Polyculture of mullets in brackishwater using compounded feed: proximate and mineral profiles in comparison with wild mullets

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ABSTRACT

Two stage rearing (nursery and grow-out) of two mullet species, *Mugil cephalus* and *Liza parsia* was demonstrated in a farmer’s pond. In the nursery phase, wild collected grey mullet (1.65±0.2 g) and gold spot mullet (1.03±0.1 g) fry numbering 450 and 4000, respectively were stocked in pen enclosures. After 30 days of nursery rearing, they were released into the same pond and reared for 7 months using dry pelleted feed produced in the feed mill of ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA), Chennai. Feed was formulated using locally available ingredients to contain 33.4% crude protein and 5.7% ether extract. The fishes were fed with crumbles (0.8 mm) and pellets (2 and 3 mm) in the nursery and grow-out phases, respectively. The production of both the mullet species together at harvest was 1262 kg ha⁻¹ (*L. parsia* 851 and *M. cephalus*, 411 kg ha⁻¹). The nutrient composition of the two farmed sympatric mullet species when compared with their respective wild samples showed a significant variation. Higher lipid content in the farmed fishes was observed compared to their respective wild originates. The effect of size was highly significant in lipid content, its value increasing proportionately with size (p<0.05) in both wild and cultured fishes. Cultured *M. cephalus* contained significantly (p<0.05) higher levels of Ca, K, Mn, Cu and Zn, while wild *M. cephalus* had significantly (p<0.05) higher concentrations of Na and Fe. The wild collected *L. parsia* had significantly (p<0.05) higher mineral content compared to the cultured fishes. The dietary value (DV%) of mullets indicates that consumption of 100 g fish could meet the daily requirements of Ca and P for adult human being. Mullet could provide 60-75% of dietary value for Se, which is an important micronutrient that plays a major role in scavenging the free radicals due to its anti-oxidative properties. The results of this study demonstrated the techno-economic viability of mullet culture in farmer’s pond. Mullets are an ideal choice for diversification of brackishwater aquaculture and could serve as an alternative livelihood source and nutritional security for the coastal population.

Keywords: Mineral composition, Mullets, Polyculture, Proximate composition

Introduction

Coastal aquaculture, synonymously known as “brackishwater aquaculture” is a rapidly expanding agro-industry which plays an important role in coastal livelihood and seafood production in India. Farming of shrimp continues to form the bulk of aquaculture activity in brackishwater sector, but for further sustainable growth, there is a need for diversification. Subsequent to failures in shrimp culture, some of the farmers are showing interest in polyculture of brackishwater finfishes. The concept of polyculture is based on the fact that culturing of two or more compatible aquatic species together in the same space will result in higher productivity compared to monoculture (Eldani and Primavera, 1981; Milstein, 2005). It is dominant mainly in Asia, providing a sustainable livelihood and source of nutrition (FAO, 2012). Traditional brackishwater polyculture was generally practiced in large impoundments known as ‘bherries’ in India (Lovatelli, 1990), ghers in Bangladesh (Shofiquzzoha et al., 2001) and pond based systems in the Philippines (Aubin et al., 2015). Polyculture of mullets with tiger shrimp was successfully demonstrated in the farm of ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA) at Kakdwip, West Bengal (Biswas et al., 2012).

