The effects of mate switching tacticon reproductive performance of the severum cichlid, *Herosseverus*

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Abstract

This study investigated the effects of mate switching on the reproductive performance of the severum cichlid, *Herosseverus*, by advancing the egg and larval production in hatcheries. Two reproductive tactic treatments of "monogamous pair" and "mate switching" were used for evaluating 4 reproductive traits of egg production, hatching rate, spawning intervals, and starvation tolerance of the larvae in 6 spawning activities. The number of eggs was not significantly different between the two reproductive tactic treatments in the 6 spawning activities, but the spawning intervals, hatching rate, and survival activity index were all significantly different. Daily average egg and larval production in the mate switching treatment were estimated to be 87.3 eggs and 43.1 larvae per pair of fish, respectively, which was 2.89 times and 1.99 times of those in the monogamous pair treatment, who produced about 30.2 eggs and 21.6 larvae per pair. Our results clearly showed that the reproductive tactic of mate switching is a suitable method for increasing the egg and larval production rate of the severum cichlid.

Keywords: Severum cichlid, Mate switching, Eggs and larvae, Mass reproduction, Monogamy

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Introduction

Ornamental fish culture is usually performed in a small indoor place containing hundreds of tanks of different sizes for all stages of fish life. This is where all of the activities related to fish propagation and culture, such as selection of brooders, pair bonding, mating, spawning, incubation of eggs, larviculture, and fish sale are carried out together. Therefore, an aquaculture practice that could take better advantage of all the available space would be more profitable. Besides, simultaneous mass production of different species of aquarium fish larvae to meet the demand of the market is crucial for the success of every newly established ornamental fish hatchery.

The production strategies which are used in the culture of many American cichlids, normally involves maintaining a group of sub-adult fish together and feeding them with high quality diets, to enhance their reproductive maturation and provide enough male and female brooders for continuous hatchery activities (Fiszbein *et al*., 2010). Severum fish, *Herosseverus*, is a monogamous cichlid from the Amazon River which both male and female parents care for their larvae. Adult males (15-20 cm) are normally bigger than females in size.In good conditions, a dominant male establishes a territory in a holding tank and if preferred by a female, a coupling pair is formed. If the female dies or is away from her mate, the male fish will couple with another female. Reproduction performance of cichlids has been the subject of many previous studies (Pandolfi *et al*., 2009; Alonso *et al*., 2011). All of these studies had an experimental origin and focused on some biological and behavioral aspects of cichlid fish. In spite of the popularity of many ornamental cichlids, there has been little attention to the development of new production techniques in hatcheries. It is worthnoting that every hatchery may apply its particular tactics on improvement of egg and larval productions, but many of them are tentative and do not have appropriate scientific knowledge to make changes.

Broodfish exchange, and using different strains of the same species, or brooders from different localities, could be some of the alternative methods to produce more eggs and larvae in fish hatcheries. It was reported that female exchange was an efficient method for higher production of *Oreochromis niloticus* in hap as culture (Little *et al*., 2000). Another study on the reproductive performance of *Pelvicachromis pulcher* by using different male reproductive strategies, showed that reproductive success of the males in a polygynous situation (harem males) were higher than those of the pair and satellite males(Martin and Tabrosky, 1997).

In this study on the severum cichlid, we tested a hypothesis that more eggs and larvae could be produced by using the reproductive strategy of switching females, compared to the traditional monogamous breeding method. Reproductive traits, such as the number of eggs, hatching rate, interval time between two spawning periods, and the survival rate of produced larvae, were investigated by using two reproductive treatment groups of severum cichlids. One treatment consisting of monogamous pairs, which were naturally selected, and another treatment with mate switching pairs, when the female has been switched. The severum fish was selected because it displays common pairing behavior and, they spawn easily in a tank system. Results of this study could be applicable to commercial scale production of cichlid fish.

