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Environmental aspects of fisheries resources
Biodiversity conservation
Open water management
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(Book/ proceedings chapter)	Wong, C.S., F.A. Whitney and W.K. Johnson, 1992. Application of different types of marine experimental enclosures to study the pathways and fate of chemical pollutants. *In:* Marine ecosystem enclosed experiments (eds. C.S. Wong and P.J. Harrison). International Development Research Centre, Ottawa, pp. 174-185.
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A Bayesian population model to estimate Hilsa, *Tenualosa ilisha*, (Hamilton, 1822) stock size in open water fishery of Bangladesh using CMSY and BSM

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Abstract

The demographic trend analyses of Hilsa (*Tenualosa ilisha*) from time series catch data using CMSY and BSM for the first time in Bangladesh. During 1986-2018, CMSY indicates average lowest production in 1993 and highest during 2014. This has been used in the estimation of prior biomass by the default rules. Possible 3195 viable trajectories for 877 r-k pairs were found by the CMSY analysis and the final estimates for intrinsic rate of population increase (r) was 0.567 year^{-1} with 95% CL=0.409-0.785 year^{-1}. The carrying capacity (k) of Hilsa was 2092×10^3 tons with 95% CL=1388×10^3-3152×10^3 tons and MSY was 296×10^3 tons year^{-1}, 95% CL=256×10^3-343×10^3 tons year^{-1}. Results from Bayesian state-space implementation of the Schaefer production model (BSM) using catch & CPUE data, found catchabilitiy coefficient (q) was 1.64×10^{-6} from lcl=1.25×10^{-6} to ucl=2.14×10^{-6} and r=0.545 year^{-1} with 95% CL=0.335-0.886 year^{-1}, k was 3296×10^3 tons with 95% CL=2242×10^3-4845×10^3tons and MSY was 450×10^3tons year^{-1} with 95% CL=298×10^3- 680×10^3tons year^{-1}. Results for Hilsa fishery management based on BSM assessment from time series catch data illustrated that, F_{msy}=0.273 with 95% CL =0.169-0.442 (if B > 1/2 B_{msy} then F_{msy} =0.5r); F_{msy}=0.273 with 95% CL =0.169-0.442 (r and F_{msy} are linearly reduced if B < 1/2B_{msy}). Biomass in 2018 was 1822 tons with 2.5th to 97.5th percentile=1169-2895tons. Relative biomass (B/B_{msy}) in last year was 1.11 from 2.5th percentile to 97.5th percentile=0.709-1.76, Fishing mortality in last year was 0.278 with 2.5th-97.5th percentile=0.175-0.433 and Exploitation F/F_{msy} was 1.02, from 2.5th to 97.5th percentile was 0.64-1.59. The reference points were at terminal tip of the optimum state (approximately linearly) for the Hilsa stock.

Key words: Carrying capacity; Catchability coefficient; MSY; F_{msy}; B/B_{msy}.

Introduction

The national fish Hilsa (*Tenualosa ilisha*) is one of largest single fishery found almost all open water bodies in Bangladesh that contributes about 12% of total fish production in this country (DoF, 2018). Hilsa production was 2.99 lakh MT in fiscal 2008-09, however the country's Hilsa output has increased by 66 percent in the last nine years, the production is beyond the expectation to exceed 5.17 lakh MT in 2017-18 fiscal (DoF 2018). In the early period, the Hilsa fishery is primarily overexploited through an open access of artisanal fishery. Destruction of habitat and overexploitation activity are key factors that affecting the Hilsa abundance. Other anthropogenic causes such as water pollution and ignorance of floodplain management are hampering the Hilsa population in a negative way (Hossain 2014). Though total catch trends creep up over period of time but the average size of Hilsa seems to have declined (Amin *et al.*, 2008). Conversely, FiSAT program illustrates a large variation in exploitation on the stock from 1992 to 2006. The optimum exploitation level is that fishing level at which MSY could be obtained without causing damage of a stock in the long run from an open water body (Miah *et al*. 1998). Worm *et al*. (2009) stated that about 63% of assessed fish stocks worldwide necessitate rebuilding; even as in the EU, 88% of assessed stocks are being caught beyond the maximum sustainable yield (MSY), being 30% of these stocks are at safe biological limits (EC 2009). Current exploitation level assessments compared to maximum economic yields (MEY) (Beverton and Holt 1957), and thus, fleet overcapacities estimation are more scarce (Dichmont *et al*. 2010). Nevertheless, a diagnostic of chronic overcapacity has been affirmed in the green paper (EC 2009). Fish stocks exploited beyond MSY and MEY are thus producing less in biologic and economic terms that, it could be achieved if they were optimally managed (Guillen *et al*. 2013).

Fisheries management endeavors to ensure the long-term sustainable use of fish stocks over a period of times. A selection of reference points are utilized as tools to relate the ecological realities of fish stock dynamics with the management objectives. Monte Carlo method (CMSY) is the modified method for estimating fisheries reference points from catch, resilience and qualitative stock status information on stocks. This also presents a Bayesian state-space implementation of the Schaefer production model (BSM), fitted to catch and biomass or catch-per-unit-of-effort (CPUE) data (Froese *et al*. 2017). MSY has been widely applied as a management objective that is said to define a functional and quantifiable goal (Holt and Talbot 1978; Mesnil 2012; Punt *et al*. 2001). Present study mainly focused on estimating intrinsic rate of population increase, Carrying capacity, maximum sustainable yield (MSY) and finally suggestion with management policy for sustainable capturing of Hilsa from natural stock in Bangladesh.

Materials and Methods

The assessment is based on long term deterministic and stochastic simulations of catch data in tons and catch per unit effort (CPUE) data in Kg/Boat/hour using CMSY and BSM analysis for the first time of Hilsa fishery in Bangladesh (23° 46' 37.8336" N, 90° 23' 58.0272"E). A time

series of maritime catch data for Hilsa fishery was collected over the period of 1986 to 2018, counting annual landing (both artisanal and industrial) including their equivalent efforts. This capture data was acquired from the Fisheries Statistical Report of Bangladesh that assembled by Fisheries Resource Survey System (FRSS), Department of Fisheries (DoF). In fact, a common log sheet has been afforded by DoF to the licensed vessel's members for reporting their catch proceeding to get a navigating approval for the forthcoming fishing trip. These secondary landing data was contained in log sheet that randomly cross-checked by the experienced resource persons of the Department of Fisheries (DoF) and compiled through a system. Catch per unit effort (CPUE) for mechanized boat in the open water was estimated using standardized fishing effort during the period of 1987-2005 that was adopted from yearly Hilsa CPUE data reported by the BFRI, Riverine station, Chandpur (Zaher *et al.* 2013).

To estimate Bayesian Schaefer model (BSM) a catch data time series and CPUE data were required. The parameters i.e. intrinsic rate of population increase (r) = r_{max} and carrying capacity (k) are estimated from (Palomares and Froese 2017).

$$B_{t+1} = B_t + r\, B_t\left(1 - \frac{Bt}{K}\right) - C_t$$

Where C_t is catch in year t, B_t is the current Biomass, B_{t+1} is the exploited biomass in the subsequent year t+1, B=CPUE/q, q is the catchability coefficient. Estimation of stock abundance as CPUE in units of number per hour of fishing or as a biomass index derived from survey catches. Abundance index is related to stock biomass by a catchability coefficient q as (Froese *et al.* 2017)

$$q = CPUE_t/B_t$$

Where $CPUE_t$ is mean catch per unit effort in year t, B_t is available biomass in year t and q is the catchability coefficient. A prior range for intrinsic rate of population increase (r) is derived from life history features, a prior range for carrying capacity (k) is derived from maximum catch and prior ranges for B_t/k (beginning and end of catch time series) are derived from expert knowledge (Palomares and Froese 2017).

$$B_{t+1} = B_t + r\, B_t\left(1 - \frac{Bt}{K}\right) - C_t$$

All r-k combinations that are similar with the life history traits r, natural mortality (M), Parameter of the von Bertalanffy growth coefficient (K), catches (C_t) and expert knowledge (B_t/K) are identified by a Monte-carlo approch. An r-k combination representative of high r values is chosen as best estimate.

To find out the viable r-k pairs, this process was applied to 10000–200000 random r-k pairs, 11–21 start-biomass values and three to six random error patterns for each r-k start biomass combination were assessed (Froese *et al*. 2017). All viable r-values are assigned to 25–100 bins of equal width in log-space. Approximate 95% confidence limits of the most probable r are obtained as 51.25^{th} and 98.75^{th} percentiles of the mid-values of occupied bins, respectively. The most possible value of k is determined from a linear regression fitted to log(k) as a function of log(r), for r-k pairs where r is larger than median of mid-values of occupied bins, with log (4MSY) as intercept and with a fixed slope of -1, based on the reshuffled Schaefer model (Schaefer, 1954).

$$MSY = \frac{rk}{4}$$

$$\text{or, } \log(k) = \log(4MSY)+(-1)\log(r)$$

It can get preliminary estimates of 'r' from the following empirical relations;
$r \approx 2M \approx 2F_{msy} \approx 3K \approx 3.3/t_{gen} \approx 9/t_{max}$ (Schaefer 1954; Gulland 1971; Jensen 1996; Roff 1984; Musick 1999)
The parameters estimated by CMSY and BSM related to standard fisheries reference points such that $MSY= \frac{rk}{4}$, the fishing mortality corresponding to MSY is F_{msy} =0.5 r, the biomass corresponding to MSY is B_{msy} = 0.5 k (Schaefer, 1954; Ricker, 1975) and the biomass below which recruitment may be compromised, is half of B_{msy} (Haddon *et al*. 2012; Carruthers *et al*. 2014; Froese 2015). Hilsa has medium range of resilience and population doubling time range from 1.4 to 4.4 years (Musick 1999). To determine prior r-ranges for the Hilsa under assessment, the proxies for resilience of the Hilsa was provided in FishBase (Froese *et al*. 2000; Froese and Pauly 2015).

Markov chain Monte Carlo (MCMC) sampling was utilized in the estimation of posterior distributions of model parameters. The model was implemented using JAGS version 4.3.0 (Just another Gibbs Sampler) (Plummer 2003).

Results

Standardized time series data of catch per unit effort (CPUE) for Hilsa (*T. ilisha*) illustrated that, CPUE fluctuated around the observed time series with maximum CPUE was 4.601±0.69 Kg/boat/hr in the year of 1998, After that it was suddenly decreased in 1999 and stabled in couple of years from 2000 to 2006 valued 0.76±0.26 Kg/boat/hr to 1.027±0.52 kg/ boat/hr.

