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Research Article
Effects of Salinity on Survival and Growth of Gurami Sago (*Osphronemus goramy*, Lacepède, 1801) Juveniles

[1]Azrita and [2]Hafrijal Syandri

[1]Department of Biology Education, Faculty of Education, Bung Hatta University, Padang, Indonesia
[2]Department of Aquaculture, Faculty of Fisheries and Marine Science, Bung Hatta University, Padang, Indonesia

Abstract

Background and Objective: Gurami Sago *Osphronemus goramy* (*O. goramy*) are an herbivorous freshwater finfish species native in Indonesia. This species has not yet been cultured commercially in brackish water. A 60-days study was conducted to evaluate the effects of salinity on survival and growth of *O. goramy*. **Materials and Methods:** Two independent experiments were performed to determine the effects of salinity on survival and growth of juvenile *O. goramy*, first one was to determine the median lethal salinity (MLS-50_{96h}) and second one was to assess the survival and growth at different sub-lethal salinities. In MLS-$50_{96\ h}$ study 0.0, 4.0, 8.0, 12.0 and 16.0 ppt salinities were used to initially find out the salinity tolerance range. Accordingly, a definitive salinity tolerance test was done in next phase to find out exact median lethal salinity by directly transferring the test species to 13.0, 14.0, 15.0 and 16.0 ppt salinity for 96 h. The median lethal salinity of *O. goramy* was estimated at 14.0 ppt. In the second experiment, survival and growth of the *O. goramy* were recorded at salinities 4.0, 8.0 and 12.0 ppt along with 0.0 ppt as control during 60 days. **Results:** *Osigni goramy* exhibited lowest final average weight at 12.0 ppt salinity and significantly highest at 4.0 ppt salinity. Highest SGR and weight gain were obtained at 4.0 ppt followed by 0 ppt, 8 ppt and 12 ppt salinity. All treatments were significantly ($p<0.05$). Survival rate of *O. goramy* varied between 76.45% (at 0.0 ppt) and 66.66% (at 12.0 ppt). **Conclusion:** The *O. goramy* grew and survived satisfactorily at 0.0 to12.0 ppt salinities, implying that the species can be cultured commercially in brackish water, in view of in Indonesia, there are many abandoned shrimp ponds.

Key words: *Osphronemus goramy*, salinity tolerance, lethal salinity, environmental factors, brackish water

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Corresponding Author: Azrita, Faculty of Education, Bung Hatta University, Padang, Indonesia Tel: +62751-7051678

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The survival, growth and distribution of many aquatic organisms very influenced by environmental factors, including salinity[1-3]. Environmental salinity is a key factor for the survival of aquatic organisms and any changes in salinity may effect various physiological processes in organisms[4,5]. Therefore, several studies have been conducted regarding the influence of salinity on survival and growth of fish, including *Oreochromis niloticus* and *O. mossambicus*[3], *Trachinotus marginatus*[2], *Cyprinus carpio*[6], *Morone saxatilis*[7] and *Epinephelus fuscoguttatus*× *E. Lanceolattus*[8]. Although many fishes exhibit some degree of euryhalinity[9], optimal salinity levels for survival, growth and production competence are often species-specific[10-13]. Thus, it is important to determine the optimum salinity level for each commercial fish species in culture systems where the salinity can be altered to suit the species.

Industrial and agricultural development caused the degradation of fish culture habitats and this posed negative impact on culture fish population[14,15]. These developments may cause acidic or alkaline water condition. Fish exposed to alkaline polluted water showed an increased in the plasma ammonia level[16,17]. The pH of most ranges between 5-10[18] and this change depends on many factors such as acid rain, pollution and CO_2 from the atmosphere and fish respiration. Thus, land use the increasingly limited inland waters, fish cultivation should be developed in brackish water. During recent decades, the freshwater fish which culture in the brackish water are Nile tilapia[19-24]. The *O. goramy* a candidat regarded as a freshwater fish species which can grows rapidly in the brackish water.

