EFFECTS OF PERIPHYTON AND MOLA (*Amblypharyngodon mola*) ON THE PRODUCTION PERFORMANCE OF MRIGAL (*Cirrhinus cirrhosus*) AND TILAPIA (*Oreochromis niloticus*)

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Abstract

The experiment was conducted to compare the production performance of Mrigal (*Cirrhinus cirrhosus*) and Tilapia (*Oreochromis niloticus*) fish in polyculture systems for a period of 124 days in the ponds at the Fisheries Field Laboratory, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. The three treatments were: Mrigal and Tilapia without Mola (*Amblypharyngodon mola*) and periphyton (T_1), Mrigal, Tilapia and Mola without periphyton (T_2), and Mrigal, Tilapia and periphyton without Mola (T_3), with three replications in each. The area of each pond was 140 m^2 having water depth of 1.5m. The stocking density of Mrigal and Tilapia were 2,143 and 20,000 fingerlings ha^{-1}, respectively in all treatments. Mola were stocked at 20,000 individuals ha^{-1} in Mrigal, Tilapia and Mola treatment. The bamboo sticks (kanchi) were added as a substrate for periphyton growth. Fertilizers like urea and triple super phosphate (TSP) were applied to the ponds at the rate of 50 kg ha^{-1} at 30 days intervals throughout the cultured period. Fish were fed with supplemental feed twice daily at a rate of 3-5% of the body weight. Periphyton biomass grew on substrate at a higher rate in early months and decreased gradually till the end of the experiment. Mean values of the water quality parameters and benthic production showed no significant differences ($P>0.05$) among the treatments. Plankton abundance was significantly different ($P<0.05$) among the treatments and the highest value were observed in T_1 (52.61±3.09 $\times 10^3$ individual l^{-1}). The net production of 1686.74 kg ha^{-1} fish in T_3 was higher than that of 1226.55 kg ha^{-1} (excluding Mola) and 1262.19 kg ha^{-1} (including Mola) and T_1 (1,233.29 kg ha^{-1}). It was concluded that, the addition of substrates for periphyton growth resulted in higher yield of fishes and good environmental condition.

***Keywords*: plankton, periphyton, benthos, production performance, polyculture.**

Introduction

Fish and fisheries play an important role in agro-based economy of Bangladesh, contributing 60% of total animal protein intake, creating employment opportunity of 17.1 million of people as a full time and part time employment, and contributing 2.01% to the total export earnings (Azad, 2014). The present fish production is unable to cope up with the ever-increasing need of fish. To fulfill the protein demand of the people; it is strongly felt that a huge quantity of fish production is essential. It is true that our

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water bodies are not properly used for fish culture. So, developing culture system is one of the most important factors to increase fish production. Periphyton based aquaculture systems offer the possibility of increasing both primary production and food availability for fish (Azim *et al.,* 2001). Periphyton is very preferable natural food for herbivorous and omnivorous fish species especially for Indian major carp (Azim *et al.,* 2002) and for Tilapia (Wahab *et al.,* 1999). Periphyton mats improved pond water quality through trapping suspended solids, oxygen production, organic matter breakdown, ammonium and nitrate and nitrification enhancement (Thompson *et al.,* 2002). The potential of periphyton-based aquaculture in Bangladesh is under investigation within an extensive research program. Like as periphyton, plankton plays a vital role in water bodies as the source of food for fishes. In environment, without supplying supplementary food the aquatic organisms depend on plankton population. Zooplankton are the main link between small phytoplankton and large carnivorous, primarily fish. In bottom sediment of a water body benthic fauna link between phytoplankton, zooplankton and the fish stock in the food chain. Benthic organisms indicate the productivity of water bodies (Latifa *et al.,* 1997). However, the objective of the present study was set to evaluate the effects of biological parameters (periphyton and Mola) on the production performance of fish (Mrigal and Tilapia) and to develop farming technology package suitable for wider adoption.

Materials and Methods

Experimental design: The experiment was conducted using a completely randomized block design with 9 ponds (140 m^2 each) having an average depth of 1.5m at the Fisheries Field Laboratory, BAU, Mymensingh, Bangladesh from 01 October, 2010 to 15 February, 2011. The experiment included three treatments namely T_1, T_2 and T_3. Each treatment had three replications. The T_1 consists of Mrigal and Tilapia without Mola and periphyton. T_2 consists of Mrigal, Tilapia and Mola without periphyton whereas T_3 consists of Mrigal, Tilapia and periphyton without Mola.