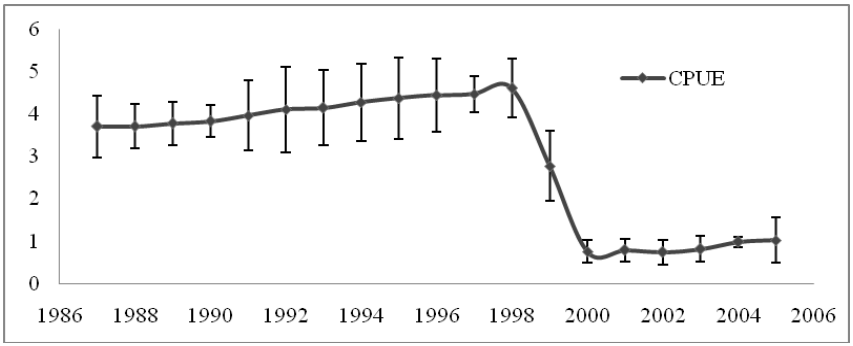

Fig. 1: Standardized time series data of CPUE with standard deviation for Hilsa (*T. ilisha*) catch in open water bodies of Bangladesh

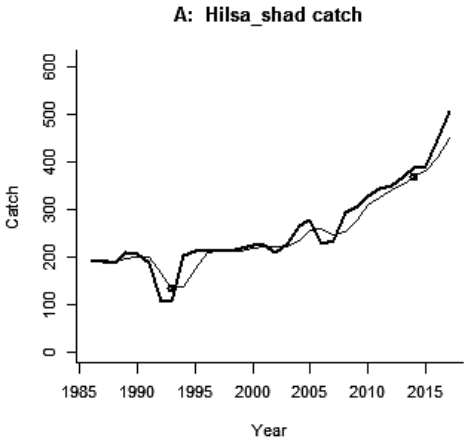

Fig. 2: Time-series of catches in 1000t for the Hilsa (*T. ilisha*) in open water bodies of Bangladesh

All R-coded (CMSY_O_7q.R) model runs, showed robust convergence diagnostics. Fig. 2 illustrates in black, the time series catches of Hilsa during 1986-2018 and in blue, the five-years moving average with indication of lowest and highest catches in 1993 and 2014 respectively. This has been used in the estimation of prior biomass by the default rules. The Heidelberger and Welch test could not reject the assumption that the Markov chain Monte Carlo (MCMC) chains were stationary at the 95% confidence level for any of the calculable parameters. Overall, the posterior distribution of the model parameters was sufficiently sampled. Fig. 3 explains the explored intrinsic rate of population increase (r) - Carrying capacity (k) log space and in dark grey the r-k pairs were found by the CMSY model that to be suitable with the catches and the prior information. Fig. 4 shows the most probable r-k pair and its approximate 95% confidence limits in blue.

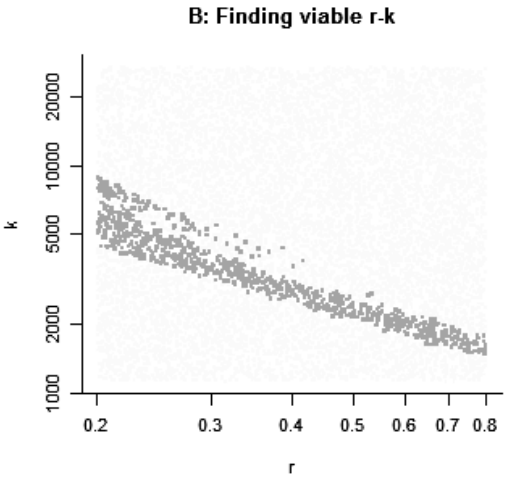

Fig. 3: *r-k* log space of Hilsa by the CMSY model

Fig. 4 shows the most probable r-k pair and its approximate 95% confidence limits in blue.

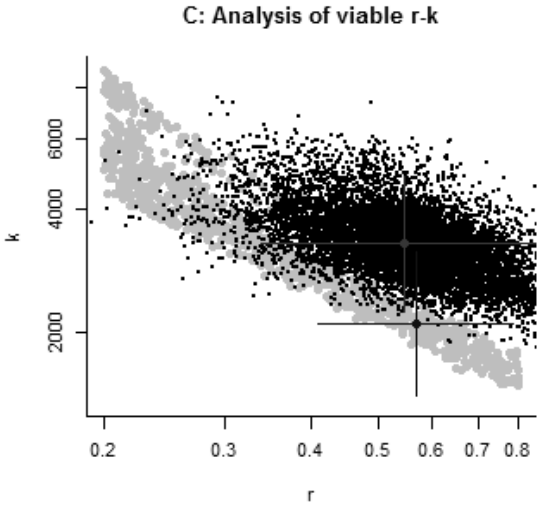

Fig. 4: Comparison of CMSY and BSM for the Hilsa base-case, showed viable r-k pairs from CMSY (grey dots) and r-k posterior values (black dots), with 95% confidence limits denoted by the blue cross for CMSY and the red cross for the BSM model

The black dots are possible r-k pairs found through the BSM model, with a red cross specifying the most suitable r-k pair and its 95% confidence limits (Fig. 4). Possible 3195 viable trajectories for 877 r-k pairs were found by the CMSY analysis and the final estimates were, intrinsic rate of population increase (r) = 0.567 year^{-1}, 95% CL=0.409-0.785 year^{-1}, carrying capacity k was 2092×10^3 tons with 95% confidence limit from 1388×10^3 to 3152×10^3 tons. From the estimation of CMSY model, Maximum sustainable yield (MSY) was 296×10^3 tons year^{-1} with 95% confidence limit 256 $\times10^3$-343×10^3 tons year^{-1}. Results from Bayesian Schaefer model (BSM) using catch & CPUE data illustrated that, catchability coefficient (q) was 1.64×10^{-6} from lower confidence limit (lcl) 1.25×10^{-6} to upper confidence limit (ucl) 2.15×10^{-6}. Intrinsic rate of population increase (r) was 0.545 yr^{-1} with 95%, (CL=0.335-0.886yr^{-1}), carrying capacity (k) was 3296×10^3 tons with 95% CL=2242×10^3 to 4845×10^3 tons and from BSM model the maximum sustainable yield (MSY) was 450×10^3 tons yr^{-1} with 95% CL = 298×10^3 to 680×10^3 tons yr^{-1}.

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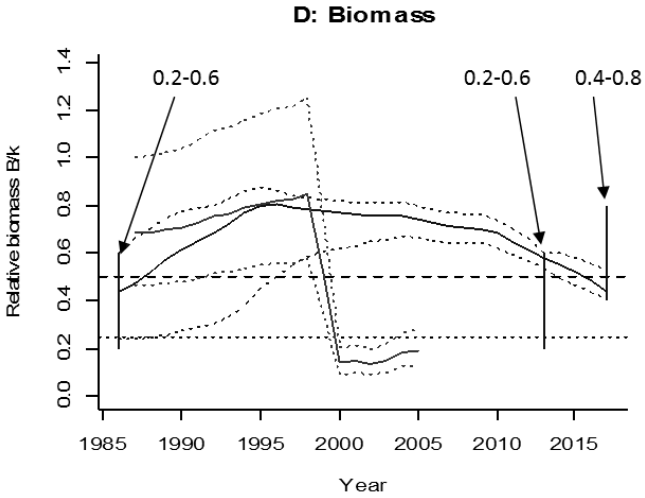

Fig. 5: Details biomass priors used as input parameters in the CMSY/BSM analysis of Hilsa, *T. ilisha* during the period of 1986-2018

The exploitation rate in Fig. 5 confirms the biomass trajectory predicted by CMSY (blue) provided a similar trend as the biomass derived from CPUE (red). Finally the exploitation rate of CMSY (blue) showed similar trend to the CPUE exploitation rate (red). Fig. 5 demonstrates the available abundance data in red, scaled to the BSM estimate of B_{msy} =0.5 k, and in blue, the biomass trajectory estimated by CMSY. Dotted lines point out the 2.5th and 97.5th percentiles. Vertical blue lines mention the prior biomass ranges. First year of estimated relative biomass (B/k) for Hilsa catch was 0.2-0.6. Intermediate biomass in 2013 found as 0.2-0.6 and final relative biomass (B/k) in the year of 2018 was 0.4-0.8.

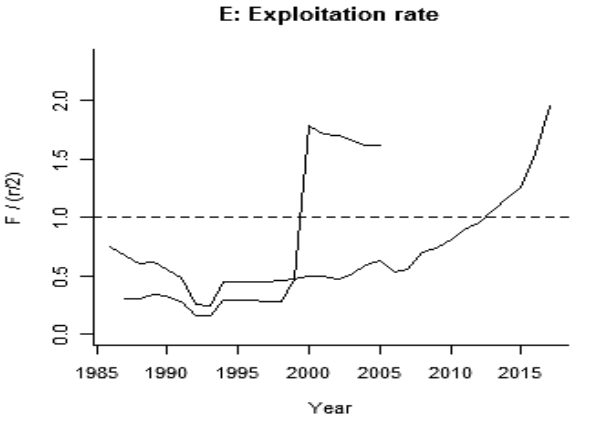

Fig. 6: Exploitation rate of *T. ilisha* from open water fishery of Bangladesh during 1986 to 2014

Fig. 6 explains in red, the harvest rate (catch/abundance) scaled to the r/2 estimate of BSM, and in blue the corresponding harvest rate from CMSY. Incorporation of CPUE for estimation of exploitation rate, the analysis results was given below:

Table 1: Relative biomass and Exploitation F/(r/2) of Hilsa during 2018

Analysis	Relative biomass in last year	2.5th percentile	97.5th percentile	Exploitation F/(r/2) in last year
CMSY	0.437k	0.402	0.524	1.95
BSM	0.553k	0.355	0.879	1.02

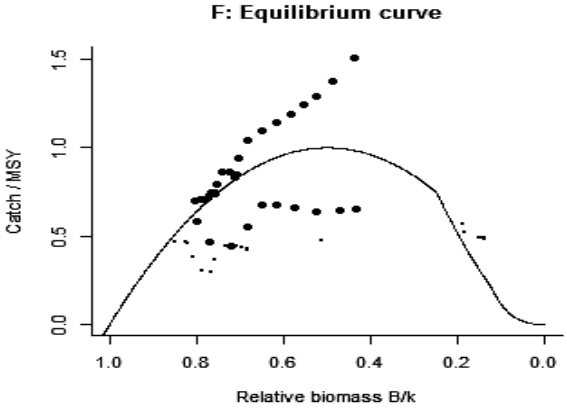

Fig. 7: Schaefer equilibrium curve of *T. ilisha* during 1986 to 2018

Fig. 7 explains the Schaefer equilibrium curve of catch/MSY relation to relative biomass B/k, here indented at B/k < 0.25 to account for creped down of recruitment at low stock sizes. The red dots are scaled by BSM calculates and the blue dots are scaled by CMSY estimates. Catch/MSY from CMSY and BSM for Hilsa in Schaefer equilibrium curve was ranged between 0.3 to 1.5. The prediction for catch relative to MSY and relative stock size showed difference between CMSY (blue) and BSM (red) and whether their distribution around the equilibrium curve makes sense. Most of CMSY points were not being fallen and remained around the equilibrium curves indicated that, the Hilsa was slightly overfished and was being shrunken of biomass. On contrary, BSM points below the curves indicated that, the Hilsa exploitation was sustainable and stock was being grown but recruitment and productivity was slightly reduced. Here the clouds of blue and red dots was overlapped and clustered around the equilibrium curve, thus was being given confidence limits in the assessment.