Mullets, belonging to the family Mugilidae, are potential candidate species for brackishwater farming due to the commercial value and tolerance to wide range of temperature and salinity (Young and Potter, 2002; Cardona, 2006; Gonzalez-Murcia et al., 2012). In many countries, grey mullet, *Mugil cephalus* is an important aquaculture species for its highly valuable roe and is called “grey gold”. Another mugilid, *Liza parsia* (Ham.), commonly called gold spot mullet is a catadromous fish. Both the species are widely distributed in the coastal waters of tropical and sub-tropical regions extending from 42°N to 42°S.
Feeding habits of mullets make them ideal species for polyculture in association with other fish and crustaceans. Recent isotopic studies on gut contents showed that gold spot mullet was not a competitor for food with grey mullet (Le Loc’h et al., 2015). Most of the earlier polyculture studies were conducted with shrimp or mud crab as one of the high valued species, along with finfishes (Shofiguzzoha et al., 2001; Biswas et al., 2012). Considering this eco-biological facts and the economic importance of these mullets, an “onfarm trial” was conducted on polyculture of grey mullet and gold spot mullet, using formulated pelleted feed in an abandoned shrimp farm after partial renovation. As a different approach, a two stage rearing (nursery and grow-out) was attempted to have good control over feed management and handling of juvenile fishes. Nutrient and mineral composition of both cultured and wild mullets were analysed to understand whether the nutritional quality of cultured fishes are equivalent or superior, which is an important determinant for acceptability of the farmed fish by the consumers as the nutrient composition vary greatly from species to species, as well as from individual to individual of the same species due to differences in size, season, location, habitat, gender, age and source of collection (i.e., wild or cultured (Alasalvar et al., 2002; Grigorakis et al., 2002; Fuentes et al., 2010). The availability of reliable nutritional data base of wild and cultured fish species not only helps in nutritional labelling and processing, but also forms the base for dietary prescriptions.

**Materials and methods**

**Culture trial**

The culture demonstration was conducted in an abandoned brackishwater shrimp pond of 0.35 ha in Nagayalanka, Diviseema region of Krishna District, Andhra Pradesh, India (15.945° N and 80.918°E). The pond was sun dried and lime (CaO) was applied to the pond bottom at 500 kg ha⁻¹. After one week, it was filled with filtered brackishwater from nearby Krishna River, to a depth of 70 cm. Subsequently, on 9th day, the pond was fertilised with organic manure, cattle dung and inorganic fertilisers, urea and single super phosphate (SSP) at 2000, 50 and 50 kg ha⁻¹, respectively and allowed for a week for the growth of natural fish food organisms (Biswas et al., 2012). Inside the pond, three pen enclosures each measuring 4 m² (2 m x 2 m) were built using nylon net of 2 mm knot to knot mesh. Mullet fry collected from shallow tidal pools in the nearby backwaters were brought in buckets and sorted for species and size. Uniform sized grey mullet (1.65±0.2 g) and gold spot mullet fry (1.03±0.1 g) numbering 450 and 4000 respectively were stocked in pen enclosures. Grey mullet seeds were stocked in one pen and gold spot mullets were stocked in two pens @ 2000 per enclosure. After 30 days of nursery rearing, the survival and average size of the fish was recorded. Juveniles of mullets (350 nos. of *M. cephalus* and 3700 nos. of *L. parsia*) were released into the same pond and cultured together for 7 months. After completing the nursery period, the pond water level was increased to >1.2 m and this level was maintained throughout the culture period. The evaporative and seepage losses were compensated and water exchange was done at 20% of the total pond water on a monthly basis. The pond soil and water quality parameters were measured once in two months for important parameters like salinity, pH, dissolved oxygen, temperature, total alkalinity, total ammonia-nitrogen (NH₃-N), nitrite-nitrogen (NO₂⁻N), nitrate-nitrogen (NO₃⁻N) and phosphate-phosphorus (PO₄³⁻P) following standard procedures (APHA, 2012). On termination, the fish were harvested using drag net and by handpicking after completely draining the pond water. The final biomass (kg ha⁻¹), average body weight (g), survival (%) and daily weight gain of both the species of mullets were calculated.

**Feed preparation and feed management**

Compounded sinking pellets were produced in the ICAR-CIBA pilot scale feed mill, Muttukadu, Chennai. All the coarse ingredients were powdered in two stages by grinding in hammer mill and micropulveriser and passed through 0.5 mm screen. The powder along with the liquid ingredients and the binder was mixed in horizontal ribbon mixture and thoroughly homogenised after adding 3 l of water per 100 kg material. The mash was pelleted in the ring-die pellet mill at 15-16% moisture and 90°C temperature under steam conditioning. The pellets were produced using 2 and 3 mm die. Crumbles of 0.8 mm were prepared for nursery feeding. Daily ration was distributed in four and two equal meals during nursery and grow-out periods, respectively. The quantity of feed was adjusted by fortnightly sampling and assumed survival percentage of 85 in grow-out culture.