Pre-stocking management

All undesirable fish were completely eradicated by applying rotenone at a rate of 2.5g/m^3. The weeds, dead fishes and other aquatic organisms were removed manually and repeated netting. All ponds were treated with lime at a rate of 250 kg ha^{-1} after one week of rotenone application. Ponds were fertilized with urea and TSP each at a rate of 50 kg ha^{-1} after three days of liming and after stocking 30 days' intervals throughout the cultured period. For periphyton growth 10 side shoots of bamboo (locally known as Kanchi) per m^2 water surface area, with a mean diameter of 2.8cm were posted vertically into the bottom mud, excluding a 0.5 meter wide perimeter before 10 days of fish stocking.

Stocking and management

Fish fingerlings were collected from a private hatchery and pond of the Fisheries Field Laboratory, BAU. The stocking density of Mrigal and Tilapia was similar in all the treatment. It was 2143 and 20000 individuals' ha^{-1} of Mrigal and Tilapia, respectively. The stocking density of Mola was 20000 individuals' ha^{-1} in Mrigal, Tilapia and Mola treatment. The length and weight of fish fingerlings in each pond was measured and recorded for estimating initial stocking biomass and to adjust initial feeding rate. Supplementary feed was prepared by mixing of rice bran and soaked mustard oil cake at a ratio of 2:1 and fed daily at a rate of 3-5% of body weight of Mrigal and Tilapia only. Fish were fed twice daily (morning and evening). The water level of ponds was maintained by adding underground water from a deep tube well to compensate loss due to evaporation and leakage. At least 10% of stocked fish were sampled from each pond to make assessment of growth trends and to readjust feeding rate. Length and weight of sampled fish were measured using a measuring scale and digital electronic balance (OHAUS, MODEL No. CT-1200-S).

Collection of water samples

Water samples were collected between 9:00 to 10:00 AM from each pond surface to a depth of 20cm by using a sampler in a manner that it is representative of all layers of the water column. Then water samples (plastic bottles volume 250ml) were carried back to the Water Quality and Pond Dynamics laboratory, BAU for chemical analysis and 100ml of water sample from each bottle was filtered through high quality glass microfibre filter paper (Whatman GF/C) with the help of vacuum pressure air pump for Chlorophyll-*a* analysis.

Measurement of water quality parameter

Temperature, transparency, pH and dissolved oxygen (DO) were measured on the spot and rests of the parameters were measured at Laboratory in fortnightly basis. A Celsius thermometer was used for water temperature measurement. Transparency was measured with a Secchi disc of 20cm diameter. DO of water samples were measured by portable digital DO meter (HACH Sension 8; p/n: 51970-00). pH of water samples were measured by a direct reading digital pH meter (METTLER TOLEDO, MP 230) at the pond site. Total alkalinity was determined by titration method using 0.02 N sulfuric acid and methyl orange indicator method (APHA, 1992).

Estimation of plankton and benthic macro-invertebrates

Plankton samples were collected monthly by pooling 10L of water from five locations in each pond and passing it through plankton net (mesh size 25µm). Each filtered sample was transferred to a measuring cylinder and made up to a standard volume of 50ml with distilled water and buffered formalin (10%), and preserved in a small, sealed plastic bottle until analysis. Plankton numbers were counted using a Sedgewick-Rafter (S-R) cell and were left to stand for 15 minutes to allow plankton to settle. Then, the plankton on 10 randomly selected fields of the chamber was counted under a binocular microscope (Olympus, M-4000D, Tokyo, Japan). Plankton abundance was calculated using the following formula (Azim *et al.,* 2001):

$$N = (P \times C \times 100)/L$$

Where,

N, is the number of plankton cells or units per liter of original water

P, the number of plankton counted in 10 fields

C, the volume of final concentrate of the sample (ml)

L, the volume (L) of the pond water sample

The benthic invertebrate samples from the pond bottom were collected monthly using a 225cm^2 Ekman dredge. The samples were taken randomly from three different locations in each pond. Mud samples were washed through a sieve (250µm mesh size) and benthic invertebrates were separated and preserved in a plastic vial containing 10% buffered formalin solution. Benthic macro-invertebrate density (individuals/m^2) was calculated using the formula (Asaduzzaman *et al.,* 2010),

$$N = Y \times 10000 / 3A$$

Where,

N = number of benthic organisms (number m^{-2})

