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Short Communication

SEX REVERSAL IN SIAMESE FIGHTING FISH LARVAE BY THERMAL MANAGEMENT*

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ABSTRACT

The present study evaluated the influence of thermal management during the larviculture of *Betta splendens* on survival and sex ratio, aiming to increase the proportion of males. Newly hatched larvae were subjected to different thermal regimes, namely, T25, T28, T30 and T33 (25, 28, 30 and 33°C, respectively). The experiment was laid out in a completely randomized design, with 4 treatments and 10 repetitions. Thermal treatment was maintained until 15 days post-hatch (DPH). Mortality was determined at the end of the thermal regime and again at 45 DPH. At the end of the experiment, the number of males and females obtained in the different thermal treatments was counted to analyze the obtained sex ratio. There was a significant effect on mortality as a function of temperature only at 15 DPH (p <0.001), with the lowest values recorded in treatments T25, T28 and T30. In terms of sex ratio, up to 65% of males were obtained in treatment T33 (p = 0.037). In conclusion, thermal management during the larval period can be a strategy to increase the proportion of males, but the increase in mortality due to the rise in temperature should be considered.

Keywords: fish farming; larviculture; masculinization; ornamental fish.

INVERSÃO SEXUAL DE LARVAS DE BETTA POR MANIPULAÇÃO TÉRMICA

RESUMO

O presente estudo avaliou a influência da manipulação térmica durante a larvicultura de *Betta splendens* na sobrevivência e razão sexual, buscando aumento na proporção de machos. Larvas recém-eclodidas foram submetidas a diferentes regimes térmicos, denominados: T25, T28, T30 e T33 (25, 28, 30 e 33°C, respectivamente). Seguiu-se um delineamento inteiramente casualizado, com 4 tratamentos e 10 repetições. O tratamento termal foi mantido até os 15 DPE (dias pós-eclosão). No encerramento do regime termal e novamente aos 45 DPE foi contabilizada a mortalidade. No final do experimento foi contabilizado o número de machos e fêmeas obtidos nos diferentes tratamentos térmicos para análise da proporção sexual obtida. Houve efeito significativo da mortalidade em função da temperatura apenas aos 15 DPE (p <0,001) e os tratamentos T25, T28 e T30 apresentaram os menores valores para tal parâmetro. Para a razão sexual, foram obtidos até 65% de machos no tratamento T33 (p = 0,037). Conclui-se que a manipulação termal durante o período larval pode ser uma estratégia para o aumento da proporção de machos, considerando o aumento da mortalidade em função da elevação da temperatura.

Palavras-chave: piscicultura; larvicultura; masculinização; peixe ornamental.

INTRODUCTION

The farming of aquatic organisms for ornamental purposes has gained prominence, with its growth leveraged by the popularization of fishkeeping around the world. The activity involves over 100 countries and 5,400 species, including marine and freshwater organisms (Zuanon et al., 2011).

In the economic aspect, the ornamental fish market moves around 70% of the global capital related to the activity in developing countries, with around 90% of these animals being produced in captivity (Dias et al., 2018). Brazil is considered one of the main exporters in South America, with a record 41 million ornamental fish exported in 2008 (SISCOMEX, 2020). Exports are restricted to native species that are obtained from fishing, which constitute the main source of income for small fishing communities

across the country (Anjos et al., 2009). In contrast, captive production is often limited to the farming of exotic species, which demand low technology and are widespread in aquariums. Thus, the ornamental fish farming segment – which today supplies the domestic market almost exclusively – is one of the branches of aquaculture with great growth potential for the coming years if better organized and coordinated (FAO, 2020).

Among the freshwater species, the Siamese fighting fish, commonly known as the "betta" (*Betta splendens*), stands out for its exuberance, exhibiting a variety of fin shapes and colors. Having originated from the Asian continent, it is easily found in nature in lakes and backwaters and can survive in environments with low oxygen content (Gomes et al., 2019). In the reproductive context, the species is extremely prolific, being easily cultivated in captivity (Faria et al., 2006).

In Brazil, the betta was introduced during the 60s and 70s, and due to its hardiness, it became popular mainly due to its low management requirements. As a tropical species, adapted well to the environment conditions in such tropical country, mainly regard to temperature conditions, witch in the nature it find to be 29.9ºC for reproduction (Jaroensutasinee and Jaroensutasinee, 2001) and in rearing conditions, 25-29°C (Harlioglu and Mise-Yonar, 2008). For larvae, recently was determined that the range for rearing was set between 25-33ºC, being 28ºC the better results for survival (Carvalho et al., 2021).