The *O. goramy* (its local name is *Gurami merah*) more popular with *Gurami sago*[25], is a herbivorous freshwater fish found in Lima Puluh Kota District, West Sumatra-Indonesia. Based on its high market value and demand, this species has not been successfully cultivated intensively in ponds and relevant aquaculture studies are completed to date. The *O. goramy* has been identified as one of the best prospects for a semi-commercial scale culture in Indonesia. These fish readily accept pelleted diets, may be tolerate captive conditions such as poor water quality and perform well in lakes and ponds freshwater[26,27] .

In Indonesia, there are many abandoned shrimp ponds which is not less than 1.2 million hectares and only used much as 37.5%[24]. The use of these ponds to culture fish species is highly possible provided that the fish can tolerate a slight saline condition. Many local species can be considered as potential candidate such as *O. goramy* as cultured species made it a likely candidate. Lack of information on the salinity tolerance has led to this study.

MATERIALS AND METHODS

Experimental species and acclimation: Thousand of *O. goramy* juveniles (5.23±0.04 g) were produced from the private hatchery located in Mungo, Lima Puluh Kota district of West Sumatra, Indonesia and transported in oxygenated polythene bag (pH 7.5, alkalinity 100 ppm as $CaCO_3$, hardness 120 ppm as $CaCO_3$) to the laboratory. The samples were acclimatized for 30 days prior the experiment. The sample were placed in concrete tank with the capacity 5,600 L. During the acclimatization, juveniles were fed with commercial feed which have proximate composition (dry weight %), 10% moisture content, 41% crude protein, 5% lipid, 6% crude fiber and 16% crude ash. Juveniles were fed ad libitum twice daily (9:00 and 16.00 h). Continuous aeration was provided along with 50% replacement of water with fresh bore well water.

Salinity tolerance (MLS_{96h}) test: In the first phase, juveniles of *O. goramy* (length: 7.07±0.17 cm, weight: 5.23±0.04 g) were directly transferred to aquaria (45×40×30 cm) with 0.0, 4.0, 8.0, 12.0 and 16.0 ppt saline water. Desired salinities were achieved by mixing freshwater with brine water collected from marine (28-30 ppt salinity). The experimental system consisted of 30-L glass aquaria stocked with 15 juveniles/aquarium for 96 h with three replicates. The pH and dissolved oxygen of the aquaria ranged from 7.0-7.4 and 5.6-6.3 mg L^{-1}, respectively. As 82.22% mortality was observed only at 16.0 ppt, a definitive salinity tolerance test was conducted in the second phase to determine the median lethal salinity concentration. Median lethal salinity ($MLS_{96\ h}$) is defined as the salinity at which survival of test species falls to 50% in 96 h following direct transfer from freshwater to various test salinities[28].

The test species (length: 7.07±0.17 cm, weight: 5.23±0.04 g) were directly subjected to 13.0,14.0,15.0 and 16.0 ppt salinities and observed for 96 h. Each aquarium was covered with a fine meshed nylon net to prevent jumping out the test juveniles. The pH and dissolved oxygen of the aquaria were ranged from 7.0-7.4 and 5.6-6.3 mg L^{-1}, respectively. Survival was recorded at 24, 48, 72 and 96 h of exposure to each salinity level. Lack of response to mechanical stimuli was the criteria to determine death of juveniles. Mortality of juveniles were removed during each observation. $MLS_{96\ h}$ was

calculated by probit method by pooling the mortality data from replicates within treatments and considered significantly different when the corresponding 95% confidence intervals did not overlap[29]. The entire experiment was carried out in Hatchery, Faculty of Fisheries and Marine Science Bung Hatta University, Padang-Indonesia.

Aquaria trials on survival and growth at different salinities: Three different sub lethal salinities, viz., 4.0, 8.0 and 12.0 ppt were chosen to assess the effects of salinity on survival and growth. Simultaneously freshwater (0.0 ppt salinity) was used as control, in three replicates for each treatment group. Different salinity gradients are made in each experimental aquarium that has been in freshwater content and adds salt water which taken from nearby seafront (average salinity of 28.0-30.0 ppt). A final water volume of 30 L was maintained throughout the experiment with salinity monitored weekly using a YSI model 30 (YSI Incorporated, Yellow Springs, OH USA) and adjusted accordingly to ensure it did not deviate by more than 1.0 ppt from the target salinity. 15 acclimatised *O. goramy* were randomly sampled, stocked in each aquaria and allowed to grow for 60 days under ideal hatchery management. The *O. goramy* were fed twice a day (9:00 h and 16:00 h) ad libitum with commercial pelleted feed (Japfa Comfeed, Indonesia, 41% crude protein, 5% lipid, 6% crude fiber).