Y = total number of benthic organisms counted in 3 samples

A = area of Ekman dredge (cm^2)

Determination of periphyton biomass

From each pond, three poles (kanchi) were selected and two 2×2 cm^2 samples of periphyton were taken at each of three depths (25, 50 and 75 cm below the water surface) per pole. One of the two samples was used to determine total dry matter (DM) and ash content. The materials from each pole were collected on pre-weighed and labeled pieces of aluminum foil, dried at 105 °C until constant weight (24h in a Memmert stove, Model UM/BM 100–800), and kept in a desiccators until weighed (BDH 100A; precision 0.0001g). Dry samples from depth and poles per pond were pooled, transferred to a muffle furnace and ash at 450 °C for 6h and weighed. The DM, ash free dry mater (AFDM) and ash content were determined by weight differences (APHA, 1992). Another sample was used to determine chlorophyll-*a* concentrations following standard methods (APHA, 1992).

Harvesting of Fish: All ponds were harvested separately by repeated netting and draining. Fishes of each pond were counted and measured weight individually.

Statistical Analysis

For the statistical analysis of the data, a one-way ANOVA and DMRT (Duncan's Multiple Range Test) were done by using the SPSS (Statistical Package for Social Science) version-16.5. Significance was assigned at the 0.05% level.

Results and Discussions

Water quality parameters: The mean values of water quality parameters are presented in the Table 1. The mean values of water temperature (°C) were 24.27±4.03, 24.29±4.25 and 24.48±4.20 in T_1, T_2 and T_3, respectively, which were within the recommended suitable range for fish culture. The findings of the present study were similar to the findings of Azim *et al.* (1995). The value of transparency in this study ranged from 38.67-58.33, 38.67-59.0 and 39.0-63.33 cm in T_1, T_2 and T_3, respectively. In the present study, the transparency values of different treatments indicated that pond waters seemed to be within the productive range for fish culture, recommended by Boyd (1990). pH ranges from 7.04 to 8.87 recorded in this study was suitable for fish culture. The pH values recorded in the experiment agreed with the findings of Ali *et al.* (2004) and Mondal *et al.* (2010). They found the ranges of pH from 6.3 to 8.9 and 7.0 to 8.9, respectively. DO concentration of the experimental ponds in the present study ranged from 1.97 to 5.37 mg l^{-1}. The concentration of DO was fairly well as stocked fish did not show any sign of oxygen deficiency throughout the study

Table 1: Mean values (±SD) and ranges (parenthesis) of water quality parameters of the ponds under three treatments.

Variables	Treatments		
	T_1	T_2	T_3
Temperature (°C)	24.27 ± 4.03 (18.67 - 31.83)	24.29 ± 4.25 (18.20 - 32.42)	24.48 ± 4.20 (18.37 - 32.33)
Transparency (cm)	49.67 ± 5.81 (38.67 - 58.33)	53.00 ± 5.81 (38.67 - 59.00)	52.07 ± 6.44 (39.00 - 63.33)
pH	7- 8.9 (7.04 - 8.85)	7.13 – 8.9 (7.18 – 8.73)	7.07- 8.89 (7.09 - 8.87)
Dissolved oxygen (mg l^{-1})	2.86 ± 1.11 (2.00 - 5.43)	2.92 ± 1.12 (1.97 - 5.37)	2.79 ± 0.88 (1.90 - 4.30)
Total alkalinity (mg l^{-1})	80.59 ± 7.66 (70.67 - 95.00)	80.37 ± 7.98 (66.33 - 90.67)	81.78 ± 7.98 (64.33 - 91.00)

period. However, the concentration of DO in the present study was lower than the findings of Ali *et al.* (2004) where they recorded DO ranged from 3.4 to 8.1 mg l^{-1}, respectively. This might be due to lower photosynthesis by the phytoplankton in winter season. Total alkalinity in the present study ranged from 66.33 to 95.00 mg l^{-1}, which is more or less similar to the findings of Kunda *et al.* (2008).

