JJBS

Volume 13, Number 2, June 2020
ISSN 1995-6673
Pages 219 - 222

Jordan Journal of Biological Sciences

Sexual Restiveness and Colouration between Partial Diallel Cross of Genetically Improved Farmed Tilapia 'GIFT' and UPM red Tilapia.

Oster F. Nwachi[1,2], Yuzine Esa[1*],Annie Christianus[1]and Salleh M. Kamarudin[1]

[1] *Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia;*[2] *Department of Fisheries, Delta State University Abraka, Asaba Campus, Nigeria*

Received July 9, 2019; Revised August 9, 2019; Accepted August 15, 2019

Abstract

The effect of colouration, number of days for reproductive success and female preference for mating between 2 Cichlids: 'Genetically Modified Farm Tilapia' (GIFT) and UPM red tilapia were examined. A total of 24 female and 12 male homozygous stock of each of UPM red tilapia and GIFT were selected, They were paired in one ton tank and labelled as: (T1) at a ratio of 4:2 GIFT female X GIFT male (G x G), T2 UPM red tilapia female X GIFT male (U x G), T3 UPM red tilapia female X UPM red tilapia male (U x U) and T4 GIFT female X UPM red tilapia male (G x U) in triplicates. The experimental units were in triplicates and the trial was conducted at four separate times using the same broodstock. The number of days for reproductive successful varied from 14.2 days for T1 to 14.72 days for T3 with T4 having a longer period at 21.93 days. The mean highest value of swim-up recorded were 154.65 for T2, an indication that female fish shows affinity to the male fish of same colour with a level of preference to male of novel colouration compared to wild type of male.

Keywords: GIFT, Cichlids, restiveness, preference, novel-colouration

1. Introduction

Production of Tilapia seed can be done through many methods, ranging from the more simple ones like pairing of parent stock in pond or hapa and harvesting their eggs after spawning, to high technological based fish ranching (Ramadan *et al.*, 2007;Vilhena *et al.*, 2014; Lu & Takeuchi, 2004). In fish production, understanding assortative mating among strains having colour variations is a way of increasing mating success(Rajaee *et al.*,2010). Generally, expressed phenotype in fish is used as an important trait for species recognition and behaviour in fish culture (Couldridge & Alexander, 2002). Male colouration is reportedly used as a criterion for selecting males for reproduction in some breeds of Cichlids (Selz *et al.*,2014); Couldridge & Alexander. (2002) with the opinion that colour pattern can also be used in understanding speciation based on the rate at which new families of Cichlids emerged.

In the past decade, little or no importance was considered to the variation in colour of Cichlids; however, observation increases the need to study and put the variation into use especially during selection of folk's fish from another region because of the consequences of this variation on breeding. Generally, the colours of Cichlids have a tendency to vary from the colours of Cichlids in other different regions, but the colours maintained by the male Cichlids during breeding in a particular region remain consistent. The implication of this is that interbreeding between Cichlids from two or more different regions will become particularly difficult as the females in a region might not readily mate with males from a different region because of the colour pattern expressed by the male fish. Hence, colour variation has the tendency to influence the pattern of actual sexual selection among closely related Cichlids. A study by Kirkpatrick & Nuismer (2004) expressed this concern, stressing that the development of entirely new species naturally, from the existing parent in the same environment, is difficult particularly for an invading male of different/novel phenotypes to be accepted by females in preference to its type.

Cichlid males' fish display high levels of variable mating behaviours, influenced by high competition among the males for access to mate with the females. The male fish employs varying persuasive, or trait based approaches such as: nest building, beautiful colour display, and even aggression in making sure that they gain the attention of female of interest (Fessehaye*et al.*,2006). Recent studies on fish production aimed at satisfying the fish needs was recommended to increase focus on the production of tilapia, such as *Oreochromis niloticus*, as a 'folks' fish due to its inherent hardy nature and other favourable traits *(Haque et al.*,2016,Lago *et al.*, 2016;Neira*et al.*, 2015).

In general *Oreochromis niloticus* is a typical 'lek' spawning fish. The males build nests to attract females and strongly put up defences against any invader, thus creating

* Corresponding author e-mail: yuzine@upm.edu.my; fish2rod@yahoo.com.

un-even ratio of females to male. Most *O. niloticus* females, like other Cichlid female fish, will select conspecific males of novel colour (Selz *etal.*, 2014); Hence, mating of the females of *O. niloticus* with conspecific males from a different region, even if the male is of novel colouration becomes particularly impossible (Rajaee *et al.*, 2010). Experimental studies byKorzan *et al.*(2008) and Fessehaye *et al.* (2006) to determine time taking for female to accept a male were conducted in monitored water bearing receptacle that made it possible for fertilized eggs to be removed from the mouth of the brooding fish.

