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Short Communication

Influence of lunar cycle in breeding of zebra fish *Danio rerio* (Hamilton-Buchanan, 1822)

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Zebra fish, *Danio rerio*, is a common and useful model organism for studies of vertebrate development and gene function. They may supplement higher vertebrate models, such as rats and mice. Zebra fish embryonic development provides advantages over other vertebrate model organisms. Although the overall generation time of zebra fish is comparable to that of mice, zebra fish embryos develop rapidly, progressing from eggs to larvae in less than 3 days. The embryos are large, robust, and transparent and develop externally to the mother; facilitating experimental manipulation and observation (*Dahm* 2006*).* Zebra fish have the ability to regenerate fins, skin, the heart, and the brain (in larval stages). Zebra fish also regenerate photoreceptors and retinal neurons following injury. All these characteristics made the species as a model for studying different biological mechanism of vertebrates. Hence it is very much needed to have sufficient numbers of seed which necessitates taking up breeding program. Though spawning of the fish is being carried out regularly in many laboratories, it remains as problematic (Markovich *et al*. 2007). Lunar cycle is a factor which influences the breeding of various fish species. Understanding the participation of the moon in the synchrony of biological activities in fishes is significant from the perspective of aquaculture as well as chronobiology (Takemura *et al*. 2004). Control of reproductive function in captivity is essential for the sustainability of commercial aquaculture production, and in many fishes it can be achieved by manipulating photoperiod, water temperature or spawning substrate (Mylonas *et al*. 2010). Hence the present study was carried out to establish the relationship between the lunar cycle and zebra fish breeding which may solve the problem of breeding of the species.

For the breeding trial, brood fishes were selected based on the body structure. Mature males are very slim and the females when ready to breed are very heavy. Females are generally larger with a more rounded belly. Both the sexes are then kept in aquarium in the wet lab at the ratio of 2 males and 1 female. Generally zebra fish breed in schools, so 3 sets of breeders were kept in an aquarium in triplicate. This facilitates sympathetic breeding amongst the breeders. If 1 set breeds, it will stimulate others also to breed. They were fed well with zooplankton and tubifex @ 4–5 times a day until they spawn. The pH of the water was maintained at a range of 6.5–7.2 with a water temperature of 18 to 24°C. While spawning, the fish shows the behaviour of going back and forth. Since the fishes feed on the eggs, a shallow water level of around 30 cm was maintained so that the eggs can fall into the rocks kept at the bottom of the aquarium before the fish can eat them. The fishes were allowed to swim in the aquarium overnight. The next day morning, they were observed whether breeding occurred or not. After spawning, the females noticeably look thin and streamlined due to dropping their eggs. The breeders were taken out of that aquarium and placed back into the conditioning tank. The spawning and the hatching percentage were calculated. The experiment was conducted for 3 months and rhythmic relationship between lunar cycle and breeding of zebra fish was studied.

In all the 3 months, the fishes spawned on the 11th day of the lunar cycle. The females were quite thinner, which indicated that the spawning is over. The fertilized eggs hatched at around 48–72 h. The fertilization and hatching percentage was around 75. Though it was reported that the fish breeds in every 10 days in favorable conditions (Markovich *et al*. 2007), in our observations the breeding occurred only after 15 days which fell on the 11th day of the lunar cycle. Breeding zebra fish are asynchronous and it breeds at various intervals depending upon the conditions and triggers which stimulate spawning. Clelland and Peng (2009) reported that adult zebra fish have asynchronous ovaries, which contain follicles of all stages of development. Eggs are spawned daily under proper environmental conditions in a population of zebra fish with individual females spawning irregularly, every 4–7 days in mixed sex conditions. In favourable conditions they spawn continuously,

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if they have reached sexual maturity (Springer and Breder 1967). Ziv and Gothilf (2006) reported that zebra fish spawning took place at dawn, although this has not been related to their circadian control. Female zebra fish can spawn almost every day, as long as they were in contact with a male zebra fish (Eaton and Farley 1974). Spence and Smith (2005) have shown that female individuals were able to make viable spawning every day during a period of about 12 days, although the quantity of eggs varied significantly.