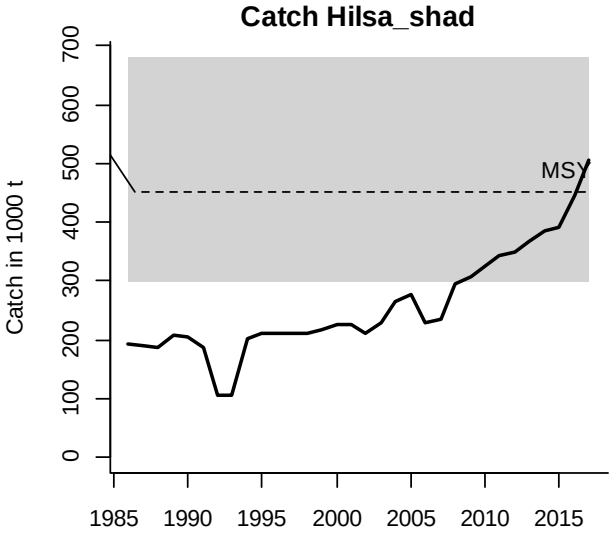

Fig. 8: Catches superimposing the maximum sustainable yield (MSY) region (95% CL) based on BSM analysis of *T. ilisha*

Fig. 8 indicates the catches relative estimation of MSY from BSM model, with an indication of 95% confidence limits in grey. The results based on BSM analysis showed that the catches were increased from 1986 and still increasing. Meanwhile, MSY for 2018 was 450×10^3 tons year^{-1} with 95% CL ranged from 298×10^3-680×10^3 tons year^{-1}.

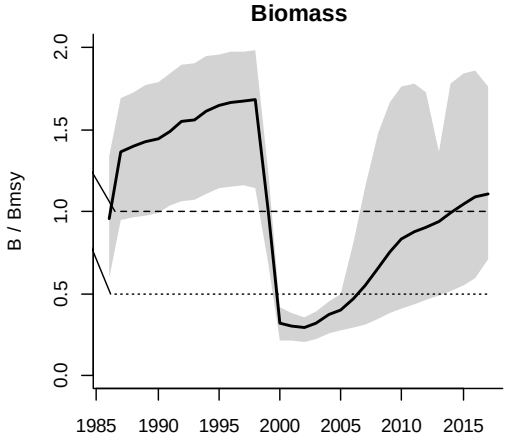

Fig. 9: Relative total biomass (B/B_{msy}) based on BSM analysis of *T. ilisha* in Bangladesh

Fig. 9 demonstrates the development of relative total biomass (B/B_{msy}), with the grey area indicate uncertainty. In the year of 2018, Biomass was estimated as 1822×10^3 with 2.5th percentile-97.5th percentile was ranged from 1169×10^3 tons to 2895×10^3 tons. Population size at the point of maximum growth rate (B_{msy}) was measured as 1648×10^3tons, 95% CL = 1121×10^3tons-2423×10^3tons.

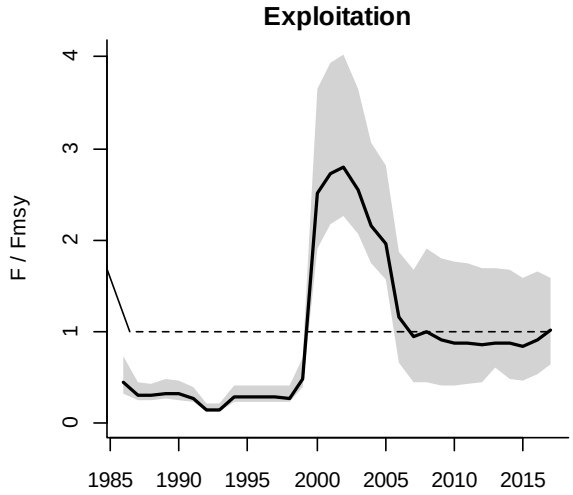

Fig. 10: Trends of Relative exploitation of *T. ilisha* based on BSM analysis from 1986 to 2018

The Fig. 10 explains relative exploitation (F/F_{msy}), with F_{msy} corrected for reduced recruitment below 0.5 B_{msy}. The relative exploitation (F/F_{msy}) was highest during 2000-2007. Maximum rate of fishing mortality (the proportion of a fish stock exploited and removed by fishing (F_{msy}) was 0.273 with 95% CL = 0.169-0.442 (if B > 1/2 B_{msy} then F_{msy} = 0.5 r) and F_{msy} = 0.273, 95% CL = 0.169 - 0.442 (r and F_{msy} are linearly reduced if B < 1/2 B_{msy}). The relative exploitation F/Fmsy for Hilsa (*Tenualosa ilisha*) was 1.02 with 2.5^{th}-97.5^{th} ranged from 0.64 to 1.59 proved that the relative exploitation was at terminal point of equilibrium position.

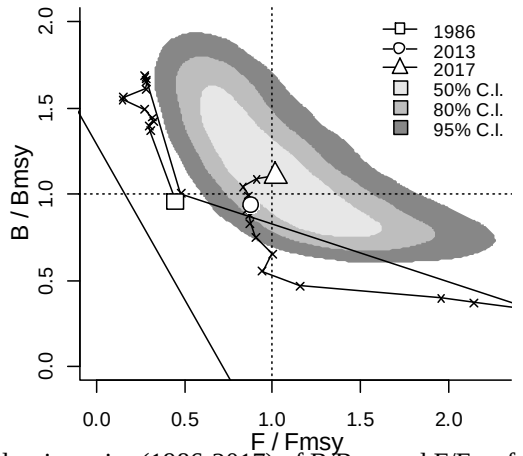

Fig. 11: The estimated trajectories (1986-2017) of B/B_{msy} and F/F_{msy} for the base-case scenario of *T. ilisha* stock. Different grey shaded areas denote the 50%, 80% and 95% credibility interval for the final assessment years

Fig. 11 provides the affiliation of relative total biomass (B/B_{msy}) over relative exploitation (F/F_{msy}). In the year of 2017, the Relative total biomass (B/B_{msy}) was 1.11 with 2.5^{th} percentile from 0.709 to 97.5^{th} percentile was 1.76. Fishing mortality in 2017 was $0.278yr^{-1}$, while 2.5^{th} percentile to 97.5th percentile was ranged between 0.175-0.433. Last year the relative total biomass (B/B_{msy}) over relative exploitation (F/F_{msy}) was just above 1 indicated that, the Hilsa stock was consequently at terminal tip of balance state. The grey areas indicate the range of uncertainty. Conversely, because of the wide ranges of uncertainty associated with stock size and exploitation rate, which overlapped with unsustainable levels, management would be well advised not to increase catches but to maintain them at the optimum level.

Discussion

Hilsa is generally one of the largest single stock in the Indian subcontinent shared by Bangladesh, India and Myanmar. Miah (2015) highlighted that, about 75% of global Hilsa was exploited from Bangladesh waters, 15% from Myanmar, 5% from India and rest 5% from other countries.

FRSS (2008) reported that, national fish Hilsa was caught within the ranged between 194,981 metric tons (MT) and 346,512 MT with an average of 237,936 MT every year during the last 26 years and it was 217,681 MT/years in 2007, appears to be more or less steady over that time. The total production of Hilsa has increased approximately 347 thousand MT, i.e. 77% from 1987 to 2012 (Hossain 2014). A study (Amin *et al*. 2002) also revealed that artisanal fishery resources have already reached a terminal level of exploitation. This is occur due to fishermen fishing in the similar areas since time immemorial because of open excess with crowding of fishing effort and due to improper management practices (Halder 2004). As a result, catch per boat has been seriously declining for the last following years for that instance; the catch of mechanized boats per year is estimated to 33 MT in 1989-90 and consequently decreased to only 9 MT in 2005-06 and 9.81 MT/year in 2012. The catch of non-mechanized boats per year were ranged from 5.3 MT to 4 MT from 1989-90 to 2005-06 and 6.34 MT in 2012 (Hossain 2014). Amin *et al.* (2006, 2008) discovered the mean catch per unit effort as 45.7 kg/boat/day, the total exploitation of Hilsa was 9,997.8MT during the peak period (September-October) in the Chandpur and the Hatia estuarine region of Bangladesh during 1998. But in the same region during 2000, mean catch was 33.0 kg/boat/day, a reduction of 12.70 kg/boat/day (about 28.2%). It is obvious that the CPUE for Hilsa catch was drastically decreased over a period of times, present study concords with the former investigations. Present study also illustrated that, the Hilsa production is subsequently increasing but size of Hilsa is decreasing over the period of times.

Although the harvest of Hilsa has increased its trends and stock is known to be annual, the exploitation level was reported by Amin *et al.* (2002) found to be much higher than the estimated maximum sustainable yield (MSY). In the year 1999, the MSY was estimated to be 162,400 MT but a total of 214,500 MT were landed (Amin *et al.* 2002) this was startling that, catch was more than 30% over and exceed the MSY. In addition, the annual stock assessment expedition conducted in 2002 estimated more than 20% catch above the MSY. However, estimates of exploitation levels between 1999 and 2008 were in the ranged 0.53–0.66 (Halder 2004; The world Bank 2009). Hilsa population in Bangladesh being overexploited and fishing mortality needs to be reduced by decreasing the fishing effort. Strictly maintaining the fishing effort would require a major change in DOF performances (Halder 2004). Until the overture of mechanized boats and nylon twine in early 1980s, the exploitations of Hilsa were mainly accumulated in the inland waters, estuaries and very little in coastal zones. Although the total Hilsa catch was more or less steady, the inland Hilsa catches have reduced by about 15% during the period 1987 to 2006 and again it is increased 26% in 2012 (Hossain 2014). This study agreed with the pervious finding that found by Hossain (2014) and Amin *et al.* (2002).

The population dynamics study for Hilsa fish was conducted before policy making (Mome 2007; Ahmed *et al*. 2008) found the MSY between 210-211 thousand metric tons (MT) during 2007-08. But after implementing the conservation method for gravid Hilsa, banning on peak spawning season and spawning ground and protection and conservation of Juvenile Hilsa activities by government from 2003-04 the Hilsa harvest increased 347 thousand MT in 2012 from 255 thousand MT in 2004. The World Bank (2009) surveyed after policy implementation

and find out that, MSY of Hilsa was around 286,000 MT and the carrying capacity (k) of Hilsa fish was 1,084,000 MT by the current bio-economics studies during 2008. Hossain (2014) also estimated intrinsic Growth rate/alpha, Carrying Capacity/Virgin Biomass and Catchability Coefficient *q* on the basis of that data by using empirical modified logistic Schaefer-Gordon model were 1.05, 1084000 MT and 0.00045 respectively. Present study showed different results due to appropriate conservation of Hilsa stock over a period of times. According to Hossain (2014), in 2012 the Maximum Sustainable Yield (MSY) for Hilsa was 510×10^3 MT and maximum economic yield (MEY) was 670×10^3 MT. Conversely the total production during 2012-2013 was 351,223 MT indicated that, the production and exploitation of Hilsa was not meet with the MSY and MEY. Miah (2015) suggested that, in the long run the Hilsa population will affect the maximum sustainable yield (MSY) from the Bay of Bengal (BoB). However, present study found that the Hilsa production has crossed the target and reached terminal point of MSY, The numbers of marine fishing boats and gears have increased by nearly 4 times since 1984-85, resulting in extreme fishing pressure on the marine Hilsa population (BOBP-IGO, 2008), so it is time to reduce the fishing effort for sustainable production of Hilsa in BOB and Rivers.

