Environment Conservation Journal 13(3) 135-139, 2012
ISSN 0972-3099 (Print) 2278-5124 (Online)
Abstracted and Indexed

Some reproductive studies in *Clarias batrachus* with reference to different thermal conditions

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Received: 10.12.2011 **Revised: 15.05.2012** **Accepted: 16.09.2012**

Abstract

Eco-physiological study indicates the Clarias batrachus is a eurythermal fish and able to tolerate the wide range of climatic conditions. The quantitative analysis of nutritional components in gonadal region, such as glucose, proteins and fats showed the variations during exposure of fish to hot and cold temperatures. The fresh water catfish exhibits distinct phases of reproduction and also showed the changes in the levels of energy precursors with change in the excretory products, with respect to adaptability towards different environmental temperature conditions. Gradual increase of glucose, proteins, and cholesterol is observed in the male gonadal tissues. Rectal gland and clasper tissues showed, decreased of urea levels during the exposure of fish to the higher tolerable temperature. However, in female fish, the levels of proteins are significantly enhanced during the high temperature exposure. The levels of urea significantly decreased in the tissues of uterus and brain during the study period, while the lowering of glucose levels in brain indicates the extent of rate of physiological activity of the brain.

Keywords*: Clarias, Temperature, Gonad, Biochemical composition*

Introduction

At present all the ecosystems are subjected to slow changes because of the increased atmospheric temperature particularly the green house effect. However, rapid changes can have undesirable consequences. According to Fry (1970), degradation of an ecosystem is a change from a more productive to less productive state. There are many environmental factors such as temperature, light, salinity, etc., which influence as limiting factors for the growth and distribution of animals and plants. It also controls the reproduction, rate of embryonic development, migration, number of behavioral and metabolic characteristics of the organisms. Out of these environmental factors, temperature factor is taken in to consideration. With the increased population and industrial development, use of water resources and recycling became invariable. The warm water if released in to fish reservoirs, may elevate the water temperature, which may prove fatal to aquatic life. Therefore besides the thermal tolerance of fishes, the reproductive and biochemical study in accordance with the environmental temperature ranges is very necessary.Fishes inhabiting heterothermal environment can regulate their internal body temperature by behavioural means, which can be achieved by swimming in to the desired habitats. On the other hand, in case of eurythermal fishes, maintenance of constant body temperature is achieved by decreasing or increasing the rate of biochemical reactions occurs in side the body tissues. However, rate of biochemical activities of body cells may affect the rate of gonadal activities of fishes, intern influence the fish reproduction. The temperature beyond which animals cannot carry their normal activities constitutes the lethal temperature. The thermal limits of an animals and the tolerance limits are mainly genetically controlled thus constituting the genetic resistance adaptation of animal, (Precht, 1973). Temperature tolerance is an experimental criterion for the demonstration of physiological changes, which has been repeatedly recorded by Nagabhushanam and Sarojini (1969) and by various workers. Such eco-physiological studies world be the important means for condensing and transmitting the information based on scientific evidence and also would be needed by the decision makers for accomplishing

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future policy decisions. (Fry *et al*, 1942).

The essence of seasonal reproduction is that animals deliver their young ones during a period of year when the conditions are maximal for the survival of the species. Breeding activities of animals are related to the climatic conditions of their habitats. Many physical environmental factors such as temperature, photoperiod, rainfall and food supply also affects the regulation of seasonal reproductive cycle. There are nevertheless certain experimental results showing temperature induced metabolic changes in some vertebrates thereby indicating that pituitary, pineal and gonadal functions are temperature dependants, however the temperature controls the neuro-endocrine axis and intern gonadal functions.

Material and methods

For the present study, river dwelling fish *Clarias batrachus* is used. The live fishes were collected from the market and acclimatized for lentic habitat in 3' x 2' sized aquarium. The acclimation for cold and hot temperature is done by gradual decrease and increase of water temperature by 1o C, by using ice flakes and thermostatically controlled heaters respectively. Lethal temperatures are recorded (10^oC-minimum and 38^oC–Maximum lethal temperature). Digital Hick made thermometer is used to record the water temperature. (APHA, AWWA and WPCF, 1975).For microscopic studies of testis and ovaries, male and female fishes were dissected to acquire the gonads. The permanent preparations of the gonads, associated organs and brain were made by micro technique slide preparation and double stating method. Ocular and stage micrometer were used to measure the size of different cells. Biochemical estimation is done by the preparation of tissue homogenate in proper quantity, by using appropriate methods of estimation given by Wybenga and Pilleggi (1979), Wybenga Donald (1971) and Crocker (1967).