This in our opinion could disrupt courtship and breeding process and give results that will not necessarily reflect the actual time at which the female accept the male fish. Therefore,this study aimed to examine the effect of colouration on the number of days of acceptance for a cross between the 'Genetically Modified Farmed Tilapia' also known as GIFT (Hybrid of *O. niloticus*) and an endemic strain of tilapia from Universiti Putra Malaysia (UPM red tilapia), which is a cross between (*Oreochromis niloticus* and *Oreochromis mossambicus*), the female preference for conspecific and novel coloured males, and the reproductive success among the strain.

2. Methodology

2.1. Selection of Brood Stock and Experimental Site

The GIFT strain is greyish/wild-colour while the UPM red tilapia (reddish colour) brood stocks were selected for this study (Figs 1a and 1b). The GIFT broodstock were obtained from the World Fish Center Penang Malaysia and maintained in a designated tank for broodstock at the Universiti Putra Malaysia (UPM) Aquaculture Center. Meanwhile, brood stocks of the UPM red tilapia was produced at the UPM Aquaculture center.

Figure1a: Genetically modified farm tilapia 'GIFT

Figure1b: UPM red tilapia

2.2. Experimental Set Up

A total of 24 females and 12 males' homozygous stocks of each of UPM red tilapia and GIFT were selected for the study. The pairing was carried out in a one ton water holding receptacle. The mouths of the males were clipped to avoid injury while mating and in-tank fighting among the males. The fish were paired in the ratio of 4:2 for the female to male per experimental unit. Tank T1 had 4 GIFT female paired with 2 GIFT male (G x G), T2had 4 GIFT females to 2 UPM red males (G x U) T3 had 4 UPM red tilapia females to 2 UPM red tilapia males (U x U) and T4 had 4 UPM red female to 2 GIFT male (U x G). All the experimental units were in triplicates, the trial was conducted at 4 separate times using the same broodstock.

In all, pairing was initiated to produce hybrid F1 from the stock (GIFT X UPM red tilapia). The males were removed from the spawning tank, allowed to rest for 15 days before re-introducing into the breeding tank, at each time a set of swim-up fry were harvested. The culture water was green by introducing 10 liters of greenish coloured stock water into the culture tank, two 1m length polyvinyl chloride (PVC pipe) was placed in each tank to provide shelter to the fish during mating.

2.3. Spawning and Larvae Collection

The spawning process was certified by looking out for swim-up fries because of the greenish coloured water of the culture tank at 3 to 4 days after hatching out from the eggs. The brood stock was fed with a commercial feed of (40% crude protein) twice daily at 09.00hrs and 05.00hrs. The 'swim up' fries were removed from the tank followed by removal and isolation of the male parent in a well aerated receptacle.

2.4. Water Quality Analysis

The one-ton tank used for the experiment was filled with aerated water and constantly supplied with extra air from a central blower. YSI (Yellow springs Ohio, USA) portable model 556 mps was used to measure the temperature, dissolve oxygen (DO), total dissolve solids and hydrogen ion concentration (pH). The concentration of ammonia was also measured using HACH test kit (HACH Company, USA).

2.5. Statistical Analysis

Data obtained was subjected to two-way analysis of variance (ANOVA) for the number of successful hatching days and the relationship between the strains of the test fish and subjected to SIGMA PLOT software (version 12.0 systat software Inc. California, USA). The means of hatching success and number of swim-up were compared using Turkey test at $p \leq 0.05$ at 95% confidence. Triplicate measures were conducted and mean ± standard error values were reported.

3. Results

3.1. Water Quality Parameters

The recorded mean temperature of the tank containing T1 during the experiment was 26.67^0C, 25.87^0C for T2 as shown in Table 1. The mean dissolved oxygen was 7.86 MgL^{1-} for T1 and 7.32 MgL^{1-} for T2 throughout the duration of this study. The mean observed hydrogen ion concentration (pH) was 6.89, 6.75, 6.76 and 6.88 MgL^{1-} for

T1, T2, T3 and T4 respectively. The highest value of total dissolve solid and ammonia was 0.243 $MgL^{1-}$for T2 and 0.3001 MgL^{1-} for T1. That coincides with Xu & Boyd (2016); Boyd & Lichtkpper (2002) who reported that these parameters contribute significantly to the culture environment of the tested fish. However, authors like Haque *et al.,* (2016) Ekasari & Maryam (2012) recommend a slightly higher value compared to what was obtained in the study.