Among the conditions for the success of the activity, prioritizing the production of male *Betta splendens* individuals is extremely important, given their accentuated development of the fins and more vibrant colors compared with females, attaining higher market values (Thongprajukaew et al., 2014). To increase the number of males from a single spawning, sex reversal techniques involving the use of hormones have been investigated in the species (Kavumpurath and Pandian, 1994; Kirankumar and Pandian, 2002; Kipouros et al., 2011; Reis et al., 2016).

Fishes are the group that has more sex determination mechanisms among vertebrates, namely, GSD – genetic sex determination – which is controlled by gens; ESD – environmental sex determination – which control by abiotic factors, as temperature; and a last mechanism which evolves an interaction between both (GSD+ESD) (Santos et al., 2017).

In bettas, recently, two papers (Kwon et al., 2021; Zhang et al., 2021) showed that the most of heterozygous individuals are male, which supports that in such specie, the male phenotype could be formed by heterogametic sexual chromosomes (XY/XX). However, even when XX *vs* XX cross was performed, 10% of offspring were male, confirming an incomplete genotypic sex determination in betta (Kwon et al., 2021). Zhang et al. (2021) also find in minor proportions homogametic males in their study, which, according with authors, suggest the influence of environmental factors in sex determination in this species.

The manipulations of sex ratio in fish farming it is a well know practice applied in aquaculture with the objective of reach better productive results, exploring characteristics such differential growth rate between male and female, minimization of unexpected reproductions, reduction of introduction of exotic species in some biomes, or yet, obtaining animals from sex with higher commercial value in the market, such betta. There are few approaches that could be applied to induce the sex reversion in fish, such as ploidy manipulation, hormonal treatments, manipulation of social factors and environmental conditions, being the temperature, the main factor used for this purpose. A complete review of this theme is provided by Budd et al. (2015).

On this basis, the present study was undertaken to examine the effect of different thermal treatments during the larval development of *Betta splendens* by analyzing the survival of the fish and the sex ratio achieved.

MATERIAL AND METHODS

Broodstock maintenance and larval hatchery

The experiment was carried out at the Marine Biology Station (EBM) of the Federal Rural University of Rio de Janeiro, located in Itacuruçá, district of Mangaratiba/RJ, Brazil (Latitude: -22.92930151; Longitude: -43.90697569). The total experimental period was 180 days.

Twenty adult couples of *B. splendens* of the longtail variety of a commercial lineage were acquired from producers registered with the Association of Aquaculturists of Patrocínio de Muriaé and Barão do Monte Alto-MG (AQUIPAM - BMA), at four months of age. The animals were identified, weighed (average weight: males = 1.78 ± 0.18 g; females = 1.03 ± 0.07 g) and were distributed individually into 2 L glass aquarium.

During the acclimation period, the water temperature was maintained at 28ºC, where the animals remained for 60 days until spawning. Every two days, approximately 50% of the water was renewed and feces and debris were removed by siphoning. A commercial extruded feed (36% Crude Protein, 1.7 mm) was supplied *ad libitum* twice daily (09h00 am and 18h00). Bloodworm was supplied twice weekly, at the rate of one larva per animal.

After acclimation period, the couples were separated and distributed into 20 (twenty) tanks for reproduction. The period of adaptation to amplexus and spawning lasted approximately 96 h. After spawning, the females were removed from aquarium and the eggs were kept with the males until hatching (24-36 h) and, subsequently, the larvae were collected for the beginning of the trial.

Experimental condition

The experiment was laid out in a completely randomized design according to the following mathematical model: $Y_{ij} = \mu + T_i + e_{ij}$, where *Yij* are the observed variables; μ is the overall mean; T_i is the fixed treatment effect at four temperatures, namely, 25, 28, 30 and 33ºC (T25, T28, T30 and T33, respectively, with 10 repetitions per treatment); and e_{ij} is the random error effect.

Each thermal treatment had 10 beakers that counting as repetitions. The spawning of each couple was identified and was

distributed in different beakers equally between each treatment. This was performed to observe and isolate the effect of breeders in the sex determination. A total of 1200 newly hatched larvae (D0) were used, at a density of 30 larvae/beaker.

