

TILAPIA AND KOI (CLIMBING PERCH) POLYCULTURE WITH *PANGASIVS* CATFISH IN BRACKISH (HYPOSALINE) WATERS OF SOUTHERN BANGLADESH

Climate Change Adaptation: Indigenous Species Development/Experiment/16IND02NC

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ABSTRACT

Pangasius catfish (*Pangasius hypophthalmus*), tilapia (*Oreochromis niloticus*), and freshwater perch (*Anabas testudineus*; locally known as Koi) are three of the most successful freshwater aquaculture species in Bangladesh. We previously established that *Pangasius* can be cultured in hyposaline waters, allowing for the use of coastal waters affected by salinity encroachment. In the present study, we assessed the potential for culturing *Pangasius* with the higher valued tilapia and freshwater Koi in hyposaline waters of coastal Bangladesh as a means of improving the food security and economic viability of these salinity affected region's poor communities. Two experiments were performed to determine the feasibility of Tilapia and Koi (climbing perch) polyculture with *Pangasius* catfish in hyposaline waters. These experiments assessed: 1) production performance and economic impacts of tilapia and Koi polycultured with *Pangasius* in brackish waters, and 2) the effect of stocking density on growth performance of tilapia and *Pangasius* in polyculture.

The first experiment was conducted in 12 farmer's ponds in the Patuakhali district of Bangladesh at a salinity of 5-6 ppt. Four culture types were examined with fish being fed commercial pellet feed (CP 28-30%) twice daily at a total rate of 10% down to 3% body weight per day. The study revealed that Koi can be cultured in saline waters as high as 6 ppt and that the highest yields could be obtained through solo culture of *Pangasius* (T1). However, due to the higher market value of tilapia and greater increases in body weight of both *Pangasius* and tilapia, the largest profit came from the co-culture of these two species (T2). This was followed by co-culture of *Pangasius* with Koi (T3). Conversely, the lowest yield and profit resulted from the culture of all three species together (T4). Thus, the results suggest that tilapia is the optimal candidate for polyculture with *Pangasius* in hyposaline waters with *Pangasius*-Koi polyculture providing the second greatest profit margin. Adoption of such aquaculture practices in coastal Bangladesh could increase earnings and improve the livelihoods of the people inhabiting these areas.

The second experiment was carried out to evaluate the effects of 3 different stocking densities on growth performance of *Pangasius* and tilapia (*O. niloticus*) in polyculture. In treatment 1 (T1), *Pangasius* and tilapia were stocked at a density of 2.5/m² and 2/m², respectively. In treatment 2 (T2), *Pangasius* and tilapia were stocked at a density of 2/m² and 2.5/m², respectively. Lastly in treatment 3 (T3), *Pangasius* and tilapia were stocked at a density of 1.5/m² and 3/m², respectively. No significant differences (P>0.05) were observed in survival rate, feed conversion ratio (FCR), or weight gain of tilapia among the treatments, however the size and overall production yields of *Pangasius* declined as the stocking density of tilapia increased. Thus, significantly higher production was observed in T1

(16,320 kg/ha), followed by T2 (21,069.05 kg/ha) and T3 (18,098.54 kg/ha). Net profit was greatest in T1 (6,751 USD/ha) than in T2 (5,798 USD/ha) and T3 (4,127 USD/ha). These results indicate that high stocking densities of tilapia are not suitable for polyculture with *Pangasius*. A higher stocking density of *Pangasius* (2.5/m²) and lower stocking density of tilapia (2/m²) is more suitable and economically profitable for *Pangasius* polyculture.

INTRODUCTION

The focus of this investigation was to assess the potential for expanding the culture of economically important finfish traditionally grown in fresh water to the hyposaline waters of coastal South and Southwest Bangladesh that are now significantly contaminated by saline waters. The regions are severely impacted by overfishing and with seawater encroachment and more frequent storms linked to global climate change, they are currently underutilized for fish production. Lands affected by salinity have increased from 83.3 million hectares in 1973 to 105.6 million hectares in 2009 and continue to rise rapidly (SRDI, 2010). GIS mapping shows 12 districts in Southwest Bangladesh alone are affected by salinity encroachment (Neogi, 2012). The gradual salinity intrusion in the coastal area of Bangladesh is damaging natural environments and threatening coastal biodiversity, aquaculture/agricultural production, and food security, all of which have negatively impacted the socioeconomic condition of fishers and farmers that are already among the poorest in the country.

