LARVICULTURE OF SIAMESE FIGHTING FISH *Betta splendens* IN LOW-SALINITY WATER

Thiago El Hadi Perez FABREGAT[1], Barbara WOSNIAK[1], Rodrigo TAKATA[2], Kleber Campos MIRANDA-FILHO[3], João Batista Kochenborger FERNANDES[4], Maria Célia PORTELLA[4]

ABSTRACT

The low-salinity water may improve live food utilization during larviculture, mainly when larvae are fed with salt water organisms. This study aimed to determine the median lethal concentration (LC_{50}) of NaCl in water for larvae of *Betta splendens*, an important ornamental species, and to evaluate the effect of low-salinity on the larviculture during the first 15 days of exogenous feeding. In the first experiment, 400 larvae were stocked in forty 250 mL aquariums, and exposed to ten saline concentrations. In the second experiment, 360 larvae were distributed in 24 1 L aquariums, in a factorial design 2x3 comprising two increasing prey densities, starting with 50 and 100 *Artemia* nauplii larva^{-1}, and three concentrations of NaCl (0, 2 and 4 g NaCl L^{-1}). After 24, 48, 72 and 96 h of exposure, the LC_{50} were 11.7, 10.1, 8.2 and 7.1 g NaCl L^{-1}, respectively. At the end of the experiment 2, larvae reared in salinity of 2 and 4 g NaCl L^{-1} and fed with the initial prey density of 100 nauplii larvae^{-1} were bigger and heavier. The use of low-saline water (2 to 4 g NaCl L^{-1}) is a safe protocol for larviculture of Siamese fighting fish as it does not affect the survival and optimizes the use of *Artemia* nauplii when higher prey densities are used.

Key words: Intensive larviculture, toxicity, live food, salinity, ornamental fish.

LARVICULTURA DO BETTA EM ÁGUA LEVEMENTE SALINIZADA

RESUMO

A água levemente salinizada melhora o aproveitamento do alimento vivo durante a larvicultura, principalmente quando as larvas são alimentadas com organismos de água salgada. Este estudo objetivou determinar a concentração letal (CL_{50}) de NaCl na água para larvas de *Betta splendens*, uma importante espécie ornamental, e avaliar os efeitos de salinidades baixas na larvicultura durante os primeiros 15 dias de alimentação exógena. No primeiro experimento, 400 larvas foram estocadas em 40 aquários (250 mL) e expostas a dez concentrações salinas. No segundo experimento, 360 larvas foram distribuídas em 24 aquários de 1 L (15 larvas aquário^{-1}), em esquema fatorial 2x3 com duas densidades crescentes de presas, começando com 50 e 100 náuplios de *Artemia* larva^{-1}, e três concentrações salinas (0, 2 e 4 g NaCl L^{-1}). Após 24, 48, 72 e 96 h de exposição, a CL_{50} foi de 11,7; 10,1; 8,2 e 7,1 g NaCl L^{-1}, respectivamente. No final do experimento 2, as larvas mantidas nas salinidades de 2 e 4 g NaCl L^{-1} e alimentadas na densidade inicial de 100 náuplios larva^{-1} apresentaram crescimento superior. O uso da água levemente salinizada (2 a 4 g NaCl L^{-1}) é um protocolo seguro para a larvicultura de *B. splendens*, não afeta a sobrevivência das larvas e otimiza o uso dos náuplios de *Artemia* quando densidades elevadas de presas são utilizadas.

Palavras-chave: alimento vivo, larvicultura intensiva, salinidade, toxicidade, peixe ornamental.