Osphronemus goramy were blot dried using a tissue paper and the body weight was measured each 30 days using a digital electronic balance (OHAUS, Model CT 1200-S, USA) and their lengths were measured using a meter ruler with 0.1 mm accuracy and these values were recorded, while mortality (if any) was noted daily. The growth performances were calculated in terms of specific growth rate (SGR, %/day), body weight gain (BWG%), average daily growth (ADG, g/day). The parameters were analyzed according to Iqbal *et al.*[30], Wang *et al.*[31] and Moutinho *et al.*[32] with the following equation:

$$(\text{SGR } \%/\text{day}) = \frac{\text{InWt} - \text{InWo}}{t} \times 100$$

where, ln represents the natural log of individual wet weight (g), Wt is the final wet weight, Wi the initial wet weight, t is the duration in day:

$$\text{BWG } (\%) = \frac{\text{Wt} - \text{Wi}}{\text{Wi}} \times 100$$

where, Wt is the final wet weight and Wi the initial wet weight:

$$\text{ADG } (\text{g/day}) = \frac{\text{Wt} - \text{Wi}}{t} \times 100$$

where, Wt is the final wet weight, Wi the initial wet weight and t is the duration in day:

$$\text{Survival } (\%) = \frac{\text{Number of species survived at end of experiment}}{\text{Number of species stocked}} \times 100$$

Water quality: The water samples were collected at a depth of 5 cm from each aquaria for the determination of the dissolved oxygen (DO) contents. An oxygen meter (YSI model 52, Yellow Spring Instrument Co., Yellow Springs, OH, USA) was used *in situ* and pH values were determined with a pH meter (Digital Mini-pH Meter, 0-14 PH, IQ Scientific, Chemo-science, Thailand) Co., Ltd, Thailand). The levels of ammonia-nitrogen, nitrite-nitrogen and alkalinity of the water in each replication were measured according to standard procedures[33].

Data analysis: Probit analysis used MINITAB 14 software. The mean values for the survival and growth performance data of *O. goramy* at each treatment were analyzed by one-way ANOVA followed by Duncan's new multiple range test[34]. All statistical analyses were performed using SPSS software (version 16.0 for Windows, SPSS Inc., Chicago, IL). The standard deviation of each parameter and treatment was determined and expressed as the Mean±SD. The treatment effects were considered to be significant at $p<0.05$.

RESULTS

Salinity tolerance ($MLS_{96\ h}$) test: 40.00±6.67% mortalities were recorded within 24 h upon exposure to 16.0 ppt. In 14.0 ppt and 15.0 ppt, survival rates after 96 h were 48.89±3.85 and 28.89±3.85%, respectively. Median lethal salinity ($MLS_{96\ h}$) and confidence limit computed using probit for *O. goramy* juvenile is presented in Table 1. The precision of the $MLS_{96\ h}$ test results for a typical sigmoid cumulative distribution dose response curve and time (h) dependent survivorship curve *O. goramy* in varied salinities has been demonstrated in Fig. 1. The 96 h Median Lethal Salinity of *O. goramy* was 14.0 ppt with confidence intervals (at 95%) of 13.47-14.40 ppt. The statistical confidence of point estimate values other than 50% can be used to characterize toxicity

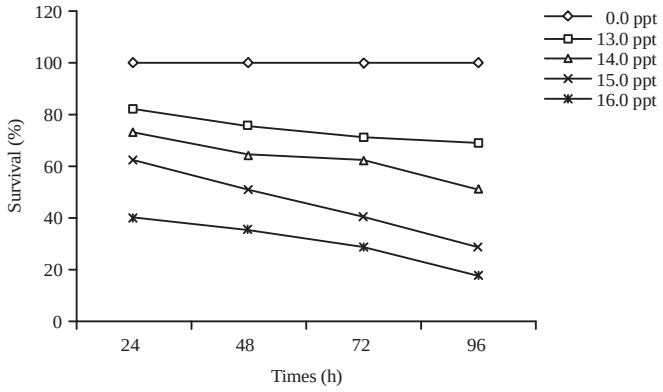

Fig. 1: Survival rate (%) of juveniles *Osphronemus goramy* in different salinities at 96 h exposure