There are several ways to mitigate the salinity problem in these areas and promote seafood production. One approach is to increase culture of marine species. However, mariculture requires considerable investment in infrastructure that is currently lacking and production of marine species is more difficult than that of freshwater fishes due, in part, to the greater complexity of larval rearing. A second approach is to promote the production of fishes that are already cultured in the country and that may tolerate hyposaline environments. The proposed research seeks to develop and establish polyculture technologies for growing *Pangasius* catfish (striped catfish, *Pangasius hypophthalmus*), Koi (climbing perch, *Anabas testudineus*), and Nile tilapia (*Oreochromis niloticus*) in hyposaline waters endemic to Southern Bangladesh.

Tilapia (*O. niloticus*) was introduced to Bangladesh over 30 years ago and is now one of the fastest growing components of the aquaculture sector, ranking 2nd to carps in total finfish production (*Pangasius* is a close 3rd; Belton et al., 2011; DoF, 2015). While its production is primarily limited to freshwater environments, tilapia possess various characteristics that make them desirable species to culture in brackish water. They are hardy, amenable for growth in numerous culture systems, and euryhaline. They can live and readily reproduce in salinities as high as 30 ppt depending on the species or strain (for review see Suresh and Lin, 1992; El-Sayed, 2006a). Nile tilapia grow well in salinities as high as 25 ppt and evidence suggest they may grow better at 5-10 ppt than in fresh water (Payne and Collinson, 1983; Suresh and Lin, 1992). Development of their culture in saline waters has received considerable attention in Asia (Dennis et al., 2004) and the growing number of Bangladesh tilapia hatcheries and availability of seed stock readily allow for integration of tilapia into brackish water farming.

Koi culture in Bangladesh has developed considerably in recent years due to its good growth, wide acceptance, and appealing taste (Kohinoor et al., 2011; DoF, 2015). Accordingly, it now constitutes 16% of farmed fish consumed by Bangladeshis (Apu, 2014). It is a hardy, air-breathing fish capable of living in low oxygen environments (Hasan et al., 2007) and recent evidence suggests Koi larvae can be raised in low salinity waters (Nadirah et al., 2014). Additionally, in short term tank trials, Koi were shown to tolerate and grow in salinities as high as 10 ppt with little impact on growth or feed conversion (Chotipuntu and Avakul, 2010; Chowdhury et al., 2014) raising the possibility that these fish could be cultured in hyposaline waters. These studies assessed for the first time the potential of culturing Koi to market size in brackish water ponds.

Pangasius catfish was introduced to Bangladesh in the 1990's, and since then it has become a thriving aquaculture industry with over 300,000 tonnes produced annually (Ali et al., 2013; Edward and Hossain, 2010; Munir, 2009). Currently, much of the *Pangasius* production comes from the North and Central regions of Bangladesh (e.g., greater Mymensingh). In these regions, *Pangasius* are cultured both intensively with commercial feeds, semi-intensively (with more limited feed), and in extensive (no feed) polyculture with both tilapia and carp (Ahmed et al., 2010). High disease resistance, along with high stocking density with greater production rates (up to 120 fish /m², average 40 tonnes / ha; UNFAO, 2010), make *Pangasius* an ideal cultivar for increasing aquaculture production in Bangladesh, particularly in regions unfamiliar with farming this species, as well as reducing the burden of population growth. The greater Barishal district is one such region, which has traditionally relied on fishing or aquaculture of marine species (e.g., shrimp) for their economic livelihoods. Through over-fishing, increased shrimp disease, and the increasing frequency of natural calamities like cyclones (e.g. Sidr, Aila), this region is nearing depletion of wild fish stocks and currently over half a million fishermen have been suffering from severe poverty. Introducing *Pangasius* as well as tilapia and Koi cultures to these coastal communities, whose water resources are largely underutilized, could enhance the dietary consumption of protein and other nutrients for low-income families, as well as provide new sources of income and employment to the area through backward and forward linkage to the value chain. In phase I of our AquaFish Innovation Lab project, we firmly established for the first time that *Pangasius* could be cultured in hyposaline ponds with salinities as high as 12 ppt with similar growth and yield as that found in freshwater pond culture (Ali et al., 2015). Further, implementation of formulated feeds and an increase in stocking densities from 2 to 3 fish/m² improved profits and production yield of fishes, respectively.