Materials and methods

Fish

This study was implemented in a private ornamental fish hatchery in Karaj, Iran. Four males and six females of mature cichlids were maintained in a 100 liter glass tank (85×30×40 cm) containing a piece of flat stone as substratum for pair bonding. There were 5 holding tanks, in which the water temperature and photoperiod was kept at 28 °C and 12D:12L respectively. In addition, there were 10 rectangular 72 l spawning tanks (60×30×40 cm), which were fitted with a sponge filter and a stone substratum for spawning. Mean body lengths ± standard errors (SEs) of the males and females were 14.09 ± 0.29 cm (n= 20) and 10.05 ± 0.21 cm (n= 30), respectively. Fish were fed 3 times daily to satiation with commercial pellets, frozen blood worms and pieces of beef heart. From this brood stock, only 10 males were selected for the experiments.

Experimental protocol

Two experimental treatment groups of breeding strategies, “monogamous pair” and “mate switching”, were designed.

(1) In monogamous pair treatment, male and female severum brooders selected each other for mating naturally. This happened when a male made a territory on the flat stone in the holding tank and then a female selected the male by entering to the male territory, thus a monogamous pair for breeding was formed. One pair of these naturally bonding fish was selected randomly and transported to a spawning tank for spawning activities.

(2) In the mate switching treatment, a male fish was randomly selected from the holding tanks and introduced to a spawning tank. Then after 24 hours, a female, being in good maturation condition and ready for mating and spawning, was introduced into the spawning tank. After spawning, the female was taken out and switched with another gravid female. The female could be re-introduced if she was well gravid again during the experimental period. In both the monogamous pair and mate switching treatments, 6 continuous spawning activities in 5replicate pairs of fish were evaluated. Means ± SEs of the body lengths in the monogamous treatment and the switching pair treatment were 14.88 ± 0.51 cm and 14.31 ± 0.79cmfor males and 10.53 ± 0.54 cm and 9.96 ± 0.30 cm for females, respectively.

The spawning substratum with eggs was transferred into a 10 l container after completion of the spawning activity. The container was pre-acclimated, by receiving constant low aeration, and being treated with methylene blue (1 ppm). A new flat stone substrate was put into the spawning tank when the old one was removed. After almost 48 hours, the eggs hatched and half of the water was changed with fresh de-chlorinated water at 28 °C. Then thirty free swimming larvae, ages of 3 to 4 days after hatching, were randomly selected and transferred to a new 10 l container for a starvation tolerance test. After transferring, the daily number of the larvae mortality and the number of days elapsed were recorded until all larvae in the container had died.

Variables

The first spawning activity in both treatments was considered as the starting point of the experiment, day 1. Reproductive traits, including total number of produced eggs (EN), hatching rate (HR), interval between each spawning in days (IBS), and quality of produced larvae, were used to evaluate the reproductive performance differences between the monogamous pair and mate switching treatments. The quality of produced larvae was checked by the larval starvation tolerance test with the survival activity index (SAI), calculated from the following equation (Furuita *et al*., 2000):

$$\mathrm{SAI} = \frac{1}{N}\sum_{i=1}^{k}(N - hi)\ \times i$$

Where N is the total number of supplied larvae, h_i is the cumulative mortality by the ith day, and k is the number of days elapsed until all larvae were dead due to starvation.

Statistics

A repeated measures analysis of variance (ANOVA) with the Green house-Geisser test (Quinn and Keough, 2002) was used in this study. Ten pairs of severum cichlids were treated with two different reproductive strategies, monogamous pair and mate switching. The second factor was the spawning activities, which were repeatedly recorded 6 times, and was applied within subjects (severum cichlids). The software used for the statistical analyses was the SPSS v. 19.0 and all statistical tests were considered significant at $p < 0.05$.