For the thermostatic baths, the beakers with the newly hatched larvae were immersed in water and then acclimated at 1ºC h^{-1}. To maintain the temperature in the T25 treatment, a BOD incubator was used. For treatments T28, T30 and T33, thermostats with a heater inside thermoboxes were used. Pumps for water recirculation were installed, maintaining the temperature uniform between the containers.

The thermal treatment lasted up to 15 days post-hatch (DPH), according with the ontogenetic period corresponding to the beginning of sexual differentiation and the appearance of the gonadal tissue in *B. splendens* (Pattiasina et al., 2021; Kwon et al., 2021). Throughout the experimental period, the temperatures were monitored four times daily using a digital multiparameter device. The recorded values were within the initially proposed ranges for each treatment (25.0 ± 0.2ºC; 27.9 ± 0.1°C; 30.0 ± 0.2°C and 33.0 ± 0.2°C).

From DPH 3 onwards, the larvae received exogenous feed, consisting of newly hatched *Artemia* nauplii. The larvae were feed *ad libtum* three times a day (08h00, 13h00 and 19h00). Every two days, 40% of the total water volume in each container was renewed, with the waste siphoned. At the end of the thermal treatment, the number of surviving larvae in each container was counted for further statistical analysis of mortality (%).

Following the heat treatment period, the fish were acclimated and kept at a temperature of 28ºC, observing the variation of 1ºC h^{-1}. The protocol for the maintenance of the animals was similar to that used in the initial stage, and from 30 DPH onwards, the fish started to be supplied with a commercial powdered feed (55%CP, 125-250 mm) in co-feeding with *Artemia* nauplii. At the end of 45 DPH, surviving animals were counted again to detect a possible residual effect of heat treatment on survival.

Obtaining data for sex ratio

At the final stage (after 45 DPH), the animals were distributed in separated net cages and identified for each thermal treatment, inside a 1000-L tank with a 40-cm water column and a submersible pump that allowed the exchange of water between the tank and the net cages. At this stage, the powdered feed was gradually replaced by an extruded commercial feed, which was supplied twice a day.

The animals that could be identified by sex were removed and counted for further statistical analysis. During this period, the sex was identified by the appearance of secondary sexual traits. Males exhibit an accentuated growth of the dorsal, caudal, and ventral fins, in addition to a characteristic aggressive behavior. Females can be determined by bulging of the belly and the appearance of a white spot on the abdomen, called ovipositor (Faria et al., 2006). The overall sex differentiation of animals lasted 135 days, with no mortalities observed during this period.

Statistical analysis

The data were initially subjected to the Shapiro-Wilk and Bartlett tests to check for normality and homoscedasticity. From this analysis, the percentage values were arcsine-transformed. Subsequently, the data were subjected to analysis of variance (ANOVA). When differences between the means were detected, Tukey's test was applied at 5% significance.

During the experiment, three replicates had a drastic drop in the proportion of males for no apparent reason. All other replicates remained with the response pattern for the proportion of males and females, so we chose to remove the data considered outliers.

Outlier removal was based on the studentized residue outside the ± 1.5 range, using the boxplot tool of R software (Schwertman et al., 2004). After such, the sample number were n = 10, n = 9, n = 10 and n = 8 for T25, T28, T30 and T33 treatments, respectively.

A contingency table was built to study the frequency of events that naturally show expected distribution. The chi-square index was used to measure the magnitude of deviations at 5% significance (Sampaio, 2010).

RESULTS

Mortality

Mortality (%) was evaluated up to 15 DPH to identify the direct effect of temperature on fish survival. At 45 DPH, mortality was measured to check the residual effect derived from the thermal treatment, as shown in the Table 1.

There was a statistical difference for mortality at 15 DPH (p <0.001). Treatments T25, T28 and T30 had a lower mortality rate than T33. For mortality at 45 DPH, corresponding to the residual effect of the thermal treatments, no statistical difference was detected (p = 0.08). Therefore, mortality was similar across the four thermal treatments (T25, T28, T30 and T33) at this stage.

Sex ratio

Table 2 describes the results for the sex ratio (%) obtained at the end of the experiment according to the tested temperatures.