Here, we expanded upon this new technology to incorporate Nile tilapia and Koi in brackish water polyculture with *Pangasius*. Both tilapia and Koi command higher prices with the market value of Koi exceeding *Pangasius* by 3 times (250-300 BDT/kg vs 80-100 BDT/kg) (Apu, 2014). Implementation of tilapia in polyculture could prove beneficial as these animals readily feed on natural productivity of ponds (plankton, plants) and on supplementary feed resulting in better utilization of resources for enhancing fish yield (Egna and Boyd, 1997; El-Sayed, 2006b). Their incorporation into *Pangasius* culture may prove more beneficial for enhancing income while also providing a more diverse crop of fishes for consumption and sale. The present research first assessed the growth and production of tilapia and Koi alone and together in polyculture with *Pangasius* in hyposaline ponds and then assessed if increasing their density could further improve fish production with the aim of enhancing the diet and economic viability of coastal communities in Southern Bangladesh.

OBJECTIVES

1. Evaluate if freshwater Koi (climbing perch) can be successfully cultured in seawater-encroached hyposaline waters of coastal Southern region of Bangladesh.
2. Assess production performance and economic impacts of tilapia and Koi polycultured with *Pangasius* in brackish waters.
3. Assess effect of increased stocking density of tilapia and Koi in brackish water *Pangasius*-Koi-tilapia polyculture.

MATERIALS AND METHODS

Location

Experiments were performed at Kalapara Upazila of the Patuakhali district, and area affected by salinity encroachment in Southern Bangladesh. Water quality analysis was conducted at Patuakhali Science & Technology University.

Feeding

Fish were fed a Mega floating feed (Lily Brand tilapia feed with CP 28-30%) at an initial rate of 10% bw/day down to 3% bw/day. Feed was applied twice daily in the morning and evening by hand. The amount of feed used was recorded for the calculation of feed conversion ratios (FCR).

Study 1- Assess production performance and economic impacts of tilapia and Koi polycultured with *Pangasius* in brackish waters

The study was conducted from March to July 2017 and consisted of a *Pangasius* monoculture (T1), a *Pangasius*-tilapia polyculture (T2), a *Pangasius*-Koi polyculture (T3), and a *Pangasius*-tilapia-Koi polyculture (T4). Ponds were stocked with fingerlings at the densities shown in Table 1 and each treatment was replicated in 3 separate ponds. Fingerlings were obtained from Sharnalata Hatchery at Trishal Upazilla in Mymensingh district with average initial weights of 6.5 g, 0.5 g, and 0.5 g for *Pangasius*, Nile tilapia, and Koi, respectively. Water quality parameters (e.g. temperature, dissolved oxygen, salinity, pH, alkalinity, ammonia) were assessed fortnightly to ensure they remained within the range appropriate for fish culture. Ten fish from all ponds were sub-sampled fortnightly for growth measurements (length and weight) and to adjust feeding rate. At the end of the 150-day culture period, all fish were harvested to determine growth and survival and an economic (cost-benefit) analysis was performed using seasonal market prices for each species (*Pangasius*- 90 BDT/kg; tilapia- 120 BDT/kg; Koi- 250 BDT/kg).

Table 1. Experimental design for Study 1.

Parameter	Treatment 1	Treatment 2	Treatment 3	Treatment 4
<i>Pangasius</i> (fish/dec)	160 (4.0/m ²)	80 (2.0/m ²)	80 (2.0/m ²)	80 (2.0/m ²)
Tilapia (fish/dec)	-	80 (2.0/m ²)	-	40 (1.0/m ²)
Koi (fish/dec)	-	-	80 (2.0/m ²)	40 (1.0/m ²)
Salinity range (ppt)	5-6	5-6	5-6	5-6
Replication	3	3	3	3

Study 2- Effect of stocking density on growth performance of tilapia and *Pangasius* in polyculture

In Study 1 we determined that *Pangasius*-tilapia polyculture was most profitable and thus we evaluated which stocking densities would provide the greatest returns. The experiment was conducted from 9th October 2017 to 9th May 2018. *Pangasius* (1.36 g) and sex-reversed Nile tilapia (0.12 g) fry obtained from Sharnalata Hatchery at Trishal Upazila in Mymensingh district were stocked at the densities indicated in Table 2 and each treatment was replicated in 4 separate ponds. Water quality was assessed and ten fish from each pond were sub-sampled for growth measurements every 15 days. At the end of the 150-day culture period, all fish were harvested for growth and survival and an economic (cost-benefit) analysis was performed using seasonal market prices for each species (*Pangasius*- 120 BDT/kg; tilapia- 130 BDT/kg).

