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Evaluation of Integrated Multi-Trophic Aquaculture System for Rearing of Grass carp, Prawns and Freshwater Mussels in a Freshwater Reservoir

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ABSTRACT: India has vast potential in the form of freshwater reservoirs and there is a need to diversify aquaculture from the traditional aquaculture of carps and catfishes. Hence, this study was conducted to evaluate the feasibility of rearing grass carp (*Ctenopharyngodon idella*), giant freshwater prawn (*Macrobrachium rosenbergii*) and freshwater mussel (*Lamellidens marginalis*) in an integrated multi-trophic aquaculture (IMTA) system using cages installed in a freshwater reservoir. The fishes were randomly (completely randomised design - CRD) distributed in 5 different combinations (treatments) in triplicates. Grass carp alone and a combination of prawn, mussels was designated as T1 (10 Nos. /0 Nos. /0 Nos.), T2 (0 Nos./10 Nos./16 Nos.), respectively. The remaining 3treatments of varying IMTA combinations of grass carp, prawn and mussels were designated as T3 (10 Nos./10 Nos./16 Nos.), T4 (15 Nos./10Nos./16 Nos.), and T5 (20 Nos./10 Nos./16 Nos.), respectively. The grass carp were fed with commercial feed of (CP: 25 - 26 %) while the prawns and mussels were not fed. After 120 days, there was no significant difference in the water quality, final weight, SGR and WG% of grass carp, survival of mussels and also quality of designer image pearls (mabe pearls) produced. However, the growth of prawns was significantly ($P<0.05$) higher in the T5- IMTA treatment (15.90 g ± 0.21) with significantly ($p<0.05$) highest specific growth rate (2.16 ± 0.07) and weight gain (%) (1225 ± 81.21) than the other treatments. Feed conversion ratio (FCR) was significantly ($p<0.05$) lowest (1.01) and the total production (5424.84 ± 53.46 g) was significantly ($p<0.05$) highest in the IMTA treatment T5. The present study demonstrates that IMTA with grass carp, freshwater prawn and freshwater mussels at a stocking density of 20 Nos. m^{-3}, 10 Nos. m^{-2} and 16 Nos. box^{-1}, respectively is a feasible technology for efficient utilisation of cages installed in freshwater reservoirs.

Keywords: Cage aquaculture, Reservoir development, Sustainable aquaculture, *Ctenopharyngodon idella*, *Macrobrachium rosenbergii*, and *Lamellidens marginalis*

INTRODUCTION

Population growth with the earth reaching for 10 billion by 2050, coupled with climate change, land scarcity, and water pollution has put the world's nations in a race to provide universal food and nutritional security (Ahmed *et al.*, 2019). Fish and other aquatic products will be critical in ensuring this goal due to their high protein and essential fatty acid content. To replace an estimated (average) 46 percent rise in meat and deliver the equal edible protein, simulation models suggest future aquaculture production (mixed and marine scenarios) must expand fourfold (Froehlich *et al.*, 2018). However, non-fed species must be included in aquaculture to efficiently use the released nutrients and the water body's diverse niches while increasing returns in comparison to conventional culture systems (Chopin and Robinson, 2006; Nobre *et al.*, 2010). This lays the groundwork for the notion of IMTA (Integrated Multi-Trophic Aquaculture), which is not polyculture in the conventional sense but a modified version of polyculture (Van Rijn, 2013). Bakhsh and Chopin (2012) called the freshwater variant of this concept as freshwater integrated multi-trophic aquaculture (FIMTA).

Cage culture is beneficial in numerous ways. In reservoirs, it provides new skill to traditional fishermen (Karnatak *et al.*, 2021). Simultaneously, integrated utilization of water resources through cage culture is the solution to address the growing concern of water scarcity for aquaculture while increasing the productivity of water especially in developing countries (Ahmed *et al.*, 2014). Developing and less developed countries, particularly Asian countries have huge potential in terms of freshwater reservoirs impounded for several purposes (De silva, 2001). Utilisation of these reservoirs will not only ensure food security, but also create employment opportunities as a means of sustainable livelihood for the people in the rural areas (Ahmed and Lorica, 2002). IMTA in freshwater reservoirs is a novel concept and it can provide a risk free culture system to the farmers as it contains different components of commercial value where one can act as an insurance against the unexpected loss of any of the other components.

