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

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

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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.

Significance

The focus of this investigation is 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 are rising 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; 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 to 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 in 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 hypopthalamus*), Koi (climbing perch, *Anabas testudineus)*, and Nile tilapia (*Oreochromis niloticus*) in hyposaline waters endemic to Southern Bangladesh.

Tilapia (*Oreochromis 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). Its production is primarily limited to freshwater environments. Tilapias 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). The 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 Bangladeshi's (Apu, 2014). It is a hardy, air-breathing fish capable of living in low oxygen environments (Hasan et al., 2007). Recent evidence suggests Koi larvae can be raised in low saline 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 will assess the first time the potential of culturing Koi to market size in brackish water ponds.

Pangasius catfish was introduced to Bangladesh in 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 /m^2, 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 aquaculture to these coastal communities, whose water resources are largely underutilized, could enhance the dietary consumption of protein for low-income families, as well as provide new sources of income and employment in an 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 with freshwater pond culture (Ali et al., 2015). Implementation of formulated feeds and an increase in stocking densities from 2 to 3 fish/m^2 improved profits and production yield of fishes, respectively.

In the proposed studies we wish to expand 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, 2006). There 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 will first assess the growth and production of tilapia and Koi alone and together in polyculture with *Pangasius* in hyposaline brackish water ponds and will then assess if increasing their density might further improve fish production. It is anticipated if *Pangasius*-tilapia-Koi polyculture can be achieved in the Southern coastal region of Bangladesh, the

production levels of these fishes could effectively double, which would significantly impact the diet and economic viability of coastal communities.

Quantified Anticipated Benefits

1. We anticipate that Koi can be cultured to market size in hyposaline, brackish water ponds.
2. Polyculture of tilapia and Koi with *Pangasius* catfish will improve economic returns over *Pangasius* monoculture alone.
3. Increasing the stocking densities of Koi and/or tilapia relative to *Pangasius* will enhance efficiency and return on investments in polyculture production of the three species.
4. Polyculture of tilapia, Koi and *Pangasius* catfish in brackish waters of Southern Bangladesh has the potential to double annual aquaculture production and will increase the diversity of aquaculture species produced and the dietary nutrition available to farming households.
5. We anticipate significant improvements in environmental water quality in the tilapia and koi polycultured with *Pangasius*.
6. We anticipate that successful development of this project will increase livelihood options and better food-security for low-income families impacted by overfishing and rises in sea level (global climate change).

Research Design and Activity Plan

Location

These studies will be performed in ponds of participating farmers in the Pauakhali district of the greater Barishal region of Southern Bangladesh.

Methods

Experiment 1. Evaluate if freshwater Koi (climbing perch) can be successfully cultured in seawater-encroached hyposaline waters of coastal Southern region of Bangladesh. Assess production performance and economic impacts of tilapia and Koi polycultured with Pangasius in brackish waters.

Null Hypothesis 1: No differences in growth efficiency, water quality, yields or economic returns are observed with Pangasius cultured together with koi or tilapia or both.
Null Hypothesis 2: No differences in growth efficiency, water quality, yields or economic returns are observed with Pangasius cultured alone versus Pangasius polycultured together with either koi, tilapia or both.

Based on our previous work we showed that *Pangasius* can be cultured in salinities as high as 10-12 ppt and that growth and production yield is similar to that of fish cultured in 5-8 ppt or in 0-0.5 ppt (freshwater). We also found that increasing the density of *Pangasius* to 3 fish/m^2 increased overall yield of fish, but caused only a mild change in return on investment. Here we will utilize 4 fish/m^2 in monoculture and test if addition of Nile tilapia or Koi alone or a combination of the two with *Pangasius* might enhance the efficiency and economic return of fishes in polyculture (fish densities will be similar for all groups). This experiment will also establish if Koi can be grown to market size in hyposaline waters. All fish will be cultured at a range of 5-8 ppt, endemic to the coastal region in Barishal and that reflects extrinsic fluctuations in salinity across the growing season. The experimental design is as follows:

Parameter	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Pangasius (fish/decimal)	160 (4.0/m^2)	80 (2.0/m^2)	80 (2.0/m^2)	80 (2.0/m^2)
Tilapia (fish/decimal)	-	80 (2.0/m^2)	-	40 (1.0/m^2)
Koi (fish/decimal)	-	-	80 (2.0/m^2)	40 (1.0/m^2)
Salinity range (ppt)	5-8	5-8	5-8	5-8

Feeding	Std. regime	Std. regime	Std. regime	Std. regime
Replication	4	4	4	4

Ponds will be selected on the basis of the surface water salinities from participating farmers in Patuakhali district. WorldFish has aided us in selection of farmers and will continue to do so. We will target women in our studies and anticipate that >50% of the pond operators will be women based on our previous studies. Ponds of ~400 m^2 will be dried, eliminated of unwanted species and then limed at 2.5 g/m^2 with CaCO3 according to standard practices. They will be filled with adjacent surface water and fertilized one week prior to stocking (28 kg N; 7 kg P/ ha).