Table 2. Experimental design for Study 2.

Parameter	Treatment 1	Treatment 2	Treatment 3
<i>Pangasius</i> (fish/dec)	100 (2.5/m ²)	80 (2/m ²)	60 (1.5/m ²)
Tilapia (fish/dec)	80 (2.0/m ²)	100 (2.5/m ²)	120 (3.0/m ²)
Salinity range (ppt)	5-8	5-8	5-8
Replication	4	4	4

Equations

$$\text{Survival Rate (\%)} = \frac{\# \text{ fish harvested}}{\# \text{ fish stocked}} \times 100$$

$$\text{Specific Growth Rate (\% bw day}^{-1}\text{)} = \frac{(\text{Log e [final weight]} - \text{Log e [initial weight]})}{\text{Time (final)} - \text{Time (initial)}} \times 100$$

$$\text{Food conversion ratio} = \frac{\text{Amount of food given (dry weight)}}{\text{Fish weight gain}}$$

Statistical Analysis

One-way analysis of variance (ANOVA) with a Duncan's New Multiple Range posthoc test was used to compare all water quality, growth, and production parameters between treatment groups (SPSS 11.5 software). Data are represented as means \pm standard deviation.

RESULTS AND DISCUSSION

Study 1- Assess production performance and economic impacts of tilapia and Koi polycultured with *Pangasius* in brackish waters

This study evaluated whether the introduction of *Pangasius* catfish to Koi and tilapia cultures in hyposaline waters could enhance production yields and earnings for farmers in Southern Bangladesh. Water quality was evaluated throughout the experimental period. Values for water temperature, dissolved oxygen, pH, alkalinity, ammonia, hardness, nitrites, and salinity were similar between treatments and within the appropriate range for fish culture (Table 3; Bhatnagar and Devi, 2013). Feed conversion ratios were also similar between treatments and at an appropriate level for fish culture (Table 4).

Gross production (kg/ha) was significantly greater in *Pangasius* monoculture (T1) compared to the 3 types of polyculture due to the larger size of this species (Table 4). However, as tilapia and Koi fetch a greater market value and require less feed input due to their smaller size, T2 (*Pangasius*-tilapia) provided the greatest net return at \$6260 USD/ha followed by T3 (*Pangasius*-Koi) at \$4127 USD/ha (Figure 1). *Pangasius* had significantly higher specific growth rates when cultured with tilapia which could increase the amount of food available for the farmers' families (Table 4). Polyculture of all 3 species together (T4) had the lowest overall production yield and profits, possibly due to the smaller size of Koi or an antagonistic effect between species that lead to slightly lower survival rates in this treatment (Table 4). These results indicate that polyculture of *Pangasius* and tilapia is the best option for enhancing the diets and incomes of farmers whose land has been affected by salinity encroachment in Southern Bangladesh, with *Pangasius*-Koi polyculture providing the second greatest profit margin.

Study 2- Effect of stocking density on growth performance of tilapia and *Pangasius* in polyculture

After determining in Study 1 that tilapia is a better candidate for polyculture with *Pangasius*, we sought to determine the optimal stocking densities to further enhance production and earnings. Water quality was deemed suitable throughout the study period and did not vary significantly between treatments (Table 5).

Growth and production parameters are presented in Table 6. The feed conversions ratios did not vary between treatments and were within the appropriate range for fish aquaculture. Survival, growth rates, and gross production (kg/ha) of tilapia did not vary between treatments. However, the final weights and gross production of *Pangasius* was significantly greater when the stocking density of tilapia was lower. Thus, T1 exhibited the greatest overall yield (16,320 kg/ha), followed by T2 (14,318 kg/ha), and T3 (12,170 kg/ha). Despite the increased production costs resulting from the higher feed requirement of *Pangasius*, T1 also provided the greatest net return at \$6,751 USD/ha (Figure 2) albeit

the benefit to cost ratio was similar between T1 (1.39) and T2 (1.37), which had a net profit of \$5,798 USD/ha.