Compatible species form the cornerstone of a successful integrated aquaculture/IMTA. Cage culture in India experimented on several species of carps (Indian major, Chinese, Indigenous), catfishes, mahseers, tilapia, *etc* but Pangasius (*Pangasionodon hypophthalmus*) has dominated the sector commercially (Karnatak *et al.*, 2021). As a new technology in freshwater cages in reservoirs, compatible species need to be chosen for IMTA in freshwater reservoir. In an effort to promote carps, grass carp was selected because among all carps, grass carp is a widely cultured species in the world and is well adapted to polyculture system (FAO, 2020). On the other hand, giant freshwater prawn is an omnivore benthic organism which can utilize the waste from the fish culture. Mussels are extractive organisms that can efficiently extract waste released from cage culture if maintained at adequate stocking densities (Cranford *et al*., 2013). They also provide additional income and financial security through pearl culture to the rural fisher folk.

Therefore, the present study was devised to assess the feasibility of IMTA in a freshwater reservoir integrating grass carp, freshwater prawn and freshwater mussels in different stocking densities using floating net cages.

MATERIALS AND METHODS

A. Experimental site animals

The experiment was carried out in floating net cages in the Dimbhe reservoir created by impounding water from the Ghod river, Ambegaon, Maharashtra, India (Fig. 1). Floating Cages (3 m × 3 m × 3 m) made of galvanised iron frames coupled with HDPE knotless netting material of 10 mm mesh installed at a water depth of >20 m were used for the present study. The efficient water depth of the cages was 2 m. Boxes used for mussels (implanted for pearl production) were made of plastic (45 × 21 × 10 cm) with perforations (1 cm). Combinations of three different species were used as experimental animals, fed species (grass carp) and extractive species (freshwater prawn- feed waste, faeces; mussel – organic components, seston). The grass carp fry, *Ctenopharyngodon idella,* were procured from the Prayas fish farm, Powarkheda, Madhya Pradesh. The giant freshwater prawn juveniles, *Macrobrachium rosenbergii*, and freshwater mussel, *Lamellidens marginalis*, were collected from the wild (Lonavala and Amba river in Maharashtra, respectively). The fishes, prawn and mussels were acclimatised in the reservoir water in separate cages and crates respectively for a week prior to stocking into different treatment groups with the respective stocking densities.

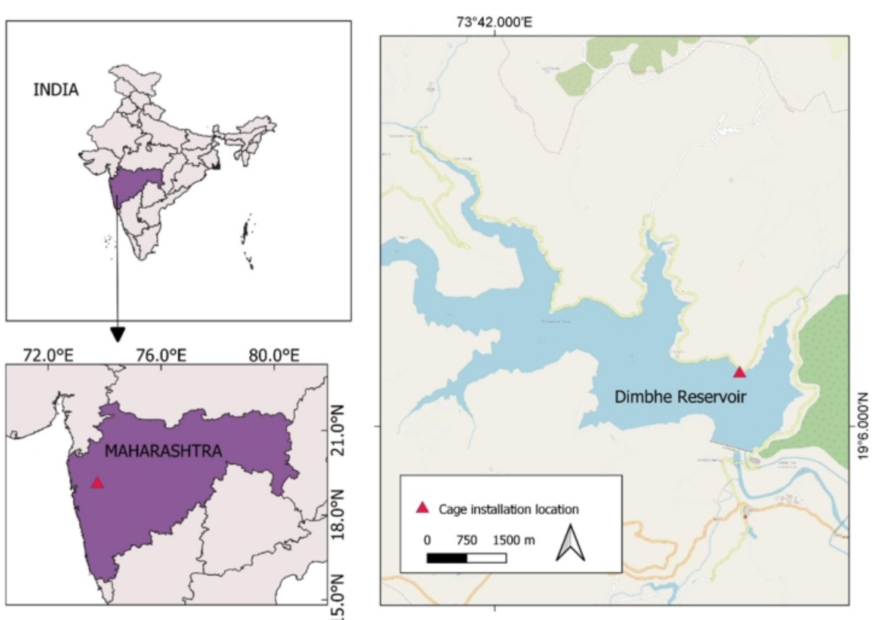

Fig. 1. Experimental cage site, Dimbhe reservoir, Maharashtra.

