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CHANGES IN OVARIAN DEVELOPMENT AND SERUM LEVELS OF VITELLOGENIN UNDER DIFFERENT ACCLIMATION TO TEMPERATURE IN *CHELA CLUPEOIDES* (BLOCH, 1795)

PRAGYAN DASH*, K. D. RAWAT, DIPANJAN KASHYAP AND A. P. SHETE
Central Institute of Fisheries Education, Versova, Andheri (W), Mumbai - 400 061, INDIA
e-mail: dipankashyap@gmail.com

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***Corresponding author**

ABSTRACT
An experiment to assess the effect of temperature on ovarian development and serum levels of vitellogenin in *Chela clupeiodes* was conducted at aquaculture division of Central Institute of Fisheries Education (CIFE), Mumbai for a period of 60 days from the month of January to February. Results showed that raising temperature to 30°C significantly enhanced GSI (6.11 ± 0.55 – 14.7 ± 0.42) but beyond that temperature the GSI increment was less (3.5 ± 0.4 - 6.4 ± 0.41). Histological examination revealed better maturity with higher percentage of mature oocytes (24.753 ± 1.366) in treatment T_3 (30°C). Elevated temperature exposure beyond 30°C showed poor maturation with lower percentage of mature oocytes (3.486 ± 0.633). Estimation of serum vitellogenin level showed elevated temperature above 30°C resulted in reduction of their capacity to be sequestered into growing oocytes. After 60 days of rearing serum level of vitellogenin attained its peak at treatment T_3 (30°C). By the present investigation it can be concluded that elevated temperature may increase ovarian maturation in *Chela clupeiodes* but up to a certain threshold limit beyond that it has a negative impact on gonadal development and vitellogenin level in serum.

INTRODUCTION

Chela clupeoides is one of the medium sized larvivorous species commonly considered as weed fish with its distribution throughout India, widely distributed in rivers, ponds, beels and inundated fields. A preliminary survey conducted by CIFE, Mumbai (2006) in Maharashtra indicates that presently a large quantum of these fish is used for human consumption and also as animal feed. In Dimbhe reservoir at Taluka Ambegaon of Pune district the catch is mostly dominated by Chela species because of its high local demand which has become a major fishery in that area.

Knowledge on reproductive biology of fish is essential for evaluating the commercial potentialities of its stock, life history, culture practice and management of its fishery (Doha and Hye, 1970). Success of reproduction depends upon normal gonadal development stimulated by favourable environmental conditions. As fish are exposed to changing environmental conditions, such as photoperiod and temperature, the external rhythm begins to dominate and its influences on the reproductive processes are transmitted by changes in the quantity of gonadotropin released from the pituitary gland (Oren, 1981).

Temperature is a major environmental factor affecting the reproductive cycle and spawning in fishes (Lam, 1983; Van der Kraak and Pankhurst, 1997). Knowledge of optimal temperature for growth and maturation has the advantage in aquaculture because it can be used to promote increased production.

Onset of vitellogenesis is timed by the environmental conditions. Temperature regulates vitellogenesis towards functional maturity (Kuo *et al.*, 1974). Study on temperature effect on reproductive development in Arctic charr, *Salvenius fontinalis* showed that thermal environment influence the deposition of lipids during oocyte development (Jobling *et al.*, 1995). Hence, quantification of serum vitellogenin could be effectively used to study the degree of maturation in female fish.

Though there has been studies related to the life-history and bionomics of *Chela phulo* (Alikunhi and Chaudhuri, 1953) on the *Oxygaster* (Syn. *Chela*) *bacaila* (Parameswaran *et al.*, 1969) and developmental morphology of *Chela dadiburjori* (Sado and Kimura, 2005), but there has been no report related to *Chela clupeoides* maturity cycle so far. Since maturation is influenced by temperature this investigation was conducted to study the effect of temperature on its maturity.

MATERIALS AND METHODS

Maintenance of fish and experimental set-up

Adult female fish, *Chela clupeoides* weighting between 3.42-3.58 g were used for the present investigation carried out at wet lab of CIFE, Mumbai. A total no. of 144 fishes were randomly distributed in six distinct experimental groups as Control (ambient temperature), T_1 (26°C), T_2 (28°C), T_3 (30°C), T_4 (32°C) and T_5 (34°C), each with three replicates. For temperature maintenance 300 w thermostat heaters (Amazon Company) of temperature range from 18° C to 34°C were installed inside the tanks.