Artigo Científico: Recebido em 17/05/2016; Aprovado em 17/04/2017

[1]*Universidade do Estado de Santa Catarina, Avenida Luis de Camões, 2090, 88520-000, Lages, SC, Brasil, thiagofabregat@hotmail.com (autor correspondente)*
[2]*Fundação Instituto de Pesca do Estado do Rio de Janeiro, Laboratório de Reprodução de Peixes Nativos e Exóticos. Cordeiro, RJ, Brazil. Cep - 28540-000.*
[3]*Universidade Federal de Minas Gerais, Escola de Veterinária, Laboratório de Aquacultura, CEP 567, 30123-970, Belo Horizonte, MG, Brazil.*
[4]*Universidade Estadual Paulista, Centro de Aquicultura, 14884-900 Jaboticabal, SP, Brazil.*

INTRODUCTION

Ornamental fish is a multimillionaire industry around the world (CHAPMAN *et al.*, 1997; ANJOS *et al.*, 2009). Nevertheless, significant number of fish is still arising from exploitation on natural stocks (TLUSTY, 2002; PELICICE and AGOSTINHO, 2005; RAGHAVAN *et al.*, 2013; SAMPAIO AND OSTRENSKY, 2013). One alternative to decrease the capture from the natural environment is the production of ornamental species in captivity (TLUSTY, 2002; BARBIERI *et al.*, 2014).

Several studies have been developed to improve the knowledge on the biology and management during the rearing of ornamental species, such as: feeding management (ZUANON *et al.*, 2004), techniques and system of production (NG *et al.*, 1992; HALACHMI, 2006; ZUANON *et al.*, 2011), nutrition (THONGPRAJUKAEW *et al.*, 2011; ZUANON *et al.*, 2011), reproduction (ROY and YANONG, 1996), behavior (HALLER and WITTENBERGER, 1987), among others. However, the number of species for ornamental purposes is high, and there is lack of information about larviculture even for those produced globally on a large scale.

The use of sodium chloride (NaCl) in water has proved to be an important rearing protocol during the production of larvae (SANTOS and LUZ, 2009; LUZ and SANTOS, 2010; LUZ *et al.*, 2012; JOM ORI *et al.*, 2012; JOMORI *et al.*, 2013) and juveniles (ZUANON *et al.*, 2009; SALARO *et al.*, 2012) of freshwater fish species. In literature, tolerance of *Betta splendens* to salinity was been reported by ZUANON *et al.* (2009) and PUELLO-CRUZ *et al.* (2010). However, there are no references of the median lethal salinity estimates to Siamese fighting fish larvae. Furthermore, information on the use of *Artemia* nauplii at first feeding in low saline water is missing for this species. In fact, the low-salinity water does not affect survival of several freshwater fish larvae and improves live food utilization, mainly when larvae are fed with *Artemia* nauplii (JOMORI *et al.*, 2013), because the saline water extends the lifetime of this microcrustacean (JOMORI *et al.*, 2012), allowing longer exposition to the larvae and improved availability.

B. splendens is a small native fish from Asia, Mekong basin, but was spread world widely. The species is one of the most important ornamental fish in United States of America (CHAPMAN *et al.*, 1997). However, information about the handling during its rearing is still scarce, mainly in larviculture, which is considered a bottleneck in fish production (PORTELLA and DABROWSKI, 2008). We hypothesize here that the use of slightly saline water can improve the larviculture of Siamese fighting fish *B. splendens*, optimizing the use of *Artemia* nauplii when higher densities of prey are used. Therefore, this study aimed to determine the salinity tolerance of Siamese fighting fish larvae, and also to improve the protocol of larviculture, by evaluating the larvae rearing in low-salinity water with different prey densities.

METHODS

The experiment was approved by the Ethics Committee of the Santa Catarina State University (protocol 4774111115). *B. splendens* larvae were obtained from natural reproduction of broodstock kept in the laboratory at 28°C. At the end of yolk-sac phase (three days post-hatching), the larvae were submitted to two experiments. In the Experiment 1, the larvae were exposed to different concentrations of NaCl in water to access the median lethal concentration (LC_{50}). In the second experiment, we evaluated the effects of low-salinity and prey density on the larviculture.

Experiment 1

Four hundred larvae of *B. splendens* (3.07 ± 0.20 mm and 0.17 ± 0.05 mg) were stocked in 40 aquariums of 250 mL, totaling 10 larvae per aquarium. The aquariums were displayed in a water bath kept at 28.4 ± 0.7°C using electric thermostat, and were supplied with low artificial aeration. The experiment was carried out in a completely randomized design, with 10 treatments (saline water) with four replicates. The commercial salt (iodine-free NaCl) was used to prepare the different saline waters.