Table 1. Water quality parameter at culture of UPM Red Tilapia and GIFT

S/N	Parameters	G x G	G x U	U x U	U x G	Standard Error
1	Temperature (^{0}C)	26.67	25.87	26.78	26.25	0.032
2	Ammonia (mgL^{1-})	0.3001	0.3000	0.2999	0.2988	0.079
3	Dissolve Oxygen (mgL^{1-})	7.86	7.32	7.87	7.77	0.004
4	pH	6.89	6.75	6.76	6.88	0.093
5	Total dissolve solids (mgL^{1-})	0.237	0.243	0.236	0.229	0.014

T1 = GIFT female X GIFT male (G x G); T2= GIFT female X UPM red tilapia male (G x U); T3 = UPM red tilapia female X UPM red tilapia male (U x U) T4= UPM red tilapia female X GIFT male (U x G). Data represents the means and 3 replicates (± standard error). Means without superscript on the same raw indicates no statistical difference by the Turkey test $p \leq 0.05$ for the water quality parameter.

3.2. Spawning Variation

A total of four trials with their means (mT1-mT4) were represented in Table 2, and statistical significance was recorded within all the batches. The highest total mean value of swim-up produced was from the cross between the GIFT and UPM red tilapia (G x U) at 154.49. A mean total of 137. 77 swim-up fries was harvested for the 4 batches of mating from the cross between UPM red tilapia and GIFT (U x G). The cross between the pure breed of the test fish yielded mean value of 149.37 and 148.16 for GIFT and UPM red tilapia at a significant difference of ($p \leq 0.05$).

Table 2. The mean number of swim-up in a successful batch mating of test fish.

Batch	G x G	G x U	U x U	U x G
mT1	138.50 ± 1.201^a	142.8 ± 0.577^b	142.41 ± 0.666^b	131.25 ± 1.154^c
mT2	151.5 ± 0.333^a	154.25 ± 0.333^a	147.75 ± 0.577^b	133.75 ± 0.333^c
mT3	154.16 ± 0.577^a	156.83 ± 0.333^a	148.91 ± 0.333^b	141.25 ± 0.333^c
mT4	153.33 ± 0.333^a	164.75 ± 0.333^b	153.48 ± 0.577^a	144.83 ± 0.577^c
Mean Total	149.37	154.65	148.16	137.77

T1 = GIFT female X GIFT male (G x G); T2 = GIFT female X UPM red tilapia male (G x U), T3 = UPM red tilapia female X UPM red tilapia male (U x U) T4= UPM red tilapia female X GIFT male (U x G). Data represents the mean and 3 replicates (± standard error). Different letters within the same raw indicate statistical difference by the Turkey test $p < 0.05$) for the number of swim-up that is harvested.

The highest number of days required for a successful mating was recorded in U x G mating (21.93 days) as seen in table 3 the time spent before successfully production of swim-up in the tank that has their mating pair varies from 14.53, 14.72 and 14.2 days for G x G, G x U and U x U respectively. The pair G x G recorded mean number of 26.58 days at mT1 compared to the mean value of 8.16 days at mT4 this observation could be seen across all the test fish. Similarly, in U x G a mean value of 15.91 days from initial value of 32.25 days at mT4 was obtained.

Table 3. The mean number of days for a successful swim-up harvest fish.

