood security due to climate change. The current generation need to decide, how should we get relief from these major problem of climate change and reduce the chances of future harm

Keywords: Global Warming; Pollution; Climate Change; Fisheries and Aquaculture.

Introduction

In twenty first century, climate change has recognized as a great problem for the entire environment around the globe. It has been forecasted that thefallout of climatic changesleads to an adversative and irreparable impacts on ecosystem. In the middle of the nineteenth century, earth's average temperature has been found to be increased at a rate of more than 0.8°C per hundred years globally but recent studies confirm that the rate of increasing temperature is now more than 0.1°C every ten years (Hansen et al., 2010). The raise of averageatmospheric temperature has concluded to cause noticeable changes like glacial retreat, shrinkage of arctic zone, sea level rise, melting of permafrost etc. Therefore, these reasons have been proven to show a great impact on fisheries and aquaculture in various aspects.

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Increase in global temperature leads to the melting of the ices present in high-altitude regions and high-latitude regions which in turn leading to the retreat and melting of glaciers, with great ramification for downstream water resources (IPCC, 2014). It has been reported that the liquefied condition of the Arctic sea has serious potential to disintegrate the global ocean tides or conveyor belt in marine ecosystem (Liu et al., 2017). The probable consequences will lead to abrupt changes in the

diversity of marine ecosystem.

Great rivers of the whole worlds have already affected by dam construction, water abstraction and regulations that are laborious to be incontestable. Besides the anthropogenic damages, it has been predicted that the melting of glaciers will surely increase the input into the river confluences in coming days (Jha et al., 2006; Siderius et al., 2013; Pervez and Henebry, 2015). This will pose some serious alteration to the productivity and diversity of cold water fisheries globally. The changing pattern of rainfall have substantially altered ecological aspects of many rivers and wetland, which causes a great influence on the diversity of aquatic environment.

Some natural processes both internal or external such as volcanic eruptions, infection of solar cycles, anthropogenic contributions to the composition of the atmosphere (viz., Green House Gases) etc have been proven to be the key behind altered climatic condition. The term "climate change" has been used and has also been described by UNFCCC (United Nations Framework Convention on Climate Change) for anthropogenic changes and 'climate variability' for other changes. According to a report by IPCC (2007) the average global atmospheric temperature has been reportedto be increased at the rate of 0.74±0.18°C (i.e., 1.33± 0.32°F).

Gradually, the occurrence of these climate changes have threatened the physical as well as biological nature of any aquatic environment. Climatic changes have imposed serious deleterious impacts on several aquatic species including aquatic plants, corals, echinoderms, shell and finfishesand aquatic mammals also (Delgado et al., 2003). The 93% additional heat caused by anthropogenic climate change has absorbed by global ocean. The melting of icecaps and snow have caused due to absorption of only three to four percent heat. The heat buffering system of oceanis thus extreme. Any little difference in the correspondence of heat between ocean and surface atmosphere have tremendous impacts on increase of average global air temperature (Reid, 2016).

Being an important source of animal protein for the human and many aquatic organisms, fishes can be collected from wild origin or can be farmed exclusively. Developing countries like India, China have incorporated their vast interest in dominating the global fish production and utilization pattern for last 25-year period (Delgado et al., 2003). Thus, fisheries and aquaculture sector is also proven to be a promising income domain for many countries. Climate change is directly exerting dreadful impact on fisheries sector and its environment. According to a report of IPCC (2007), human race is highly influenced by climate change in various aspects including food security.Among all primary food production sectors, fish production is expected to be greatly influenced by varying degrees of climate change and the manifestations can be conventionally present in varying forms in different parts of the world up to varying extent. The objective of the review work is to comprehend the potential role of global climate change on fisheries and aquaculture domain.

Review Methodology

It was a mammoth work in compile the available data on the research topic "Impact of Climate Change on Fisheries and aquaculture". In order to make an utmost consolidate manuscript on the topic extensive searching has been done using some key words. Data have been collected from different Science Journal of repute, published data sources or reports from various international agencies. Articles includingabstruse working methodologies have let off fastidiously. During data screening, importance have been paid exclusively to the reproducible articles, that are indexed in Science Journal database. Keywords have been meticulously scrutinized and searched based on standard scientific methodologies. Keywords, for searching; were as follows: Global warming, pollution, climate change, Fisheries and aquaculture. Data related to the research topic have chosen in such a way that a comprehensive manuscript can be provided to the future researches.