Plankton abundance

Phytoplankton abundance was found to range from 40000-46000, 27330-42500 and 28170-43330 individual l^{-1} in T_1, T_2 and T_3, respectively. The mean zooplankton concentration was 9.01±2.58, 9.30±3.58 and 9.46±2.27 ($\times 10^3$ individual l^{-1}) in T_1, T_2 and T_3, respectively. The mean value of total plankton differed significantly ($P<0.05$) among the treatments (Table 2) and the highest value observed in T_1. Since Mola is a plankton feeder, their absence in T_1 facilitated the accumulation of higher phytoplankton in the ponds. Though in T_3 there was no Mola but producing periphyton substrate helped Tilapia to actively consume attached algal aggregates. Plankton populations in the water of the experimental ponds were found to be consisted of 97 genera belonging to 6 planktonic groups. Phytoplankton composed of 81 genera belonging to 4 major groups: Bacillariophyceae (36), Chlorophyceae (25), Cyanophyceae (13) and Euglenophyceae (7) and Zooplankton composed of 16 genera belonging to 2 groups: Rotifera (6) and Crustacea (10). Phytoplankton density was higher than zooplankton population. Chlorophyceae showed both qualitative and quantitative dominance over other plankton groups in T_1 and T_3. Shahin *et al.* (2011) identified 45 genera of phytoplankton and 13 genera of zooplankton in carp-prawn-mola polyculture system with tilapia, which is lower than the present study.

Benthic production

Benthic organisms are very important food items for various fish species. In this study the abundance of benthic organisms were reduced with time. Benthos population of the fish ponds was composed of four major groups (Table 3): Chironomidae, Oligochaeta, Mollusca and Miscellaneous. Chironomide was the most dominant benthic organism.

Table 2: Mean abundance of plankton (×10³ individual l⁻¹) recorded from the ponds under the three treatments. The range of observed values is given in the parenthesis.

Plankton Group	Treatment		
	T_1	T_2	T_3
Bacillariophyceae	12.17± 2.17[a] (10.00 - 14.50)	9.31 ± 3.66[b] (5.5 - 13.50)	9.93 ± 2.97[b] (6.83 - 14.00)
Chlorophyceae	17.48 ± 1.89[a] (14.67 - 19.33)	13.11 ± 2.58[b] (9.17 - 14.67)	14.13 ± 4.20[b] (8.17 - 18.00)
Cyanophyceae	9.11 ± 2.24[a] (7.17 - 13.17)	7.33 ± 1.97[b] (5.67 - 9.50)	6.63 ± 1.96[b] (5.00 - 9.83)
Euglenophyceae	4.83 ± 2.15[a] (2.83 - 8.83)	3.70 ± 2.47[ab] (2.00 - 7.67)	3.22 ± 2.20[b] (1.00 - 7.50)
Total phytoplankton	43.59 ± 2.75[a] (40.00 - 46.00)	33.46 ± 5.90[b] (27.33 - 42.50)	33.90 ± 6.43[b] (28.17 - 43.33)
Rotifera	2.70 ± 0.90 (1.67 - 3.67)	3.28 ± 1.98 (2.00 - 7.17)	3.56 ± 1.50 (2.1 - 4.00)
Crustacea	6.31 ± 3.3 (2.50 - 12.33)	6.02 ± 2.94 (2.33 - 12.17)	5.91 ± 2.49 (2.50 - 9.67)
Total zooplankton	9.01 ± 2.58 (4.17-15.17)	9.30 ± 3.58 (4.67-14.67)	9.46 ± 2.27 (4.67-13.50)
Total plankton	52.61 ± 3.09[a] (49.17 - 57.83)	42.76 ± 6.75[b] (33.83 - 56.67)	43.37 ± 6.73[b] (37.83 - 56.83)

The mean values of Chironomidae was 684.44±1720.06, 343.70±282.83 and 294.32±510.10 individuals m⁻² for T_1, T_2 and T_3, respectively. Total benthos production was higher in T_1. No significant difference in benthos production among the treatments was found.

Periphyton biomass and chlorophyll-*a*

The quantity of periphyton DM ranged from 1.57 to 5.19 mg cm⁻² during the experiment. Azim *et al.* (2001) found the DM concentrations of periphyton ranged from 3.12±0.20 to 4.89±0.26 mg cm⁻² which is similar to the present study. The mean values of AFDM ranged from 0.65 to 3.80 mg cm⁻² while the concentration of ash ranges from 0.93 to 1.39 mg cm⁻² throughout the experimental period which is more or less similar to Azim *et al.* (2001). The chlorophyll-*a* content was higher in October and lower in February. In the present study, the level of chlorophyll-*a* was found 6.63 to 8.04 µg cm⁻². The values of chlorophyll-*a* indirectly express the abundance of phytoplankton of the water body. The presence of Tilapia decreased periphytic phytoplankton and biomass per unit surface area. It indicates that Tilapia takes periphyton as food. Azim *et al.* (2003) already studied and revealed that Tilapias are omnivores capable of feeding benthic and attached (periphyton) algal and detrital aggregates. Since it was already revealed, no gut analysis was performed in this study. There is also evidence that Tilapia grows better grazing on periphyton than filtering suspended algae from water column.

