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Chronic Exposure to Moderate Hypoxia Impairs Reproductive Success in the Mosquitofish *Gambusia Affinis*

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Abstract

Increasing incidences of hypoxic environment in aquatic systems is a serious threat to the life of fish. In the present study, chronic effect of moderate hypoxia on reproductive performance was studied in the viviparous fish Gambusia affinis. Exposure of female fish to mild hypoxic condition for a period of 25 days resulted in a general decrease in the body weight and absence of vitellogenic follicles in the ovary compared to controls. Further, the mean number of early embryos (eye-spot stage) did not differ significantly, whereas the mean number of late embryos (yolk sac stage) was higher in hypoxic fish compared to controls. However, the mean number of juveniles produced from hypoxia alone and hypoxic recovery groups remained significantly lower compared to controls. These results suggest that exposure to moderate hypoxia delays the embryonic development at late stage thereby impairs hatching success of juveniles and that exposure to normoxia does not improve the detrimental effect of hypoxia in the viviparous fish G. affinis.

Keywords: Hypoxia, normoxia, embryonic development, viviparous fish, dissolved oxygen, *Gambusia affinis*.

Introduction

Environmental cues play a crucial role in controlling the endocrine system of reproduction in fish by regulating the hypothalamo-pituitary-gonad (HPG) axis. Interruption in this neuroendocrine axis can result in alteration of reproductive functions. Dissolved oxygen (DO) is necessary to many forms of aquatic life including fish, which use oxygen in respiration, similar to organisms on land[1]. DO is one of the environmental cues known to play an important role in maintaining the normal reproductive activity in the water breathing fishes[2]. Low concentration of DO (about 2 mg/L) in natural waters can lead to a condition called hypoxia for most fish[3]. Hypoxia is an endocrine disrupter and has negative impacts on growth and reproduction in fish[4]. Hypoxia impairs sex steroid levels, gonadal maturation, egg production and gamete fertility and inhibits ovulation by reducing luteinizing hormone (LH) levels in the common carp *Cyprinus carpio*[5], affects the gonadal growth and gamete production in the Atlantic croaker *Micropogonias undulates*[2, 6], and delays spawning in the gulf killifish *Fundulus grandis*[7]. However, reproduction and hatching success in relation to more moderate degrees of oxygen deprivation has not received much attention in fish. Furthermore, majority of the studies have focused on the effect of hypoxia in oviparous fish species and very little consideration has been given on viviparous species. In the vertebrate line, viviparity first evolved among the fishes and in particular, the ovary of viviparous teleosts is unique among vertebrates, since it is the site of both egg production and gestation[8]. Thus far, effect of hypoxia is reported in one viviparous eelpout *Zoarces viviparous,* but limited to respiration and behaviour[9]. The mosquitofish *Gambusia affinis* is a viviparous teleost, which produces offsprings at 10-20 weeks of age, and it continues to reproduce until 20-34 months of age. The development period for fry inside the mother is approximately 28 days, which may fluctuate depending on stress and environmental conditions[10]. The aim of the present study is to determine the effect of moderate hypoxia on reproduction and hatching success in *G. affinis*.

Material and Methods

Experimental procedure: Adult *G. affinis* weighing 0.25-0.27g were collected from the ponds in Karnatak University Campus, Dharwad and reared at a sex ratio of ten females and five males in separate plastic tubs (60 cm diameter and 30 cm depth), each consisting of four liters of water, for one month prior to the commencement of experimentation. The natural photoperiod and temperature were 11.45 ± 0.5 L: 12.15 ± 0.5 D and 25.15 ± 2.25^{o}C respectively. The fish were fed daily twice with commercially available food pellets (Taiya pet feed, Chennai, India). Fifty female fish with regressed abdomen (after parturition) were identified and used for the experimentation. A group of fish (n=10) were sacrificed on the day of commencement of the experiment and served as initial controls. The remaining fish were divided into four groups. The first group was maintained in normoxic condition (7.2 ± 0.2 mg/L) for a period of 25 days (25d controls), whereas the fish in second group were exposed to moderate hypoxic condition (4.0 ± 0.08) for a period of 25 days. The fish in the third group were also exposed to hypoxia initially for 25 days and then kept in normoxic condition until 40 days (hypoxic recovery group). Parallel controls were maintained for 40 days in normoxia (40d controls). The tubs were provided with aerator and aquatic

plants to ensure the supply of oxygen for 25d and 40d controls and recovery group (between 26-40 days). For these groups, recirculation of water was done on alternate days to keep up normoxia, whereas for the hypoxic fish, the low DO level was achieved by not providing aerators to water.