The larvae were submitted to increased concentrations of saline water at 0 (S0 -freshwater), 2 (S2), 4 (S4), 6 (S6), 8 (S8), 10 (S10), 15 (S15), 20 (S20), 25 (S25) and 30 (S30) g of NaCl L^{-1} for 96 h, and the survival was evaluated every 24 h. In all treatments, the water was renewed every day at the proportion of 90% and during the trial the larvae were not fed.

Data of cumulative mortality were used to estimate median lethal concentration (LC_{50}) of NaCl in water and their respective confidence intervals (95%) for safe salinity concentration.

Experiment 2

Three hundred and sixty larvae of *B. splendens* (3.12 ± 0.21 mm and 0.25 ± 0.04 mg) were distributed in 24 1 L aquariums (15 larvae aquarium^{-1}) supplied with low artificial aeration. The temperature was maintained constant at 27.4 ± 1.7°C using water bath, and the other water quality variables were: dissolved oxygen 6.0 ± 0.3 mg L^{-1}, pH 7.65 ± 0.24 and total ammonia 0.09 ± 0.05 mg L^{-1}.

The study was carried out during 15 days in factorial design 3 x 2, with three low-salinity concentrations (0, 2 and 4 g NaCl L^{-1}) and two increasing levels of prey densities (N_{50} and N_{100}), with four replicates. Larvae were fed with freshly hatched *Artemia* nauplii (24-h post cyst incubation) and the live food amount was divided in three meals a day, at 8 am, 12pm and 4pm. At the beginning of exogenous feeding, larvae were fed with *Artemia* nauplii at daily density of 50 and 100 nauplii larvae^{-1} from the first to the third day in N_{50} and N_{100} levels, respectively. The prey densities increased to 100 and 200 nauplii larvae^{-1} per day from the fourth to sixth day, 150 and 300 nauplii larvae^{-1} per day from the seventh to ninth day, 200 and 400 nauplii larvae^{-1} from the tenth to twelfth day and 250 and 500 nauplii larvae^{-1} from the thirteenth to fifteenth day, in N_{50} and N_{100} levels, respectively. During the experiment, after the last meal, the residues accumulated on the bottom and dead larvae were removed by siphoning, and approximately 90% of the volume was renewed with clean water.

The total length and weigh of the *B. splendens* larvae were evaluated using a digital calliper (Starret®) and an electronic scale (Marte®), respectively. The growth data were used to calculate the weight gain (WG = final weight – initial weight), final biomass (B = final number of larvae x final weight) and specific growth rate [SGR = (ln final mean weight - ln initial mean weight) 100/ time interval (in days)]. The survival at the end of Experiment 2 was expressed in percentage.

Statistical analysis

Statistical analyses were performed using the software Statistical Analysis System – SAS Institute, version 8.0. Percent results (survival rates and SGR) were arcsine transformed before being analyzed but only the original data are presented. Tests were performed to verify error normality (Cramer-von Mises) and homoscedasticity of the variance (Levene). In experiment 1 comparison among survival rates were made by one-way ANOVA followed by the Tukey's Test. In addition, the median lethal concentration (LC_{50}) of NaCl in water and their respective confidence intervals (95%) for safe salinity level, were estimated using the software Trimmed Spearman Karber (HAMILTON *et al.*, 1977). The data from Experiment 2 were compared by parametric Factorial ANOVA (salinity x prey density), and means were compared using Tukey's multiple range tests, at 5% probability level.

RESULTS

Experiment 1

Total mortality was observed in salinities above 10 g NaCl L^{-1} after 24 h of exposure. The salinity of 10 g NaCl L^{-1} provided decreasing survival ($P<0.05$) after 24 and 48 hours of exposure. After 72 and 96 hours, larvae exposed to salinity of 10 g NaCl L^{-1} showed the lower survival ($P<0.05$) and the salinities of 0, 2 and 4 g NaCl L^{-1} resulted in higher survival, with no statistic differences among them. Salinities of 6 and 8 g NaCl L^{-1} showed intermediate survival rates ($P<0.05$) (Table 1).