Batch (Days)	G x G	G x U	U x U	U x G
mT1	26.58 ± 0.378^a	26.58 ± 0.312^a	26.25 ± 0.371^a	32.25 ± 0.350^b
mT2	12.58 ± 0.192^a	13.41 ± 0.228^b	12.00 ± 0.213^a	22.50 ± 0.261^c
mT3	10.83 ± 0.270^a	11.00 ± 0.275^a	10.75 ± 0.278^a	17.08 ± 0.259^b
mT4	8.16 ± 0.259^a	7.91 ± 0.259^b	7.83 ± 0.207^b	15.91 ± 0.287^c
Mean Total	14.53	14.72	14.2	21.93

T1 = GIFT female X GIFT male (G x G); T2 = GIFT female X UPM red tilapia male (G x U); T3 = UPM red tilapia female X UPM red tilapia male (U x U) T4= UPM red tilapia female X GIFT male (U x G) Data represents the mean and 3 replicates (± standard error). Different letters within the same raw indicate statistical difference by the Turkey test $p \leq 0.05$) for observed successful days

4. Discussion

4.1. Means of Successful Days

A partial diallel crossing in Table 2 examines the hypothesis of no differences on the reproductive success with the number of days for the pairings of the test fish at ($p \leq 0.05$); however, deductions infer that there was a significant difference between the days that the fish record successful reproduction that is associated with the day fries swim up. The reddish coloured UPM red tilapia female and the wild coloured GIFT female readily accept the male in a shorter number of days compared to the pair of the reddish coloured fish to the wild type (GIFT). However, the pair of the GIFT female to the UPM red tilapia (G x U) produced the highest number of swim-up throughout the study.

Breeding success in a 'lekspawner' is related to individual fish success in fertilizing a batch of egg. Logically, *Oreochromis niloticus* strain is known to prepare breeding space and attract female of choice. Preparing and protecting this space, however, depends on the individual's ability to undergo assortative mating, which is an important features in a fish that readily speciates. In this study, two males of similar morphology were paired with females although the mouth of the males were clipped as a deterrent to aggression during mating because males with the same pattern or colour tend to attack each other but hardly attack males of novel phenotypic traits, in line with Seehausen & Schluter (2004) who reported 'a negatively frequency-dependent fitness advantage' which is an indices that affects the distribution of males of same species in a particular territory. Similarly sound of particular frequency can be a source of attraction to fish (Verzijden *et al.*,2010).

The female's individual mating success in this work skewed towards crossing of female fish with novel colour and or conspecific male. This assertion is observed in the

number of days swim-up was detected in the G x U pair compared to U x G pairing. The G x U pairing reveals great affinity for reddish coloured (UPM red tilapia) female compared to those of wild coloured GIFT strain. In Table 3, the average successful days for the pair between conspecific UPM red tilapia female with UPM red tilapia male (U x U), GIFT female pairing with GIFT male (G x G) were not significant. However, when compared to the heterospecific pair between UPM red tilapia (novel coloured female) with wild coloured GIFT male (U x G) a level of significance is observed.

This assertion is in line with the Karen et al. (2005); Plenderleith et al. (2005); and Couldridge & Alexander (2002)findings on crossing of fishes with different express phenotypic traits. Likewise, Selz *et al.* (2014) and Reichard & Polačik (2010)reported findings on the female fish preference for conspecific males of the same colour and or novel colouration to wild type. Studies by Dijkstra *et al.*(2008) and Plenderleith et al. (2005) infer that in addition to colour, olfactory organ and ability of males to claim and protect territory also increase the chances of being selected by female. In general, the main underling factor is the exhibition of the preferred colour of interest that will attract females because of the urge to choose male of novel coloration

5. Conclusion

This study examined the spawning success from pairing of GIFT and UPM red tilapia. The results show that when fishes of different colouration are paired, the time period for spawning success to be achieved depends on the female affinity to the male, the female fish has the tendency to copulate with male of same colour and male of novel colouration (Reddish) compared to wild type of male.

Acknowledgement

The authors would like to extend their sincere appreciation to Educational Trust Fund (TETFUND) Nigeria. Delta State University Abraka and Universiti Putra Malaysia Aquaculture center.

Declaration

There is no conflict of interest to be declared.

References

Boyd CE and Lichtkpper F. 2002. Water quality management in pond culture. In *Research and Development series* (22nd ed.).

Couldridge VCK and Alexander GJ. 2002. Color patterns and species recognition in four closely related species of Lake Malawi cichlid. *Behav.Ecol.*, **13**: 59-64.

Dijkstra PD, Van Der Zee EM and Groothuis TGG. 2008. Territory quality affects female Preference in a Lake Victoria cichlid fish. *Behavioral Ecology and Sociobiology*, ***62*(5)**, 747–755.

Ekasari J and Maryam S. 2012. Evaluation of Biofloc Technology Application on Water Quality and Production Performance of Red Tilapia *Oreochromis sp*. Cultured at Different Stocking Densities. *HAYATI Journal of Biosciences* ***19*(2)**, 73–80.