B. Implantation of Freshwater mussel

Freshwater mussels of average length (10.55±0.56 cm) and weight (58.42 ± 1.20) g were incorporated into the system and implanted with designer images (the images for nacre secretion to accumulate) to form image pearls (designer mabe pearls). A dough was prepared using a mixture of powdered mussel shells, an adhesive (Araldite® Standard) and coconut oil. This dough was pressed into metallic dies of images to get an impression of the images on the dough. The resulting image was sun dried, edges were cut and smoothened and used as nuclei for implantation. Such images were implanted in the acclimatised mussels by following mantle cavity insertion as per the method of Misra *et al.* (2009).

C. Experimental design and rearing

The experiment was done using acclimatised grass carp, prawns and implanted mussels in 5 treatments (Table 1). The grass carp (Initial avg. body weight 0.52 ± 0.02 g), freshwater prawn (Initial avg. body weight 1.15 ± 0.02 g) and mussel (Initial avg. body weight 58.42 ± 1.20 g) were stocked in floating net cages as per the experimental design (T1, T2, T3, T4, T5) in triplicates following the completely randomised design (CRD). The experimental animals were stocked with the stocking density as follows (Grass carp.m^{-3}/prawns.m^{-2}/mussels.box^{-1}), T1 (10/0/0), T2 (0/10/16), T3 (10/10/16), T4 (15/10/16) and T5 (20/10/16). The stocked fish were reared for a period of 120 days. Grass carp was fed with a commercial feed (crude protein 25-26 %), twice a day at apparent satiation, while no feed was given to the prawns and mussels. The nets of the cages were periodically cleaned to maintain the water quality inside the cages.

D. Sampling

Physico-chemical characteristics of water in the cages such as temperature, dissolved oxygen, pH, total alkalinity, hardness, TAN (Total Ammonia– N), nitrate - N, nitrite - N, phosphate were checked fortnightly as per standard protocols (APHA, 2005). The water samples were collected between 10 AM and 10:30 AM.

The fish and prawn were sampled fortnightly to record the growth performance. Similarly, implanted mussels were also checked for image rejection, mortality and overall condition. 10% of the stock from fish and prawn were sampled each time.

On completion of the experimental trial, the animals were harvested and required data was collected and calculated as per formulae mentioned below.

Mussels were assessed for pearl formation and quality of nacre deposition. They were graded visually for lustre and any malformations using a 3 point scale modified from Li *et al*. (2017). 3 signified “bright” with “no cracks or blemishes”; 2 for “medium” with/without 1 or 2 spots or cracks; 1 for “dull” with/without more than 2 spots/cracks.

Total yield = Yield of grass carp + yield of prawn (in treatments where applicable)

E. Ethics statement

All the ethical guidelines as per the ethical committee of the institute has been duly followed in this research work.

F. Statistical analysis

The data was analysed in IBM-SPSS (Statistical Package for Social Sciences) version 22. Treatment mean values were tested in one way ANOVA and post-hoc analysis was done using Duncan’s multiple range test to analyse the significant difference among the mean values. The level of significance was set at 95% confidence level ($P<0.05$).

RESULTS AND DISCUSSION

IMTA can aid in better utilisation of reservoirs because of its environmental sustainability and remediating capacities. Generally it is a bioremediation technology for the intensive culture of high value species in cages in developed countries (Fang *et al*., 2016; Ellis and Tiller, 2019). But, implementation in a freshwater reservoir will help in establishing a low-intensive cage aquaculture in reservoirs for increasing productivity from the cages. It can also increase the economic yield from cage culture in rural areas.

The water quality parameters (Table 2) viz. temperature, dissolved oxygen, pH, alkalinity, hardness, NH4-N, NO_3-N, NO_2-N and PO_4-P did not display any significant difference among treatments in the course of the experiment. The insignificance of physico-chemical characteristics of the water among all the cages was because this experiment was done in a small scale in comparison to the size of the reservoir and the wastes were flushed out due to wave action. However, there was a variation in the temperature over the culture period which might be due to the influence of the seasons (Sehgal *et al.,* 2013). Nevertheless, all water quality parameters were found to be in the optimal range for aquaculture in general (Boyd and Tucker, 2014; Pillay and Kutty, 2005) and cage culture in reservoirs in particular (Devi *et al*., 2017). As far as IMTA systems in freshwater is concerned, Kibria and Haque (2018) also observed no changes in the physicochemical parameters of the culture water in their experiments comparing IMTA and monoculture in ponds. Thus, it can be affirmed that the water quality was maintained well in all cages and there could have been no negative effect of water quality on any of the cages that could have reduced the growth or yield of any of the cages in comparison to other treatment cages.