**Fish samples for nutrient and mineral analysis**

The salinity of the pond water and the area in Krishna River from where wild fish sample collection was made ranged between 10 and 22‰. The fishes were collected from the pond and river at regular intervals until harvest in such a way as to have three size groups (50-100 g, 101-150 g and >150 g for *L. parsia* and 100-250 g, 251-500 g and >500 g for *M. cephalus*) in both cultured and wild systems. They were dressed by descaling and degutting. Except the head and tail portion of the fish, the remaining portions were cut into pieces and macerated along with skin and spines for further analysis. Triplicate
from each group (5-8 fishes) of wild and cultured fish were stored at -20°C until analysis. Harvested fish samples with six replications were used for mineral analysis.

**Laboratory analysis**

The nutrient composition of fish feed and fish tissue samples was determined following standard methods (AOAC, 1997). Minerals in the fish samples were analysed after digestion with Anton-Par microwave system using nitric acid and hydrochloric acid. The contents were centrifuged at 10000 rpm for 5 min and filtered by 0.2 μm membrane syringe filter in case of turbid filtrate. Mineral contents were determined by ICP-OES (Agilent 5100 VDV). The calibration curve was plotted and checked for linearity at five different concentrations of 2, 4, 6, 8 and 10 mg l⁻¹ with 23 element standard mix (Merck, Cat No: 1.11355.0100). The analytical conditions were maintained at 0.61 min⁻¹ nebuliser flow, 0.2 l min⁻¹ auxiliary flow and 15 l min⁻¹ plasma flow.

**Statistical analysis**

Experimental data with two factors viz., source of fish (wild and cultured) and size group (three size groups) with three levels in each factor were analysed using 2x3 factorial design. Mineral analysis data was compared by two sample t-test. Descriptive statistical measures (mean±standard error) were calculated for the two main factors and their interactions. All the data analyses were done using SPSS version 17.0. The *post-hoc* analyses were done using least significance difference. Comparison of means was carried out at 5% significance level (p<0.05).

**Results and discussion**

**Production performance**

The pelleted feed contained 33.4, 5.7, 3.8, 11.87 and 37% crude protein, crude fat (ether extract), crude fibre, total ash and nitrogen free extract (NFE), respectively (Table 1). The water quality parameters (Table 2) were within the optimum levels for brackishwater finfish culture.

Growth, survival and production data of the two mullet species is presented in Table 3. Results indicate the ability of the two mullet species to grow in brackishwater pond.

Table 2. Soil and water quality parameters of mullet polyculture pond during the culture trial

| Water parameters | Value (±SE) |
|------------------|-------------|
| Temperature (°C) | 30±1.3      |
| Salinity (%)     | 17.5±5.3    |
| pH               | 7.85±0.28   |
| Dissolved oxygen (mg l⁻¹) | 7.32±0.6 |
| Turbidity (NTU)  | 12.25±1.71  |
| Total alkalinity (mg l⁻¹ as CaCO₃) | 133±10.42 |
| Total hardness (mg l⁻¹ as CaCO₃) | 2394±943 |
| Total ammonia N (mg l⁻¹) | 0.199±0.11 |
| Nitrite-N (mg l⁻¹) | 0.011±0.004 |
| Nitrate-N (mg l⁻¹) | 0.02±0.008 |
| Phosphate-P (mg l⁻¹) | 0.16±0.08 |