Experimentation

Approximately every 30 days, one fish from each aquarium was randomly sampled and sacrificed for serum and gonad tissue collection. Blood samples were collected from caudal vein of fish using 1 ml syringe in 0.5 ml eppendorf tubes, which were kept in slanting position for about 2 hr for serum preparation. After centrifugation the resulting serum was stored at -20°c prior to analysis of vitellogenin levels. Gonadal maturity was assessed by calculating gonado- somatic index, average frequency distribution of different developmental stages of oocytes and histological examination of gonadal tissues. The ovary were weighed and fixed in 10% Neutral buffer formalin (NBF), hydrated and embedded in paraffin. Sectioning of 5μm thickness was carried out on ovaries prior to staining with haematoxylin and eosin. The oocytes stages were determined under light microscope according to the method adopted by Yon *et al*. (2008).

Statistical analysis

Data were statistically analyzed by statistical package SPSS version 16 in which one way ANOVA and Duncan's multiple range tests were used to determine the significant differences between the means. Comparisons were made at the 5% probability level.

RESULTS

Maturation in female Chela clupeoides

Several stages of oocytes development were observed in matured female *Chela clupeoides*. This might indicates the fish is a asynchronize spawner and could possibly reproduce several times in a year. Histological sections of ovary of the experimental fish contained highly distinct oogonia at all stages of development throughout the period of observation. Progressive changes in cellular diameters during oogenesis are given in Table 1. Developmental stages of oocytes growth are shown in Fig. 1.

Maturation in female *Chela clupeoides* with respect to temperature variation

Mean gonado somatic index (GSI) of female *Chela clupeoides* with respect to different temperatures are shown in Fig. 2. After 60 days of rearing, GSI value of T_2 and T_3 group were statistically similar but significantly higher ($p < 0.01$) than other groups, whereas; T_4 and T_5 group showed significantly lower value ($p < 0.01$) than others. The highest GSI (12.7%) was recorded at 30°C temperature (T_3) while lowest (6.4%) in 34°C (T_5). Histological changes of oocytes development with respect to temperature variation are shown in Fig. 3. The percentage of individual oocytes in different stages of maturity observed during different treatments was computed and depicted in Fig. 4.

Hormonal profile of female *Chela clupeoides*

Serum Vitellogenin level (ng/ml) in female *Chela clupeiodes* in different temperatures was estimated and depicted in Fig. 5. There was no significant difference ($p > 0.05$) of Vitellogenin level at the beginning of experiment. On 60th day of rearing highest Vitellogenin level was recorded in T_5 treatment exposed

Table 1: Changes in cellular diameters during oogenesis

Stages	N	Oocyte diameter (mm)	Criteria
Primary growth stage	30	0.06-0.18	Multiple nucleoli were observed in the nucleus of the (germinal vesicles) oocytes.
Cortical alveolus stage	30	0.18-0.48	The cortical alveoli proliferated and follicle increased in size and the oocytes became opaque in the area that surrounded the nucleus.
Vitellogenic stage	30	0.46-0.68	Number and size of the yolk vesicle increased. Granular structures appeared in the cortical alveolar phase were larger and the nucleus was irregular in the shape. Vitellus density in the oocyte was extended towards the centre from the cortical alveolar area.
Mature stage	30	0.65-0.73	Nucleus could not be observed due to the fact the granular structures filled up the entire cytoplasm. The membrane of the nucleus dissolved.