Result and Discussion

The principal aim of the review article is to consolidate the evidence on the impact of climate change on the domain of fisheries and aquaculturein the published literature. The objective of the article is also to evaluate the superiority of the scientific objectivity and quality of the evidence on the topic. Fisheries and aquaculture have acquired only little awareness in the principle analysis of climate change persuadedeffects on shell and fin fish production. It is vital to remember that significant attention to climate change issues on fisheries and aquaculture domain have been constructed almost a decade ago (Wood and McDonald, 1997).

Allowance of fisheries and aquaculture to food sector

Fisheries and aquaculture is noticeably a fast

growing sector related to food security and continue to emerge more speedily than other animal food-producing sector. From capture fisheries (both freshwater and marine) and aquaculture, fish products contribute about 1/10th of total agricultural exports of the world. The amount of global fish trade beat the total amount of international trade in all other animal food sources (World Bank, 2011). Normally, fish production and trading is responsible for about 0.5%-2.5% of total GDP of the world but these contribution is more than 10% for countries like Mauritania and Vietnam (Allison, 2011). In some small island states ofPacific Ocean, fisheries sector contributes about 25% of total GDP due to their geographical position (Gillett, 2009).

Presently, millions of people directly or indirectly depends on fisheries and aquaculture for their livelihoods around the globe. Fish products can afford 15% or more of the total intake of protein dominate for about 3 billion people of the world. It also supports economy and livelihoods of about 520 million people around the world, interestingly most of them are women (FAO 2009).

Global change in climate is now recognized as highly influencer of a wide range of environmental variables like rain fall, average temperature, volume of river water, ocean nutrients, severity and frequency of oceanic storm, harmful algal bloom and acidification of oceans (Fleming et al., 2006; Feely et al., 2009). These unusual changes of climate directly or indirectly affect the fish ecology and production of fisheries and aquaculture posing severe threat to the economy ofdependent communities like fishers, food-processor, retailer etc also to the wider economy (Cochrane et al., 2009; Merino et al., 2012).

The literature indicates the growing importance of fisheries and aquaculture is continuously contributing to fill the gap between demand and supply of fish (Merino et al., 2012). The growth also ensures the improvement of fisheries and aquaculture sectorlessening of rural poverty and safeguardingfood security.

Impact of climatic changes on environment related to fisheries and aquaculture

Impact on Ocean temperature

In 1960s, it has observed that many anthropogenic forces built a considerable contribution to raise up the temperature of the ocean surface (above 700 m) (Cheung et al., 2016). It has also found that there is an increase in the temperature of the surface waters by an average of 0.7°C per hundred years globally during a time span of 116 years (from 1900 to 2016) (Huang et al., 2015). Due to the variation in Oceanic current, various changes in fisheries and aquaculture may happen through changes in water temperature, primary productivity, food availability increasing rate of disease transmissions andgrowing toxic algal blooms (Handisyde et al., 2008).

Variation in Water Level

Global changes in average temperature of the world lead to melting of arctic ice caps and high altitude ice which finally result in rise in sea level and altering oceanic current. Mean sea level rise is a long term consequence which would happen over a long time period scale. It has been presumed that rise in sea level may affect distribution and migration patternof fish (Bakshi and Panigrahi, 2015).

Because of climatic and non-climatic factors, recorded mean sea level rise was about 3.1 mm/ year in recent years (Dangendorf et al., 2017). The projected global mean sea level rise will extend approximately(90% probability) between0.3m to 0.8m under RCP2.6, 0.4 m to 0.9 m under RCP4.5, and 0.5m and 1.2m under RCP8.5 in 21st century(Kopp et al., 2014).