| Soil parameters | Value (±SE) |
|-----------------|-------------|
| pH              | 7.1±0.05    |
| Organic carbon (%) | 0.235±0.07 |
pond in a polyculture mode by consuming formulated feed as the major nutrient and energy source. Though the seed of both the mullet species were of almost similar initial weights, the final average weight of *M. cephalus* was almost 5.2 times higher than that of *L. parsia*, indicating significant difference in genetic potential of sympatric mullet species grown under same conditions. Higher growth rate was observed for *M. cephalus* compared to *L. parsia* when reared in polyculture mode with three mullet species viz., *M. cephalus, L. parsia* and *L. Tade*, along with tiger shrimp (Biswa et al., 2012). In the present study, daily weight gain (DWG) of 0.36 and 1.92 as well as SGR of 1.85 and 2.35, respectively for *L. parsia* and *M. cephalus* were higher compared to the results of a farm trial conducted at the Kakdip Research Centre of ICAR-CIBA (Biswa et al., 2012). This may be due to the longer culture period, less stocking density and lower crude fibre content in the feed used in the present study. The growth performance of both the mullet species based on the periodic samplings is presented in Fig. 1. Mortality of both the mullet species was significantly high in the nursery phase (9.4%) compared to the grow-out phase (7.9%). This reflects adaptation of the juveniles to pond environment after initial acclimatisation in pen enclosures.

Production of both the species of mullets together was 1262 kg ha\(^{-1}\), with production of *L. parsia* and *M. cephalus* being 851 and 411 kg ha\(^{-1}\), respectively. Production was higher than that reported in earlier studies for polyculture systems (Eldani and Primavera, 1981; Shofiquzzoha et al., 2001; Biswas et al., 2012) which might be due to better survival and longer culture period.

**Nutritional composition of the mullets**

The high price for even small sized mullets indicates that they are very popular food fish in India. Because of the high demand, most of the fish are sold fresh and the price normally ranges from ₹200 to 250 kg\(^{-1}\). In the present study, proximate analysis (Table 4 and 5) of the two farmed sympatric mullet species showed a significant variation when compared with their respective wild samples, specifically in total lipid content which was higher in the farmed fishes. The effect of size was highly significant on lipid content, its value increasing proportionately with size (p<0.05) in both wild and cultured fishes. The interactions of source of fish and size were also significantly different (p<0.05). Lipid content of wild mullets was similar to the level reported for *Mugil* sp. (Karakoltsidis et al., 1995; Menezes et al., 2008). Marais and Erasmus (1977) rated mugilids as “fatty fish” which is also appropriate for farmed mullets in the present study. Higher lipid content of farmed mullets could be mainly attributed to the type of food consumed. Compared to the wild and cultured populations

| Main effects and interactions | Crude protein | Ether extract | Total ash |
|------------------------------|--------------|--------------|-----------|
| Source                       |              |              |           |
| Wild                         | 20.19\(a\)  | 2.47\(b\)  | 2.51\(b\) |
| Cultured                     | 19.74\(a\)  | 4.17\(a\)  | 2.61\(a\) |
| Size                         |              |              |           |
| 100-250 g                    | 20.22\(a\)  | 2.04\(a\)  | 2.38\(c\) |
| 250-500 g                    | 19.87\(a\)  | 3.52\(a\)  | 2.62\(c\) |
| >500 g                       | 19.80\(a\)  | 4.41\(a\)  | 2.68\(a\) |
| Source x Size                |              |              |           |
| Wild x 100-250 g             | 20.26\(a\)  | 0.53\(c\)  | 2.26\(c\) |
| Wild x 251-500 g             | 19.92\(a\)  | 2.68\(b\)  | 2.56\(c\) |
| Wild x >500 g                | 20.37\(a\)  | 4.22\(b\)  | 2.72\(a\) |
| Cultured x 100-250 g         | 20.17\(a\)  | 3.56\(ab\)| 2.50\(c\) |
| Cultured x 251-500 g         | 19.81\(a\)  | 4.35\(a\)  | 2.69\(ab\)|
| Cultured x >500 g            | 19.23\(a\)  | 4.61\(a\)  | 2.65\(b\) |
| Pooled SEM (±)               | 0.120        | 0.324       | 0.0001    |