The study of relative biomass analysis for Hilsa, the start biomass was 0.2-0.6 B/k seems reasonable, as catches were already high in the first year. The intermediate biomass was 0.2-0.4 in 2013 seem also reasonable, because that was a period similar catches prior to starting year. The final biomass of 0.4-0.8 in 2018 seems also reasonable, because catches had recovered and the length frequency data for that period also suggested healthy biomass. Al-Mamun *et al*. (2017) found starting biomass for Hilsa was 0.4-0.8 during 1990 and final biomass was 0.4-0.8 in 2016, present study finds out the similar result proved that, the stock was recovered and remained in healthy condition.

During 1997, 1998, 1999 the Hilsa fishing level F was $1.36yr^{-1}$ (where the Fmsy=0.6 yr^{-1}) and exploitation rate E was 0.70 (Emsy>0.5). The data of exploitation rate illustrated that the Hilsa population was in over exploitation level and the fishing level was doubled than the F_{msy} at the spawning ground (Miah *et al*. 1998; Miah *et al*. 1999; Miah *et al*. 1997). Amin *et al.* (2006) observed that, the fishing mortality of Hilsa was varied as ($1.43yr^{-1}$ to 2.49 yr^{-1}) during 1995 to 2003. The highest fishing mortality was 2.49 yr^{-1} during 1999 while the lowest was $1.43yr^{-1}$ during 1995. During the year of 2002, the calculated annual yield, standing stock and MSY were 256,902 MT, 148,498.27 MT and 210,125.04 MT respectively (Hossain, 2014). It was alarming that, the annual total catch of Hilsa was 256,902 MT in 2002, much higher than the estimated MSY as 210,125.04 MT. Amin *et al*. (2002) also reported that, in the year of 1999 the fishing pressure on the stock i.e., the fishing pressure (2.49 yr^{-1}) needs to be reduced near to 1.88 yr^{-1} to obtain MSY from the stock. However, the current landing in 2016 for Hilsa was 278,948 MT indicating above the MSY level. This has led to decline in biomass (Miah *et al*. 1997). Fernandes *et al*. (2016) projected Hilsa catch MSY in the Exclusive economic zone for Bangladesh were $220.6\times 10^3\pm14.5\times 10^3$ MT and $239.0\times10^3\pm20.1\times10^3$ MT for 2020s and 2050s respectively from different fisheries management scenarios (MSY or Sustainable exploitation, BaU or Business as Usual, and OF or Overfishing scenario). Present study finds the similar

than the target (F_{opt}=0.37) and limit (F_{limit}=0.5) Biological reference points suggested that the stock was heavily over exploited. However present study found that, fishing mortality for Hilsa was at optimum level. Das *et al*. (2018) realized that, at the value of fishing mortality (F = 2.34 $year^{-1}$), the maximum sustainable yield (MSY) limit for Hilsa was 25,440 MT per year with the corresponding effort (F_{msy}) that may be deployed to achieve the target exploitations in the northern Bay of Bengal, India. Hashemi *et al*. (2010) calculated the MSY as 3247.19 tons from Northwest of Persian Gulf and Roomiani and Jamili (2011) calculated the MSY was 2653 tons from Khuzestan Province, concluded that, the Hilsa was overexploited. Rahman *et al*. (2018) summarized that, fishing mortality of Hilsa during July 2015 to June 2016 was 2.83 $year^{-1}$ and the maximum sustainable yield (MSY) was estimated as 526,000 metric tons/year specify that, the Hilsa achieved it target's with MSY through sustainable management of fisheries by regulating mesh size and protecting brood stock. Present study concluded that, the stock has been over exploited in the past but current year it has recovered and likely to be at the terminal point of MSY.

Conclusion and recommendations

The results clearly mention that *T. ilisha* species is lightly over-exploited in Bangladesh. In order to promote more awareness and some management strategies need to be taken near future for sustainable management of Hilsa stock in Bangladesh through:

1. Ensuring legal size of Hilsa during capture i.e. above 30 cm length of Hilsa catch;
2. Exactly maintaining the mesh size i.e. above 6.5 cm for Hilsa catch;
3. Reducing industrial trawling boat or licensing certain amount of industrial trawler in Bay of Bengal (BOB);
4. More emphasize on demarcation of juvenile Hilsa grounds for seasonal closures;
5. Proper documentation of log book for Marine Hilsa catches.
6. Reduction of fishing effort of Hilsa for industrial trawling in BOB.
7. Providing more awareness on jatka catch among the fishers.

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Catfish diversity, reproductive biology and development of induced breeding techniques for riverine catfishes of Bangladesh

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Abstract

Species diversity, research status, and induced breeding techniques of catfishes of Bangladesh have been presented in this paper. A total of 55 catfish species have so far been recorded from different aquatic habitats of Bangladesh. Abundance and commercial importance have also been highlighted. The significant achievements in catfish research in Bangladesh so far been made is also stated. Catfishes being important food fishes of the people of Bangladesh and because recently their production is decreasing from inland openwater, induce breeding techniques of commercially important riverine catfishes have been developed. Among the different species, *Pangasius hypophthalmus* and *Pangasius pangasius* have successfully been induced by cPGE and HCG in 1993 and 2004, respectively. The breeding season, fecundity, hatching rate, % survival and nursery rearing techniques have also been discussed. Except for the *P. hypophthalmus* and *P. pangasius* none of the riverine catfishes could bred by applying inducing agents.

Key words: Diversity, Catfishes, Research status, Induced breeding, Bangladesh

Introduction

Fisheries are an important sector of economy, playing a dominant role in nutrition, employment generation and foreign exchange earning of Bangladesh. It contributes nearly 60% of the total animal intake, 4.37% to the GDP and 5-6% to country's exports earnings. About 1.2 million people are employed full time in the sector and 12.20 million people are partially dependent for their livelihood or employment.

The present fish production of the country is about 4.277 million tons of which catfish contribute a significant amount. From inland open water about 50,000 tons of catfishes are being harvested every year. Recent introduction of catfishes as culture species and due to development of their induced breeding, the contribution of catfishes is more than 4,41,600 tons from the pond culture system. Overall, the contribution of catfishes is about 10-15% of the country's total production. Instead of increase of catfish production from the culture, the

production is being depleted from natural waters because of improper management, over fishing and increasing unfavorable environmental conditions. Due to these and other man-induced changes, the natural spawning areas of these fishes have decreased rapidly limiting the natural recruitment. As a result, some of the species are now endangered and need protection from being extinct.

With the above background, a programme to study the reproductive biology and development of induced breeding technique of important riverine catfishes such as *Bagarius bagarius, Eutropiichthys vacha, Mystus aor, Pangasius pangasius, P. hypophthalmus* (*sutchi*), *Rita rita, Silonia silondia,* and *Wallago attu* was undertaken at Riverine Station, Chandpur from 1988. Research on riverine catfishes is being continuing to refine induced breeding technique and to develop nursery rearing method.

Although, some important work with the fishes belonging to the family Clariidae, and Heteropneustidae done both in Bangladesh and India, but the work with riverine species is more or less new in this sub-continent. The pond culture feasibility study of *Pangasius pangasius* was done earlier by Hannan *et al.* (1988) and Rahman *et al*. (1991 & 1992). Due to problem with induce breeding of local *P. pangasius,* fingerlings (3-5 cm size) of exotic *P. hypophthalmus*, whose artificial breeding in captivity is possible (Potaros & Sitasit 1976) was imported from Thailand in 27 August 1990. The species was successfully introduced and induce breeding technique was developed in Bangladesh in 8 May 1993 (Rahman *et al*. 1993). *Pangasius pangasius* was bred successfully for the first time in Bangladesh with cPGE at Riverine Station, Chandpur in 30 June 2004 (Rahman *et al*. 2006).

Materials and Method

To review the species diversity and state of catfish research, the published journals, books and references were consulted and compiled. The classification and arrangement of families up to genera were done in accordance with Rahman (2005). Taxonomic features were taken from Joyaram (1981) and Rahman (2005) and remarks on abundance and commercial importance of the species were done in accordance with Rahman (2005), authors own publications (Rahman *et al*. 1991, 1992, 1993, 1994a, 1994b, 1995 & 2006, Haldar & Sorowardi 2000) and from field observations. Most of the materials of reproductive biology and breeding technique study are the results of the riverine catfish project conducted at Riverine Station, Chandpur, during 1988-2006.

Moreover, published papers and articles on different aspects of biology, induced breeding and rearing of various catfishes have been reported by Hamilton (1822), Hora (1939), David (1963 & 1962), Bardach *et al*. (1972), Aizam *et al.* (1983), Ali *et al.* (1985), Hannan *et al*. (1988), Meenakaran (1986), Varikul & Boonsom (1968), Vinci (1984), Saxena (1972), Afser (1992), Devi *et al* (1991, Khan (1924 & 1934), Raj (1940), Chacko & Kuriyan (1948), Saigal & Motwani (1962), Azadi *et al*. (1985, 1988, 1990a, 1990b & 1992), Hossain *et al*. (1991 & 1992), Mollah & Tan (1982 & 1983), Mollah & Karim (1990), Zaman & Seng (1987), Bhatt

(1971a, 1971b, 1970 & 1968), Pantulu (1961 & 1962), Ahmad (1944), Anwar & Siddiqui. (1992), Khumar (1985), Lal & Dwivedi (1965 & 1969) and Doha (1974) were also consulted.

Brood management for gonadal development of *P. pangasius*

Experiments on the gonadal development of *P. pangasius* were conducted under different environmental conditions. A total of 80 fishes weighing 8-12 kg each, collected from the River Meghna during 1989-1991 and were stocked in three ponds of 0.17ha each. The stocking density was maintained at 1,025 kg/ha. Fish were reared with a non-pelleted feed containing fish meal 15%, mustard oil cake 30%, rice bran 30%, wheat bran 20%, wheat flour 3% and molasses 2% at the rate of 5% (wet weight basis) daily. Total daily ration was divided into two equal splits and was administered twice daily in each pond. Besides pond, 10 pairs of adult fish were reared with the same feed in a cage of 10 x 5 x 4 m size in the River Dakatia. In addition to the above brood stock, juveniles of Pangas (average 3-5 cm size) were collected during 1990 and 1991 and were reared in a separate pond with the above mentioned non-pelleted feed to develop a new brood stock.

A total of 40 old broods of *P. hypophthalmus* were reared in a pond while a new brood stock of about 100 individuals have been raised since 1993 from the fingerlings of the first generation of the old stock. Fishes were reared with a non-pelleted feed as done for *P. pangasius*. Observations on the maturity stages of ovaries and testes of *P. hypophthalmus* were made according to the methods prescribed by Nikolsky (1963).

