



## CYCLIC LONG TERM HYPOXIA IMPAIRS ENDOCRINE AND REPRODUCTIVE FUNCTIONS IN GOLDFISH, *CARASSIUS AURATUS*

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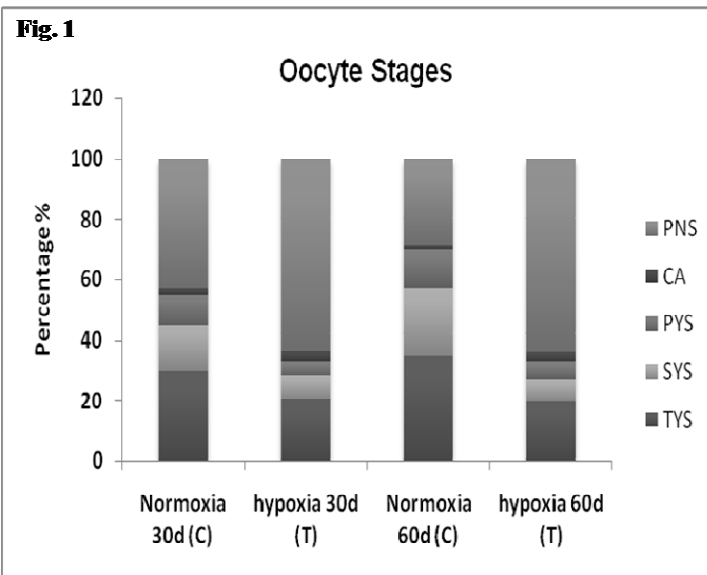
### Introduction:

Aquatic hypoxia has been considered as a major environmental concern in the new millennium. Recent studies clearly revealed that hypoxia acts as an endocrine disruptor and reduced reproductive performance of the fish. Wild fish populations generally face hypoxia for variable duration with or without different frequency of daily hypoxic periods. The effect of such cyclic hypoxia on reproduction is not yet elucidated in any fish. Therefore, the present study we have investigated the effect of cyclic hypoxia on gametogenesis and reproductive endocrine function in goldfish, *Carassius auratus*, a well established freshwater model for reproductive endocrinology.

**Methods:** Adult goldfish were exposed to hypoxia (T, 1.2 mg/l DO) for different durations such as 6h, 9h, 12h, 24h followed by normoxic (C, DO 6.0 mg/l) recovery daily for a total period of 60 days. Gonads were collected for calculation of gonado-somatic index (GSI) and for histological examination. Plasma total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were estimated by enzymatic colourimetric tests (Merck India Ltd.). Plasma levels of 17 alpha hydroxyprogesterone, testosterone, estradiol, 11 ketotestosterone and vitellogenin were measured by ELISA. Sperm production was estimated by calculating the sperm number and motility score.

### Results and Discussion:

The cyclic hypoxia for different duration inhibited gonadal growth by suppressing gametogenesis in both male and female. It significantly reduced GSI values more than 50% which was mainly attributed to reduction of number of vitellogenic oocytes, particularly the tertiary yolk oocytes in the fish exposed to hypoxia (Fig. 1). The hypoxia caused a sharp decline in plasma profile of two key reproductive steroids: estradiol in female and 11 ketotestosterone (11-KT) in male (Fig. 2, 3). The overall inhibition in steroid production pathway played a crucial role in retarding the process of gametogenesis in both the sexes. Plasma vitellogenin (Vg) which is a biomarker of oocyte development was also significantly decreased in female fish resulting hindrance in oocyte growth. On the other hand, significant increase in plasma estradiol may be attributed to higher plasma vitellogenin level in hypoxic male (Fig. 4). The total cholesterol, HDL and LDL cholesterol were significantly reduced in the fish exposed to different duration of cyclic hypoxia (Fig. 5). As cholesterol remains the starting precursor molecule for steroid synthesis, the decrease in its production may be attributed at least to certain extent to the inhibition of steroidogenesis pathway. There was a marked decrease in production of sperm and their motility compared with controls in hypoxic groups, which was associated with decreased 11-KT signaling regulating spermatogenesis. The degree of endocrine disruption and impairment of the reproductive activity



Parameters		30 Days	60 Days
<b>GSI Female</b>	<b>C</b>	<b>10.54 ± .469</b>	<b>9.21 ± .503</b>
	<b>T</b>	<b>4.63* ± .248</b>	<b>3.99* ± .124</b>
<b>GSI Male</b>	<b>C</b>	<b>3.37 ± .181</b>	<b>3.20 ± .200</b>
	<b>T</b>	<b>1.67* ± .067</b>	<b>1.25* ± .049</b>
<b>Sperm Count (X 10<sup>6</sup>)</b>	<b>C</b>	<b>3.38 ± .135</b>	<b>3.15 ± .076</b>
	<b>T</b>	<b>2.03* ± .122</b>	<b>1.88* ± .06</b>
<b>Sperm Motility</b>	<b>C</b>	<b>4.66 ± .210</b>	<b>4.16 ± .307</b>
	<b>T</b>	<b>3.83* ± .307</b>	<b>2.33* ± .210</b>



was highest in 24h hypoxia groups followed by 12h and 9h hypoxia groups, whereas 6h hypoxia had significantly lower effect compared to other hypoxia treatments.

adaptive strategy to cope up under cyclic hypoxia by reducing the cholesterol supply for production of sex steroids during active reproductive phase.