Impact of sea level rise

The consequence of sea level rise is continuously posing severe threat to the halotolerant mangroves in Sundarbans and other mangrove forests. The unique ecosystem is very much vulnerable to the increase of sea water. Increased sea level will ensure the increased penetration of salt water into the land confirming decrease of habitat space for fresh water fishes. This could also ensure the increase of brackish water area along with its diversity (De Silva and Soto, 2009). From the report of 2000 to 2008, it has been found that there is arise in the level of sea of Sundarbans. The amount of sea level rise from the Sagar island observatory has observed at the rate of about 12 mm/year. Within the period of 2001-2009 the rate of coastal erosion in the Indian Sundarbans for the sea level rise has found to be about 5.50 km 2/year (Bakshi and Panigrahi, 2015), in the year 2001 a total land area of 6402.09 sq. km of Sundarbans has reduced to 6358.05 sq. km in 2009.

Turbidity of the aquatic ecosystem also changes due to the effect of climate change. Due to changing turbidity of riverine water there is decreasing in the production of the estuarine ecosystem. Therefore, these altered turbidity pattern is responsible for the effect of erosion of coastal region (Bakshi and

Panigrahi, 2015). The growth of phytoplankton has inhibited due to less penetration of sunlight and affecting the productivity of the whole ecosystem.

Impact on inland water

According to Ficke et al., (2007), the rate of increasing temperature of the atmosphere is certainly bringing about some changes in aquaculture that could affect the fisheries activities in both lentic and lotic waters. Even the temperature of any pond water has depended upon by atmospheric temperature,velocity ofwind, solar radiation, turbidity of water,humidity and pond geo-morphometry. The raise of the atmospheric temperature will surely cause an expansion in evaporation and cover ofcloud (IPCC, 2007).

The inland aquaculture in India is mostly depended upon Indian major carps or IMCs (i.e., Labeorohita or Rohu, Catlacatlaor Catla fish, andCirrhinusmrigalaor Mrigale). The spawning of IMCstake place during the time of monsoon (June-July) and extends until September. Now a peculiar phenomenon of Indian major carp maturation and spawning havealso observed in March enhancing the chance of breeding to twice a year. So, the breeding activity has noticeably found to differ in comparison with the last few decades(Dey et al., 2007).

Impact on net primary productivity

The productivity of any water body is determined by net primary production of the system. A good valuation of net primary productivity (NPP) in respect to the consequences of climate change may alter the future trend of marine as well as fresh water production. Change in temperature may affect the natural phenomenon of thermal stratification of any aquatic system; these also may put some distress the amount of respiratory gases exchange andalso the amount of bio-nutrients at the water surface altering the primary productivity. Therefore, the consequences may lead to alteration in food chain and food web resulting ultimate change in the species accumulation pattern (Dangendorf et al., 2017; Behrenfeldet al., 2018)

The base volume of marine food web is determined by the productivity of phytoplankton. It could regulate the energy flow and availability of food to the higher trophic levels even in fishes. According to Earth system model, primary production of marine ecosystem, as a result of climate change, is indeterminate. The model is alsosticking out both surges (Taucher and Oschiles., 2011) and declination of marine primary productivity up to about 20% by the year 2100 (Bopp et al., 2013). In some Arctic and boreal lakes, primary production has detected to increase (Michelutti et al., 2005) though exception has been detected in Lake Tanganyika in the tropics with a declinein primary productivity level (O'Reilly et al., 2003).