Means bearing different superscripts differ significantly (p<0.05) within main factors (source, size) and interactions

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**Table 3. Growth and production performance of mullets in polyculture pond**

| Performance parameters | Liza parsia | Mugil cephalus |
|------------------------|------------|---------------|
| Initial weight (g)     | 1.03±0.10  | 1.65±0.20     |
| Final weight after nursery phase (g) | 5.1±0.46 | 11.3±0.76 |
| Survival after nursery phase (%) | 92        | 78            |
| Stocking density (nos. ha\(^{-1}\)) | 10570     | 1000          |
| Final average weight at harvest (g) | 87.64±15.2 | 464±159 |
| Daily weight gain (g)  | 0.36       | 1.92          |
| SGR (% day\(^{-1}\))   | 1.85       | 2.35          |
| Survival at harvest (%)| 92         | 88            |
| Total biomass harvested (kg ha\(^{-1}\)) | 851      | 411           |

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**Table 4. Nutrient composition (% on wet weight basis) of *Mugil cephalus* of different size groups collected from wild and cultured populations**

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**Fig. 1. Growth curve of two sympatric mullet species under same grow-out conditions**
natural food such as algae and detritus consumed by wild fishes, the compounded feed offered to the cultured fishes was more nutrient dense (Grigorakis et al., 2002). Further, reduced activity (Alasalvar et al., 2002) compared to wild fish which are also prone to periods of starvation (Haard, 1992) would also have resulted in higher fat in farmed fishes. These results corroborate with the findings of other workers, who compared captive and farmed finfishes (George and Bophal, 1995; Grigorakis et al., 2002; Zhao et al., 2010). In contrast to the present results, Cox and Karahadian (1998) did not find a significant difference in lipid contents of wild and farmed yellow perch.

Positive relationship was observed between fish size and fat content, irrespective of the source and feeding history, indicating that this as intrinsic physiological trait and may also be related to reproductive physiology of the fish (Rheaman et al., 2002). Similar observations have been reported in sebass and gilthead bream (Poli et al., 2001; Grigorakis and Alexis, 2005). Morshita et al. (1989) found increased fish muscle lipid values in cultured seabream with increase in size of the fish. Argyrosomus regius contained a lower fat level in the bigger fishes (1600 g) than smaller ones (830 g) and the authors attributed this to a different lipid metabolism of the fish and the feed offered during farming (Grigorakis et al., 2011; Giogios et al., 2013). The crude protein values were not significantly different between wild and cultured fishes in the present study and this finding is in agreement with the earlier reports (Nettlon and Exler, 1992). The wild collected L. parsia had higher total ash values, whereas cultured M. cephalus contained higher ash values which may be due to the size and species differences.

Minerals play an important role in human metabolism (Belitz et al., 2008). Minerals present in food can be essential, non-essential or toxic to human beings. Lack of essential minerals in the diet may lead to improper enzyme-mediated metabolic functions, organ malfunctions, chronic diseases and ultimately death. Minerals might have an influence on fillet flavour, thus increasing the importance on mineral comparisons between wild and farmed fish. Mineral content of mullets was affected by the source of collection (Fig. 2). Cultured M. cephalus contained significantly (p<0.05) higher levels of Ca, K, Mn, Cu and Zn, while wild M. cephalus had significantly (p<0.05) higher concentrations of Na and Fe. Wild collected L. parsia contained significantly (p<0.05) higher mineral content compared to cultured fishes. Selenium concentration did not differ (p>0.05) between wild and farmed fish of both the species. Cultured yellow perch contained higher Mg, P and K, while wild samples had significantly higher concentrations of Na (p<0.05). Significantly higher concentrations (p<0.05) of Mn and lower concentrations of Selenium were recorded in farmed yellow perch when compared to the wild fish (Gonzalez et al., 2006). Ćalvi et al. (2006) observed higher levels of Zn in farm-raised eel and higher levels of Ca in the wild-caught samples. The variation in mineral composition of marine fish can occur due to seasonal and biological differences (species, size, dark/white muscle, age, sex and sexual maturity), area of catch, processing method, food source and environmental conditions (water chemistry, salinity, temperature and contaminants) (Rodrigo et al., 1998; Alasalvar et al., 2002; Turhan et al., 2004).