Average monthly variation of DM, AFDM and Chlorophyll-*a* in T_3 are shown in the following Figure 1, 2 & 3.

Table 3: Abundance of benthos individual m^{-2} (mean ±SD) with their different groups recorded in different treatments.

Benthos group	Treatment		
	T_1	T_2	T_3
Chironomidae	684.44±1720.06	343.70 ± 282.83	294.32 ± 510.10
Oligochaeta	341.73 ± 292.97	341.73 ± 278.43	300.25 ± 284.55
Mollusca	238.02 ± 225.75	213.33 ± 180.65	197.53 ±148.85
Un-identified	48.40 ± 76.26	49.38± 76.09	28.64 ± 32.39
Total benthos	1312.59±1832.94	948.15 ± 525.78	820.74 ± 689.14

Table 4: Means of monthly periphyton quantitative parameters recorded from T_3.

Parameters	Treatment T_3
DM (mg cm^{-2})	1.57–5.19
AFDM (mg cm^{-2})	0.65–3.80
AC (mg cm^{-2})	0.93–1.39
Chlorophyll-a (µg cm^{-2})	6.63–8.04

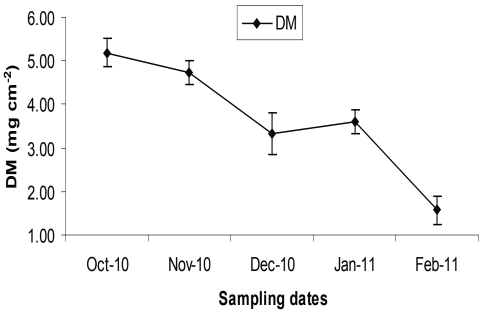

Figure 1: Average monthly variation of dry matter (DM) mg cm^{-2} in T_3.

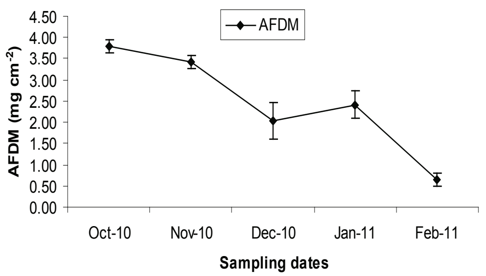

Figure 2: Average monthly variation of ash free dry matter (AFDM) mg cm^{-2} in T_3.

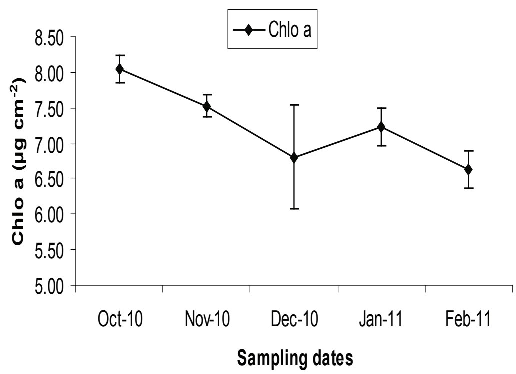

Figure 3: Average monthly variation of Chlorophyll–a (µg cm^{-2}) in T_3.

Production performance of fishes

Both gross and net production varied significantly (P<0.05) among the treatments. The combined net fish production were significantly higher in T_3 (1686.74±28.88 kg ha^{-1}) than T_1 (1233±33.79 kg ha^{-1}) and T_2 (1226.55±28.05 kg ha^{-1}, if combine of three fish species then 1262.19±28.05 kg ha^{-1}) in the crop. This might be due to the presence of substrate as an additional source of food for fishes. The combined net production of fishes is more or less similar with Azim *et al.* (2002) they recorded 1,650 kg ha^{-1} $crop^{-1}$ in a polyculture of Indian major carp with bamboo poles as substrate. In T_2, three fish species Mrigal, Tilapia and Mola were present but their combine production was lower the T_3.