Climate Change Scenario in Twenty-first century

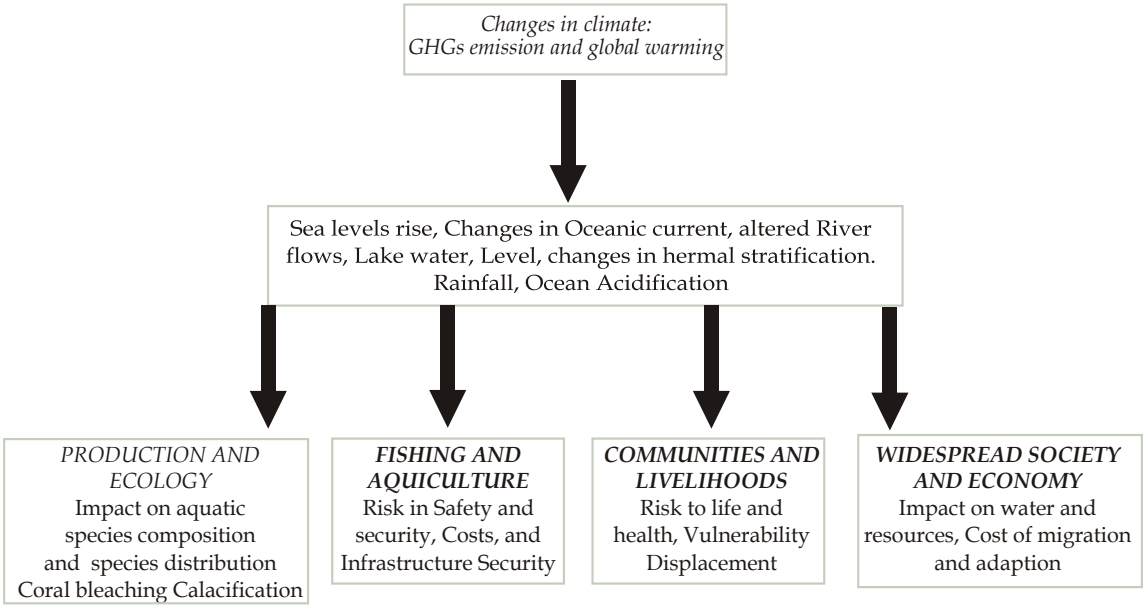

Fig. 1: Consequences of climate change on the fisheries and aquaculture

Therefore, we can summarize the total situation in the following way: increase of GHG and other harmful gases is the main reason behind the climate change which results in melting of polar ice, sea level rise, alteration in river flows, oceanic currents etc. These factors put direct impact on fisheries and its dependants (Fig 1).

Combustion of fossil fuel and other anthropogenic activities are directly related to the increase of greenhouse gases in the atmosphere which in turn responsible for increase of water temperature, decrease in oxygen content of water bodies, acidification of ocean water confirming the large-scale change in marine species diversity (Portner et al., 2014).These variations particularly ensure the alteration in marine primary productivity (Bopp et al., 2013) as well as marine biodiversity (Jones and Cheung, 2015). Human race is highly depended on fisheries sector for the source of protein thus these changes may surely hamper the promises of food security in the later part of 21stcentury (Lam et al., 2016; Cheung et al., 2016; Golden et al., 2016).

Conclusion

Fisheries and aquaculture has effectivelyrecognized as a major food sector over last two to three decades. It also providesa major portion of total animal protein requirements across most of thehuman communities of the world. Like other food producing sectors, fisheries and aquaculture have faced major challenge due to climate change. A number of climatic changes are natural and inevitable which also responsible for the change in aquatic flora and fauna diversity. Human race is heavily dependent up on the products of aquatic origin for food security in coming decade which is unfortunately under the threat of climate changes.

The predicted pattern of increases in water temperatures are often well understooddue to its natural occurrence. The temperature remains within a definite range particularly in the tropics and subtropics enhancing the chances of survival of cultured species. Thus it can be understood that warming of marine water would actually improve growth of cultured stocks in tropic and sub tropic regions increasing the fish production. The pattern of sea level rise is highly associated with the change in water temperature of ocean. Salt-water intrusion is also associated with the rise of water temperature confirming the constriction of natural habitat of fresh water fishes. The impression of climate change on fish populations is directly related to the growth of fisheries and aquaculture. Seasonal availability of the fish population altered with the pattern of climatic alteration affecting capture fisheries production very much.Naturalaccessibility of the raw materials and availability of suitable environmental condition for production of dry fishmeal and fish oil are also facing serious trouble therefore it can be said that unpredicted climatic changes are the reason behind peculiar constrains on fisheries. Coral bleaching due to changes in aquatic environment is putting serious threat to towards the loss of marine diversity which in turn affecting the capture fisheries sector indirectly. Global warming is proven to be the main cause of climate change. Therefore, the key solution is to reduce the emission of GHGs into the environment. Domain of fisheries and aquaculture must require exclusive adaptation and vindication actions like refining the management strategies of the sector to mitigate the problem of global warming as well as GHG emission. These improvements arevery much needed as threats to food security and livelihood is continuously increasing due to change in climatic condition.