Results

Egg number (EN)

The results indicated that both of the reproductive strategies ($F_{1,8}$= 0.012, P= 0.916) and the spawning activities (Greenhouse-Geisser epsilon= 0.625, F= 1.245, p= 0.315) were insignificant on the number of produced eggs (EN). The interaction was also insignificant (F= 1.003, p= 0.410). Therefore, there was no significant differences in the mean ENs produced in the 6 spawning activities between the monogamous pair (mean ± SE, 764.1 ± 44.8) and the mate switching treatments (751.1 ±32.2; Fig. 1A).The total number of eggs produced in the 6 spawning activities in the monogamous pair and mate switching treatments were 22,924 and 22,532, respectively. During the experimental period, the EN was not significantly different among the 6 spawning activities between the 2 reproductive strategies. The EN also did not change significantly among the 6 spawning activities during the experimental period (Fig. 2).

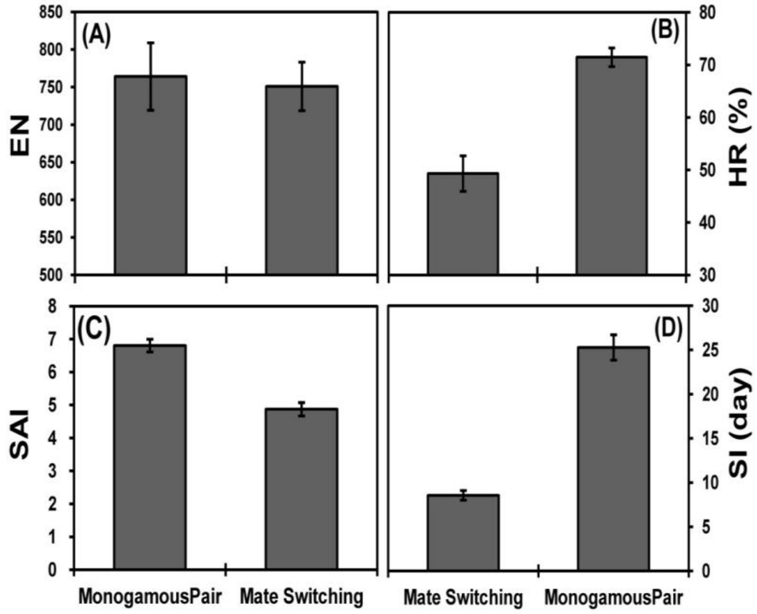

Figure 1: Means and standard errors (vertical line) of the reproductive traits, number of produced eggs (A), hatching rate (B), interval between each spawning in days (C), and the survival activity index (D) among the 6 spawning activities of *H. severus*(each n= 30).

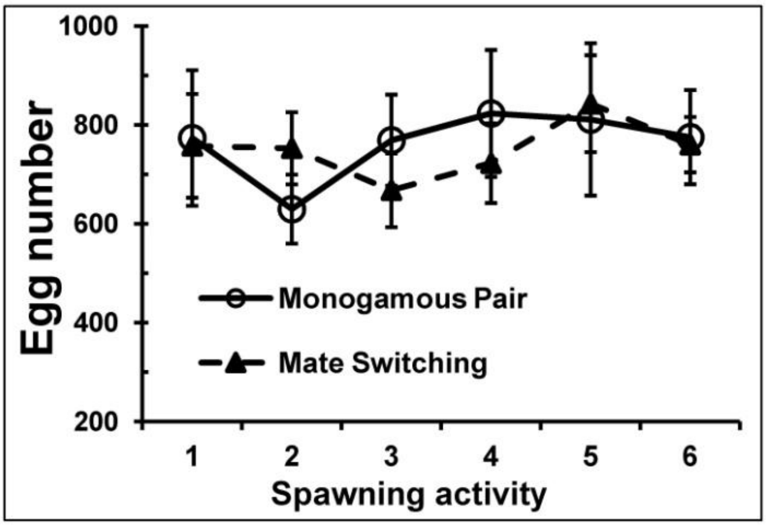

Figure 2: Changes in egg numbers produced by each pair of *H. severus* during the 6 spawning activities (n= 5; Error bars indicate SE of means).