The median lethal salinity concentrations (LC_{50}) for *B. splendens* larvae were estimated after 24, 48, 72 and 96 h of exposure, as well as the respective safe salinities. The results demonstrate that toxicity of NaCl increased over time. The LC_{50} for *B. splendens* larvae changed from 11.7 at 24 h to 7.1 g NaCl L^{-1} at 96 h (Table 2).

Experiment 2

Larvae of *B. splendens* reared at different salinities and prey densities showed distinct growth parameters. The mean body weight, total length, final biomass and SGR showed interactions ($P<0.05$) between salinity levels and prey densities. On the other hand, survival did not show interaction between the factors (Table 3).

At the higher prey density (N_{100}), the mean body weight, total length (Table 4), final biomass and SGR (Table 5) were, in general, higher ($P<0.05$). When analyzing the saline influence, we observed that the lower prey density (N_{50}) resulted in similar mean values of all parameters ($P>0.05$), except total length in freshwater (Table 4).

However, the higher level of prey density (N_{100}) resulted in higher ($P<0.05$) weight and length to *B. splendens* larvae reared at salinities of 2 and 4 g NaCl L^{-1} than in freshwater (Table 4).

Final biomass and SGR were higher (P<0.05) in the N_{100} treatments, independently of the salinity used. However, in N_{50} treatments, there were no statistic differences in final biomass and SGR of *B. splendens* larvae reared at the different salinities. On the other hand, the larvae at N_{100} showed higher (P<0.05) average values when reared at salinities of 2 and 4 g NaCl L^{-1} compared to those reared in freshwater (0 g NaCl L^{-1}). The *B. splendens* larvae survival did not differ amongst the treatments (Table 6).

Table 1. Survival (%) of *Betta splendens* larvae exposed to different salinities for 96 hours.

	24h	48h	72h	96h
S0	100.0±0.0^{a}	100.0±0,0^{a}	100.0±0.0^{a}	100.0±0.0^{a}
S2	100.0±0.0^{a}	100.0±0,0^{a}	92.5±9.6^{ab}	92.5±9.6^{ab}
S4	100.0±0.0^{a}	100.0±0,0^{a}	90.0±11.6^{ab}	82.5±12.6^{ab}
S6	100.0±0.0^{a}	100.0±0,0^{a}	82.5±5.0^{b}	77.5±5.0^{b}
S8	97.5±5.0^{a}	97.5±5,0^{a}	82.5±5.0^{b}	57.5±18.9^{b}
S10	87.5±12.6^{b}	40.0±18,3^{b}	7.5±0.5^{c}	2.5±0.5^{c}
CV (%)	6.9	26.4	42.8	50,1

Means followed by the same letters on the columns did not differ by Tukey's test (p<0.05)
S0, S2, S4, S6, S8 and S10 mean 0, 2, 4, 6, 8 and 10 g NaCl L^{-1}, respectively.

Table 2. Median lethal salinity concentrations (LC_{50}) and safe salinities (confidence interval) throughout the 96 h test with *Betta splendens* larvae.

Time (h)	Salinity (g L^{-1})	
	LC_{50}	Confidence interval
24	11.7	11.3-12.1
48	10.1	9.6-10.6
72	8.2	7.5-9.0
96	7.1	6.3-7.9

B. splendens larvae exposed to salinities of 0, 2, 4, 6, 8, 10, 15, 20 and 30 g of NaCl L^{-1}.

Table 3. F and average values of weight, total length (TL), final biomass, specific growth rate (SGR), and survival during the larviculture of *Betta splendens* at different salinities and prey densities.

Statistics	F values				
	Weight	TL	Biomass	SRG	Survival
Salinity (S)	57.6 **	143.2 **	34.3 **	45.6 **	0.18 ^{ns}
Prey density (P)	399.9 **	360.5 **	263.2 **	365 **	0.55 ^{ns}
Interaction S x P	39.0 **	25.7 **	24.0 **	22.2 **	1.4 ^{ns}
Significance: * p<0.05 ** p<0.01 and "ns" not significant					

Table 4. Interaction (Salinity × Prey density) average values (± standard deviation) of weight and total length after fifteen days of active feeding for *Betta splendens* larvae.