Breeding of the zebra fish in the interval of 15 days in the present study that too on a particular day of the lunar cycle established a relationship between spawning of the fish and the lunar cycle. Already there are few reports on inter-relationship between lunar cycle and reproduction. It has been reported that many reef fishes utilize such rhythmic changes for synchrony of recruitment, development and release of gametes (Rahman *et al*. 2003). However, some of the evidences related to lunar related reproductive rhythm are unconfirmed, because most studies have been carried out based upon field observations of spawning behaviour and occurrence of spawned eggs. The lack of definitive physiological studies adds to the problems for determining the lunar synchrony of reproduction. For photosensory-neurosecretory cells, such a minimal module would have directly coupled the secretion of neuromodulators and neurohormones to particular light conditions, for example to adjust metabolism, growth or other physiological processes to circadian or seasonal rhythms (Tessmar-Raible 2007). For these photosensory-neurosecretory cell types conserved between annelids and vertebrates, the specific presence of a non- peptide hormone acting in gonadal development and function suggests that reproductive control could also have been ancestrally correlated with photic input (Tessmar-Raible *et al*. 2007). Indeed, synchronized reproduction is common to externally fertilizing marine animals (Babcock *et al*. 1986, Babcock and Mundy 1992 and Lamare and Stewart 1998) and often governed by lunar light or day length (Taylor 1986 and Bentley *et al.* 2001). Blanco-Vives and Sánchez-Vázquez (2009) confirmed the existence of a rhythm in the biological processes of zebra fish.

The breeding of this fish is affected by different factors. Effect of container size on spawning of the fish (Goolish *et al*. 1998) was earlier described. The role of pheromone on zebra fish breeding was studied by Whitlock (2006). Feed is another factor, which regulates the spawning of zebra fish (Lie and Mangor-Jensen 1993, Brooks *et al*. 1997 and Markovich *et al.* 2007). There are few studies on effect of light on spawning and it was found that light condition or photoperiod effect the spawning rhythm (Blanco-Vives and Sánchez-Vázquez 2009). The photo period has got an effect on locomotor activity, which also has an impact on spawning (López-Olmeda *et al.* 2006, Hurd *et al*. 1998). Oliveira *et al*. (2009) studied the spawning rhythm of Senegal sole (*Solea senegalensis*) and reported that they spawn during the night and that there is a relation between locomotor activity and spawning rhythms, which coincide with the highest moment of greatest locomotor activity (Bayarri *et al*. 2004). On the other hand, Herrero *et al*. (2004) reported that lunar cycle affect the locomotor behaviour of tench and highest activity was recorded under full moon. In the present study also the fish bred during night in moonlight. Hence the inter relationship between lunar period and spawning is established. Blanco-Vives and Sánchez-Vázquez (2009) reported that the zebra fish showed the moment of greatest activity just before spawning in the light phase, regardless of the type of photoperiod and the reproduction rhythms were strongly synchronized by light. Spence *et al*. (2006) reported that zebra fish spawn during the first hours of light in their natural environment. The hormones involved in maturation and ovulation showed a different pattern of fish on exposure to light (Wang and Ge 2004). This explains the importance of light on reproduction of the fish. So *et al*. (2005) observed that the gonadotropins (GTHs) levels, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) increased before the start of ovulation. They showed that these hormones not only had an important role in the last stages in follicle development, but they also have a direct action on the postvitellogenic follicles. However specific influence of moonlight on the spawning of the zebra fish has not yet been described. In fact, there are very few studies on lunar synchronization in teleost fishes from physiological aspects although lunar-related synchrony is an important rhythm. To understand the way of perceiving these cues from the moon and the way of transmission of such cues as endogenous stimuli, a detailed investigation is needed. The changes in plasma melatonin concentration have been reported based on the changes in moonlight intensity (Yamada *et al*. 2002). Exposing the fish to moonlight intensity at the midnight of the new and the full moon resulted in rapid and significant decreases in the plasma melatonin concentrations suggesting that the fish possibly perceives the 'brightness' of the night. Oliveira *et al*. (2007) suggest that moonlight may affect melatonin rhythms, encouraging synchronization to the lunar cycle. On the other hand the melatonin-mediated photoperiodic control of neuroendocrine functions might involve a direct effect on the pituitary in fish (Gaildrat and Falcón 2000). In rabbit fishes and groupers, the synchronous spawning occurs around a specific lunar phase (Rahman *et al*. 2003). These findings suggested a synchrony between the lunar periodicity and gonadal maturation which in turn increase the opportunity for encounter with appropriate partners for successful spawning.

This endogenous reproduction rhythm of zebra fish is controlled by light. Better understanding of the circadian physiology and the environmental conditions synchronising zebra fish reproduction rhythms, may be useful for improving the breeding of zebra fish which is used as a model for different physiological studies.

SUMMARY

Lunar cycle is one of such stimuli affecting breeding of many species. Hence a trial on breeding of *Danio rerio* was performed in glass aquaria to know the effect of lunar cycle on spawning. For this purpose, the zebra fish were allowed to breed in moonlight and was monitored for 3 months. It was observed that the breeding of the fish is having a rhythmic relation with the lunar period. The fishes bred on the 11th day of the lunar cycle which has established the relationship between spawning of the zebra fish and the lunar cycle.

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