Hatching rate (HR)

Both of the reproductive strategies ($F_{1,8}$= 42.553, P<0.001) and the spawning activities (Greenhouse-Geisser epsilon= 0.480, F= 5.581, p=0.009) had significant effects on the hatching rate (HR). The interaction was also significant (F= 16.370, p< 0.001). The mean HR in the monogamous pair treatment for the 6 spawning activities (71.5 ± 1.8%) was higher than that of the mate switching treatment (49.3 ± 3.4%; Fig.1B). The difference between hatching rates among treatments became significantly greater with each spawning activity onwards (Fig. 3). During the experimental period, the hatching rate in monogamous pair increased progressively from the first spawning, (HR 65.0 ± 3.5%) to the sixth spawning, (HR 78.5 ± 3.4%). Conversely in mate switching treatment, the hatching rate steadily decreased from the first spawning, (HR 67.6 ± 3.5%), to the sixth spawning activity (HR 24.8 ± 4.5%).

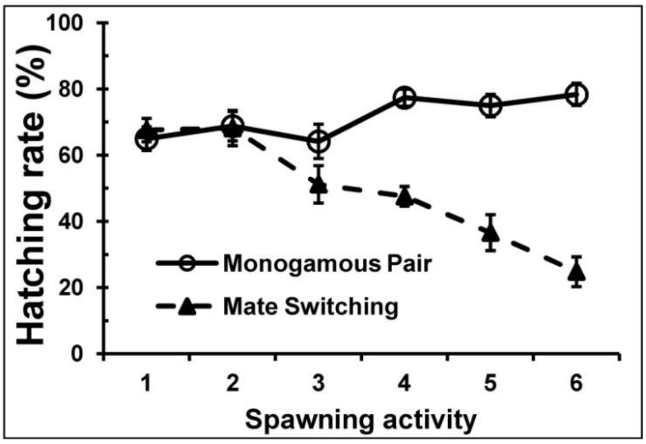

Figure 3: Changes in hatching rate of *H. severus* during the 6 spawning activities (n= 5; Error bars indicate SE of means).

Larval survival activity index (SAI)

Both of the reproductive strategies ($F_{1,8}$= 22.208, *p*= 0.002) and the spawning activities (Greenhouse-Geisser epsilon= 0.586, *F*= 7.316, *p*= 0.001) had a significant effect on the SAI. The interaction was insignificant (*F*= 2.599, *p*= 0.077). The mean SAIs of the larva produced in the monogamous pair treatment (6.81 ± 0.19) was more than that of the mate switching treatment (4.87 ± 0.20; Fig.1C). During the experimental period, the SAI did not change with successive spawning activities in the monogamous pair treatment, whereas it steadily decreased with each spawning activity in the mate switching treatment from 6.26 ± 0.28 in the first spawning activity to 3.91 ± 0.15 in the sixth (Fig. 4).

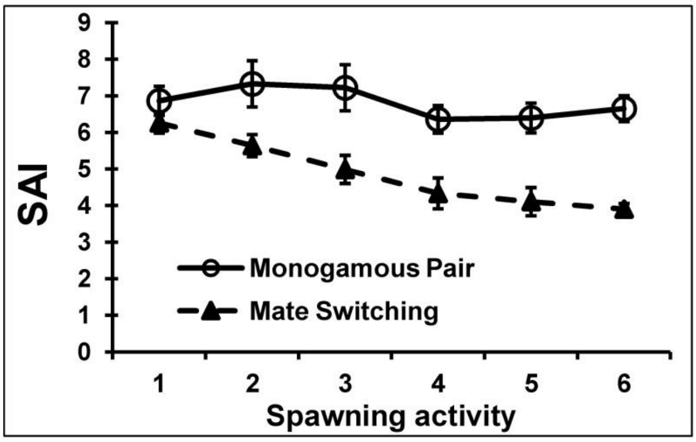

Figure 4: Changes in larvae survival index of *H. severus* during the 6 spawning activities (n= 5; Error bars indicate SE of means).