Histology: At the time of each autopsy, the fish were anaesthetized with 2-phenoxy ethanol (Sigma, USA; 1:1500), weights of the body mass were recorded, and killed by decapitation method. The ovaries were dissected out, fixed in Bouin's fluid and processed for histological studies. Photographs of the representative ovaries/embryos were taken by using the stereozoom attached with digital camera (SZX-16 C3, Olympus, Japan). Then serial paraffin sections of the ovaries were cut at 5 µm thickness and stained with hematoxylin and eosin. The follicles and embryos belonging to different categories were classified based on the previous report for *G. affinis*[11]. The photographs of cross sections of the ovary were taken by using BX 41 microscope attached with E-330 camera (Olympus, Japan).

Statistics: The data on DO levels and number of embryos in all experimental groups are expressed as means ± SE. The mean number of embryos in different experimental groups were compared using one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test. Significant differences were determined at $P<0.05$ level.

Results and Discussion

The ovaries in the *G. affinis* are of gymnoovarian type (i.e. the follicles are exposed to the body cavity and carried through the oviducal) consisting of developing follicles at different stages. Figure – 1 shows the presence of ovarian follicles, early and late embryos and juveniles in *G. affinis*. Early embryo is a yolk filled cavity in which developing eye spot, blood vessels and heart beat are noticed. Late embryo is well developed as a juvenile, but is still attached to the yolk sac and enclosed within the embryo sac. Juveniles are the fully developed free swimming hatchlings. Previous studies have shown that under hypoxic conditions (below 2 mg/L), fertility and hatching success were decreased in common carp *C. carpio*[5] and Atlantic croaker *M. undulates*[6]. However, exposure of *C. carpio* to hypoxia (1.0 ± 0.2mg/L) for more than two months resulted in retardation of final oocyte maturation concomitant with suppression of LH levels and failure of spawning despite oocyte development[12]. In the present study, the ovary consisting of previtellogenic and vitellogenic follicles in initial controls, 25d and 40d controls are shown in figure – 2. In fish exposed to hypoxia, only previtellogenic follicles along with some atretic follicles were present, whereas the vitellogenic follicles were completely absent. Furthermore, exposure of hypoxic fish to normoxia from day 26 to 40 did not promote vitellogenic follicular development as shown by the absence of these follicles in this group in contrast to 40d controls. These results indicate that exposure of fish to mild hypoxia significantly affects the process of vitellogenic follicular development and that this inhibitory effect is not recovered following reestablishment of normoxic condition, for the first time in a viviparous fish *G. affinis*.

Table-1
Effect of hypoxia on body weight of the fish *G. affinis*

Experimental Groups	Initial body weight (g)	Final body weight (g)	Weight gained (g)
25d controls	0.30 ± 0.02	0.32 ±0.01	0.02
Hypoxia group	0.32 ± 0.01	0.33± 0.06	0.01
40d controls	0.34 ±0.01	0.37±0.02	0.03
Recovery group	0.30 ±0.01	0.32±0.01	0.02

Table – 2
Effect of hypoxia on embryonic developmental stages in the fish *G. affinis*

Experimental Groups	**Mean number of embryos at different stages ± SE**		
	Early embryo	**Late embryo**	**Juveniles**
Initial control	-	-	-
25d controls	1.50 ± 0.82 [a]	-	4.54 ± 0.84 [b]
Hypoxia group	1.75 ± 0.67 [a]	0.80 ± 0.08 [a]	1.00 ± 0.44 [a]
40d controls	1.00 ± 0.40 [a]	0.33 ± 0.2 [a]	5.50 ± 0.35 [b]
Recovery group	0.50 ± 0.19 [a]	0.50 ± 0.35 [a]	1.45 ± 0.56 [a]

Effect of hypoxia on body weight of *G. affinis* is shown in table-1. There was a decrease in the mean body weight gain in fish exposed to hypoxia compared to 25d and 40d controls. Increasing the DO levels in rearing tubs of hypoxic recovery group after 25 days resulted in body weight gain similar to that of 25d controls. The body weight gain was maximum in 40d controls. These results are not surprising, given that oxygen is required to metabolize fats and metabolic rate may decline with decrease in available oxygen[13].