The results of this experiment revealed that high stocking densities of tilapia are not suitable for polyculture with *Pangasius*. As such, polycultures employing a higher stocking density of *Pangasius* (2.5/m²) and lower stocking density of tilapia (2/m²) are more suitable and economically profitable for fish farmers in Southern Bangladesh.



CONCLUSIONS

This investigation revealed that Koi can be successfully cultured in hyposaline waters of up to 6 ppt. Further, we showed that Nile tilapia are the optimal candidate for polyculture with *Pangasius* catfish as this combination yielded the highest overall production and net returns. Koi also proved to be an acceptable candidate for polyculture with *Pangasius*, yielding a greater profit margin than *Pangasius* monoculture, however antagonistic effects were observed when all 3 species were cultured together. The adoption of *Pangasius* polycultures with tilapia or Koi could increase household food availability and earnings for farmers inhabiting areas affected by salinity encroachment in Southern Bangladesh.

The optimal stocking densities for *Pangasius*-tilapia polyculture were also evaluated and the results indicate that higher densities of tilapia have detrimental effects on the growth of *Pangasius*. Thus, it is recommended that these species be stocked at a ratio of 2.5 *Pangasius* to 2.0 tilapia for pond cultures using hyposaline waters.

ADDITIONAL INFORMATION

Training

Two MS graduate students were trained under this project. Additionally, as part of Investigation 16MNE02NC two short term training workshops on *Pangasius*, Tilapia and Koi culture in hyposaline waters were organized. Approximately 100 farmers received training on the new aquaculture production technology.

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TABLES AND FIGURES

Table 3. Average water quality parameters for Study 1. Data are means \pm SD.

	<i>Treatment 1</i>	<i>Treatment 2</i>	<i>Treatment 3</i>	<i>Treatment 4</i>
Water Temperature ($^{\circ}\text{C}$)	30.18 \pm 0.95	30.10 \pm 0.42	30.52 \pm 0.4	30.22 \pm 0.6
Dissolved Oxygen (mg/L)	5.16 \pm 0.12	5.24 \pm 0.13	5.23 \pm 0.21	5.33 \pm 0.11
pH	7.77 \pm 0.37	7.49 \pm 0.26	7.67 \pm 0.14	7.77 \pm 0.24
Alkalinity (mg/L)	90.5 \pm 6.12	91.5 \pm 6.61	94 \pm 5.15	93 \pm 5.85
Ammonia (mg/L)	0.67 \pm 0.02	0.64 \pm 0.03	0.71 \pm 0.03	0.73 \pm 0.05
Nitrites (mg/L)	0.37 \pm 0.02	0.24 \pm 0.23	0.29 \pm 0.28	0.28 \pm 0.08
Hardness (mg/L)	560 \pm 4.12	540 \pm 5.12	534 \pm 4.92	542 \pm 4.72
Salinity (ppt)	5.50 \pm 0.15	5.7 \pm 0.12	5.5 \pm 0.13	5.9 \pm 0.17

Table 4. Growth and production parameters for Study 1. Data are means \pm SD. Different letters indicate significant differences (One-way ANOVA; $P < 0.05$).