Interval between each spawning (IBS)

Both of the reproductive strategies ($F_{1,8}$= 79.420, p<0.001) and the spawning activities (Greenhouse-Geisser epsilon= 0.586, F= 11.835, p<0.001) had a significant effect on the IBS. The interaction was insignificant (F= 2.749, p=0.083). The mean IBS in the monogamous pair treatment (25.3 ± 1.4 days) was more than what was recorded for the mate switching treatment (8.6 ± 0.5 days; Fig. 1D). The 6 spawning activities took 127.4 ± 9.1 days in the monogamous pair treatment, while the mate switching treatment took only 43.8 ± 3.2 days to achieve 6 spawning activities. During the experimental period, the IBS increased with each spawning activity in both the monogamous pair treatment and the mate switching treatment from 19.2 ± 1.3 days and 5.8 ± 0.7 days for the first spawning interval to 33.0 ± 3.2 days and 11.6 ± 1.5 days for the fifth spawning interval respectively (Fig. 5). The IBS in the monogamous pair treatment was about 3 times more than the mate switching treatment.

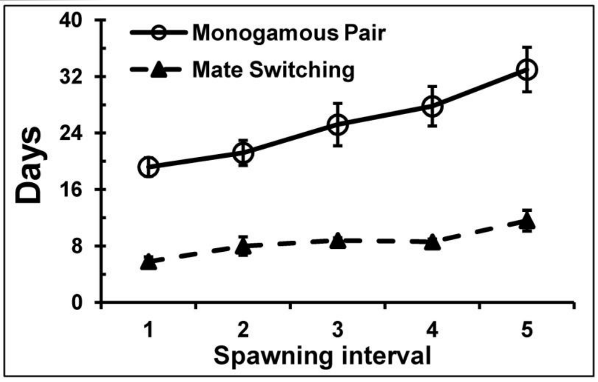

Figure 5: Changes in interval between spawning among the 6 spawning activities of *H. severus* (n= 5; Error bars indicate SE of means).

Discussion

This study investigated how the reproductive performance, including four traits (EN, HR, IBS, and SAI), of severum cichlids respond to the reproductive tactic of mate switching. Based on the results, if the mate switching method is used, the daily egg and larval production of *H. severus* increases. Therefore, application of the mate switching method on the monogamous cichlid fish, *H. severus*, could produce distinctly more eggs and larvae on a daily mean basis. In practice, both male and female exchange was the best mating tactic for egg production in Nile tilapia (Lovshin and Ibrahim, 1988). Ridha and Cruz (2003) tendered schedules of broodstock exchange for higher egg production in Nile tilapia. However, there is a trade-off that quality of the eggs and larvae, indicated by the HR and SAI, respectively, produced by the mate switching method were lower than those by the traditional monogamous pair method.