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Review Article

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Reactive Oxygen Species (ROS) and Antioxidant Machinery of the Plant System

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Abstract

Environmental fluctuation leads to overproduction of ROS, which are extremely toxic and damages cellular biomolecules i.e. nucleic acid, DNA, lipid, and protein. However, the delicate balance between ROS production and antioxidant machinery of the plant is always maintained under normal condition but during stress, this balance comes to a halt. To ensure the plant survival under stressful environment, plants have adopted well equiped antioxidant defense machinery having two forms: (i) Enzymatic such as Superoxide Dismutase (SOD), Ascorbate Peroxidase (APX), Catalase (CAT), Dehydroascorbate Reductase (DHAR), Monodehydroascorbate Reductase (MDHAR), Glutathione Reductase (GR) and Guaiacol Peroxidase (GPX); and (ii) Non-enzymatic such as reduced glutathione (GSH), Ascorbic Acid (AA), Carotenoids, α-tocopherol, proline and flavonoids. In this review, a summarized form of ROS action and their regulation via antioxidant machinery of plant cells have been discussed.

Keywords: Antioxidants, Oxidative stress, Reactive Oxygen speices, Redox status.

Introduction

About 2.7 billion years ago, molecular oxygen (O_2) was introduced into the atmosphere by O_2 evolving photosynthetic organisms, which lead to the evolvement of undesirable by-products i.e. reactive oxygen species (ROS) which mainly includes $^1O_2$, $O_2^{\bullet -}$, H_2O_2 and $OH^{\bullet}$ (Halliwell, 2006). Under the favourable condition, the primary ROS production (various cellular compartments i.e. chloroplast, mitochondria, apoplast and peroxisomes) is constantly maintained to regulate the cellular metabolism; however, stressful environment (pathogen infection, drought, salinity, heavy metals, high temperatures, pollution etc.) disturb the delicate balance between ROS production and their scavenging by antioxidant defense system of plants (Foyer and Noctor, 2005). These ROS are lethal and cause damage to DNA, lipids and proteins, thereby disturbing cell functioning (Foyer and Noctor, 2005). The redox homeostasis is maintained by two types of antioxidants machinery- (i) Enzymatic antioxidants involving Superoxide Dismutase (SOD), Peroxidase (POD), Catalase (CAT), Glutathione-S-transferase (GST), Ascorbate Peroxidase (APX), Dehydroascorbate Reductase (DHAR) and Glutathione Reductase (GR) and, (ii) Non-enzymatic Antioxidants (low molecular) involving Ascorbate (AA), Reduced Glutathione (GSH), Cysteine (Cys), Proline (Pro), Non-Protein Thiols (NPTs), Carotenoids, α-tocopherol, flavonoids and Phenolics (Gill and Tuteja, 2010;

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Miller et al., 2010; Gill et al., 2011, Singh, 2019). In this review, a deeper insight into ROS toxicity and their detoxification by well-equipped antioxidant machinery of plants have been discussed.

ROS defense machinery

The ROS defense machinery has been categorized into enzymatic and non-enzymatic antioxidants.

Enzymatic antioxidants

Superoxide dismutase (SOD; E.C 1.15.1.1)

The metalloenzyme SOD is considered as the first line of defense, which catalyzes the dismutation of O_2•− into O_2 and H_2O_2, thereby eliminating the opportunity of OH• formation by Haber-Weiss reaction. Based on metal ion binding, there are three isoenzymes of SODs; Fe-SOD (confined in chloroplasts), Mn-SOD (confined in mitochondria) and Cu/Zn-SOD (confined in the cytosol, chloroplasts, and peroxisomes) (Mittler, 2002).

$$O_2•− + O_2•− + 2H+ \rightarrow 2H_2O_2 + O_2$$

Peroxidase (POD; E.C.1.11.1.7)

The heme-containing POD enzyme removes excess H_2O_2 in both healthy and stressful environment. It is essential in lignin biosynthesis and removes H_2O_2 from the cell by using guaiacol and pyrogallol as electron donors, and degrading indole acetic acid (IAA) against stressful condition (Asada, 1999). It functions both intracellularly (cytosol, vacuole), in the cell wall and extracellularly, with higher Km value.