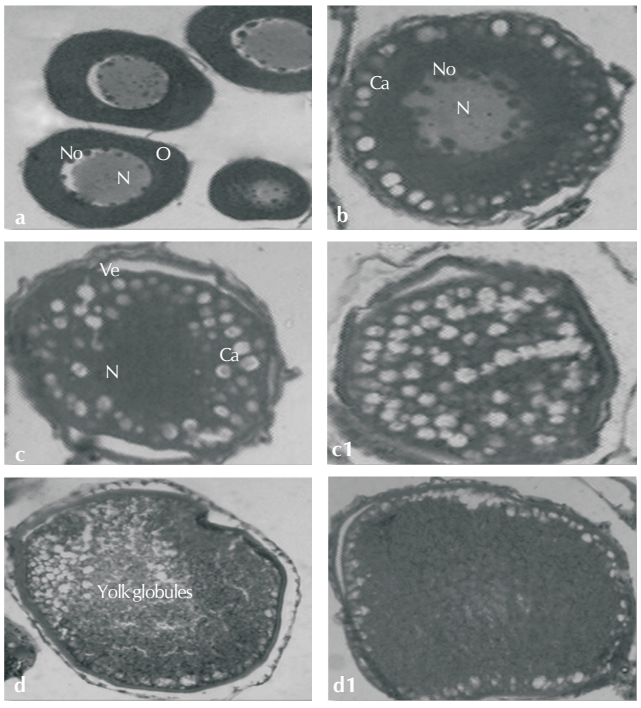

Figure 1: Developmental stages of oocytes growth in *Chela clupeoides*: (a) Primary Oocyte, Several nucleoli appear at the periphery of nucleus. (b) Cortical alveolus stage, Cortical alveoli fill the oocyte of nucleus. (c) & (c1) Vitellogenic Oocyte , Vitellus density extended towards the center from cortical alveolar area. Vitelline membrane develop at this stage. (d) & (d1) Mature Oocyte, All cortical alveoli integrated and form yolk. The granular structure filled up the entire cytoplasm. H & E (LM - 40 X). (N) Nucleus; (No) Nucleoli; (Ca) Cortical alveoli ; (O) Ooplasm; (Ve) Vitelline membrane

with 34°c temperature at 4.4±0.2 ng/ml. It has been also recorded that T_3 group fish (30°c) showed lower serum vitellogenin level than Control, T_1 and T_2 group fish.

DISCUSSION

The primary aim of the present study was to delineate the effect of temperature on the maturation of *Chela clupeoides*. Gonadosomatic index (GSI) is generally used as a reliable criterion for expression of gonadal development and reproductive effort in fishes (Calow, 1979; Saksena, 1987). In present study, the highest GSI was recorded at treatment T_3 exposed with 30°C temperature compared to other treatments. Higher GSI indicated better maturation in fishes. T_2 (28°C) and T_3 (30°C) group showed similar result. This result is in agreement with the finding of Kohinoor *et al*. (2003) who have reported highest GSI value of *Chela cachius* in the month of June.

The study also indicated that gonado-somatic index was highest in T_3 treatment exposed to 30°C temperature, whereas