Fessehaye Y, El-bialy Z, Rezk MA, Crooijmans R, Bovenhuis H and Komen H. 2006. Mating systems and male reproductive success in Nile tilapia (*Oreochromis niloticus*) in breeding hapas: A microsatellite analysis. *Aquaculture*, ***256*(**1–4), 148–158.

Haque MR, Islam MA, Wahab MA, Hoq ME, Rahman MM and Azim ME. 2016. Evaluation of Production Performance and Profitability of Hybrid Red tilapia and Genetically Improved Farmed tilapia (GIFT) strains in the carbon/nitrogen Controlled Periphyton-based (C/N- CP) on-farm Prawn Culture system in Bangladesh. *Aquaculture Reports*, ***4,*** 101–111.

Karen lC, Juliet WL, Parry JK, Bowmaker DMH and Seehausen O. 2005. Colour vision and speciation in Lake Victoria cichlids of the genus Pundamilia. *Molecular Ecology*, 4341–4353.

Kirkpatrick M and Nuismer SL. 2004. Sexual selection can constrain sympatric speciation. *Proceedings of the Royal Society B: Biological Sciences*, ***27(1***), 687-693.

Korzan WJ, Robison RR, Zhao S and Fernald RD. 2008. Color change as a potential behavioral strategy. *Hormones and Behavior*, ***54*(3)**, 463–470.

Lago A, De A, Rezende TT, Dias MAD, Freitas RTF and Hilsdorf AWS. 2016. The development of genetically improved red tilapia lines through the backcross breeding of two Oreochromis niloticus strains. *Aquaculture*. DOI: 10.1016/j.aquaculture.2016.06.042

Lu J and Takeuchi T. 2004. Spawning and egg quality of the tilapia Oreochromis niloticus fed solely on raw Spirulina throughout three generations. *Aquaculture*, ***234***: 625–640.

Neira R, García X, Lhorente JP, Filp M, Yáñez JM and Cascante AM. 2015. Evaluation of the growth and carcass quality of diallel crosses of four strains of Nile tilapia (Oerochromis niloticus). *Aquaculture*. https://doi.org/10.1016/j.aquaculture.

Plenderleith M, Van Oosterhout C, Robinson RL and Turner GF. 2005. Female preference for conspecific males based on olfactory cues in a Lake Malawi cichlid fish. *Biology Letters*, ***1*(4)**, 411–414.

Rajaee AH, Huntingford FA, Ranson KJ, McAndrew BJ and Penman DJ. 2010. The effect of male colouration on reproductive success in Nile tilapia (Oreochromis niloticus). *Aquaculture*, ***308***, 119–S123.

Ramadan M, Abou-Zied and Ahmed AA. (2007). Evaluation of Nile tilapia commercial hatcheries systems in Fayoum governorate 2 1-. *Egypt. J. Aquat. Boil. & Fish.*,***11***: 230–238.

Reichard M and Polačik, M. 2010. Reproductive isolating barriers between colour-differentiated populations of an African annual killifish, Nothobranchius korthausae (Cyprinodontiformes). *Biological Journal of the Linnean Society*, ***100***: 62–72.

Seehausen O and Schluter D. 2004. Male-male competition and nuptial-colour displacement as a diversifying force in Lake Victoria cichlid fishes. *Proceedings of the Royal Society B: Biological Sciences*, ***271*(1546)**, 1345–1353.

Selz OM, Pierotti MER, Maan ME, Schmid C and Seehausen O. 2014. Female preference for male color is necessary and sufficient for assortative mating in 2 Cichlid sister species. *Behavioral Ecology*, ***25*(3)**, 612–626.

Verzijden MN, Van Heusden J, Bouton, N, Witte F, Ten Cate C and Slabbekoorn H. 2010. Sounds of male Lake Victoria cichlids vary within and between species and affect female mate preferences. *Behavioral Ecology*, ***21*(3)**, 548–555

Vilhena R, Neto R, Antônio C, Oliveira L, De-Ribeiro RP, Tadeu R and Freitas FD. 2014. Genetic parameters and trends of morphometric traits of GIFT tilapia under selection for weight gain. *Scientia Agricola*, ***71*(4)**, 259–265.

Xu Z and Boyd CE. 2016. Reducing the monitoring parameters of fish pond water quality. *Aquaculture*, ***465***, 359–366.