Brood management for gonadal development of *Rita rita*

Rita rita, a bottom dwelling riverine catfish, is found in the large rivers of Bangladesh. Experiment on the rearing of broods of *R. rita* was carried in the freshwater ponds of the Riverine Station, Chandpur in 1990-1996. A total of 110 broods of *R. rita* averaging 1.0 kg were collected from the river Meghna during 1990 and 1996. All these fishes were reared in a pond of 0.17 ha at a stocking density of 650 kg/ha. Fishes were reared with a non-pelleted feed as supplied for *P. pangasius*. Monthly sampling was done to monitor the development of secondary sexual characters.

Brood management for gonadal development of *Mystus aor*

M. aor, a bottom dweller and nest builder, is found to breed in freshly inundated river banks. A total of 85 adult fish of *M. aor* averaging 2.5 were collected from the River Meghna in 1993 and 1995. The fish were reared in a pond of 0.17 ha along with *R. rita* at a stocking density of 700 kg/ha. A similar type of feed and feeding regime of *P. pangasius* was employed for their rearing. Monthly sampling was done to monitor their gonadal development and development of the secondary sexual characters.

Brood management for gonadal development of *Silonia silondia*

S. silondia is highly sensitive to pollution and oxygen depletion. Only 34 adult individuals of *S. silondia* weighing 2 to 4 kg were collected in December 1995 and were reared in a pond with other riverine catfishes. During May-July 1996, a total of 52 juveniles of *S. silondia* averaging 100-150 g were also collected from the River Meghna. Feed and feeding regime were similar to those of *P. pangasius*. Gonadal development was monitored monthly.

Brood management for gonadal development of *Bagarius bagarius*

Bagarius bagarius, a giant, voracious and deep water riverine catfish was collected from the River Meghna. It is a bottom dweller having carnivorous feeding habit. Only 27 adult individuals of *B. bagarius* averaging 5 to 7 kg were collected in December 1994 and reared in a pond with other catfishes. During May-July 1996, a total of 22 juveniles of *B. bagarius* averaging 200-300 g were also collected from the River Meghna. Feeding management and monitoring schedule were similar to those of other riverine catfishes were followed for *B. bagarious* also.

Artificial breeding

Experiments on the standardization of the hormone doses required for artificial breeding for above mentioned species were conducted with both carp pituitary extract (cPGE) and human chrionic gonadotropin (HCG). cPGE were prepared instantly during hormone administration from locally procured carp pituitary glands while imported HCG (Pregnyl of Organon and Profasi of Serono) were used.

Results

Species Diversity of Freshwater Catfishes

Bangladesh is rich with its fish fauna. About 260 species of freshwater fishes, 20 species of exotic fish including three catfishes occur in freshwater of Bangladesh. Accordingly, the species diversity of catfishes is also rich. About 50 species of catfishes were so far been registered. The species names of freshwater catfishes along with their genus and family are given below:

Family, Genus and Species	Key Characters and Remarks
Family Clariidae Genus *Clarias* Scopli, 1777 1. *Clarias batrachus* (Linnaeus)	Air breathing catfish, four pairs of barbels, rayed dorsal fin long, 66-71 rays and without any spine. Single species, confined to stagnant and muddy water. Culture and induced breeding technique developed. High priced commercial fish.

Family Siluridae Genus *Wallago* Bleeker, 1851 2. *Wallago attu* (Bloch)	Freshwater shark, mouth sub-terminal up to anterior border of eyes. Dorsal fin inserted above half of the pectoral fin, without a spine, pectoral fin with smooth spine. Single species, commercially important, found almost in all rivers and flood plain. Voracious feeder highly predatory behavior.
Genus *Ompok* Lacèpéde, 1803 3. *Ompok pabda* (Hamilton) 4. *O. bimaculatus* (Bloch) 5. *O, pabo* (Hamilton)	Butter catfishes, two pairs of barbels, maxillary and mandibular, latter rudimentary or small. Rayed dorsal fin, 4-5 rays without spine, pectoral fins with feebly serrated or smooth spine. Commercially important, high priced fish. Induced breeding developed, Occasionally mixed culture practiced. DO sensitive, Carnivorous.
Family Heteropneustidae Genus *Heteropneustes* Müller, 1840 6. *Heteropneustes fossilis* (Bloch)	Stinging catfishes, rayed dorsal fin short, without spine, adipose dorsal absent, pectoral fin with seven or eight rays and a strong spine serrated along the inner edge. Single species, primarily occur in freshwater pond, ditches swamps and marshy land. High priced commercial fish. Induced breeding developed.
Family Olyridae Genus Olyra McLelland, 1842 7. *Olyra Kempi* (Chaudhuri)	Barbels four pairs, dorsal fin without spine, pectoral with a short stout spine. Found occasionally in hill streams. DO sensitive.
Family Plotosidae Genus *Plotosus* Lacèpéde, 1803 8. *Plotosus canius* (Hamilton)	River eel catfish, rayed dorsal fins with both edges serrated pungent spine, pectoral fins laterally inserted with a strong serrated spine. Found in the estuaries and bay, ascend in rivers. Little commercial importance. Endangered.
Family Chacidae Genus *Chaca* Gray, 1831 9. *Chaca chaca* (Hamilton)	Square-head catfish, rayed dorsal fin with pungent spine, pectoral fins with strong serrated spine, six barbels. Found in rivers and flood plains. No commercial importance, even not eaten. Deep water bottom dweller, carnivorous. Endangered.
Family Schilbeidae Genus *Silonia* Swainson, 1839 10. *Silonia silondia* (Hamilton)	Two pairs of barbels; rayed dorsal fin with a spine. Pectoral with a strong spine serrated along both edges. Found in rivers and estuary, commercially important, landing decreasing.

Genus *Pangasius* Valenciennes, 1840 11. *Pangasius pangasius* (Hamilton)	Large size fishes, two pairs of barbels, rayed dorsal fin with a spine, pectoral fin with strongly serrated spine. Commercially important high priced fish, found in big rivers and estuaries sometimes in flood plains. One species present.
Genus *Clupisoma* Swainson, 1839 12. *Clupisoma garua* (Hamilton) 13. *C. murius* (Hamilton)	Four pairs of barbels, rayed dorsal fin, seven rays and a spine, pectoral fin with a spine serrated along inner edge. Two species, *C. garua* is dominant, have commercial importance, found in rivers, streams and canals.
Genus *Pseudeutropius* Bleeker, 1863 14. *Pseudeutropius atherinoides* (Bloch)	Four pair of barbels, rayed dorsal fin with a spine, pectoral fins with a spine serrated along both margin. Vary small size, have some commercial importance, found in rivers and floodplains.
Genus *Eutropiichthys* Bleeker, 1862 15. *Eutropiichthys vacha* (Hamilton)	Four pairs of barbels, rayed dorsal fin with a spine, pectoral fins with a smooth spine. Found in rivers and flood plains, have commercial importance.
Genus *Ailia* Gray, 1830 16. *Ailia coila* (Hamilton)	Four pairs of uniform barbels, longer than head. Rayed dorsal fin absent, pectoral fin with a smooth or serrated spine. Found mainly in the rivers, have commercial importance.
Genus *Ailiichthys* Day, 1871 17. *Ailiichthys punctata* Day	Barbels four pairs, shorter than half of the standard length, dorsal fin absent, pectoral well developed, occur mainly in river, little commercial value.
Family Amblycipitidae (Torrent Catfishes) Genus *Amblyceps* Blyth, 1858 18. *Amblyceps mangois* (Hamilton)	Four fairs of barbels, rayed dorsal and pectoral fin with a week spine, very rarely found, little commercial value
Family Bagridae Genus *Mystus* Scopoli, 1777 19. *Mystus aor* (Hamilton) 20. *M. seenghala* (Sykes) 21. *M. menoda* (Hamilton) 22. *M. gulio* (Hamilton) 23. *M. tengara* (Hamilton) 24. *M. vittatus* (Bloch) 25. *M. cavasius* (Hamilton) 26. *M. bleekeri* (Day)	Four pairs of barbels, generally longer than head, rayed dorsal fin with a spine, pectoral fins spine serrated along the inner edge. Most important food fishes of Bangladesh. Represented by nine species, these fishes are very numerous in the inland water of Bangladesh, exception is the *M. gulio* which is abundant in the estuaries and the Bay. *M. tengara* is the commonest of all available species. *Mystus armatus* is rare.

27. *Mystus armatus* (Day)	*Mystus seenghala* and *Mystus aor* are the largest members of the group. Jayaram (1955) proposed the sub-genus *Osteobagrus* for possessing an interneutral shield between occipital process and the basal bone of dorsal fin and elevated to genus *Aorichthys.* Commercially important.
Genus Bleeker, 1858 28. *Rita rita* (Hamilton)	Three pairs of barbels, dorsal fin rayed with a spine, pectoral fins with a serrated spine along the both edges. Found in river and estuaries. Moderate size fish, have commercial importance, but now endangered.
Genus *Chandramara* Jayaram, 1972 29. *Chandramara chandramara* (Hamilton)	Barbels four pairs, unserrated dorsal spine moderately strong, pectoral spine stout, small size, found in ditches, streams and canals, little commercial value.
Genus *Batasio* Blyth, 1860 30. *Batasio tengana* (Hamilton) 31. *B. batasio* (Hamilton)	Four pairs of barbels, rayed dorsal fin with a spine, rayed pectoral fin with a serrated spine along inner edge. Small size fish, have little commercial importance, found in rivers and canals.
Family Sisoridae Genus *Sisor* Hamilton, 1822 32. *Sisor rhabdophorus* (Hamilton)	Sucker catfishes, elongate, tapering posteriorly. Five pairs of barbels, dorsal spine week, finely serrated, pectoral spine serrated on both edge. Found in rivers of northern region of Bangladesh, very rare, little commercial importance.
Genus *Bagarius* Bleeker, 1853 33. *Bagarius bagarius* (Hamilton)	Four pairs of barbels, rayed dorsal fin with a smooth spine, pectoral fins' spine serrated along inner edge. Found through out Bangladesh, voracious and predatory, very big size (180 cm recorded) have commercial importance.
Genus *Gagata* Bleeker, 1858 34. *Gagata gagata* (Hamilton) 35. *G. cenia* (Hamilton) 36. *G. viridescens* (Hamilton) 37. *G. nangra* (Hamilton) 38. *G. youssoufi* (Rahman)	All the species have four pairs barbels, with dorsal and pectoral spines, found in rivers. Very small size, little commercial importance.