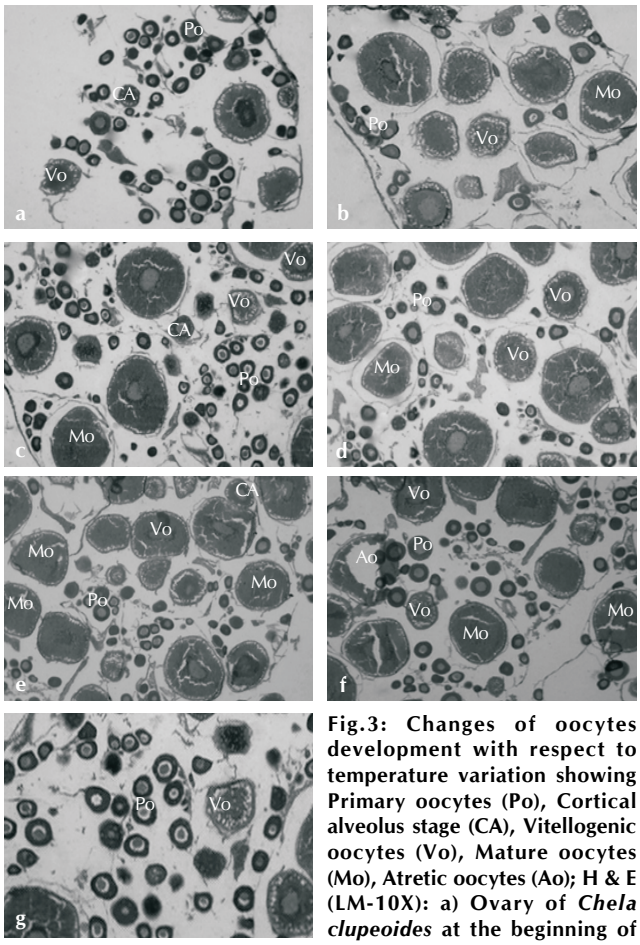

Fig.3: Changes of oocytes development with respect to temperature variation showing Primary oocytes (Po), Cortical alveolus stage (CA), Vitellogenic oocytes (Vo), Mature oocytes (Mo), Atretic oocytes (Ao); H & E (LM-10X): a) Ovary of *Chela clupeoides* at the beginning of experiment. b) Ovary of *Chela clupeoides* after 60 days of rearing in Control. c) Ovary of *Chela clupeoides* after 60 days of rearing in T1(26°c) d) Ovary of *Chela clupeoides* after 60 days of rearing in T2(28°c). e) Ovary of *Chela clupeoides* after 60 days of rearing in T_3 (30°c). f) Ovary of *Chela clupeoides* after 60 days of rearing in T_4 (32°c). g) Ovary of *Chela clupeoides* after 60 days of rearing in T_5 (34°c)

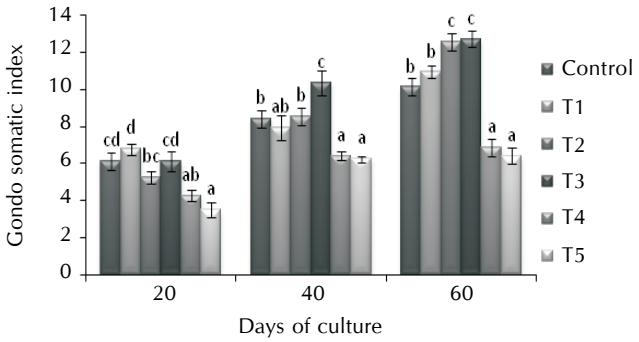

Fig. 2: Gonado somatic index of female *Chela clupeoides* acclimatized to different temperatures

vitellogenin level was lowest in T_3 (30°C) treatment. This is supported by the findings of Carnevali *et al*. (2003) that plasma vitellogenin and estradiol are widely accepted parameters related with oocyte vitellogenin growth and maturation, and when compared with GSI, found well correlated. He further suggested that during spawning, plasma vitellogenin, sequestered from the circulation, rapidly decreased, and therefore GSI values were found still high. The high molecular protein vitellogenin is then incorporated into the developing enlarged oocytes in the ovary via circulation resulting into formation of yolky oocytes, and thereby accounts for a tremendous increase in the values of ovarian weight and gonadosomatic index of the concerned fish during the peak breeding period (July-August) in an annual cycle (Nagahama, 1994). Earlier studies (Nath and Sundararaj, 1981) also indicated that two consecutive physiological events are involved in the completion of an ovarian cycle in fish in general. The first event is enlargement of the ovary with concomitant formation of the yolky oocytes, i.e., vitellogenesis, which is followed by the second event that includes maturation of oocytes, ovulation and spawning.

Study from the average frequency distribution of different developmental stages of oocytes after 60 days of rearing showed that in all the treatments primary oogonia percentage was more as compared to other developing oocytes. Therefore, it can be concluded that maturation process was under progress. The study also showed that highest percentage of primary oogonia in the ovary was recorded in treatment T_5 exposed at temperature 34°C; while the highest percentage of vitellogenic oocytes occupied most areas of the ovaries in

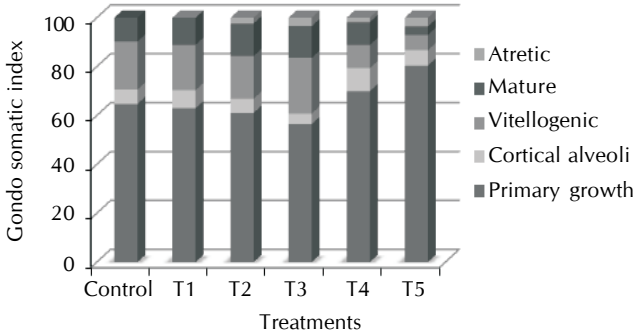

Figure 4: Average frequency distribution of different developmental stages of oocytes in ovaries of *Chela clupeoides* acclimatized to different temperatures