There was no significant difference among the treatments for final weight (16 g approx.), SGR, WG% and survival of grass carp. This is due to the low stocking density used in this experiment in comparison to suggested stocking density (Mane *et al.,* 2019) for carps. It would have aided for the growth of grass carp fry in a stress free environment. The growth of grass carp in this study can be corroborated to the findings of Taher *et al.* (2017) who studied the growth of grass carp under different feeding regimes and obtained a growth of 14.05 g when fed with pelleted feed of 20 % protein. Fishes in this study were fed a commercial diet having higher protein (25-26 %). Moreover in IMTA systems also, the growth of primary species i.e. the fed species among various stocking densities was reported to be insignificant in freshwater (Kibria and Haque, 2018) and brackish water (Biswas *et al*., 2019). Therefore it can be seen from this experiment that the integration of grass carp (maximum density of 20 m^{-3}) with prawn and mussels had no negative or positive effect on the growth and survival of the grass carp making grass carp a suitable species for integration with prawns and mussels in an IMTA system.

Growth of prawn juveniles in the IMTA treatments (T3, T4, T5) showed significance ($p<0.05$) than those in control (T2) with highest final body weight in T5 (15.90 g ± 0.21) (Table 3) even though they were not fed. This was because the prawn in the IMTA groups was nourished by the uneaten feed and waste that was produced by the grass carps in the cages in the water column. Highest growth of prawn was in the IMTA cages with higher density of grass carp. More fish meant more feed waste and faeces for prawn to feed. IMTA is a modified form of polyculture where the nourishment of the prawn depends entirely on the feed given to the fish and the remaining feed and faeces have high nutrient content as per findings of Kibria *et. al.* (1997). Integrated aquaculture experiments on Amazon River prawn and Tambaqui fish conducted by Flickinger *et al.* (2019) reported that the extractive animals (unfed prawn) in the IMTA had 53-75 % higher efficiency in conversion of feed to finished products than that in monoculture. This is further supported by the results of Dong *et al.* (2018) who maintained different treatments of IMTA with freshwater prawn and obtained highest growth in the IMTA with higher number of fish. The survival of prawns in various treatments showed no significant difference ($P>0.05$) among the treatments. It is also reported that in a polyculture setting, prawn shows better growth (Costa-pierce *et al.,* 1984) because prawn and carp can utilize different niche efficiently. Thus, the compatibility of prawn with grass carp in an IMTA system can be seen from the higher growth of prawns in the IMTA treatments of this experiment.

At the end of the experimental period, the average survival of mussels in the treatments was~94 %. The images implanted in the IMTA treatments developed into image pearls displaying a whitish and silvery tinge with no significance between treatments. However, based on the scale used, "medium" pearls were obtained in IMTA treatments T4 and T5 and "dull" were obtained in the treatment T2 with prawns and mussels only (Table 3). The implanted mussels in this experiment showed a final survival of ~94% at the end of 120 days experiment. Hossain *et al.* (2004) after working with different species of mussels concluded that the freshwater Mussel *L. marginalis* showed highest survival and pearl formation (pearl sacs were formed) in a period of 3 months and hence suggested them to be the ideal species for freshwater pearl production. Similarly, Pandey and Singh (2015) also got a survival between 20-85 % in different methods of implantation with higher survival rate after mantle insertion. The implanted mussels were placed in boxes hung along the sides of the cages, in order to utilise the water in the cages which is expected to have better plankton availability supported by the nutrients released from the cages than other parts of the open water body (Degefu *et al.,* 2011). The nutrients released from the cages would have stimulated algal growth and increased plankton in their study. Bivalves are highly efficient filter feeders with capacity to filter 10 – 100% of the water in the column in a day (Strayer *et al.,* 1999), they filter and feed on alga, zooplankton and also particulate organic matter such left over feed (Janakiram, 2003) this makes them highly suitable for the IMTA system as they can filter and extract the nutrients released from the cages. On harvest of the image pearls from mussels, it was observed that the images started developing a shiny layer of the nacre of whitish silver colour and on evaluation showed no significant difference among them. In a study conducted by Tanu *et al.* (2019), pearls with nacre of 4.17-5.19 mm were obtained only after culturing them for a period of 3 years in ponds with regular fertilisation and mineral supplementation. Cranford *et al*. (2013) suggests, in order to obtain an economical product from IMTA, stocking density must be higher. Hence, it is advisable to keep the density higher than 16 per cage as per availability. Thus, freshwater mussel, *Lamellidens marginalis* is an efficient and valuable component for integrated multi-trophic aquaculture in freshwater reservoirs.