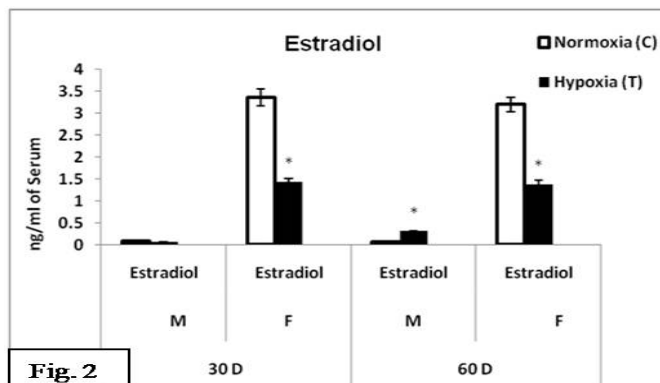


Fig. 2

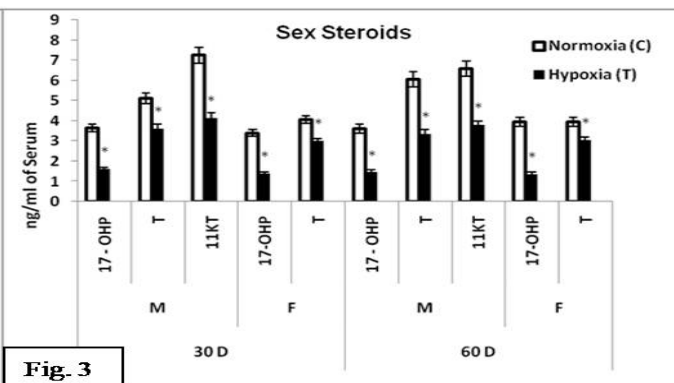


Fig. 3

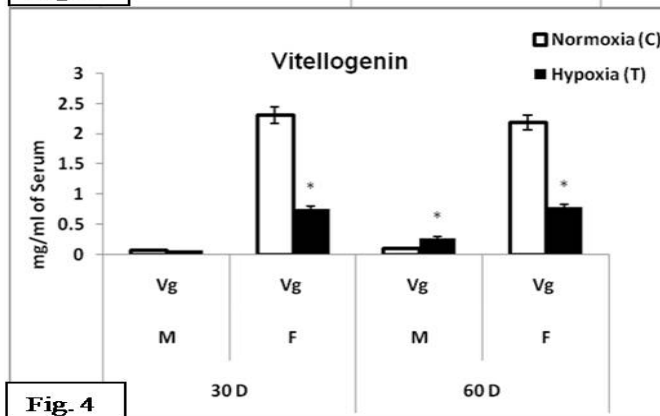


Fig. 4

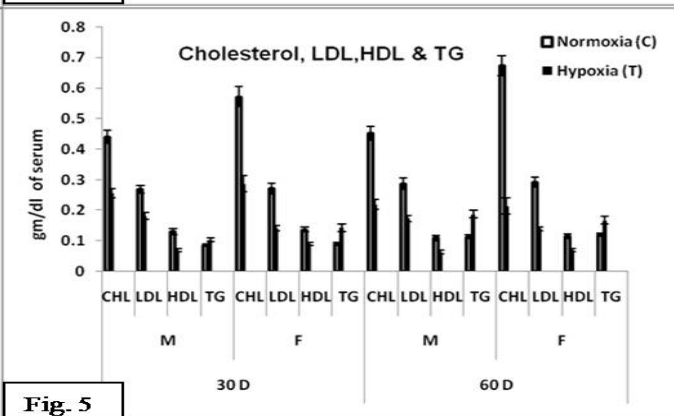


Fig. 5

**Conclusion:**

The study clearly reveals that cyclic hypoxia caused impairment in reproduction similarly as reported in other fishes exposed to continuous hypoxia (1, 2). However the extent of reproductive suppression under higher hypoxia duration in a cycle was found to be severe similar to continuous hypoxia. Inhibition in steroid synthesis was a prime attributor retarding the gametogenesis process, mainly to reduce the energy expenditure towards reproduction under anaerobic condition. Moreover hypocholesterolemia may be an

**References:**

[1]WU, R., ZHOU, B., WOO, N., LAM, P., 2003. Aquatic hypoxia is an endocrine disruptor and impairs fish reproduction. *Environ. Sci. Technol.*, 37: 137–1141.  
 [2]THOMAS, P., RAHMAN, S.M., KHAN, I.A., KUMMER, J.A.2007. Widespread endocrine disruption and reproductive impairment in an estuarine fish population exposed to seasonal hypoxia. *Proc. R. Soc. B*, 274: 2693-2702.