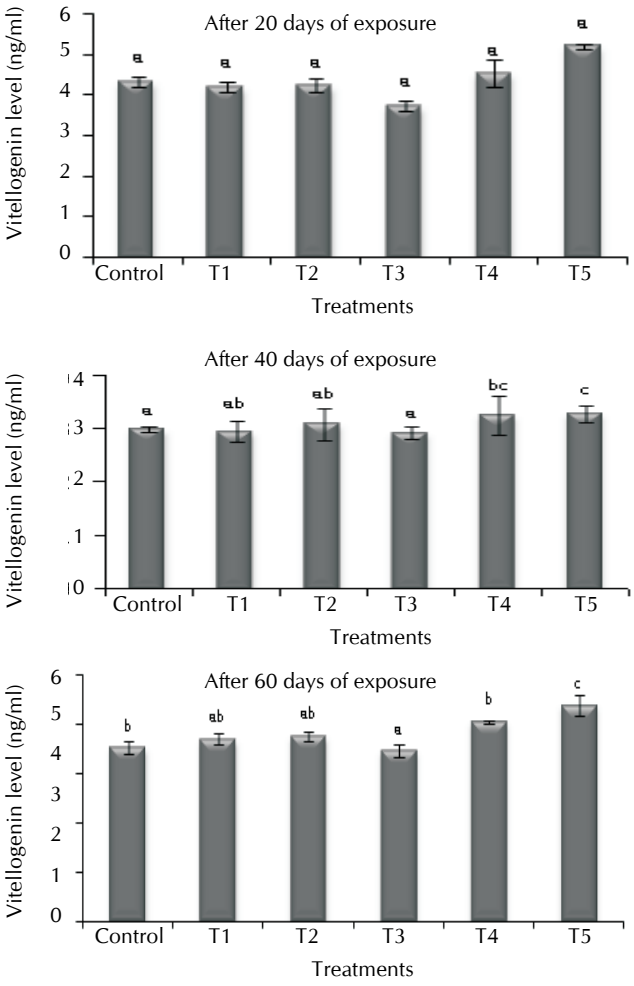

Figure 5: Serum Vitellogenin level (ng/ml) in female *Chela clupeiodes* acclimatized to different temperatures

treatment T_3 (30°C). From this, it can be concluded that at very high temperature maturation occured of a slower pace.

Histological study of ovary revealed that at beginning of the experiment, ovary was mostly filled with primary oocytes and after 60 days of rearing, it was predominantly filled with vitellogenic and mature oocytes. In T_3 (30°C) group vitellogenic oocytes were seen in higher number as compared to other treatments. Observations of histological examination are also supported by the higher GSI and higher percentage of vitellogenic oocytes in treatment T_3 (30°C).

Normal changes in environmental temperature have the capacity to affect endocrine function and either advance or retard maturation but above normal temperatures have deleterious effects on reproductive processes. Exposure of female *Chela clupeoides* to different temperatures showed that vitellogenin sequestration to oocytes is associated with the maintenance of water temperature in rearing system. After 60 days of rearing, it has been noticed that at elevated temperature beyond 30°C showed very high level in serum Vitellogenin concentration. This suggests that the principle effect of thermal stress is to reduce the capacity for Vitellogenin to be sequestered into growing oocytes which has also observed by Pankhurst *et al., 2010*. It is known that environmental temperature determines the rate of metabolic and enzymatic reactions. According to the Van't Hoff rule, every 10 °C increase in water temperature provokes a 2–3-fold increase in biochemical or enzymatic activity (Reid *et al.,* 1997 and Caissie, 2006). Hence, it can be suggested that elevated vitellogenin levels at elevated temperatures (up to a certain level) are the result of a higher synthesis rate.

CONCLUSION

It can be concluded that increasing level of ovarian development appeared positively correlated with increasing temperature up to a certain level (30°C) and above that temperature, maturation was affected negatively. Hence, in captive condition this temperature can be taken as guideline for successful spawning and culture of fish.

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