There was a statistical difference for the proportion of males and females obtained at the end of the experiment (p = 0.037). Treatment T33 had a significantly higher proportion of males than T25, the latter of which did not differ from treatments T28 and T30.

Table 3, below, shows the results referring to the chi-square test (X^2). The number of males and females was equal only for the treatment T30, while in the treatments T25 and T28 had a greater number of females and the treatment T33 high number of males.

Table 1. Average mortality values (%) as a function of different temperature levels at 15 and 45 DPH.

Variable	Thermal Treatments (ºC)				SEM	p-value
	T25 (n = 10)	T28 (n = 9)	T30 (n = 10)	T33 (n = 8)		
MORT (15 DPH)	17.7±4.88[a]	6.29±2.32[a]	15.27±4.15[a]	45.00±5.30[b]	2.95	<0.001
MORT (45 DPH)	4.00±0.81	0.43±1.38	1.63±0.1	4.41±0.62	0.49	0.08

MORT = mortality (%); 15 DPH = 15 days after hatching; 45 DAH = 45 days after hatching; T25 = 25ºC; T28 = 28ºC; T30 = 30ºC; T33 = 33ºC; SEM = Standard Error of Mean. Means followed by the same letter do not differ from each other by the Tukey test at 5% significance

Table 2. Mean values of the males and females (%) as a function of different temperature levels, after 45 DPH.

Sex	Thermal Treatments (ºC)				SEM	p-value
	T25 (n = 10)	T28 (n = 9)	T30 (n = 10)	T33 (n = 8)		
MALE (%)	43.43±6.73[b]	45.74±4.29[ab]	51.14±5.06[ab]	67.21±8.14[a]	3.349	0.037
FAMALE (%)	56.57±6.736[a]	54.26±4.296[ab]	48.86±5.06[ab]	32.79±8.14[b]	3.349	0.037

T25 = 25ºC; T28 = 28ºC; T30 = 30ºC; T33 = 33ºC; SEM = Standard Error of Mean. Means on the same line followed by the same letter do not differ from each other by the Tukey test at 5% significance

Table 3. Chi-square test (X^2) for the number of males and females obtained as a function of different temperatures, after 45 DPH.

Treatment	nº male	nº female	X^2 calculated
T25	103	132	16.29
T28	101	115	11.9
T30	118	112	2.1
T33	82	40	23.1

* The value of Chi-square distribution for $p = 0.05$ and 1 degree of freedom (3.84).

DISCUSSION

Temperature is one of the main environmental parameters that affect the aquatic environment, directly and indirectly influencing other variables such as dissolved oxygen content, non-ionized ammonia concentration, distribution of organisms, natural productivity in water, reproduction, growth, and even sex ratio in fish (Budd et al., 2015; Boyd and Tucker, 2012).

In the present study, fish larvae survival was directly influenced by the proposed thermal regimes, with the lowest mortality rates occurring in treatment T28 during the period of exposure. This result corroborates literature data that demonstrate that the ideal temperature for rearing adult *Betta splendens* is around 27.5°C (Faria et al., 2006).

Based on the results, it can be stated that the ideal temperature range for the larviculture phase is between 25 and 30ºC. These results are similar those reported by researchers that investigated the ideal temperature for survival in other species with ornamental purposes, e.g. goldfish, *Carassius auratus* (Kestemont, 1995); angelfish, *Pterophyllum scalare* (Zuanon, et al., 2006); and the guppy *Poecilia reticulata* (Dzikowski et al., 2001).

In addition, there was no significant difference in mortality values between 15 and 45 DPH, which shows that the thermal adaptation protocol of 1°C h^{-1} to 28°C was effective, even in the treatment in which there was greater thermal variation (33-28°C).

As regards the influence of temperature, there are several factors that affect the sex ratio in fish, such genetic, epigenic and environmental, as reviewed by Budd et al. (2015). Zhang et al. (2021) find evidence that betta is a heterogametic specie (male XY and female XX), which indicate the existence of genotype sex determination (GSD) mechanism, even finding in less proportion (~8,8%) males with homogametic female genotype between the fish analyzed in their study. According with authors, this may suggest also the influence of environmental factors in sex determination in this specie.