Reduction in egg production, gamete fertility and hatching success due to hypoxia is reported in the carp *C. carpio*[5]. Studies on other teleost species such as *Danio rerio*[14] and *Oncorhynchus mykiss*[15] have documented developmental delay during embryogenesis following hypoxia exposure. Similarly, exposure of zebra fish to sub lethal levels of hypoxia significantly increased malformation in embryonic development[16]. In the present study, table–2 reveals the effect of moderate hypoxia on specific stages of embryonic development in a viviparous species. In the current study, no embryos were noticed in the ovary of initial controls on the day of autopsy indicating the complete release of developed juveniles. No significant differences were observed in the mean number of early embryos among 25d and 40d controls, hypoxia and hypoxic recovery groups. The late embryos were found in hypoxia group in contrast to their absence in 25d controls. However, the number of juveniles were significantly ($P<0.05$) lower in these fish compared to those of 25d controls suggesting retardation in the rate of conversion of late embryos to juveniles

due to low DO. This possibility is further substantiated by the presence few late embryos and a significantly higher number of juveniles in 40d controls compared to those of hypoxic fish and hypoxia recovery group.

Conclusion

The results of the present investigation suggests that chronic exposure to moderate hypoxia condition interferes with vitellogenic follicular development, and impairs the rate of conversion of late embryos to juveniles, and that these detrimental effects are not restored following re-exposure to normoxic condition in the viviparous species *G. affinis.*

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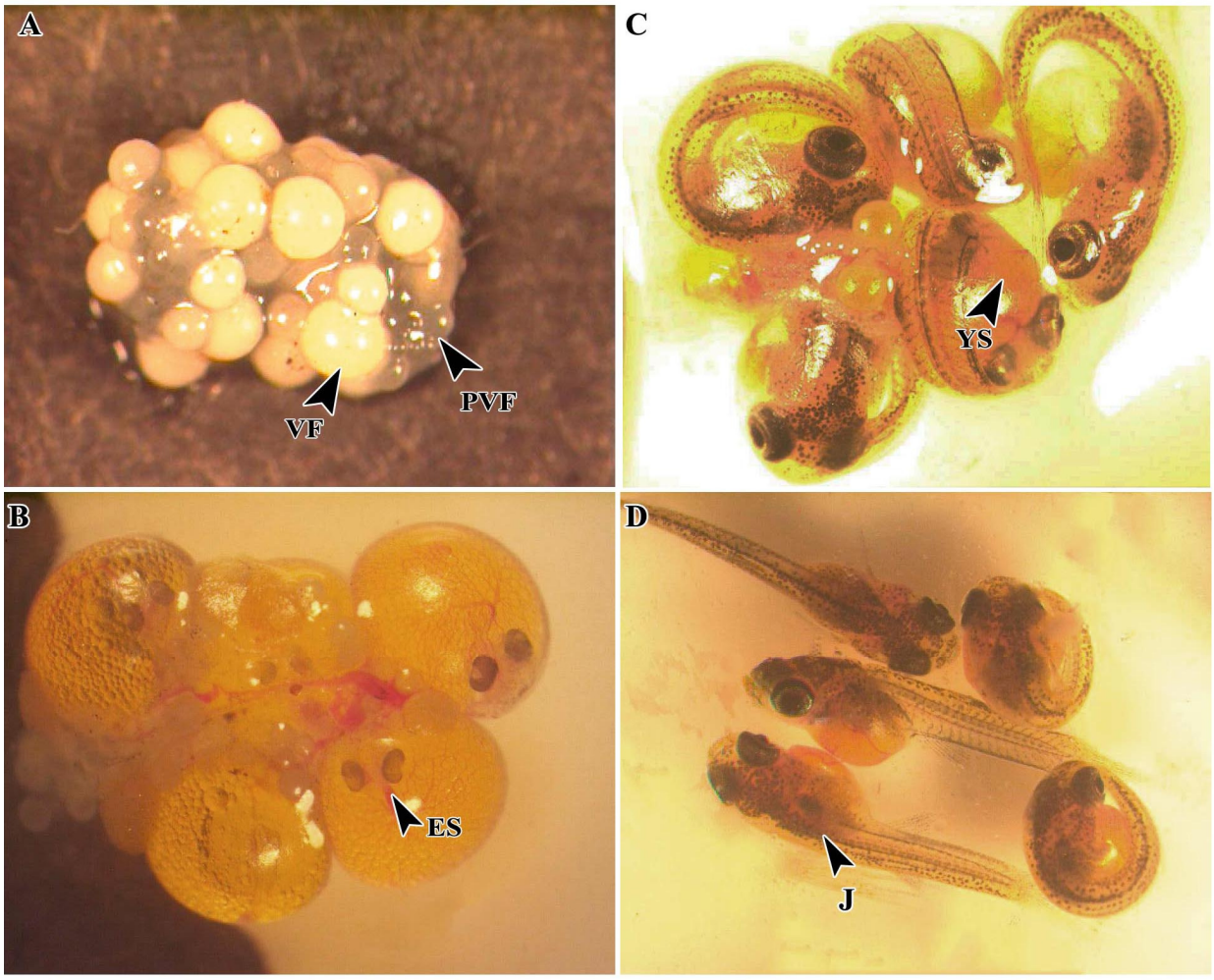