Salinity	Weigth (mg)		Total length (mm)	
	Prey density		Prey density	
(g NaCl L^{-1})	N_{50}	N_{100}	N_{50}	N_{100}
0	13.0±1.2^{Ba}	17.9±2.5^{Ab}	10.3±0.1^{Bb}	11.4±0.4^{Ab}
2	13.9±0.4^{Ba}	30.7±2.1^{Aa}	11.3±0.3^{Ba}	14.3±0.2^{Aa}
4	14.6±0.7^{Ba}	31.9±1.6^{Aa}	11.8±0.3^{Ba}	14.6±0.3^{Aa}

Means followed by the same letters (A, B in the rows and a, b, in the columns) for each parameter did not differ by Tukey's test (p<0.05) N_{50} and N_{100}: Larvae fed initially with 50 and 100 nauplii $larvae^{-1}$ per day, respectively.

Table 5. Interaction (Salinity × Prey density) average values (±standard deviation) of final biomass specific growth rate (SGR) after fifteen days of active feeding for *Betta splendens* larvae.

	Final biomass (mg)		SGR	
Salinity	Prey density		Prey density	
(g NaCl L^{-1})	N_{50}	N_{100}	N_{50}	N_{100}
0	178.9 ± 22.9^{Ba}	254.8 ± 34.2^{Ab}	0.26 ± 0.01^{Ba}	0.28 ± 0.01^{Ab}
2	198.3 ± 12.7^{Ba}	422.0 ± 41.7^{Aa}	0.27 ± 0.01^{Ba}	0.32 ± 0.001^{Aa}
4	194.0 ± 6.2^{Ba}	545.3 ± 33.5^{Aa}	0.27 ± 0.01^{Ba}	0.32 ± 0.01^{Aa}

Means followed by the same letters (A, B in the rows and a, b, in the columns) for each parameter did not differ by Tukey's test ($p<0.05$) N_{50} and N_{100}: Larvae fed initially with 50 and 100 nauplii $larvae^{-1}$ per day, respectively.

Table 6. Average values (± standard deviation) of *Betta splendens* survival rates after 15 days of exogenous feeding.

Salinity (g NaCl L^{-1})	Survival (%)
0	93.3±6.2
2	93.3±7.1
4	91.7±5.9
Prey density	
N_{50}	91.7±6.4
N_{100}	93.9±6.0
c.v. [1]	6.67

[1] c.v.: coefficient of variation. N_{50} and N_{100}: Larvae fed initially with 50 and 100 nauplii $larvae^{-1}$ per day, respectively.

DISCUSION

The larvae of the ornamental fish *B. splendens* showed susceptibility to high-salinity water. However, the intensive larviculture of this species could be carried out successfully in low-salinity conditions. In such case, a series of factors may be improved during the larviculture, as the physiological condition by decreasing the energetic requirements for ionic and osmotic regulation (ALTINOK and GRIZZLE, 2001), the prevention of diseases (GARCIA *et al.*, 2007) and the increased lifetime and availability of the live food (*Artemia* nauplii) (JOMORI *et al.*, 2012).

The median lethal salinity (LC_{50}) for *B. splendens* larvae changed during the time, from 11.7 at 24 h to 7.1 g NaCl L^{-1} at 96 h. The longer the exposure time, more larvae were dying. A possible explanation for the increased toxicity over time may be that mortality occurred as the energy reserves of the larvae were depleted by the energy expenditure of the osmoregulation. For instance, salinity of 10 g NaCl L^{-1} killed only 12.5% of *B. splendens* larvae during the first 24 hours, but after 96 hours it ended up being lethal for 97.5%. However, the LC_{50} 96 h for *B. splendens* larvae was lower than the reported for adult of the same species, 11.88 g NaCl L^{-1} (ZUANON *et al.*, 2009). The saline tolerance may change during fish development, as already showed for bonefish (Albulidae: *Albula*) (PFEILER, 1981) and mangrove red snapper (*Lutjanus argentimaculatus*) (ESTUDILLO *et al.*, 2000).