In the present study was observed an increasing male number in response to elevation of the temperature (43-67%), suggesting that in betta, besides the GSD, also there is a thermal effect in sex determination (GSD+TE). The GSD+TE mechanism it already determined in a few fish species, including ornamental zebrafish (*Danio rerio*) and kinguio (*Carassius auratus*), as in wide world Nile tilapia (*Oreochromis niloticus*) (Ospina-Álvarez and Piferrer, 2008). According to the authors, in all previous species, there is evidence of existence of sexual chromosomes (GSD) and previous work showing that higher temperatures lead to high male: female ratio (GSD+TE), as is observed in the present study with betta.

Even through that was not a complete sex reversion, which is common in GSD+TE sex determination mechanism, others genetic and molecular cues are well known to be evolved in the mechanism (Budd et al., 2015), and they should be further investigated in this specie.

In this study, the characteristics of sexual dimorphism could be observed from 45 DPH onwards. With the rise in temperature, the proportion of males increased, with just over 65% males obtained in treatment T33. Furthermore, there was no presence of external anatomical abnormalities that could be attributed to the thermal treatments.

Researchers have obtained superior results in the masculinization of Nile tilapia, *O. niloticus*, with the treatments of 32 and 34ºC, which led to 98 and 91% males, respectively (Zanoni et al., 2013). For this species, reversal to male sex is essential in the production aspect, as males have an accelerated development in comparison to females (Reis et al., 2016). Comparatively, sex reversal in *B. splendens* is also essential in the production

aspect, and the differentiated morphological traits of males are an important commercial factor for the species.

Experiments with the crucian carp, *Carassius carassius*, demonstrated the effect on larvae exposed to two different temperatures (24 and 30ºC), which were obtained from a cross between hormone-induced neomales and homogametic females. The temperature of 30ºC favored the appearance of male individuals (21.7% males) as compared with the treatment using 24ºC (0% males) (Fujioka, 2002). A similar result was described with goldfish, *C. auratus* (Goto-Kazeto et al., 2006), corroborating the crucial role that temperature plays in sex ratio in fish, overlaying primary genetic mechanisms.

Factors other than environmental and genetic can modulate the male proportion in the progenies. There is evidence that parental factors have a lesser effect on sex determination in fish, as demonstrated by Baroiller et al. (2009). According to the authors, some animals provide offspring that are more sensitive to heat treatment than others, and this characteristic should be explored as a quantitative trait in offspring. Although in the present study the separation of each offspring of the couples was carried out in different containers distributed in all heat treatments, a longer study with their progenies will be necessary to ensure that the other animals can have greater success in reverting with the heat treatment.

CONCLUSION

Based on the results obtained in this study, the temperature range of 25 to 30ºC provided satisfactory survival in *B. splendens* larviculture. In addition, the temperature of 33ºC during the 15 DPH led to a ratio of up to 67% males at the end of the experiment.

Further studies are recommended considering the feasibility of the technique, since, despite the higher market value of males, the high mortality of animals observed in the treatment with a higher proportion of males can be a risk factor for producers.

REFERENCES

Anjos, H.D.B.; De Souza Amorim; R.M.; Siqueira; J.A.; Anjos, C.R. 2009. Exportação de peixes ornamentais do estado do Amazonas, Bacia Amazônica, Brasil. Boletim do Instituto de Pesca, 35(2): 259-274.

Baroiller, J.F.; D'cotta, H.; Saillant, E. 2009. Environmental effects on fish sex determination and differentiation. Sexual Development: Genetics, Molecular Biology, Evolution, Endocrinology, Embryology, and Pathology of Sex Determination and Differentiation, 3(2-3): 118-135. https://doi.org/**10.1159/000223077**.

Boyd, C.E.; Tucker, C.S. 2012. Pond aquaculture water quality management. USA: Springer Science & Business Media. 700p.

Budd, A.M.; Banh, Q.Q.; Domingos, J.A.; Jerry, D.R. 2015. Sex control in fish: approaches, challenges and opportunities for aquaculture. Journal of Marine Science and Engineering, 3(2): 329-355. https://doi.org/**10.3390/jmse3020329**.

Carvalho, R.S.; Mello, P.T.S.; Figueiredo, P.G.P.; Sousa, Q.R.R.B.; Silva, L.R.; Silva, R.R.; Lopes, J.M.; Ramos, L.R.V. 2021. Determination of the upper and lower critical limits of temperature in *Betta splendens* (Regan, 1910) larviculture. In: Cordeiro, C.A.M.; Sampaio, D.S.; Holanda, F.C.A.F. (eds). Engenharia de pesca, aspectos teóricos e práticos. Guarujá, SP: Editora Científica, p. 145-154. https://doi.org/**10.37885/210203414**.