$$H_2O_2 + GSH \rightarrow H_2O + GSSG$$

Catalase (CAT; E.C.1.11.1.6)

The tetrameric heme-containing CAT enzyme catalyzes the dismutation of H_2O_2 into H_2O and O_2 in peroxisome, cytosol, mitochondria and chloroplast. CAT is unique among the antioxidants; with a high turnover rate. It does not require a reducing equivalent (Mittler, 2002). CAT has three genes; CAT1 which is restricted in the cytosol and peroxisome and express in seeds and pollens; CAT2 is restricted in peroxisome and cytosol and express in root, seed and photosynthetic tissues and CAT 3 is restricted in mitochondria and express in leaves and vascular tissues of angiosperms.

$$H_2O_2 \rightarrow H_2O + O_2(1/2)$$

Ascorbate peroxidase (APX; E.C.1.1.11.1)

Being the immense part of AsA-GSH cycle, APX predominantly scavenges H_2O_2 in chloroplast and cytosol, while CAT performs the same function in peroxisomes. APX reduces H_2O_2 to H_2O and dehydroascorbate (DHA) using AA as an electron donor (reducing agent). APX have five isoforms located in cytosol, peroxisomes, mitochondria, and chloroplast (Sharma and Dubey, 2004). Due to its higher affinity for H_2O_2 and wide distribution, it is a more efficient scavenger of H_2O_2 than CAT.

$$H_2O_2 + AA \rightarrow 2H_2O + DHA$$

Dehydroascorbatereductase (DHAR; M.C.1.8.5.1)

DHAR enzyme reduces DHA into AA using GSH as an electron donor, which means regenerates AA pool in both apoplast and symplast; therefore, it maintains the redox status of the cell (Eltayeb et al., 2007). It is predominantly found in root, shoot and seeds.

$$DHA + 2GSH \rightarrow AA + GSSG$$

Glutathione reductase (GR; E.C.1.6.4.2)

The flavoprotein oxidoreductase enzyme GR, reduce oxidised glutathione (GSSG) to GSH using NADPH as an electron donor. GR catalyzes the formation of disulfide bond in GSSG and maintains the high GSH/GSSG ratio, mainly in the chloroplast. Its small amount is also present in cytosol and mitochondria.

$$GSSG + NADPH \rightarrow 2GSH + NADP^+$$

Nonenzymatic antioxidants

Ascorbate (AA)

AA is a powerful electron donor, mainly comes from L-galactano-γ-lactone dehydrogenase catalysed Smirnoff-Wheeler pathway of mitochondria and D-galacturonic acid. It is oxidized into succeeding steps, starting with oxidation into MDHA, which if not immediately reduced into AA, disproportionate to AA and DHA. AA protects the membrane by directly scavenging O_2•− and OH• and regenerating α-tocopherol from tocopheroxyl radical (Shao et al., 2005). In its reduced state, AA acts as a cofactor of violaxanthin de-epoxidase and maintains the dissipation of excess excitation energy (Smirnoff, 2000), prevents the photo-oxidation of PS II and its down-regulation is associated with zeaxanthin formation.

Glutathione (GSH)

The low molecular weight thioltripeptide

(γ-glutamyl-cysteinyl-glycine) GSH is found in almost all the cellular compartments and participates in a wide range of processes (Mullineaux and Rausch, 2005), as it has high reductive potential due to centrally located Cys residues with nucleophilic character. GSH protects various biomolecules by scavenging ($^1O_2$, O_2•−), H_2O_2 and OH• and forms GSSG as by-product. It is also essential in (i) regenerating AA and forms GSSG, which with the help of GR enzyme converted back in to GSH and (ii) Synthesizing PCs by PC synthase, which is necessary for heavy metals chelation and ROS scavenging (Roy Choudhury et al., 2012). Thus, the delicate balance between GSH and GSSG is essential for maintaining the redox status of the cells.