The differences observed in median lethal salinity between the larval and adult stages may be related to the ontogeny of osmoregulation in postembryonic fish, since during the larval phase the osmoregulatory function shifts from the skin to the gills, which become the main osmoregulatory site (VARSAMOS *et al.*, 2005). According to the authors, the main difference is due to Na^+/K^+-ATPase activity, which varies ontogenetically in the tegument and gill. Future studies should be developed to characterize the Na^+/K^+-ATPase activity during the initial development of stenohaline fish species.

The rearing of *B. splendens* larvae in low-salinity conditions resulted in better or similar growth and survival rates compared to freshwater rearing conditions, as previously observed for other stenohaline species (BORODE *et al.*, 2002; SANTOS and LUZ, 2009; LUZ *et al.*, 2012; JOMORI *et al.*, 2012; 2013). For instance, the growth of *Colossoma macropomum*, *Astronotus ocellatus*, *Brycon amazonicus* and *Leporinus macrocephalus* larvae was positively affected when they were reared in low-salinity water (JOMORI *et al.*, 2013). The growth and survival of *A. ocellatus* and *B. amazonicus* were improved in freshwater and at 2 g NaCl L^{-1} in the water. The species *C. macropomum* and *L. macrocephalus* grew better in water at 2 and 4 g NaCl L^{-1}; however, the survival of *C. macropomum* was lower at 4 g NaCl L^{-1} whereas the survival of *L. macrocephalus* larvae was not affected. Taken together, it is possible to say that *B. splendens* larvae follow the tendency observed in larvae of other stenohaline fish species.

Artemia nauplii has been used successfully as live feed during the intensive larviculture of freshwater species (PORTELLA and DABROWSKI, 2008; LUZ and SANTOS, 2010; JOMORI *et al.*, 2012; PORTELLA *et al.*, 2014), and also in Siamese fighting fish larval rearing (OGATA and KUROKURA, 2012; FOSSE *et al.*, 2013). However, the lifetime of the *Artemia* nauplii in freshwater is reduced, and the use of low-salinity water may improve its lifetime (JOMORI *et al.*, 2012), providing longer period of live prey exposure to the fish larvae. In this situation, the higher prey density in low-salinity water may favor the possibility of larvae feeding, by extending the period of feeding activity and, by having a higher amount of live prey available, resulting in higher growth rate. In the present study, this was evidenced by the interaction between the factors salinity and density of prey. The higher density of prey resulted in higher weight and length of *B. splandens* larvae reared at salinities of 2 and 4 g NaCl L^{-1} than in freshwater. Similar situation was observed during the intensive larviculture of other stenohaline fish species, such as *Prochilodus costatus*, *Lophiosilurus alexandri* (SANTOS and LUZ, 2009), *Astronotus ocellatus*, *Brycon amazonicus* and *Leporinus macrocephalus* (JOMORI *et al.*, 2013). This is the first report on the use of common salt to improve the *Artemia* nauplii utilization in the larviculture of *B. splendens*.

Our findings confirmed that low-saline water is a safe protocol and can be used during *B. splendens* larviculture. In addition, the results of the interaction between the factors NaCl concentration and prey density show that the longer availability of *Artemia* nauplii improved the growth performance of *B. splendens* larvae.

CONCLUSION

The CL_{50} for *B. splendens* larvae ranges from 11.7 to 7.1 g NaCl L^{-1} during the first 96 h of observation. The use of low-saline water does not affect the survival of *B. splendens* larvae and optimizes the use of *Artemia* nauplii when higher prey concentrations are used. To improve the larviculture of *B. splendens* we can recommend the use of low-salinity water (2 to 4 g NaCl L^{-1}) and an initial prey density of 100 nauplii $larvae^{-1}$, followed by periodic increasing amounts during the first 15 days of exogenous feeding.

REFERENCES

ALTINOK, I.; GRIZZLE, J.M. 2001 Effects of brackish water on growth, feed conversion and energy absorption efficiency by juveniles euryhaline and freshwater stenohaline fishes. Journal of Fish Biology, 59(5): 1142-1152.

ANJOS, H.D.B.; AMORIM, R.M.S.; 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.