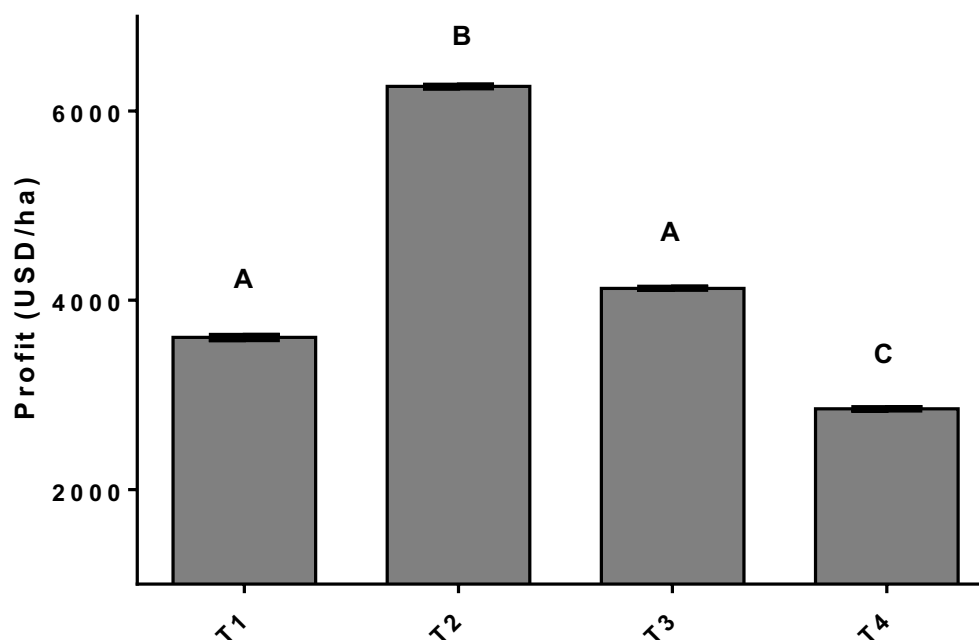
	<i>Treatment 1</i>	<i>Treatment 2</i>	<i>Treatment 3</i>	<i>Treatment 4</i>
Pangasius				
Weight (g)	637 \pm 6.4 ^a	687 \pm 6.6 ^b	646 \pm 8.1 ^a	672 \pm 7.6 ^c
Survival Rate (%)	94.38 \pm 2.76 ^a	94.58 \pm 2.98 ^a	94.17 \pm 2.12 ^a	92.50 \pm 2.33 ^a
Specific Growth Rate (% bw d ⁻¹)	3.05 \pm 0.19 ^a	3.11 \pm 0.32 ^b	3.07 \pm 0.23 ^a	3.09 \pm 0.12 ^{ab}
Gross Production (kg/ha)	22,292 \pm 141	12,837 \pm 62	12,065 \pm 52	6,138 \pm 87
Tilapia				
Weight (g)	NA	282 \pm 6.1	NA	276 \pm 4.2
Survival Rate (%)	NA	90.83 \pm 2.13	NA	87.5 \pm 2.23
Specific Growth Rate (% bw d ⁻¹)	NA	4.23 \pm 0.24	NA	4.21 \pm 0.17
Gross Production (kg/ha)	NA	5,064 \pm 123	NA	2,431 \pm 12
Koi				
Weight (g)	NA	NA	169 \pm 6.5	123 \pm 4.9
Survival Rate (%)	NA	NA	87.92 \pm 2.76	85.83 \pm 2.76
Specific Growth Rate (% bw d ⁻¹)	NA	NA	3.89 \pm 0.12	3.67 \pm 0.10
Gross Production (kg/ha)	NA	NA	2,980 \pm 53	1,022 \pm 13
Total				
Feed Conversion Ratio	1.35 \pm 0.04	1.30 \pm 0.05	1.33 \pm 0.08	1.33 \pm 0.06
Gross Production (kg/ha)	22,292 \pm 141 ^a	17,901 \pm 66 ^b	15,045 \pm 73 ^c	9,590 \pm 65 ^d
Gross Return (USD/ha)	23,884 \pm 123 ^a	23,441 \pm 56 ^a	21,726 \pm 65 ^b	13,089 \pm 59 ^c
Cost (USD/ha)	20,277 \pm 112 ^a	17,181 \pm 45 ^b	17,599 \pm 56 ^b	10,089 \pm 49 ^c
Net Return (USD/ha)	3,607 \pm 33 ^a	6,260 \pm 24 ^b	4,127 \pm 21 ^a	2,853 \pm 23 ^c
Benefit Cost Ratio	1.17	1.36	1.23	1.29

Table 5. Water quality parameters for Study 2. Data are means \pm SD.

	<i>Treatment 1</i>	<i>Treatment 2</i>	<i>Treatment 3</i>
Water Temperature ($^{\circ}\text{C}$)	28.58 \pm 0.25	28.34 \pm 0.32	28.52 \pm 0.41
Dissolved Oxygen (mg/L)	5.76 \pm 0.12	5.84 \pm 0.13	5.91 \pm 0.21
pH	8.17 \pm 0.37	8.29 \pm 0.26	8.27 \pm 0.14
Alkalinity (mg/L)	145.5 \pm 6.15	140 \pm 5.61	142 \pm 4.15
Ammonia (mg/L)	0.27 \pm 0.02	0.34 \pm 0.03	0.29 \pm 0.03
Hardness (mg/L)	240 \pm 6.12	235 \pm 7.12	242 \pm 6.92
Salinity (ppt)	5.22 \pm 0.62	5.28 \pm 0.87	5.17 \pm 0.51

Table 6. Growth and production parameters for Study 2. Data are means \pm SD. Different letters indicate significant differences (One-way ANOVA; $P < 0.05$).