Dias, J.A.R.; Abe, H.A.; Sousa, N.C.; Ramos, F.M.; Cordeiro, C.A.M.; Fujimoto, R.Y. 2018. Uso do sal comum (NaCl) e densidade de estocagem durante a larvicultura de *Betta splendens.* Boletim do Instituto de Pesca, 42(3): 719-726. https://doi.org/**10.20950/1678-2305.2016v42n3p719**.

Dzikowski, R.; Hulata, G.; Karplus, I.; Harpaz, S. 2001. Effect of temperature and dietary L-carnitine supplementation on reproductive performance of female guppy (*Poecilia reticulata*). Aquaculture, 199(3-4): 323-332. https://doi.org/**10.1016/S0044-8486(01)00561-0**.

FAO – Food and Agriculture Organization of the United Nations 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in Action. Rome, Italy: FAO. 244p. https://doi.org/**10.4060/ca9229en**.

Faria, P.M.C.; Crepaldi, D.V.; Teixeira, E.A.; Ribeiro, L.P.; Souza, A.B.; Carvalho, D.C.; Melo, D.C.; Saliba, E.O.S. 2006. Criação, manejo e reprodução do peixe *Betta splendens* (Regan 1910). Revista Brasileira de Reprodução Animal, 30(3-4): 134-149.

Fujioka, Y. 2002. Effects of hormone treatments and temperature on sex-reversal of Nigorobuna *Carassius carassius grandoculis.* Fisheries Science, 68(4): 889-893. https://doi.org/**10.1046/j.1444-2906.2002.00507.x**.

Gomes, V.D.S.; De Lima Amâncio, A.L.; Jordão Filho, J.; Cavalcanti, C.R.; Batista, J.M.M.; Da Silva, J.H.V. 2019. Índices de desenvolvimento em juvenis *Betta splendens* alimentados com aditivos enzimáticos. Visão Acadêmica, 19(4): 46-54. http://dx.doi.org/**10.5380/acd.v19i1.61302**.

Goto-Kazeto, R.; Abe, Y.; Masai, K.; Yamaha, E.; Adachi, S.; Yamauchi, K. 2006. Temperature-dependent sex differentiation in goldfish: establishing the temperature-sensitive period and effect of constant and fluctuating water temperatures. Aquaculture, 254(1-4): 617-624. https://doi.org/**10.1016/j.aquaculture.2005.10.009**.

Harlioglu, M.M.; Mise-Yonar, S. 2008. The importance of temperature, individual size and habitat arrangement on the bubble nest construction of Siamese fighting fish (*Betta splendens* Regan, 1910). International Journal of Science & Technology, 3(1): 53-58.

Jaroensutasinee, M.; Jaroensutasinee, K. 2001. Bubble nest habitat characteristics of wild Siamese fighting fish. Journal of Fish Biology, 58(5): 1311-1319. https://doi.org/**10.1111/j.1095-8649.2001.tb02288.x**.

Kavumpurath, S.; Pandian, T.J. 1994. Masculinization of fighting fish, *Betta splendens* Regan, using synthetic or natural androgens. Aquaculture Research, 25(4): 373-381. https://doi.org/**10.1111/j.1365-2109.1994.tb00702.x**.

Kestemont, P. 1995. Influence of feed supply, temperature and body size on the growth of goldfish *Carassius auratus* larvae. Aquaculture, 136(3-4): 341-349. https://doi.org/**10.1016/0044-8486(95)00060-7**.

Kipouros, K.; Paschos, I.; Gouva, E.; Ergolavou, A.; Perdikaris, C. 2011. Masculinization of the ornamental Siamese fighting fish with oral hormonal administration. Science Asia, 37(3): 277-280. https://doi.org/**10.2306/scienceasia1513-1874.2011.37.277**.

Kirankumar, S.; Pandian, T.J. 2002. Effect on growth and reproduction of hormone immersed and masculinized fighting fish *Betta splendens*. The Journal of Experimental Zoology, 293(6): 606-616. https://doi.org/**10.1002/jez.10181**.