Genus *Hara* Blyth, 1860. 39. *Hara hara* (Hamilton) 40. *Hara jerdoni* (Day)	Very small size, no commercial importance. Bottom dweller, Clear and highly oxygenated water. Carnivorous. Pollution and DO sensitive. Endangered.
Genus *Erethistes* Müller and Troschel, 1845 41. *Erethistes pusillus* Müller & Troschel	Very small size, no commercial importance. Bottom dweller, carnivorous. Endangered.
Genus *Conta* Hora, 1949 42. *Conta conta* (Hamilton)	Barbels four pairs, dorsal and pectoral spine serrated both edges, found in flowing stream with sandy bottom, no commercial importance. Bottom dweller, Clear and highly oxygenated water. Carnivorous. Endangered. Pollution and DO sensitive.
Genus *Glyptothorax* Blyth, 1860 43. *Glyptothorax horai* Shaw and Shebbeare 44. *G. telchitta* (Hamilton) 45. *G. shawi* (Hora) 46. *G. rebeiroi* (Hora)	All the four species found in the Northern and Eastern rivers, little commercial importance. Pollution and DO sensitive.
Family Tachysuridae Genus *Tachysurus* Lacèpéde, 1803 47. *Tachysurus gagora* (Hamilton) 48. *T. nenga* (Hamilton)	Marine and estuarine, enters rivers, have little contribution in inland catch but contributes significantly along with other catfishes in marine catch (about 1% of the country total).
Genus *Batrachocephalus* Bleeker, 1846 49. *Batrachocephalus mino* (Hamilton)	Found in estuarine and Bay of Bengal, enters rivers, little commercial importance.
Genus *Osteogeneoiosus* Bleeker, 1846 50. *Osteogeneiosus militaris* (Linnaeus)	Found in estuarine and Bay of Bengal, enters rivers, very small size, little commercial importance.

Exotic catfish introduced in Bangladesh

Family, Genus and Species	Key Characters and Remarks
51. *Pangasius hypophthalmus* (Fowler)	Introduced from Thailand in 1990, adapted well, induce breeding and culture technique developed.
52. *Clarias gariepinus* (Burchell)	as above
53. *C. macrocephalus* (Güther)	as above
54. *Pangasianodon gigas* Chevey	Introduced from Thailand in 2006
55. *P. bocourti* Fowler	Introduced from Thailand in 2009

State of Catfish Research in Bangladesh

Bangladesh is abounding with a large variety of fishes but very little scientific studies have been done on fish and fisheries. Although the systematic studies of freshwater fishes fauna of this continent goes back to the work of Francis Hamilton, 1822. With other fish species of the sub-continent, Francis Day in 1878, published scientific account of the catfishes. Many useful taxonomic contributions on the fish fauna of India including Burma and Sri Lanka were done by many authors. Among them taxonomic account of freshwater fishes published by Jayaram (1981) is an excellent work. The most outstanding work on taxonomy of the fishes of Bangladesh was done by Rahman (1989). Among other contribution, the works of Hussain (1970), Doha (1973) and Rahman *et al*. 1991, 1992, 1993, 1995 & 2006 are important. The concepts of induced breeding and culture of freshwater catfishes of Bangladesh started with the establishment of Bangladesh Fisheries Research Institute in 1984. Both from its two stations, the Freshwater Station, Mymensingh and Riverine Station, Chandpur, a considerable amount of work have been carried out (Table 1). Some important works on catfishes were also carried out from different universities of Bangladesh (Table 1).

Table 1: Important research work done on catfishes of Bangladesh

Clarias macrocephalus:

Authors	Works done
1. Hussain A.K.M.A. 1988	Studied first feeding of *C. macrocephalus*
2. Mollah and E.S.P. Tan 1982	Studied reproductive biology of *Clarias macrocephalus*
3. Mollah and E.S.P. Tan 1983	Induce spawning of *Clarias macrocephalus* by HCG
4. Mollah M.F.A. 1984	Studied the effect of water temperature on the growth and survival of *C. macrocephalus* larvae
5. Mollah M.F.A. 1985	Studied the effect of stocking density and water depth on growth and survival *C. macrocephalus*
6. Mollah M.F.A. 1986	Cyclic changes in the ovary of *Clarias macrocephalus*

Clarias gariepinus

Authors	Works done
7. Ahmed *et al*. 1997	Studied the culture feasibility of *C. gariepinus* fry in glass tank and synthetic hapa
8. Alam *et al*. 1998	Studied the effect of testosterone propionate on growth, survival and sex ratio of African Catfish, *C. gariepinus*
9. Hussain *et al*. 1998	Studied on the optimum protein energy-ratio of *C. gariepinus*
10. Mollah and Karim 1990	First induced breeding of African catfish, *Clarias gariepinus* with mixture of HCG and cPGE

Clarias batrachus

11. Ahmed *et al*. 1980	Studied the food and feeding habit of *Clarias batrachus*
12. Ahmed *et al*. 1981	Tried for induce spawning of *Clarias batrachus*
13. Alam and Mollah 1988	Formulated artificial dry feed for nursing of *Clarias batrachus*
14. Alam and Mollah 1989	Experimented moist diets for rearing *C. batrachus* larvae
15. Bairage *et al.* 1988	Compared the different feed for *C. batrachus* fry rearing.
16. Barua *et al*. 1988	Studied L-W relationship and condition of *Clarias batrachus*
17. Barua *et al*. 1988 & 1986	Studied the reproductive biology of *Clarias batrachus*
18. Barua G. 1990	Studied gonadal development of *Clarias batrachus*
19. Islam *et al*. 1986	Determine the influence of stimulant, size and stock of fishes on the success of induce breeding of *C. batrachus*
20. Kamal Y. M. 1987	Standardized the breeding technique of *C. batrachus* and *Heteopneuestes fossilis*
21. Mollah and Nurullah 1988	Studied on the effects of feeding frequency on the growth and survival of *Clarias batrachus* larvae
22. Mollah M. F. A. 1987	Developed mass production and rearing of *C. batrachus* fry
23. Paul *et al*. 1989	Attempted to breed *Clarias batrachus* by PG extract
24. Rahman *et al.* 1987	Formulated quality feed from indigenous raw materials for culture of *Clarias batrachus*
25. Rahman *et al*. 1995	Produced hybrid vigor through cross breeding between *Clarias batrachus* and *Clarias gariepinus*
26. Rahman *et al*. 1997	Studied the effect of supplemental feeds on survival and growth of *Clarias batrachus* fry
27. Rahman *et al*. 1997	Experimented on breeding of *Clarias batrachus* in paddy field
28. Rahmatullah *et al*. 1983	Attempted to breed *C. batrachus* by pituitary hormone
29. Rashid *et al*. 1983	Recorded some metazoan parasites of *Clarias batrachus*
30. Saha *et al*. 1998	Developed rearing technique of *C. batrachus* larvae with formulated diets

Heteropneustes fossilis

Authors	Works done
31. Mollah *et al*. 1973	Experimented on the feeding of *H. fossilis* fry
32. Ahmed & Sanaullah, 1976	Studied metazoan parasites in *H. fossilis* and *C. batrachus*
33. Ahmed & Sanaullah, 1977	Studied infestation of helminths in *H. fossilis* and *C. batrachus*
34. Huq M. F. 1977	Determined the sexes of catfishes of Bangladesh through external characters
35. Mia G. K. 1984	Studied L-W relationship and condition factor of *H. fossilis*
36. Azadi & Siddique 1986.	Estimated the fecundity of *Heteropneustes fossilis*
37. Haque *et al*. 1988	Studies culture prospects of *H. fossilis* in floating net cages
38. Latifa and Begum 1989	Studied sex ratio and size frequency distribution of *H. fossilis*
39. Chandra and Khatun 1993	Identified a new species of *Caryophallacid cestoda* from *H. fossilis*

Pangasius pangasius* and *P. hypophthalmus

40. Ali *et al*. 1985	Food and feeding habits of *Pangasius pangasius*
41. Hannan *et al*. 1988	Studied culture feasibility of *Pangasius pangasius* in pond.
42. Rahman M. K. 1989	Formulation of feeds for *Pangasius pangasius*.
43. Rahman *et al*. 1992	Studied comparative growth rate and survival of two *Pangasius* species, *P. pangasius* and *P. sutchi*
44. Rahman *et al*. 1993	Developed induced breeding of *P. sutchi* for the first time
45. Rahman *et al*. 1994	Studies on embryonic and larval development of *P. pangasius*
46. Rahman *et al*. 1995	Studied the biology of *P. pangasius* of natural waters
47. Rahman *et al.* 1995	Produced hybrid pangas (*P. sutchi* ♀ X *P. pangasius* ♂)
48. Rahman *et al*. 2006	Developed induced breeding of *P. pangasius*

Rita rita

49. Rahman *et al*. 1991	Studied pond culture feasibility of *Rita rita*

Mystus cavasius

50. Akhtaruzzaman *et al*. 1991	Developed induced breeding technique of *Mystus cavasius*
51. Hossain *et al*. 1998	Studied on polyculture feasibility of *M. cavasius* with *Puntius gonionotus* and *Hypophthalmichthys molitrix*
52. Kohinoor *et al*. 1994	Studied monoculture feasibility of *M. cavasius* in pond

Mystus tengara

53. Alam S.M.N. 1989	Studied fecundity of *Mystus tengara*

Mystus aor* and *Mystus seenghala

54. Azadi *et al*. 1990	Established relationships between body measurements and some internal organs of *Mystus aor*
55. Azadi *et al*. 1992	Studied biology and fishery of *Mystus aor* in Kaptai Reservoir

Ompok bimaculatus* and *Ompok pabda

56. Hossain *et al*. 1991	Studied food and feeding habits of *Ompok pabda*
57. Hossain & Rahman 1992	Studied reproduction and fecundity of *Ompok pabda*.
58. BFRI 1994	Developed induce breeding technique of *Ompok bimaculatus*

Wallago attu

59. Anon 1997	Studied some aspects of biology of *Wallago attu*
60. Haldar & Sorowardy 2000	Studied food, feeding habit, predation rate, induced breeding and culture feasibility of boal fishes, *Wallago attu*

Eutropiichthys vacha

61. Azadi *et al*. 1988	Established length-weight and girth-weight relationships and condition factor of *Eutropiichthys vacha*
62. Azadi *et al*. 1990	Studied reproductive biology of *Eutropiichthys vacha*
63. Azadi *et al*. 1991	Studied food and feeding habits of *Eutropiichthys vacha*

Reproductive biology and development of breeding techniques

Gonadal development and induced breeding of *P. pangasius*

Microscopic examination revealed that the male fish developed secondary sexual characters by May which could be identified by their oozing condition of milt. Females though were found having slightly bulging belly yet they did not develop well. Artificial propagation were tried respectively with cPGE @ of 12 mg/kg of body weight (bw) and/or HCG @ 5,000 IU/kg bw during August to September, but ovulation did not took place except a few in 1992. Using the ovulated eggs embryonic and larval development was studied (Rahmat *et al*. 1994). All the fishes of the new stock seemed matured by May 1996 attaining an average weight of 3-4 kg. Most of the males were found at oozing condition but the females did not show the sign of ripeness. During August-September attempts were again made to induce them with cPGE and HCG at the earlier mentioned doses. There was no ovulation success with these stocks as well. In 1996-1998, a group of adult fishes (4-8 kg sizes) were kept under extreme feeding care both in the pond and riverine condition (floating cages hanged in the river Dakatia) and fed them as discussed earlier. Fishes kept in the ponds were given primary hormone dose with cPGE @ 2.0 mg/kg of bw, once monthly during January to July for expedition their gonadal development. Besides, another group of pond reared fishes were given priming hormone dose with LHRH-A_3 @ 4.0 and 10.0 µg/kg of bw, once monthly during January-July period for expediting their gonadal development.