Table 1: Salinity tolerance with confidence interval for *Osphronemus goramy* estimated using probit

		Confidence interval (95%)	
Probability	Salinity (ppt)	Lower bound	Upper bound
10	11.36	9.51	12.25
20	12.27	10.92	12.94
30	12.92	11.92	13.46
40	13.48	12.75	13.92
50	14.00	13.47	14.40
60	14.52	14.11	14.97
70	15.08	14.67	15.71
80	15.73	15.23	16.66
90	16.63	15.94	18.05
95	17.38	16.50	19.21
99	18.78	17.55	21.42

Table 2: Initial weight (g), final weight (g), weight gain (g), average daily growth (g/day), specific growth rate (%/day), body weight gain (%) and survival (%) of *Osphronemus goramy* reared in different salinities for 60 days

	Salinity (ppt)			
Variables	0 (control)	4	8	12
Initial weight	5.26±0.09^{a}	5.25±0.10^{a}	5.21±0.04^{a}	5.18±0.03^{a}
Final weight	15.63±0.51^{a}	17.53±1.21^{b}	13.67±0.11^{c}	9.03±0.15^{d}
Weight gain	10.18±0.64^{a}	12.28±1.14^{b}	8.47±0.13^{c}	3.86±0.13^{d}
Average daily growth	0.17±0.01^{a}	0.20±0.02^{b}	0.14±0.00^{c}	0.06±0.00^{d}
Specific growth rate	1.73±0.10^{a}	2.00±0.10^{b}	1.60±0.02^{c}	0.93±0.02^{d}
Body weight gain	197.53±13.70^{a}	233.70±19.00^{b}	162.63±3.25^{c}	74.49±2.15^{d}
Survival	76.45±3.39^{a}	88.78±1.82^{b}	75.55±2.18^{a}	66.66±6.67^{d}

Data are presented as Mean±SD of three replicates. Different superscripts in same row were significantly different ($p<0.05$)

(lethal salinity). Survival rate of *O. goramy* each treatment from 24 h until 96 h in varied salinities has been demonstrated in Fig. 1, respectively. There was a strong linear relationship between salinities level and survival rate at 24, 48, 72 and 96 h were ($r^2 = 0.95, 0.99, 0.98$ and 0.98), respectively.

Survival and growth at different sub-lethal salinities: The initial average body mass of the *O. goramy* were not significantly different ($p>0.05$) at the commencement of the experiment. Significant differences in monthly average body mass were observed in different salinity treatment (Fig. 2). At the end of 60 days culture period, *O. goramy* exhibited the lowest average growth (9.03±0.15 g) at 12 ppt and the highest average growth (17.53±1.21 g) at 4.0 ppt. During the experimental period the growth performance of *O. goramy* at 60 days showed significant differences ($p<0.05$). The highest weight gain was obtained *O. goramy* in 4.0 ppt (12.28±1.14 g) followed by 0.0 ppt (10.18±0.64 g), 8.0 ppt (8.47±0.13 g) and 12.0 ppt (3.86±0.13 g). This growth trend was also true for daily weight gains (Table 2). The specific

growth rates (SGR) of *O. goramy* were also highest when cultured in 4,0 ppt (2.0±0.10%/day) followed by freshwater (0.0 ppt), 8.0 ppt salinities and 12.0 ppt. Each treatments also different were significant ($p<0.05$). Significantly lower SGR was obtained in 12.0 ppt salinity treatment (0.93±0.02%/day). This growth trend was also similar in case of the percentage body weight gain (BWG%). The SGR in first month (30 days) and second month (60 days) differed significantly ($p<0.05$). In case of 30 days SGR, highest growth rate were obtained in 4.0 ppt, followed by freshwater (0 ppt), 8.0 and 12.0 ppt salinities, this trend was same in 60 days SGR (Fig. 3).