Because Mola is a plankton feeder so it might compete with other two species for plankton which could result in less overall production. So, it is concluded that the addition of substrates for periphyton growth served as a food source for fishes in polyculture. It is clear that periphyton as a food source for fishes in polyculture has more significant effect on production than plankton or benthos or addition of other fish species.

Conclusion

This study shows the effects of periphyton and Mola on the production of fishes in polyculture system. Highest production of fishes was observed in treatment with periphyton which clarifies the choice of fish species selected for periphyton as a food source. From the above discussion it may be concluded that the addition of substrates for periphyton growth plays an important role as food source for fishes in polyculture system.

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Table 5. Comparison of production parameters (mean ± SD) of Mrigal and Tilapia in different treatments. Values in each column with different superscript are significantly different (P<0.05).

Species/parameters	Treatments		
	T_1	T_2	T_3
Mrigal			
Individual stocking weight (g)	9.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00
Individual weight at harvest (g)	75.33±2.52^b	77.67±2.52^b	85.67±2.08^a
Individual weight gain (g)	66.33±2.517^b	68.67±2.517^b	76.67±2.082^a
SGR (%)	1.71±0.03^b	1.74±0.03^b	1.82±0.02^a
Survival (%)	82.22±1.92^b	81.11±1.92^b	92.22±1.92^a
Gross production (kg ha^{-1} 124 d^{-1})	132.67±2.00^b	134.93±1.92^b	169.33±7.02^a
Net production (kg ha^{-1} 124 d^{-1})	116.81±2.20^b	119.29±2.11^b	151.55±6.69^a
Tilapia			
Individual stocking weight (g)	0.40±0.00	0.40±0.00	0.40±0.00
Individual harvesting weight (g)	92.00±2.65^b	92.00±4.00^b	107.00±2.65^a
Individual weight gain (g)	91.60±2.65^b	91.60±4.00^b	106.60±2.65^a
Specific growth rate (% bw d^{-1})	4.39±0.02^b	4.39±0.04^b	4.51±0.02^a
Survival (%)	60.95±1.44^b	60.48±1.35^b	72.02±1.25^a
Gross yield (kg ha^{-1} 124 d^{-1})	1121.36±34.18^b	1112.10±27.08^b	1540.95±22.5^a
Net yield (kg ha^{-1} 124 d^{-1})	1116.48±34.13^b	1107.26±27.16^b	1535.19±22.6^a

Table 6. Mean (± SD) values of production parameters of Mola.

Parameters	Treatments		
	T_1	T_2	T_3
Individual stocking weight (g)	0.00±0.00	0.30±0.00	0.00±0.00
Individual harvesting weight (g)	0.00±0.00	3.53±0.04	0.00±0.00
Individual weight gain (g)	0.00±0.00	3.23± 0.04	0.00±0.00
Specific growth rate (% bw d^{-1})	0.00±0.00	1.99±0.01	0.00±0.00
Survival (%)	0.00±0.00	55.24±1.25	0.00±0.00
Gross yield (kg ha^{-1} 124 d^{-1})	0.00±0.00	38.96±0.77	0.00±0.00
Net yield (kg ha^{-1} 124 d^{-1})	0.00±0.00	35.64±0.70	0.00±0.00

Table 7: Mean (± SD) combined production (kg ha^{-1} $crop^{-1}$) of all species. Values in each column with different superscript are significantly different (P<0.05).

Parameters	Treatments		
	T_1	T_2	T_3
Gross production	1254.02 ±34.16^b	1285.98±28.17^b	1710.29±29.22^a
Net production	1233.29±33.79^b	1262.19±28.05^b	1686.74±28.88^a

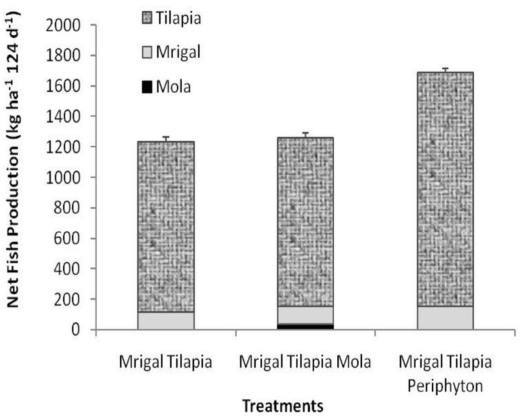

Figure 4: Relative contribution of different species in combined net production in different treatments.

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