Females which showed little swelling of the abdomen were injected with a preparatory dose of Damperidon @ 5.0 mg/kg of bw + Salmon gonadotropine 10.0 µg/kg of bw and a resolving dose of cPGE @ 5.0 mg/kg of bw at an interval of 12 hours during June to July. Yet, there was no ovulation. Males showed development of milt with injection of cPGE at 2.0 mg/kg of bw.In 2004, *Pangasius pangasius* was bred successfully with cPGE at the rate of 9 mg per kg of bw and the male received 3.0 mg of cPGE. Food and feeding rate are two important factors regulating optimum maturation of *P. pangasius*.

Gonadal development of *P. hypophthalmus*

Gross microscopic examination revealed that the ovaries of *P. sutchi* could be placed in six maturity stages (Table 2) and the testes in five (Table 3).

Table 2: Descriptions of maturity stages of the ovary of *Pangasius hypophthalmus*

Maturity stages	Descriptions
1. Immature virgin	Ovary colorless to translucent-cream, lanceolate and lobular in appearance. No oocytes are visible in naked eyes.
2. Developing virgin	Ovary translucent, yellowish in color and occupies 1/2 of the length of peritoneal cavity. Individual oocytes as tiny specks are visible in naked eyes.

3. Maturing or ripening	Ovary opaque-yellowish-brown in color, occupying about 1/2 of the ventral cavity. Eggs are visible in naked eyes as brownish-yellow granules. Blood capillaries are visible around the ovary.
4. Mature or ripe	Ovary large, opaque, brownish-yellow in color. Egg-yolk laden and are clearly visible by the naked eyes. Ovary occupies 4/5 of the peritoneal cavity. Blood capillary networks are highly developed around the organ.
5. Running or spawning	Eggs translucent and easily are extruded on slight pressure after cPGE or HCG treatment. Maximum sized eggs.
6. Spent	Ovary flaccid, flabby and blood-shot with thick whitish tough walls. Genital aperture of female looks inflamed. Some translucent and opaque (residual) eggs visible by the naked eyes.

Table 3: Descriptions of maturity stages of the testes of *P. hypophthalmus*

Maturity stages	**Descriptions**
1. Immature virgin	A pair of small, thread-like elongated colorless organ with slightly serrated edges.
2. Developing virgin	Testes translucent opaque white color, occupying about 1/3 of the length of the body cavity. Serration at edges becomes more sharp and pronounced.
3. Maturing or ripening	Testes more enlarged, occupying 1/2 of the length of the body cavity, opaque and dirty-white in color.
4. Mature or ripe	Turgid, greasy-white in color, greatly enlarged, occupying about 2/3 of ventral cavity. Finger-like structures become full of milt and are easily extruded by soft pressure as in other fishes.
5. Spent	Testes shrunken and flaccid. Finger-like structures revert to original sharp condition.

Breeding season of *P. hypophthalmus*

Observations were made for determining the breeding season of *P. hypophthalmus.* It was found that the breeding season starts from the late March and continues up to late August. However, peak spawning season lies between May and July. Spent and immature virgin fishes were found during September and October while developing virgin and maturing fishes were found between November and January. Mature fishes were found between February and March while running or spawning fish were found during the period of late March through mid September. It was also observed that spent male recover their milt after 2 months of stripping while female require 3 months to regain their eggs.

Fecundity of *P. hypophthalmus*

Relative fecundity (number of eggs per unit of fish) of *P. hypophthalmus* was estimated by gravimetric method. Total weight of the ovary was taken after removing excess water. Then sub-samples of eggs of 5g each were taken from the anterior, posterior and middle portion of the ovary. Each sub-sample was counted separately and the mean number was taken. Total numbers of eggs were calculated by multiplying the mean number of eggs in a sub-sample with the total weight of the ovary. *Pangasius sutchi* fishes matured at a minimum size of 2.0 kg. Mature and berried females had a mean (±sd) of 146.7±38.84 g of eggs/kg of bw with a mean number of 1350.5±272.8/g of eggs. Mean relative fecundity was 1936.5±503.2 eggs/g of bw. Smaller fishes had higher number of eggs/g of bw than those of the larger fishes and the ratio was 1.25:1. It is a quite high fecund fish in comparison to the other.

Egg colour and size of *P. hypophthalmus*

The color of unfertilized egg was yellowish-gray. The size of the unfertilized and fertilized eggs was 0.9 and 1.7 mm in diameter, respectively. Both fertilized and unfertilized eggs were adhesive, demersal and spherical in shape.

Induced breeding of *P. hypophthalmus*

On the basis of maturity of the fish and the breeding season, females were injected with cPGE @ of 6-10 mg/kg of bw and a dose of 8.0 mg/kg of bw was found very effective (Table 4). In case of HCG, females were given @ 1,000-3,000 IU/kg of bw, while males were given @ 100 to 300 IU/kg of bw. For females, 1/3 of the total dose of the required hormone was applied as preparatory dose after 6h of interval the rest 2/3 was injected as decisive dose.

Males received single hormone dose of cPGE @ 1-3 mg/kg of bw (usually 2.0 mg 3.0 kg of bw) during the decisive injection of the females. Females, were found ready for stripping after 6±1h of the final injection. The ready females were stripped and jets of yellowish-gray eggs were collected in a dry enamel bowl. Immediately after this, the males were stripped and the milt was mixed with the eggs for fertilization. Dry method of fertilization was followed to fertilize the eggs. Ratio of the male and female spawners was maintained at 1:1 and often 2:1. Though milt from one male is sufficient to fertilize the eggs of one female, yet milt of two males were generally used during fertilization. The eggs and milt were mixed together very gently using a bird feather for one and half minute and then rinsed with water for 2-3 minutes, to remove excess milt and the mucus. Before the eggs develop stickness, they were transferred to galvanized iron trays, round circular tank and cisterns, with water showers. Higher doses of hormone were required during the early and the late breeding season while low dose was required in the peak breeding season.

In all trials, *P. hypophthalmus* injected either with cPGE or HCG responded positively and ovulated within 5-7h after the final injection. During early and late breeding season, ovulation took little longer period of up to 10 h while it was shorter of up to 4 h during the peak season. Both cPGE and HCG were found effective for inducing *P. hypophthalmus* in captive condition. However, genital opening may be clogged due to improper doses of hormone during early and late seasons. Fertilization rate of eggs ranged from 75 to 95% depending on the season and the latency period. If the latency period is longer than normal, the fertilization rate was low. It may be due to accumulation of water within the ovary and the eggs shell.

Hatching of *P. hypophthalmus*

The fertilized eggs of *P. hypophthalmus* were found sticky and were reared in galvanized iron trays of 2.13 x 0.61 x 0.31 m (7 x 2 x 1 feet) size at a density of 30,000 per tray for 5 days. Continuous water shower was provided in each tray at the rate of 5.0 1/min. Hatching of the eggs started within 18 h and mostly completed by 24 h after fertilization, ranging between 27.1 and 29.20 day degrees (Table 4). The suitable water temperature for incubation ranged between 27°C and 29°C. Hatching often did not took place at water temperatures below 25°C and beyond 32°C, respectively. Hatching rate was satisfactory and ranged between 80 and 90% while survival of hatchling varied between 55 and 75%. Surface water from the nearby pond had to be used for hatchery operations that contained silts, debris, plankton and other aquatic organisms. Yolk-sac absorption usually completed with 48 h after hatching, ranging between 54.10 and 58.4 day degrees (Table 4, Fig. 1).

First feeding starts within 15-18 h after hatching although the fry contains yolk-sac at that time. Hatchlings were fed boiled egg yolk mixed with water or fish muscle paste from 18 h after hatching up to the next 36 h and then *Artemia* nauplii and live zooplankton were supplied as supplemental feed. It was observed that the hatchling became extremely cannibalistic if sufficient food was unavailable after 2nd day of hatching. A total of 4,50,000 to 5,50,000 numbers of 2 days old larvae were calculated from one kg of spawn. After this period their survival was recorded as 15%. Overall, low survival was probably due to the poor quality of water and inappropriate food at the early rearing stage. Use of only *Artemia* nauplii (Red Jungle brand) even after the 5th days after hatching (DAH) showed different rates of survival with different feeding and incubation regimes. An incubation rates of 1.0g egg/L of water and feeding with nauplii produced from 15.0, 35.0 and 40.0g or 5.0, 7.5 and 10.0g of *Artemia* after 5, 8 and 11 DAH produced the highest numbers (38 and 27 respectively) of fry/l of water (Table 4). Administration of minced but live *Tubifex* sp. every 6 h interval from the 4th day onwards increased the larval survival to a greater extent. After every feeding uneaten food stuff were siphoned out to prevent water fowling. After 5 to 10 days of rearing, fry were ready for stocking in the nursery pond. Further experiments are needed to increase the rates of survival in the hatching units or to develop other rearing methods.

Table 4: Mean numbers of 12 days old fry of *P, sutchi* produced/L of water (% in the parenthesis) at different incubation and feeding regimes, Bangladesh (degree days = days x mean water temp. in ^{O}C, DAH = days after hatching).

Egg incubation (g/l water)	Hatching duration (daydegrees)	Yolk-sac absorption (daydegrees)	Feeding upto 5 DAH with nauplii produced from	Feeding upto 8 DAH with nauplii produced from	Feeding upto 11 DAH with nauplii produced from	Fry produced (nos/L water)
0.75	27.10	58.10	10 g of *Artemia*	10 g of *Artemia*	20 g of *Artemia*	3.35 (2%)
1.0	29.20	58.15	5 g of *Artemia*	7.5 g of *Artemia*	10 g of *Artemia*	27.0 (12%)
1.0	28.87	57.80	10 g of *Artemia*	15 g of *Artemia*	20 g of *Artemia*	8.3 (4%)
1.0	29.07	58.40	15 g of *Artemia*	35 g of *Artemia*	40 g of *Artemia*	38 (17%)
1.25	28.90	58.00	10 g of *Artemia*	15 g of *Artemia*	20 g of *Artemia*	22.4 (8%)
1.5	28.90	58.00	10 g of *Artemia*	15 g of *Artemia*	20 g of *Artemia*	14.2 (4%)

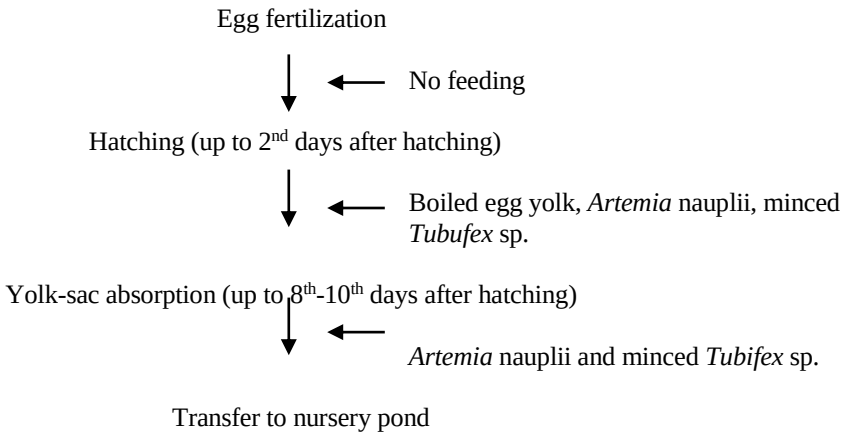

Fig.1: Schematic presentation of egg fertilization and larvae production in *Pangasius hypophthalmus*

Rearing technique of *P. hypophthalmus* in nursery pond

Fry of 5-7 days old were stocked in the nursery ponds at the rate of 2,000 to 3,000 individuals/decimal (40 m^2). Before stocking, ponds were prepared with lime and cow-dung respectively at the rate of 1 and 4 kg/decimal. Fry were reared for one month in the nursery ponds in the beginning alternately with live and minced *Tubifex* sp. and oil cake pest with 4

times feeding daily @ 10-12% of their bw. Later on a locally prepared non-pelleted diet containing 50% oil cake and 25% rice polish and 25% wheat flour was fed @ 10% of their bw daily. The total daily ration was divided into two equal portions and was given 2 times a day. Gradually feeding was reduced to 6-8% of the bw, daily. The fry attained a length of 5.5 to 8.0 cm after 15 days of rearing. The survival ranged from 10 to 15% only. The low survival might be due to poor quality of water and pond condition. Further investigations are required to find out the optimum stocking density and to increase the survival in the nursery ponds.