With regard to feed conversion ratio (FCR), lowest significant FCR of 1.01 was obtained in the IMTA treatment T5similar to the results obtained in other IMTA experiments by Dantas *et al*. (2020).

The highest significant total yield of grass carp and prawns (5424.84 ± 53.46 g) was also obtained in the IMTA treatment T5 (Table 3). This is in agreement to the reports on the growth of freshwater prawn and tambaqi fish in IMTA treatments by Flickinger *et al.* (2019, 2020); Dantas *et al.* (2020). As a whole, only the total yield in the IMTA (T5) treatment varied significantly in comparison to other treatments and it was the growth of the prawns that contributed significantly to the overall yield of the other treatments. Similar results obtained by Biswas *et al.* (2019) in their experiment of Brackish water IMTA (BIMTA). In their study, there was no significant difference in the growth of mullets between the control and the treatment groups but it did affect growth of *P. monodon*. This proves that integration has a positive influence on the growth of the extractive organisms as in the present experimental trial. Work on IMTA by Kibria and Haque (2018) also reported similar results. This is because the animals in the lower trophic level utilise the waste feed and assimilate the inorganic nutrients efficiently (Chopin and Robinson, 2006) which leads to maintenance of optimal water quality, prevents growth of obnoxious algae and other unwanted planktonic organisms while giving higher production when compared to a monoculture.

Table 1: Species combination and stocking density in various IMTA treatments.

Species	T1	T2	T3	T4	T5
Grass carp *Ctenopharyngodon idella* (no.m^{-3})	10	-	10	15	20
Macrobrachium rosenbergii (no.m^{-2})	-	10	10	10	10
Lamellidens marginalis (no.box^{-1})	-	16	16	16	16

Table 2: Physico-chemical characteristics of water collected between 10:00-10:30 AM at 15 days intervals from various treatment of IMTA (T3,T4,T5) and controls (T1,T2) in floating net cages during the experimental period.

	Range	T1	T2	T3	T4	T5
Temperature (°C)	25-28	25.02 ± 0.13	25.08 ± 0.02	25.12 ± 0.03	25.11 ± 0.00	25.01 ± 0.13
Dissolved Oxygen (mg/l)	5-7	5.8 ± 0.13	6.02 ± .13	6.12 ± 0.03	6.13 ± 0.00	6.08 ± 0.08
pH	7.5	7.5 ± 0.00	7.5 ± 0.01	7.5 ± 0.01	7.5 ± 0.00	7.5 ± 0.00
Hardness (mg/l)	65-70	68.08 ± 0.30	68.25 ± 0.52	67.91 ± 0.08	67.41 ± 0.74	67 ± 0.08
Alkalinity (mg/l)	54-58	55.25 ± 0.01	55.00 ±0.00	55.33 ±0.02	55.38 ± 0.17	55.17 ± 0.12
Nitrite-N (mg/l)	0.01- 0.02	0.0193 ± 0.00	0.0190 ± 0.01	0.0187 ± 0.02	0.0193 ± 0.03	0.0190 ± 0.01
Nitrate – N (mg/l)	0.04-0.05	0.0491 ± 0.00	0.0491 ± 0.00	0.0490 ±0.00	0.0491 ± 0.00	0.0493 ± 0.00
Ammonia - N (mg/l)	0.01-0.02	0.01886 ± 0.00	0.01910 ± 0.02	0.01922 ± 0.23	0.0187 ± 0.24	0.01895 ± 0.00
Phosphate (mg/l)	0.02-0.03	0.0379 ± 0.00	0.0370 ± 0.00	0.0308 ± 0.01	0.0307 ± 0.02	0.0310 ± 0.22

Values are (Mean ± S.E.); n=3:

Table 3: Comparison of growth, survival, mean yield, production performance and FCR of Grass carp and giant freshwater prawn and survival, image pearl quality in mussels in different treatments.