Results and Discussion

The data harvested during the present study indicate single layer of primary gonial elements are present in the form of large cells in the gonads of one week old fish. Each cells are characterized by predominant oval and vesicular nucleus having distinct nucleolus and granular chromatin matter. Some of the cells showed active mitotic divisions. After one week the proliferation of preceding stage by mitosis, forms a double layer of cells. The sexes are distinguished at first time this stage. The germinal cells proliferated and increase in number and undergo differentiation. The present investigation on the gonads showed that progressive maturational changes occurs during the first three weeks and the differentiation of mitotic cellular pattern occurs in one month. The hypothalamic neurosecretary system becomes differentiated relatively late in the development as described by Olivereau, (1967). The occurrence of mitotic process in the gonads is dependent on the hypothalamic nurosecretory control.After two months of age spermatogenetic wave becomes advanced up to the formation of spermatids. Spermatids are very small cells with 1.75 to 1.4 µ diameter. The primary and secondary spermatocytes are having more cell size than spermatids. (Table- 1.4). The ovary is characterized by the presence of several primary and secondary oogonia and numerous primary oocytes and secondary oocytes. Secondary oocytes are having well marked nuclei with one or two nucleoli in nucleoplasm towards the periphery, (Table-1.3). Vivian (1939) have studied the similar pattern in male and female Gobius paganellus.A great proliferation of the germinal elements with four and five fold increase of primary and secondary spermatogonia are observed in three month old fish. Infact the secondary spermatocytes increased double in number than the primary spermatocytes. The newly formed spermatozoa are evident as a aggregation of smaller cells of size 1.05 µ. In ovary the secondary oocytes are quite predominant and underwent multiplication are observed.Testis of six month old fish showed marked degree of maturation a evidenced by the reduction in percentage of pre-spermiogenic elements and appreciable increase in the number of spermatids and spermatozoa. But the testis was not considered as completely mature as the testicular endocrine cells and leydig cells were indistinguishable. In female of this age group showed noticeable increase of secondary oocytes with presence of small lipoid vesicles in their cytoplasm. In adult fish the size of testis greatly increased. A great depletion of spermatogonia was noticed. Although the spermatocyte and spermatids multiplied in number.

Table – 1.1:Effect of Temperature on Biochemical Composition of Different Reproductive organs in Male *Clarias batrachus*, exposed to different temperature conditions.

Organs	Temp. Range	Glucose	Total Protein	Cholesterol	Urea
Testis	22 to 24 ^{o}C	0.0054±0.0039	7.61 ± 2.49	.020 ± 0.02	0.0035±0.0004
	33.5 to 36 ^{o}C	.0073 ± .0085	8.21 ± 2.27	0.064 ± 0.05	0.0059±0.004
	10 to 13 ^{o}C	0.0045±0.003	2.40 ± 2.02	0.048 ± 0.034	0.003 ± 0.001
Seminal vesicle	22 to 24 ^{o}C	0.031 ± 0.006	19.31 ± 8.80	0.19 ± 0.069	0.014 ± 0.003
	33.5 to 36 ^{o}C	0.034 ± 0.029	20.47 ± 4.87	0.20 ± 0.08	0.015 ± 0.01
	10 to 13 ^{o}C	0.027 ± 0.024	16.07 ± 5.63	0.24 ± 0.10	0.010 ± 0.004
Vas deference	22 to 24 ^{o}C	0.026± 0.0053	17.23 ± 3.52	0.34 ± 0.004	0.021 ± 0.001
	33.5 to 36 ^{o}C	0.067 ± 0.010	20.79 ± 6.1	0.39 ± 0.05	0.029 ± 0.01
	10 to 13 ^{o}C	0.023± 0.0034	13.23 ± 2.19	0.34 ± 0.05	0.018 ± 0.002
Rectal gland	22 to 24 ^{o}C	0.054 ± 0.008	14.04 ± 4.07	0.044 ± 0.017	0.0048±0.0007
	33.5 to 36 ^{o}C	0.066 ± 0.0021	15.68 ± 9.25	0.26 ± 0.14	0.021 ± 0.01
	10 to 13 ^{o}C	0.045 ± 0.016	12.90 ± 5.43	0.034 ± 0.004	0.04 ± 0.006
Clasper	22 to 24 ^{o}C	0.027 ± 0.0071	10.89 ± 2.05	0.044 ± 0.01	0.014 ± 0.01
	33.5 to 36 ^{o}C	0.039 ± 0.0085	11.89 ± 3.02	0.018± 0.036	0.017 ± 0.002
	10 to 13 ^{o}C	0.023 ± 0.0051	9.75 ± 0.46	0.026 ± 0.003	0.0088±0.0006
Brain	22 to 24 ^{o}C	0.0031±0.0025	35.15 ± 21.09	0.015 ± 0.012	0.0046±0.002
	33.5 to 36 ^{o}C	0.0043±0.0022	35.99 ± 0.14	0.016 ± 0.007	0.0074±0.0003
	10 to 13 ^{o}C	0.0011±0.0005	16.05 ± 0.44	0.010 ± 0.004	0.0027±0.002