Figure - 1(A-D)

Photomicrographs showing ovarian follicles (A), early embryo (B), late embryo (C) and juvenile (D) stages in the fish *G. affinis*. VF, vitellogenic follicle; ES, eye spot stage; YS, yolk sac; J, juvenile. All figures × 12.5

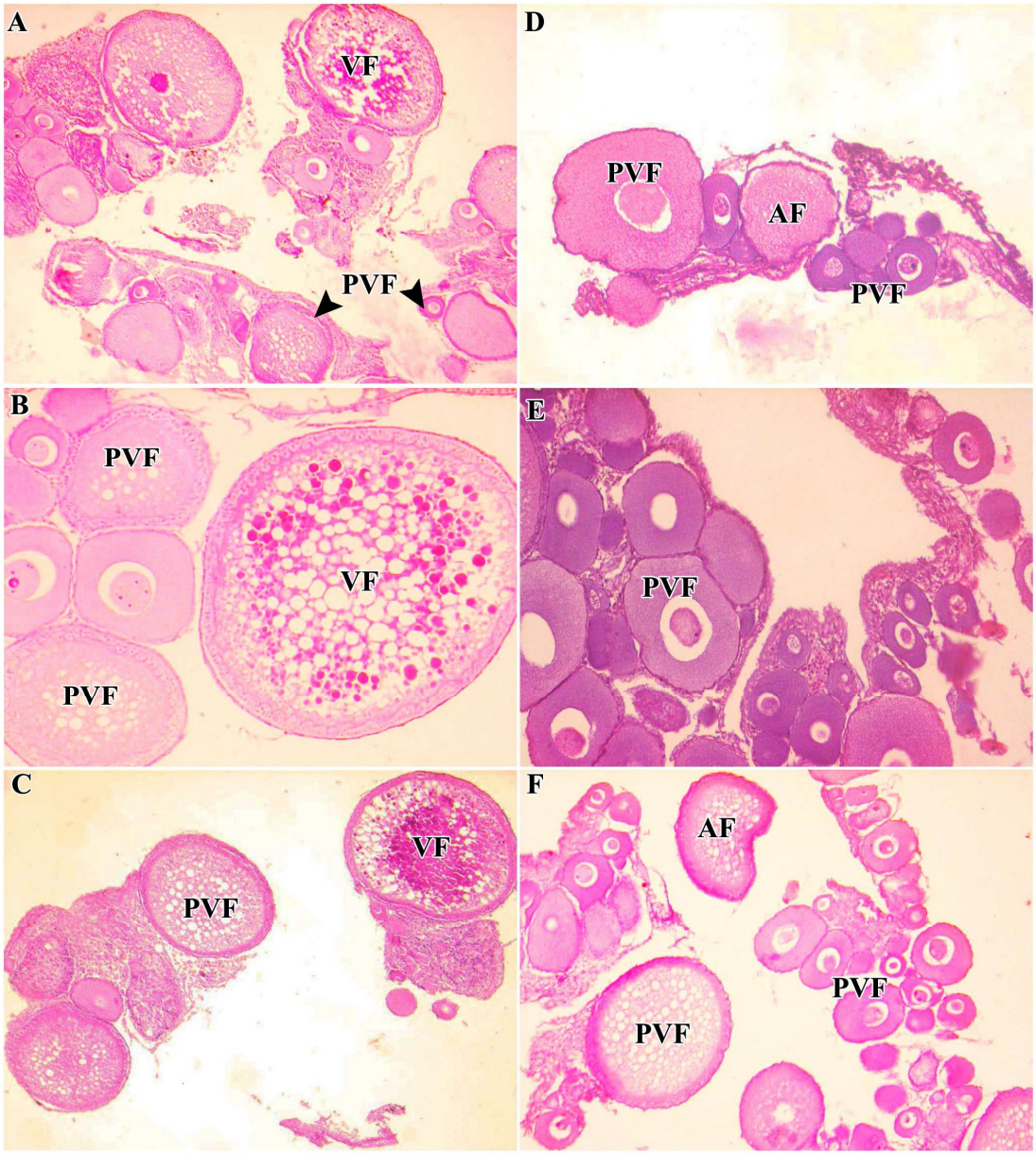

Figure-2 (A-F)

Photomicrographs showing the ovarian follicles in initial control (A), controls (25 days, B; 40 days, C), fish exposed to hypoxia (D and E) and hypoxic recovery group (F). AF, atretic follicle; PVF, previtellogenic follicle, VF, vitellogenic follicle; Hematoxylin and Eosin. Fig. B × 200, others × 100

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