Kwon, Y.M.; Vranken, N.; Hoge, C.; Lichak, M.R.; Francis, K.X.; Camacho-Garcia, J.; Bista, I.; Wood, J.; McCarthy, S.; Chow, W.; Tan, H.H.; Howe, K.; Bandara, S.; von Lintig, J.; Durbin, R.; Svardal, H.; Bendesky, A. 2021. Genomic consequences of domestication of the Siamese fighting fish. bioRxiv 2021:1-30. Pre-print. https://doi.org/**10.1101/2021.04.29.442030**.

Ospina-Álvarez, N.; Piferrer, F. 2008. Temperature-dependent sex determination in fish revisited: prevalence, a single sex ration response pattern, and possible effects of climate change. PLoS One, 3(7): e2837. https://doi.org/**10.1371/journal.pone.0002837**.

Pattiasina, B.J.; Pattinasarany, M.M.; Manuputty, M.M.D.; Kokmesa, E.R. 2021. Masculinization of beta fish larvae *Betta splendens* through the different treatment immersion of honey solution and larval age. IOP Conference Series: Earth and Environment Sciences, 797(1): 012016. https://doi.org/**10.1088/1755-1315/797/1/012016**.

Reis, V.R.; De Almeida, F.L.; Piferrer, F. 2016. Produção de populações monossexo em peixes. Revista Brasileira de Reprodução Animal, 40(1): 22-28.

Sampaio, I.B.M. 2010. Estatística aplicada a experimentação animal. 3ª ed. Belo Horizonte: Fundação de Estudo e Pesquisa em Medicina Veterinária e Zootecnia. 264 p.

Santos, D.; Luzio, A.; Coimbra, A.M. 2017. Zebrafish sex differentiation and gonad development: a review on the impact of environmental factors. Aquatic Toxicology, 191: 141-163. https://doi.org/**10.1016/j.aquatox.2017.08.005**.

Schwertman, N.C.; Owens, M.A.; Adnan, R. 2004. A simple more general boxplot method for identifying outliers. Computational Statistics & Data Analysis, 47(1): 165-174. https://doi.org/**10.1016/j.csda.2003.10.012**.

SISCOMEX – Sistema Integrado de Comércio Exterior. 2020 [online] URL: http://comexstat.mdic.gov.br/pt/geral

Thongprajukaew, K.; Kovitvadhi, S.; Kovitvadhi, U.; Rungruangsak-Torrissen, K. 2014. Pigment deposition and in vitro screening of natural pigment sources for enhancing pigmentation in male Siamese fighting fish (*Betta splendens* Regan, 1910). Aquaculture Research, 45(4): 709-719. https://doi.org/**10.1111/are.12009**.

Zanoni, M.A.; Leal, T.V.; Caetano Filho, M.; de Oliveira, C.A.L.; Ribeiro, R.P. 2013. Sex reversal of Nile tilapia (*Oreochromis niloticus*) Supreme variety, fingerlings submitted to different temperatures for sexual differentiation phase. Semina: Ciências Agrárias, 34(1): 455-466. https://doi.org/**10.5433/1679-0359.2013v34n1p455**.

Zhang, W.; Wang, H.; Brandt, D.Y.C.; Hu, B.; Sheng, J.; Wang, M.; Luo, H.; Guo, S.; Sheng, B.; Zeng, Q.; Peng, K.; Zhao, D.; Jian, S.; Wu, D.; Wang, J.; van Esch, J.H.M.; Shi, W.; Ren, J.; Nielsen, R.; Hong, Y. 2021. The genetic architecture of phenotypic diversity in the betta fish (*Betta splendens*). bioRxiv. https://doi.org/**10.1101/2021.05.10.443352**.

Zuanon, J.A.S.; Salaro, A.L.; Balbino, E.M.; Saraiva, A.; Quadros, M.; Fontanari, R.L. 2006. Níveis de proteína bruta em dietas para alevinos de acará-bandeira. Revista Brasileira de Zootecnia, 35(5): 1893-1896. https://doi.org/**10.1590/S1516-35982006000700003**.

Zuanon, J.A.S.; Salaro, A.L.; Furuya, W.M. 2011. Produção e nutrição de peixes ornamentais. Revista Brasileira de Zootecnia, 40(1): 165-174.