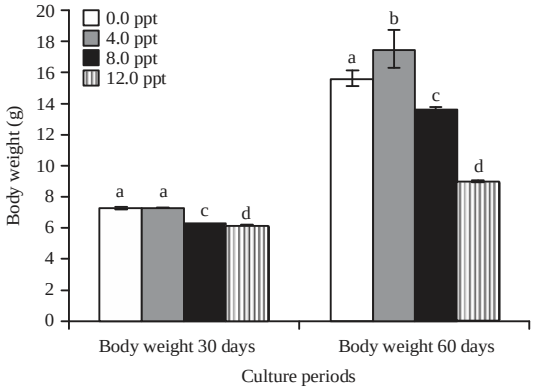

Fig. 2: Monthly average body weight (g) of *Osphronemus goramy* cultured in different salinities for 60 days. The results are expressed as Mean±SD of three replicates
Different superscripts indicate significantly differences ($p<0.05$)

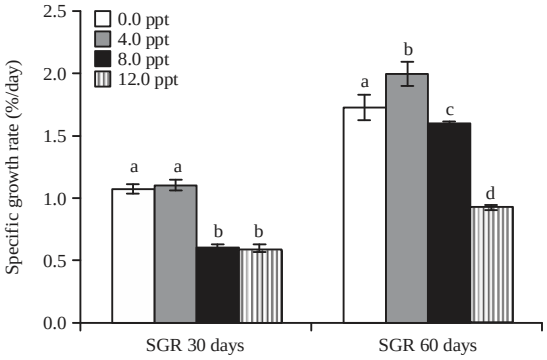

Fig. 3: Monthly average specific growth rate (%/day) of *Osphronemus goramy* cultured in different salinities for 60 days. The results are expressed as Mean±SD of three replicates
Different superscripts indicate significantly differences ($p<0.05$)

The survival rate of *O. goramy* after 60 days trial period was significantly high ($p<0.05$) in freshwater (0 ppt) and decreased as salinity increased. The differences in survival rate between treatments were significant ($p<0.05$) (Table 2). The physico-chemical parameters of aquaria water measured during trial period were depicted in Table 3.

DISCUSSION

Osphronemus goramy is one of Indonesia's most widely cultured and has played an important role in the Indonesian economy through income generation, livelihood diversification and supply of animal protein for rural and urban communities[27,26]. Juveniles or sub-adults of *O. goramy* occur naturally live in freshwater areas. Meanwhile, may be can survival and grown up in brackish water thus must adapted to an environment in which salinity levels vary constantly. Results of this study also indicated that the median lethal salinity value of *O. goramy* is 14.0 ppt and it supports that the species exhibits less a tolerance to abrupt changes in salinity, may be *O. goramy* is stenohaline. This tolerance lower compared than *Oreochromis niloticus* and *Oreochromis mossambicus*[3,19]. According to Watanabe *et al.*,[35] that Nile tilapias are far less tolerant to high salinities and do not easily acclimate to salinities exceeding 25.0 ppt. Meanwhile, that *Trachinotus marginatus* juvenile displayed good survival rate and growth in a wide range of salinities (3.0-32.0 ppt), at least for up to 28 days, due this species are euryhaline to a considerable degree and tolerated salinities up to 21.0 ppt[2]. Salinity is specific to the aquatic environment and in most aquatic organisms survival and growth were dependent on the salinity level[2,3,6] .

Table 3: Water quality parameters analyzed in different salinity treatments during 60 days growth trial of *Osphronemus goramy*

	Salinity (ppt)			
Parameters	0.0 (control)	4.0	8.0	12.0
Temperature (°C)	28.66±0.57^a	30.33±0.57^b	30.66±0.57^b	30.66±0.57^b
pH	7.63±0.23^a	7.90±0.10^b	7.97±0.05^b	7.97±0.05^b
Dissolved oxygen (mg L^{-1})	6.26±0.05^a	6.29±0.03^b	6.35±0.05^b	6.35±0.05^b
NH-N (mg L^{-1})	0.19±0.10^a	0.21±0.01^b	0.23±0.01^c	0.26±0.01^d
N-$N0_2$ (mg L^{-1})	0.09±0.01^a	0.10±0.02^a	0.11±0.07^a	0.12±0.06^a
Alkalinity (mg L^{-1})	89.00±3.00^a	104.66±2.51^b	111.33±2.08^c	121.33± 2.51^d