Proline (Pro)

The osmolyte Pro is a powerful antioxidant, which efficiently scavenges $^1O_2$ and OH• and can prevent the damage due to LPO. Pro is synthesized via pyrroline 5-carboxylate (P5C) using glutamic acid as a substrate; the step in plants completed by two enzymes: ð1-pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR). During stress, Pro accumulation could either be due to enhanced synthesis or reduced degradation (Verbruggen and Hermans, 2008).

a-Tocopherol

The α-tocopherol is the active member of the lipophilic antioxidants' family, which is presents in green tissues of the plants because it is synthesized only by the photosynthetic organisms. They protect lipid and other elements of chloroplasts by reacting with O_2 and quenching its excess energy, thus protects PSII, both structurally and functionally (Kiffin et al., 2006). Tocopherol traps free radical by hampering chain proliferation of the LPO cycle. The α-tocopherol reacts with lipid radicals i.e., ROO•, RO• and RO, by reducing them and itself converted into TOH•, which then recycled to get its reduced form by interacting with AA and GSH (Igamberdiev et al., 2004).

Carotenoids (Car)

The Car also belongs to lipophilic antioxidants family and localized into plastid of plant tissues, which absorbs 450–570nm wavelength of light and transfers energy to chlorophyll (Chl) molecules. Car reveals their antioxidative property by protecting the photosynthetic machinery by (i) Scavenging $^1O_2$ and generating heat as a by-product, (ii) reacting with LPO products to end the chain reactions, (iii) dissipating excess excitation energy via xanthophyll cycle, and (iv) preventing $^1O_2$ formation by reacting with 3 Chl* and excited Chl*.

Flavonoids

Flavonoids mainly found in the floral organs, leaves, and pollen grains in four forms i.e. flavones, isoflavones, flavonols, and anthocyanins and provides pigmentation to seed, fruit, and flowers and defend plants against stress. These are mainly secondary ROS scavenging compounds and function when photosynthetic apparatus experiences damage due to excess excitation energy and protects outer envelope of chloroplast membrane by scavenging $^1O_2$ (Fini et al., 2011; Agati et al., 2012).

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State the background of the study and purpose of the study and summarize the rationale for the study or observation.

Methods

The methods section should include only information that was available at the time the plan or protocol for the study was written such as study approach, design, type of sample, sample size, sampling technique, setting of the study, description of data collection tools and methods; all information obtained during the conduct of the study belongs in the Results section.

Reports of randomized clinical trials should be based on the CONSORT Statement (http:// www. consort-statement. org). When reporting experiments on human subjects, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000 (available at http://www.wma. net/e/policy/l 7-c_e.html).

Results

Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. Extra or supplementary materials and technical details can be placed in an appendix where it will be accessible but will not interrupt the flow of the text; alternatively, it can be published only in the electronic version of the journal.

Discussion

Include summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); Strengths and limitations of the study (study question, study design, data collection, analysis and interpretation); Interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now?, What this study adds to the available evidence, effects on patient care and health policy, possible mechanisms)? Controversies raised by this study; and Future research directions (for this particular research collaboration, underlying mechanisms, clinical research). Do not repeat in detail data or other material given in the Introduction or the Results section.

References

List references in alphabetical order. Each listed reference should be cited in text (not in alphabetic order), and each text citation should be listed in the References section. Identify references in text, tables, and legends by Arabic numerals in square bracket (e.g. [10]). Please refer to ICMJE Guidelines (http://www.nlm.nih.gov/bsd/uniform_ requirements.html) for more examples.

Standard journal article

[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540–7.

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003; 61: 347–55.

Article in supplement or special issue

[3] Fleischer W, Reimer K. Povidone-iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3–9.

Corporate (collective) author

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792–801.

Unpublished article

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

Personal author(s)

[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

Chapter in book

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O,

Kidd EAM, editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. pp 7–27.

No author given

[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

Reference from electronic media

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979–2001. www.statistics.gov.uk/downloads/theme_health/HSQ20.pdf (accessed Jan 24, 2005): 7–18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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Tables should be self-explanatory and should not duplicate textual material.

Tables with more than 10 columns and 25 rows are not acceptable.

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Explain in footnotes all non-standard abbreviations that are used in each table.

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