BARBIERI, E.; BONDIOLI, A. C. V.; MELO, C. B.; HENRIQUES, M. H. 2014 Effects of low salinity on juvenile pink shrimp (Perez-Farfante 1967, Crustacea). Marine and Freshwater Behaviour and Physiology, 47(4): 273-283.

BORODE, A.O.; BALOGUM, A.M.; OMOYENI, B.A. 2002 Effect of salinity on embryonic development, hatchability, and growth of *African catfish*, *Clarias gariepinus*, eggs and larvae. Journal of Applied Aquaculture, 12(4): 89-93.

CHAPMAN, F.A.; FITZ-COY, S.A.; THUMBERG, E.M.; ADAMS, C.M. 1997 United States of America trade in ornamental fish. Journal of the World Aquaculture Society, 28(1): 1-10.

ESTUDILLO, C.B.; DURAY, M.N.; MARASIGAN, E.T.; EMATA, A.C. 2000 Salinity tolerance of larvae of the mangrove red snapper (*Lutjanus argentimaculatus*) during ontogeny. Aquaculture, 190(1-2): 155-167.

FOSSE, P.J.; MATTOS, D.C.; CARDOSO, L.D.; MOTTA, J.H.S.; JASPER, A.P.S.; RADAEL, M.C.; ANDRADE, D.R.; VIDAL-JÚNIOR, M.V. 2013 Estratégia de coalimentação na sobrevivência e no crescimento de larvas de *Betta splendens* durante a transição alimentar. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 65(6): 1801-1807.

GARCIA, L.O.; BECKER, A.G.; COPATTI, C.E.; BALDISSEROTTO, B.; NETO, J.R. 2007 Salt in the food and water as a supportive therapy for *Ichthyophthirius multifiliis* infestation on silver catfish, Rhamdia quelen, fingerlings. Journal of World Aquaculture Society, 38(1): 1-11.

HALACHMI, I. 2006 Systems engineering for ornamental fish production in a recirculating aquaculture system. Aquaculture, 259(1-4): 300-314.

HALLER, J.; WITTENBERGER, C. 1987 Biochemical energetic of hierarchy formation in *Betta splendens*. Physiology and Behavior, 43(4): 447-450.

HAMILTON, M.A.; RUSSO, R.C.; THURSON, R.V. 1997 Trimmed Spearman–Karber method for estimating median-lethal concentrations in toxicity bioassays. Environmental Science Technology, 11(7): 714-719.

JOMORI, R.K.; LUZ, R.K.; PORTELLA, M.C. 2012 Effect of salinity on larval rearing of pacu, *Piaractus mesopotamicus*, a freshwater species. Journal of the World Aquaculture Society, 43(3): 423-432.

JOMORI, R.K.; LUZ, R.K.; TAKATA, R.; FABREGAT, T.E.H.P.; PORTELLA, M.C. 2013 Água levemente salinizada aumenta a eficiência da larvicultura de peixes neotropicais. Pesquisa Agropecuária Brasileira, 48(8): 809-815.

LUZ, R.K.; SANTOS, J.C.E. 2010 Effect of salt addition and feeding frequency on cascudo preto *Rhinelepis aspera* (Pisces: Loricariidae) larviculture. Journal of Applied Ichthyology, 26(3): 453-455.

LUZ, R.K.; SILVA, W.S.; MELILLO-FILHO, R.; SANTOS, A.E.H.; RODRIGUES, L.A.; TAKATA, R.; ALVARENGA, É.R.; TURRA, E.M. 2012 Stocking density in the larviculture of Nile tilapia in saline water. Revista Brasileira de Zootecnia, 41(12): 2385-2389.

NG, W.J.; KHO, K.; HO, L.M.; ONG, S.L.; SIM, T.S.; TAY, S.H.; GOH, C.C.; CHEONG, L. 1992 Water quality within a recirculation system for tropical ornamental fish culture. Aquaculture, 103(2): 123-134.

OGATA, Y.; KUROKURA, H. 2012 Use of the freshwater rotifer *Brachionus angularis* as the first food for larvae of the Siamese fighting fish Betta splendens. Fisheries Science, 78(1): 109-112.

PELICICE, F.M.; AGOSTINHO, A.A. 2005 Perspectives on ornamental fisheries in the upper Paraná River floodplain, Brazil. Fisheries Research, 72(1): 109-119.