Data are presented as Mean±SD of three replicates. Different superscripts in same row were significantly different ($p<0.05$)

Current results revealed that effect of level salinity on growth rate of *O. goramy* can be separated into two different phases and may explain the significant differences found. In the first phase, covering between days 0 and 30 of the experiment, the growth rate showed a similar trend in all experimental groups, however, in the second phase (from day 30-60) increased level salinity had a negative effect on mean body weight and mean specific growth rate (Fig. 1 and 2). A similar response has been previously observed in *Macrobrachium rosenbergii* in different stages of development[1]. In this study, food consumption was not a limiting factor during the experimental time because fish were observed and controlled to ensure that feeding occurred two times per day proportionally to body mass.

Water quality parameters like temperature, pH, dissolved oxygen, ammonia-nitrogen, nitrate-nitrogen and alkalinity (Table 3) during growth trial period were found within acceptable range for freshwater fish rearing[26,36]. Though survival of *O. goramy* was higher in freshwater (0.0 ppt) in the study, the highest growth was achieved in 4.0 ppt. Salinity beyond 12.0 ppt was not suitable for growth of *O. goramy*. However, for growth of *O. goramy* more tolerance to salinity compared than *Cyprinus carpio*[6]. It was suggested that *O. goramy* can be cultured in brackish water (up to a salinity of 12.0 ppt), although better production, individual size and survival of the stock were observed at a salinity of 4 ppt. Nirmala and Rasmawan[37] indicated that juvenile of *O. goramy* grew more rapidly in slight brackish water (<3.0 ppt) when compared to more brackish water up to 9.0 ppt. In contrary to the present investigation[38] demonstrated that *Oreochromis niloticus* were able to grow in salinity up to 30.0 ppt with highest growth achieved at salinity between 0.0 ppt and 20.0 ppt. Specific growth rates showed a steady enhancement with the relative increase in total biomass as the *O. goramy* become larger (Fig. 3). Noteworthy observation in this trial is that after 30 days, SGR raised at lower salinity levels (Fig. 3) revealing that *O. goramy* were acclimatising and recovering stress in lower salinity as the culture period progressed.

The freshwater fish in culture on the salinity media need the process of osmoregulation to overcome changes in salinity, especially in the brackish water. In this study, *O. goramy* hyper osmoregulated when salinity was above its iso-osmotic point. This statement support by Li *et al*.[39] and Garcia-Santos *et al*.[40] that freshwater animals winch live in different degrees of salinity will maintain their body fluids for survival. Siira *et al*.[41] reported that the major precondition for a successful selective fishery is that released fish would survive and continue their normal physiological functions. The physiological functions are generally influenced by environmental variables such as water salinity in fish growth[3,6].

This study indicate that the lower salinity had better growth. However, it is important to emphasize that the goal of this study was not to test crowding stocking situations for *O. goramy*, since no previous information is available for this species, so even the higher salinity can be considered low when compared with studies with other fish species. Therefore, additional experiments to evaluate these physiological parameters, survival and growth performance and bio-economic analysis in aquaculture operations of *O. goramy* at field trials very important.

CONCLUSION

The results of the present experiments indicated that salinity plays a significant role in the culture of *O. goramy* and this species showed satisfactory growth and survival at salinity range (0.0-12.0 ppt). In view of in Indonesia, there are many abandoned shrimp ponds and more coastal areas of Indonesia are going to be vulnerable to brackish water inundation. Under such scenario, *O. goramy* can be considered as an ideal species to promote. Noteworthy this conclusion has significant implications for *O. goramy* aquaculture, as it can be utilized in farm site selection and salinity maintenance to maximize commercial productivity in coastal area.

SIGNIFICANCE STATEMENT

This study discover that *Osphronemus goramy* has tolerance for survival and growth up to 12.0 ppt salinity. This study will help the researcher to uncover that *O. goramy* which culture on salinities 4.0-8.0 ppt have the survival and higher growth rate which previous research were not able to explore. Thus, the new experiments have advantage for aquaculture operation of *O. goramy* in brackish water, in view of in Indonesia, there are many abandoned shrimp ponds.

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