Hatching rate is a factor which is affected by broodstock condition (Brooks *et al*., 1997). The majority of studies reported a direct relationship between broodstock condition and HR as a good indicator of the reproductive function (e.g., Brooks *et al*., 1997; Izquierdo *et al*., 2001). In this study, male fish from the mate switching treatment continuously ventured to mate 6 times in a short period of time (43-49 days). This large number of mating led to a decrease of the male’s condition; even effecting sperm storage and ejaculate size (Hetteyey *et al*., 2009). Probably, reduction of male libido is the main reason for decreased egg performance. For example, successful reproductive rates of male diamondback moth, *Plutella xylostella*, were reduced with increased mating frequency (Wang *et al*., 2005). In a mouth brooding cichlid, *Eretmodus cyanostictus*, it has been reported that by artificial reduction of male body condition, the embryos were taken later into the mouth (Steinegger and Taborsky, 2007). Rurangwa *et al*. (2004) indicated that any parameter which affects sperm fertilization capacity can be considered as a potential parameter to reduce sperm quality, and the decline in sperm quality is a limiting factor for successful reproduction. Therefore, in this study, multiple mating by the severum male could cause reduction in his sperm availability and the reproductive quality of the males gradually decreased. This shows that continuous spawning is costly for male severum cichlid and results in decreasing the hatching rate. In addition to the reduction of egg hatching rate in the mate switching treatment, survival of larvae also decreased significantly in this group. Briefly, the low quality eggs produced in the mate switching treatment is a disadvantage of trade-off in aquaculture practice. However, despite this decline of egg quality, Brooks *et al*. (1997) indicated that factors affecting egg quality are not specified, though survival and hatching rate are practical ways to determine ultimate egg quality. There are many factors which can influence the fish egg quality, such as, the conditions in which eggs are fertilized (Brooks *et al*., 1997), the physiology and hormonal situation of broodstock (Schreck *et al*., 2001), genetic background of broodfish (Stoddard *et al*., 2006) and environmental conditions. Nutrition is one of the main factors in spawning quality of different fish species (Izquierdo *et al*., 2001; Morais *et al*., 2014). In addition, the effect and hormonal pathway of broodstock exchange strategy on reproductive performance in an African mouth brooding cichlid, *O. niloticus*, was evaluated and determined (Little *et al*., 2000). Therefore, a better diet supply and/or hormonal treatment could be of the solutions to reduce the disadvantage of the male impotence in the mate switching practice. No significant difference was found between the monogamous pair and mate switching treatments produced eggs in this study. It is clear that in many fish species, female body size is one of the main factors in fecundity (Heinimaa and Heinimaa, 2004). Also, a positive correlation was found between egg numbers and male sizes in the paradise fish, *Macropodus opercularis* (Huang and Chang, 2011). However, the parent body sizes of the severum males used in both the reproductive tactic treatments of our study were close (14.88 ± 0.51cm and 14.31 ± 0.79 cm for the monogamous pair and mate switching treatments, respectively) as well as those of the severum females (10.53 ± 0.54 cm and 9.96 ± 0.30 cm, respectively). This design could reduce the interference of the body size effect on the reproductive performance of the severum cichlid, so that the body sizes were not affecting factor in this study. Inter-spawning intervals among the 6 spawning activities were significantly different between the monogamous pair and mate switching treatments. The less time needed for spawning in the switching pair treatment indicates the great tendency of the severum for mating and shows that this fish is a highly efficient ornamental fish in the hatcheries. From the first spawning activity onwards, the mean number of days needed for spawning with new females in the mate switching treatment increased significantly 2.1 times from 5.8 to 11.6, showing the increasing male impotence for mating. In conclusion, if the mate switching method is used, a daily egg and larval production of *H. severus* could increase 2.89 times and 1.99 times, respectively, in comparison with the traditional monogamous pair method. Disadvantageous effects of low quality eggs and larvae from the mate switching method, caused by the male impotence, should be noticed. In practice, it is worth noting that female switching is a viable method for advancing the daily egg and larval production of the severum cichlid and could be applied to other monogamous cichlids in hatcheries.

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References

Alonso, F., Cánepa, M., Moreira, R.G. and Pandolfi, M., 2011. Social and reproductive physiology and behavior of the Neotropical cichlid fish *Cichlasoma dimerus* under laboratory conditions. *Neotropical Ichthyology,* 9, 559-570.

Brooks, S., Tyler, C.R. and Sumpter, J.P., 1997. Egg quality in fish: What makes a good egg? *Reviews in Fish Biology and Fisheries,* 7, 387-416.

Fiszbein, A., Cánepa, M., Vázquez, G.R., Maggese, C. and Pandolfi, M., 2010. Photoperiodic modulation of reproductive physiology and behaviour in the cichlid fish *Cichlasoma dimerus*. *Physiology and Behavior,* 99, 425-432.

Furuita, H., Tanaka, H., Yamamoto, T., Shiraishi, M., and Takeuchi, T., 2000. Effects of n-3 HUFA levels in broodstock diet on the reproductive performance and egg and larval quality of the Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, 187, 387-398.