Based on the Recommended Daily Allowance (RDA), the dietary values (DV%) for minerals were calculated for 100 g fish (Dayal et al., 2013; Mohanty et al., 2016). The DV% indicates that consumption of
Table 6. Mineral composition (mg 100 g⁻¹ wet weight) of *Mugil cephalus* and *Liza parsia* collected from wild and cultured populations

| Particulars | Ca     | P      | Na    | K     | Mg     | Mn     | Fe     | Cu     | Zn     | Se     |
|-------------|--------|--------|-------|-------|--------|--------|--------|--------|--------|--------|
| *M. cephalus* |        |        |       |       |        |        |        |        |        |        |
| Wild        | 611.67±0.33 | 467.37±3.92 | 62.27±2.33 | 205.11±4.42 | 30.63±0.60 | 0.24±0.002 | 4.18±0.131 | 0.27±0.013 | 0.81±0.032 | 0.03±0.002 |
| Cultured    | 663.61±7.26 | 458.14±6.80 | 54.81±1.22 | 279.29±7.37 | 30.48±0.30 | 0.34±0.017 | 3.74±0.106 | 0.34±0.015 | 0.98±0.035 | 0.03±0.001 |
| *L. parsia*  |        |        |       |       |        |        |        |        |        |        |
| Wild        | 778.32±5.68 | 549.23±3.81 | 70.45±1.35 | 252.91±4.32 | 38.95±0.02 | 0.82±0.006 | 2.89±0.057 | 0.31±0.016 | 1.04±0.025 | 0.02±0.002 |
| Cultured    | 594.46±5.14 | 467.15±5.65 | 66.16±2.81 | 169.68±4.23 | 35.16±0.71 | 0.29±0.046 | 1.86±0.095 | 0.25±0.016 | 1.05±0.037 | 0.02±0.001 |

Means bearing different superscripts within the species differ significantly (p<0.05)

100 g fish could meet the requirements for Ca and P. Mullet could provide 60-75% of dietary value for Se which is an important micronutrient that plays a major role in scavenging free radicals with its anti-oxidative properties and is an essential trace element required in small amounts for the basic functions of life and nutrition. The Se level in both the species of mullets was far below the FDA recommended safe level of 70 µg (FDA, 2009). The lower Na content is another positive aspect of mullets, especially for hypertension patients. These mineral values are calculated for raw fish; however, dietary bioavailability is dependent on many factors. Ca, Mg, Cu and Se are relatively well absorbed, with reported fractional absorption values from mixed diets in human beings. The less absorbed trace elements include Fe, Zn and Mn, with absorption varying widely according to the nutritional status (including body reserves) of the individual and the composition of the diet (Tait and Hurrell, 1996).

Economic analysis

The economic analysis was carried out for testing the profitability of mullet polyculture (Table 7). The net income and benefit cost ratio (BCR) were better than the reported results at the Institute farm (Biswas et al., 2012). This is because of the higher production performance of mullets in the present study and the contribution of farm resources and labour by the farmer. Though the net income cannot be compared with that of shrimp aquaculture (~5-6 lakhs per culture cycle), risks involved in the polyculture of finfish is minimal compared to shrimp farming. The profitability can be further increased by increasing the stocking density and productivity.

The results of this study indicate the techno-economic feasibility of mullet polyculture which could be considered as an ideal option for diversification of brackishwater aquaculture and it could serve as an alternative livelihood and provide nutritional security for the coastal population.

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