	<i>Treatment 1</i>	<i>Treatment 2</i>	<i>Treatment 3</i>
<i>Pangasius</i>			
Weight (g)	501 \pm 68 ^a	492 \pm 18 ^b	443 \pm 44 ^c
Survival Rate (%)	95.38 \pm 3.16	94.58 \pm 3.06	94.17 \pm 3.23
Specific Growth Rate (% bw d ⁻¹)	3.01 \pm 0.09	3.05 \pm 0.11	3.07 \pm 0.13
Gross Production (kg/ha)	11,806 \pm 121 ^a	9,193 \pm 109 ^b	6,184 \pm 116 ^c
<i>Tilapia</i>			
Weight (g)	233 \pm 63	229 \pm 22	225 \pm 54
Survival Rate (%)	90.13 \pm 3.16	90.83 \pm 3.16	89.92 \pm 3.16
Specific Growth Rate (% bw d ⁻¹)	4.43 \pm 0.12	4.23 \pm 0.14	3.89 \pm 0.11
Gross Production (kg/ha)	4,514 \pm 91 ^a	5,124 \pm 85 ^b	5,986 \pm 79 ^c
Total			
Feed Conversion Ratio	1.35 \pm 0.06	1.30 \pm 0.04	1.33 \pm 0.07
Gross Production (kg/ha)	16,320 \pm 135 ^a	14,318 \pm 128 ^b	12,170 \pm 132 ^c
Gross Return (USD/ha)	23,853 \pm 132 ^a	21,069 \pm 114 ^b	18,098 \pm 127 ^c
Cost (USD/ha)	17,102 \pm 112 ^a	15,271 \pm 112 ^b	13,971 \pm 112 ^c
Net Return (USD/ha)	6,751 \pm 31 ^a	5,798 \pm 43 ^b	4,127 \pm 38 ^c
Benefit Cost Ratio	1.39	1.37	1.29

**Figure 1.** Net returns (USD/ha) for the culture of *Pangasius*, tilapia, and Koi in Study 1. T1, *Pangasius* monoculture; T2, *Pangasius*-tilapia polyculture; T3, *Pangasius*-Koi polyculture; T4, *Pangasius*-tilapia-Koi polyculture.

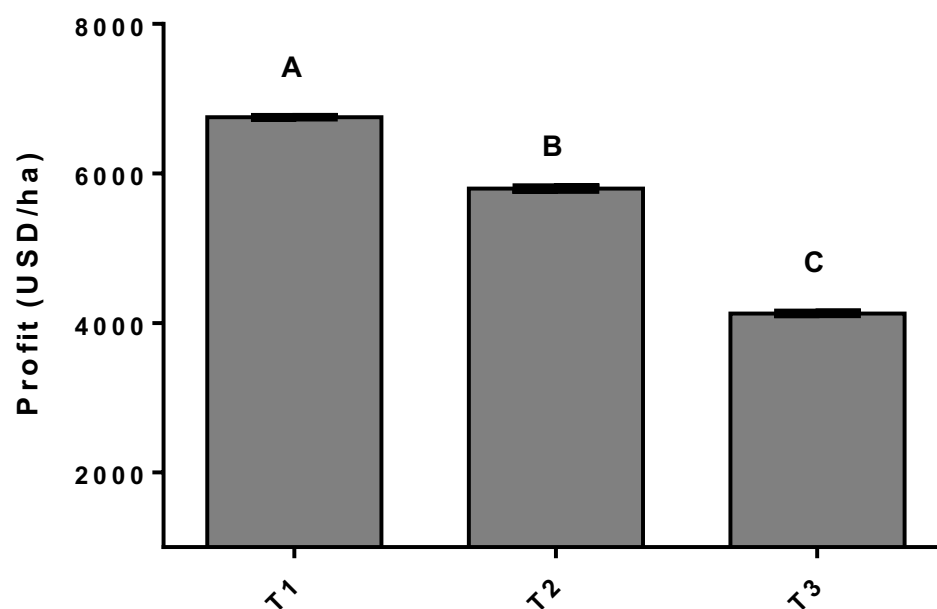


Figure 2. Net returns (USD/ha) for the culture of *Pangasius* and tilapia at different stocking densities in Study 2.