Pangasius, Koi, and sex-reversed Nile tilapia fingerlings (~10 g) will be stocked in all ponds for a 160-day growout period. Fish will be fed a Mega floating feed (28% CP, 7% Fat; Spectra Hexa Feeds, Ltd.) at the rate of 10% of bw/day down to 3% bw/day. Feed will be applied twice daily at 9.00 and 16.00.

Fortnightly subsampling of the experimental fish will be done using a cast net to measure growth performance of experimental fish and to adjust feeding rate. After completion of the growth trial, a subsample of fish weights and lengths and the total number of fish harvested for each pond will be determined for assessing survival rates, total yield, and final weights and lengths.

Water quality parameters including water temperature, dissolved oxygen, pH, alkalinity, hardness, ammonia, nitrite, and salinity will be measured fortnightly.

Production parameters including % weight gain, specific growth rate, and feed conversion will be calculated. Production yields (market weight, kg), estimated market returns, feed input costs (feed, fertilizers, labor, fingerlings), and labor costs will be gathered for all treatment groups at the end of experiment for marginal cost-benefit analysis.

All treatments will be tested for significant differences in growth (mean length, weight X time), growth efficiency (specific growth rate, feed conversion ratio), yield and water quality using Analysis of Variance ($p < 0.05$; preplanned contrasts).

Experiment 2. Assess effect of increased stocking density of tilapia and Koi in brackish water Pangasius-Koi-tilapia polyculture.
Null Hypothesis: No improvement in growth, water quality, yield or economic returns is observed by increasing the proportion of tilapia, Koi or both in polyculture with Pangasius.

We expect that that polyculture of either tilapia (Treatment 2, T2), or Koi (Treatment 3, T3) or both (Treatment 4, T4) with *Pangasius* from Experiment 1 will enhance economic returns over *Pangasius* monoculture, primarily because of the higher value of tilapia and Koi. It is also likely that tilapia will utilize the natural productivity in ponds, which could increase overall yields or food conversion efficiency while improving water quality. We will take the best result from Treatments 2-4 of the Experiment 1, and conduct a second experiment to determine if increasing the proportion of either tilapia, koi, or both relative to *Pangasius* might further improve production efficiency and economic returns. Ponds (N = 15, 5 replicates per treatment) from operators used in Experiment 1 will be used. Total stocking densities of fishes will remain the same among groups, but the proportion of tilapia or Koi or their combination will be increased. The experiment design is as follows:

Parameter	Treatment 1	Treatment 2	Treatment 3
Pangasius (fish/decimal)	100 (2.0/m^2)	80 (1.5/m^2)	60 (1.0/m^2)
Best of T2-T4 in Experiment 1 (fish/decimal)	80 (2.0/m^2)	100 (2.5/m^2)	120 (3.0/m^2)
Salinity range (ppt)	5-8	5-8	5-8
Feeding	Std. regime	Std. regime	Std. regime
Replication	5	5	5

The preparation and fertilization of ponds and feeding regimen for fishes will be performed as described in Experiment 1.

Growth and water quality measurements will be collected as described previously, with food amounts recorded daily for economic analysis. All treatment groups will be tested for significant differences in growth (mean length and weight), growth efficiency (specific growth rates, feed conversion ratio), and water quality using ANOVA. A marginal cost-benefit analysis will be determined incorporating total production yields (kg), expected market returns, feed and labor costs for these treatments.

Trainings and Deliverables

1. Two students will receive training on tilapia and koi polyculture with *Pangasius* in hyposaline waters and its economic impacts as part of their MSc thesis work.
2. The findings from these studies will be documented through the Technical Reports of the AquaFish Innovation Lab (FIR).
3. The work will be presented at Patuakhali Science & Technology University workshop (in Investigation 5), and may also be presented at a regional Aquaculture Conference.
4. If the technologies for tilapia-koi polyculture with *Pangasius* in hyposaline prove effective, then results will also be disseminated through production of an extension factsheet in the local language for wider outreach to farmers, extension agencies of the government, and NGOs (in Investigation 5). A paper reflecting the results should they be promising will also be prepared for publication in a peer-reviewed journal.

Schedule

June 2016 to February 2017: Experiment 1; Assess production performance and economic impacts of tilapia and Koi polycultured with *Pangasius* in brackish waters. Complete analyses and preparation of report.

April 17 to December 2017: Experiment 2; Effects of stocking density on growth performance of *Pangasius* and Tilapia/ Climbing perch in hyposaline water polyculture pond.

January 2018 to February 2018: Complete Final Technical Report and begin preparing manuscript.