Table – 1.2:Effect of Temperature on Biochemical Composition of Different Reproductiveorgans in Female *Clarias batrachus* exposed to different temperature conditions.

Organs	Temp. Range	Glucose	Total Protein	Cholesterol	Urea
Overy	22 to 24 ^{o}C	0.036 ± 0.01	13.15 ± 4.87	0.15 ± 0.10	0.018 ± 0.015
	33.5 to 36 ^{o}C	0.039 ± 0.01	20.22 ± 4.71	0.30 ± 0.11	0.020 ± 0.002
	10 to 13 ^{o}C	0.015 ± 0.01	12.21 ± 5.34	0.12 ± 0.08	0.017 ± 0.005
Epigonal Organ	22 to 24 ^{o}C	0.006 ± 0.004	0.88 ± 0.011	0.011 ± 0.009	0.0025±0.0031
	33.5 to 36 ^{o}C	0.008 ± 0.006	13.44 ± 4.42	0.022 ± 0.002	0.0045±0.0011
	10 to 13 ^{o}C	0.002 ± 0.001	0.64 ± 0.36	0.01 ± 0.002	0.001 ± 0.0002
Uterus	22 to 24 ^{o}C	0.017 ± 0.008	5.82 ± 1.38	0.062 ± 0.03	0.0068±0.0006
	33.5 to 36 ^{o}C	0.017 ± 0.003	23.17 ± 3.12	0.12 ± 0.03	0.0084±0.0016
	10 to 13 ^{o}C	0.014 ± 0.007	4.21 ± 1.67	0.044 ± 0.02	0.0059±0.0022
Rectal Gland	22 to 24 ^{o}C	0.041 ± 0.01	10.98 ± 2.74	0.18 ± 0.05	0.016 ± 0.005
	33.5 to 36 ^{o}C	0.050 ± 0.01	21.32 ± 10.03	0.24 ± 0.013	0.017 ± 0.010
	10 to 13 ^{o}C	0.039 ± 0.016	10.40 ± 6.22	0.048 ± 0.021	0.011 ± 0.005
Brain	22 to 24 ^{o}C	0.0046±0.0016	2.42 ± 1.18	0.024 ± 0.012	0.003 ± 0.001
	33.5 to 36 ^{o}C	0.0053±0.0015	5.9 ± 2.73	0.030 ± 0.016	0.0045±0.0026
	10 to 13 ^{o}C	0.0020±0.0008	0.62 ± 0.19	0.17 ± 0.002	0.0008±0.0001

Table 1.3: Gonadal cell size during the progressive growth of gonads in female *Clarias batrachus*

Age of fish	*Diameter of overy*	*Size of cytes / ovum*	*Size of nucleus*
30 days	40.6 µ	POG – 17.5 µ	10.5 µ
60 days	91.46 µ	POG – 37.16 µ, SOG – 62.2 µ	26.6 µ, 38.9 µ
90 days	118.3 µ	SCO – 100 µ	49.6 µ
180 days	725.3 µ	Follicle – 183.5 µ	56.75 µ
Adult fish	2708.1 µ	Follicle – 395.3 µ	93.3 µ
Mature fish	2708.1 µ	Follicle – 802.4 µ	99.3 µ