Treatments[1]	Initial body weight (g)	Final body weight (g)	SGR[2]	WG[3](%)		
Grass carp						
T1	0.51 ± 0.02	16.61 ± 0.14	2.88 ± 0.01	3156.01 ± 52.07		
T2	-	-	-	-		
T3	0.52 ± 0.00	16.55 ± 0.64	2.89 ± 0.02	3082.69 ± 47.57		
T4	0.53 ± 0.01	16.10 ± 0.27	2.87 ± 0.03	2937.73 ± 57.04		
T5	0.53 ± 0.04	16.37 ± 0.40	2.86 ± 0.02	2988.67 ± 32.26		
p value	>0.05	>0.05	>0.05	>0.05		
Prawn						
T1	-	-	-	-		
T2	1.10 ± 0.06	7.10^a ± 0.66	1.55^a ± 0.04	545.45^a ± 31.53		
T3	1.23 ± 0.03	9.57^b ± 0.23	1.71^a ± 0.07	678.04^a ± 63.99		
T4	1.07 ± 0.07	11.87^c ± 0.19	2.01^b ± 0.07	1009.34^b ± 84.21		
T5	1.20 ± 0.06	15.90^d ± 0.21	2.16^b ± 0.07	1225^c ± 81.21		
P value	>0.05	<0.05	<0.05	<0.05		
Total yield						
	Grass carp yield (g)	Prawn yield (g)	Pooled survival[4](%)	Total yield[5] (g)	FCR[6]	Image pearl quality based on scale[7]/ survival%
T1	2492.56^a ± 31.65	-	86.11 ± 0.56	2492.56^b ± 31.65	1.61^d ± 0.02	-
T2	-	455.80^a ± 24.94	83.07 ± 1.33	455.80^a ± 24.94	-	1.33 ± 0.21/93.00 ± 0.12
T3	2453.42^a ± 18.32	630.13^b ± 14.94	84.53 ± 0.14	3083.56^c± 32.39	1.29^c ± 0.02	1.83 ± 0.30/94.20 ± 0.06
T4	3626.70^b ± 101.94	810.09^c ± 30.33	84.75 ± 1.22	4436.78^d± 33.23	1.13^b ± 0.02	2.00 ± 0.25/ 94.11 ± 0.26
T5	4337.09^c ± 98.49	1087.74^d± 18.32	84.33 ± 1.05	5424.84^e ± 53.46	1.01^a ± 0.15	2.00 ± 0.36/ 94.00 ± 11
P value	<0.05	<0.05	>0.05	<0.05	<0.05	>0.05

[1]Treatments: T1-$G_{10}P_0M_0$: T2-G_0P_{10} M_{16}: T3-$G_{10}P_{10}$ M_{16}: T4-$G_{15}P_{10}$ M_{16}: T5-$G_{20}P_{10}$ M_{16}: G-Grass carp; P-Prawn; M - Mussel
Values are (Mean ± S.E.); n=3:

Mean in the column with different superscript letters are significantly different

[2]Specific growth rate (%) = $\frac{\text{(ln final weight – ln initial weight)}}{\text{Number of days}} \times 100$

[3]Percentage weight gain (%) = $\frac{\text{Final weight (g) – Initial weight (g)}}{\text{Initial weight (g)}} \times 100$

[4]Pooled Survival (%) = $\frac{\text{Total number of animal (grass carp + prawn) harvested}}{\text{Total number animal (grass carp + prawn) stocked}} \times 100$

[5]Total yield = Yield of grass carp + yield of prawn (in treatments where applicable)

[6]FCR = $\frac{\text{Feed intake}}{\text{Weight gain}}$

[7]Image pearl quality scale: 3- bright; 2-medium; 1- dull

CONCLUSION

Thus, based on the findings of this study, the authors indicate that grass carp, freshwater prawn, and freshwater mussel can be successfully integrated into an IMTA system in cages in a freshwater reservoir. Use of new technologies like IMTA in the numerous underutilised water bodies will promote aquaculture and increase water productivity globally and nationally. IMTA can provide returns from two or more species while providing inputs for only one. Thus, as an alternative to standard cage farming, IMTA in cages can generate revenue for those who rely on tropical freshwater reservoirs for their livelihood.

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Conflict of Interest. None.

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