Heinimaa, S. and Heinimaa, P., 2004. Effect of the female size on egg quality and fecundity of the wild Atlantic salmon in the sub-arctic River Teno. *Boreal Environment Research*, 9,55-62.

Hetteyey, A., Vagi, B., Hevizi, G. and Toeroek, J., 2009. Changes in sperm stores, ejaculate size, fertilization success, and sexual motivation over repeated mating in the common toad, *Bufo bufo* (Anura: Bufonidae). *Biological Journal of the Linnean Society,* 96, 361-371.

Haung, W.B. and Chang, C.C., 2011. Effects of parental care and body size on the reproductive success of the paradise fish *Macropodus opercularis* in a small area. *Zoological Studies,* 50, 401-408.

Izquierdo, M.S., Fernandez-Palacios, H. and Tacon, A.G.J., 2001. Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture,* 197, 25-42.

Little, D.C., Coward, K., Bhujel, R.C., Phama, T.A. and Bromage, N.R., 2000. Effect of broodfish exchange strategy on the spawning performance and sex steroid hormone levels of *Oreochromis niloticus* brood fish in hapas. *Aquaculture,* 186, 77-88.

Lovshin, L.L. and Ibrahim, H.H., 1988. Effect of broodstock exchange on *Oreochromis niloticus* egg and fry production in net enclosures, p. 231-236. In R.S.V Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) The second international symposium on Tilapia in aquaculture. ICLARM conference proceedings 15, 623 p. Department of fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Martin, E. and Taborsky, M., 1997. Alternative male mating tactics in a cichlid, *Pelvicachromis pulcher*: A comparison of reproductive effort and success. *Behavioral Ecology and Sociobiology,* 41, 311-319.

Morais, S., Mendes, A.C., Castanheira, M.F., Coutinho, J., Bandarra, N., Dias, J., Conceição, L.E.C and Pousão-Ferreira, P.,2014. New formulated diets for *Solea senegalensis* broodstock: Effects of parental nutrition on biosynthesis of long-chain polyunsaturated fatty acids and performance of early larval stages and juvenile fish. *Aquaculture,* 432, 374-382.

Pandolfi, M., Cánepa, M.M., Meijide, F.J., Alonso, F., Rey Vázquez, C., Maggese, M.C. and Vissio, P.G., 2009. Studies on the reproductive and developmental biology of *Cichlasoma dimerus* (Percifomes, Cichlidae). *Biocell,* 33, 1-18.

Quinn, G.P. and Keough, M.J., 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge.

Ridha, M.T. and Cruz, E.M., 2003. Effect of different schedules for broodstock exchange on the seed production of Nile tilapia *Oreochromis niloticus* in freshwater. *Aquaculture International,* 11, 267-276.

Rurangwa, E., Kime, D.E., Ollevier, F. and Nash, J.P., 2004. The measurement of sperm motility and factors affecting sperm quality in cultured fish. *Aquaculture,* 234, 1-28.

Schreck, C.B., Contreras-Sanchez, W. and Fitzpatrick, M.S., 2001. Effects of stress on fish reproduction, gamete quality, and progeny. *Aquaculture,* 197, 3-24.

Steinegger, M. and Taborsky, B., 2007. Asymmetric sexual conflict over parental care in a biparental cichlid. *Behavioral Ecology and Sociobiology,* 61, 933-941.

Stoddard, J.W., Parsons, J.E. and Nagler, J.J. 2006. Early onset of embryonic mortality in sub-fertile families of rainbow trout (*Oncorhynchus mykiss*). *Reproduction, Fertility and Development,* 17, 785-790.

Wang, X.P., Fang, Y.L. and Zhang, Z.N., 2005. Effect of male and female multiple mating on the fecundity, fertility, and longevity of diamondback moth, *Plutella xylostella*. *Journal of Applied Entomology,* 129, 39-42.