PFEILER, E. 1981 Salinity tolerance of leptocephalous larvae and juveniles of the bonefish (Albulidae: Albula) from the Gulf of California. Journal of Experimental Marine Biology and Ecology, 52(1): 37-45.

PORTELLA, M.C.; DABROWSKI, K. Diets. 2008, physiology, biochemistry and digestive tract development of freshwater fish larvae. In: CYRINO, J.E.P.; BUREAU, D.P.; KAPOOR, B.G. Feeding and digestive functions of fishes. Science Publishers, New Hampshire. p. 227-279.

PORTELLA, M.C.; JOMORI, R.K.; LEITÃO, N.J.; MENOSSI, O.C.C.; FREITAS, T.M.; KOJIMA, J.T.; LOPES, T.S.; CLAVIJO-AYALA, J.A.; CARNEIRO, D.J. 2014 Larval development of indigenous South American freshwater fish species, with particular reference to pacu (*Piaractus mesopotamicus*): A review. Aquaculture, 432(1): 402-417.

PUELLO-CRUZ, A.C.; VELASCO-BLANCO, G.; MARTÍNEZ-RODRÍGUEZ, I.E. 2010 Growth

and survival of Siamese fighting fish, *Betta splendens*, larvae at low salinity and with different diets. *Journal of the World Aquaculture Society, 41*(5): 823-828.

RAGHAVAN, R.; DAHANUKAR, N.; TLUSTY, M.F.; RHYNE, A.L.; KUMAR, K.K.; MOLUR, S.; ROSSER, A.M. 2013 Uncovering an obscure trade: Threatened freshwater fishes and the aquarium pet markets. *Biological Conservation, 164*(1): 158–169.

ROY, P.E.; YANONG, V.M.D. 1996 Reproductive management of freshwater ornamental fish. *Seminars in Avian and Exotic Pet Medicine, 5*(4): 222-235.

SALARO, A.L.; CAMPELO, D.A.V.; PONTES, M.D.; TAVARES, M.M.; ZUANON, J.A.S.; LUZ, R.K. 2012 Saline water for juvenile giant traira during feed training. *Revista Brasileira de Zootecnia, 41*(6): 1342-1345.

SAMPAIO, F.D.F.; OSTRENSKY, A. 2013 Brazilian environmental legislation as tool to conserve marine ornamental fish. *Marine Policy, 42*(1): 280–285.

SANTOS, J.C.E.; LUZ, R.K. 2009 Effect of salinity and prey concentrations on *Pseudoplatystoma corruscans*, *Prochilodus costatus* and *Lophiosilurus alexandri* larviculture. *Aquaculture, 287*(3-4): 324–328.

THONGPRAJUKAEW, K.; KOVITVADHI, U.; KOVITVADHI, S.; SOMSUEB, P.; RUNGRUANGSAK-TORRISEN, K. 2011 Effects of different modified diets on growth, digestive enzyme activities and muscle compositions in juvenile Siamese fighting fish (*Betta splendens* Regan, 1910). *Aquaculture, 322-323*(1): 1-9.

TLUSTY, M. 2002 The benefits and risks of aquacultural production for the aquarium trade. *Aquaculture, 205*(3-4): 203-219.

VARSAMOS, S.; NEBEL, C.; CHARMANTIER, G. 2005 Ontogeny of osmoregulation in postembryonic fish: A review. *Comparative Biochemistry and Physiology, Part A 141*(4): 401-429.

ZUANON, J.A.S.; ASSANO, M.; FERNANDES, J.B.K. 2004 Desempenho de tricogaster (*Trichogaster trichopterus*) submetido a diferentes níveis de arraçoamento e densidades de estocagem. *Revista Brasileira de Zootecnia, 33*(6): 1639-1645.

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.

ZUANON, J.A.S.; SALARO, A.L.; VERAS, G.C.; TAVARES, M.M.; CHAVES, W. 2009 Tolerância aguda e crônica de adultos de beta, *Betta splendens*, à salinidade da água. *Revista Brasileira de Zootecnia, 38*(11): 2106-2110.