Table 1.4: Gonadal cell size during the progressive growth of gonads in male *Clarias batrachus*

Age of fish	**Diameter of Testis**	**Size of cytes / spermatozoa**	**Size of nucleus**
30 days	42.28 µ	PSC – 7.56 µ, SSC – 8.75 µ	4.38 µ, 7.0 µ
60 days	76.54 µ	PSC – 5.95 µ, ST – 1.75 µ	3.15 µ, 1.4 µ
90 days	99.23 µ	SZ – 1.06 µ	1.05 µ
180 days	424.8 µ	SZ – 2.1 µ	1.98 µ
Adult fish	1420.6 µ	SZ – 3.6 µ	3.41 µ
Mature fish	1438.9 µ	SZ – 3.6 µ	3.62 µ

The leydig cells were distinguished for the first time and occupying interlobular areas up to 9 cells in thickness. These cells are having round to oval nuclei and basophilic cytoplasm. Dadzie (1969) has studied the testis of mature fish Clarias batrachus. In female ovary, maturing oocytes are observed with appearance of minute yolk globules in the cytoplasm .

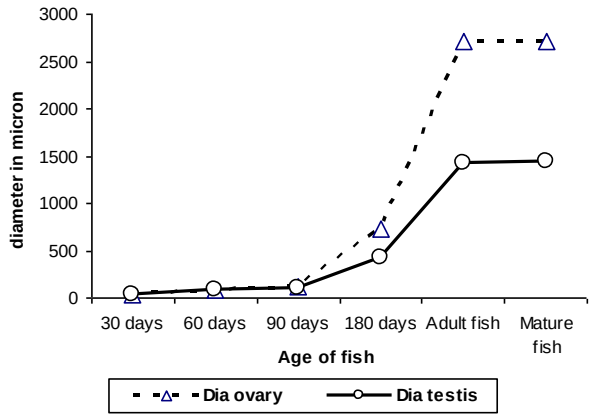

Fig.1 - Rate of development of gonads in *Clarias batrachus.*

The spermiogenesis in C. batrachus is completed at the end of three months, the male is in sexual readiness only when it attains the adult stage. Similarly in the females, although oocytes continue to enlarge the mature ova are formed in the adult condition. In the process of vitallogenesis storage of nutritive material in the age is involved. It is possible that such extra nutritive material cannot be made available to the ova till the maturational changes in them, Vivian (1939). However, the actual rate of growth of testis and ovary enhances, when, male and female attains the age of 90 days. It is evidenced by the microscopic studies and from the increased size of gonads after 90 days. (Fig. & Graph). The gonadal activities are influenced by the hypothalamus, however before the two month age of fish the activity of adeno-hypophysis is not seen apparent due complete development of hypothalamo – pituitary axis. After 90 days age of fish complete development of adeno-hypophysial function intern enhance the rapid development of gonads.

Effect of Temperature on Biochemical Composition :

Present investigation indicates that the Indian cat fish Clarias batrachus is a eurythermal fish as it is able to tolerate a wide range of temperature change, due to natural climatic changes between summer and winter. There is much relationship between temperature and composition of glucose, protein, cholesterol and urea. (Kulkarni, 1987) Glucose and Proteins are the chief nutritional constituents of any tissues. The actions of several cellular enzymes influence the synthesis of glucose, protein, fats and urea. Certain seasonal changes of

nutritional constituents in H. fossilis were recorded by Shrivatava and Shrivastava, (1994). Increased levels of glucose and proteins during the hot exposure of fish may attribute to increased cellular enzymatic activities, also enhance basal metabolism. The cellular byproducts in the rapid enzymatic activities may also increased during the hot environmental conditions, that intern enhance the formation of urea. The organs having active cellular activities such as gonadal cells and brain cells exhibit higher nutritional components. On the other hand various enzymes shows less activity during the cold condition caused the depletion of glucose, protein, cholesterol and urea levels in the reproductive organ tissues. The lesser rate of metabolic activities in the tissues with less quantity of cellular byproducts may lower the urea levels in the tissues in male and female fishes. Ananthakrishnan and Kutti, (1979) has carried the similar type of studies on Channa punctatus. During the reproductive cycle of C. batrachus, the biochemical levels of glucose, protein, cholesterol and urea increases with respect to environmental temperature conditions from low to high temperatures. (Lal, and Singh, 1987).

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