Gonadal development and induced breeding of *R. rita*

During the breeding season (May-July) adults never show secondary sexual character but females were found having soft belly with reddish oval genital opening and male were found with protruded genital organ but no milt. Females were injected with cPGE @ 3.0 kg/kg of bw. One third of the total hormone was injected as preparatory and the rest as decisive dose. Interval between the two injections was 6h. Males were injected with cPGE @ 3.0 mg/kg of bw at the time of final injection of the females. Other pairs of females were injected with HCG at a total dose of 3,000 IU/kg of bw. One third of the total hormone was injected as preparatory dose and the rest two third as final dose with 6 h intervals between the two injections. Males were injected once, @ 300 IU/kg of bw at the time of final injection of the female. The fishes were checked for 6 h after final dose. No sign of ovulation and oozing of milt were observed.

After that these fish were dissected to examine the maturity of the gonad. It was found that the fish with projected genital organ contained highly branched, thread like testes with milt, and the fish with oval shaped genital organ contained ovary with light yellowish ova.

Their annual cycle of GSI need to be studied both from the wild and culture origin to understand the state of maturity of ova and the breeding period.

Gonadal development and induced breeding of *M. aor*

During their breeding season (late February-June) females and males did not show external sign of gonadal development. Regular monthly monitoring of their maturity did not show any sign of swollen abdomen in the females and presence of milt in the males. Yet females with slightly distended abdomen were injected with cPGE @ 20.0 mg/kg of bw. One third of the total hormone was injected as preparatory dose and the rest two third as final dose. Interval between two injections was 6 h. Males were injected with cPGE @ of 5 mg/kg of bw once, at the time of final injection of the female. Other females were injected with HCG @ of 5,000 IU/kg of bw. One third of the total dose was injected as preparatory and the rest as final with 6 h interval between the two injections. Males were injected once with HCG at the time of final injection of the females @ of 500 IU/kg of bw. The fishes were checked up to 6 h after the final injection. There were no sign of ovulation and oozing of milt in all these cases too.

However, natural breeding of *M. aor* was observed in the pond during the first shower of monsoon. Small fingerlings of 0.5-0.7 inch sizes were found in the pond during late March onwards. Besides bowl shaped fine and smooth nests (quite different from those built by Tilapia) found in the pond bottom were though to be built by the *M. aor*. This feature indicates that *M. aor* is a low fecund fish. These features indicate that their state of maturity can not be confirmed by their external appearance. Their annual cycle of GSI need to be studied both from the wild and culture origin to understand the state of maturity of ova.

Gonadal development and induced breeding of *Silonia silondia*

This species also did not show secondary sexual characters during their breeding season (April-July). Yet, some females with soft, distended belly, reddish oval genital opening and males with protruded genital organ were selected for breeding under controlled conditions. Females were injected with cPGE @12.0 mg/g of bw. Similarly, one third of the total hormone was injected as preparatory and the rest as final dose. Interval between the two injections was 6 h. Males were injected @ of 3.0 mg/kg of bw once, at the time of final injection of the female. The fishes were checked up to 6 h after the final. No sign of ovulation and oozing of milt were observed. *S. silondia* was found to be very sensitive to handling an dissolved oxygen content of water. They were often found dead when handled and dissolved oxygen content of water goes <4.0 mg/L. Their annual cycle of GSI need to be studied both from the wild and culture origin to understand the state of maturity of ova and the breeding period.

Probable reasons for failure in induced breeding of local riverine catfishes

Poor gonadal development was observed in *B. bagarius, E. vacha, M. aor, R. rita* and *S. silondia* which might be due to environmental and/ or nutritional factors.

Problems in the understanding of the state of gonadal maturity of these fishes by their external appearance.

Sufficient number of adults could not be sacrificed and the state of ovarian maturity and GSI could not be ascertained due to shortage of fish and often funds.

Conclusion

Brood management, artificial breeding and nursery rearing techniques of *P. hypophthalmus* were established. Induced breeding techniques of *Pangasius pangasius* were developed with cPGE that needs refinement for mass seed production. While artificial breeding and consequent larval and nursery rearing techniques of *B. bagarius, E. vacha, M. aor, R. rita* and *S. silondia* could not be established.

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Cage culture of walking catfish, *Clarias batrachus* in the lotic habitat: Optimization of stocking density

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Abstract

A study was conducted to optimize the stocking density for developing production technique of indigenous walking catfish, *Clarias batrachus* in cage in the lotic habitat. For this purpose, two months old fingerlings of fishes (5.10±1.54g) were stocked in the floating net cages at four stocking densities *viz.*, 100, 150, 200 and 250 fishes/m^3 each with three replications. Fishes were fed with floating feed containing 30% crude protein. After rearing for six months from November to April, all fishes were harvested and it was observed that growth of fishes was higher in lower stocking cages with the mean weights of 148±4.21g, 136±3.95g, 125±4.68g and 106±3.96g at the above densities, respectively. But both production and net return per cubic meter were highest of 15.50 kg and Tk. 2258.33, respectively at 200 fishes/m^3 density.

Key words: Cage, *Clarias batrachus*, Production, Stocking density

Introduction

Culture of fish in cages has gained much popularity throughout the world due to a number of advantages over the conventional methods of fish farming. The major advantages of fish culture in cage are: flexibility in the use of resource, comparably low capital cost, simplified husbandry practices, easy harvesting of fish and multi use of water resources. Though the country has vast water resources and climatic conditions favorable for cage culture of fishes, the aquaculture practices in Bangladesh are still concentrated largely on rearing of fishes in earthen ponds. Hasan *et al.* (1982) also mentioned that there is vast scope of increasing fish production in inland waterbodies through cage culture in Bangladesh and it would be a very profitable industry like other Asian countries. But production of fishes in cages on commercial basis in Bangladesh is yet to be popularized due to lack of knowledge on selection of species, determination of appropriate stocking density and management practices. Some social problems are also a constraint for expansion of this fish production system.

Unlike other Asian countries such as Indonesia, Thailand and Vietnam where commercial cage culture for a number of species is a well established practice, cage culture in Bangladesh is still limited. Some individuals and entrepreneurs started commercial culture of tilapia in cages in the river ecosystem. But due to high cost of feed and high mortality caused by disease, net return from this practice was not that much encouraging (Sarker, 2010). Later on, Bangladesh Fisheries Research Institute has started research to overcome these problems through introducing some high valued fishes. In this context, Kohinoor and Rahman (2013 and 2015) conducted experiments on the cage culture of *Cyprinus carpio* and *Mystus cavasius* in the river ecosystem and obtained encouraging findings. Later on, initiative has been taken to develop cage culture of high valued walking catfish, *Clarias batrachus*, locally called Magur.
Clarias batrachus is particularly important for its growth, protein quality, tastiness and market stocky demand. Due to having aerial respiratory habit, this fish can be cultured at high density and marketed in live condition and hence fetches higher net return than carps (Thakur and Das, 1986). As suggested by Borthakur and Goswami (2007), this fish can be successfully reared in cages. Determination of stocking density is a prime need for developing culture practice of any fish. Because, higher density may cause crowding effects and reduction in growth rate, while lower stocking density will not be commercially profitable (Bachiel and Le Cren, 1978). Therefore, the present study has been conducted for the optimization of stocking density of *Clarias batrachus* in cage culture system in a lotic habitat. A simple economical analysis to determine effect of stocking density on the cage culture economics has also been included in the study.

Materials and methods

Site and design of the experiment

The study was conducted in the lotic habitat of the old Brahmaputra river at the vicinity of Mymensingh town for six months during November 2016 to April 2017 period crossing both winter and summer seasons. The experiment was conducted with four stocking densities *i.e.*, treatments (T) *viz.*, 100 fishes/m^3 (T_1), 150 fishes/m^3 (T_2), 200 fishes/m^3 (T_3), and 250 fishes/m^3 fishes (T_4), each with three replications.

Construction and installation of cages

For the experiment, twelve rectangular cages of 3 m^3 (2m x 1.5m x 1m) each were made by encircling an iron made rectangular frame with knotless polyethylene net of 10 mm mesh size. The cages were set in water with the help of bamboo poles inserted into the river bottom. For easy management, the cages were numbered as 1 to 12 and were divided into four treatment groups according to the design of the experiment.

Collection and stocking of fishes

Two months old fingerlings of indigenous walking catfish, *C. batrachus* were collected from a local private hatchery. The collected fingerlings were acclimatized with the river water for seven days in a separate cage. After then, required quantity of good quality fingerlings were

stocked in the respective cage according to the design of the experiment. The mean weight of the stocked fingerlings was 5.10±1.54g.

Feeding of Fish

The stocked fishes were fed with commercial floating feed containing 30% crude protein twice (09.00h and 16.00h) daily of six days per week. Fishes were fed with starter-1 feed for the first and second months and then with starter-2 for the rest of culture period. Feeding was done initially @ 15% of average body wt. of fish and then gradually reduced to 5% at the end of the culture period. Feed was adjusted fortnightly. For this, average weight of fishes was determined by bulk weighing of 50 fishes in each cage and accordingly, quantity of feed to be supplied in each cage was estimated.

Water quality monitoring

Water quality parameters *viz.*, temperature (°C), pH, transparency (cm), dissolved oxygen (mg/l) and unionized ammonia (mg/l) of the sub-surface water were determined weekly at 9.00 to 10.00 am following standard methods as mentioned by APHA (1992).

Harvesting

After 180 days (6 months) of rearing, all cages were shifted from the river to the river bank area. The fishes of all cages were harvested by hand picking. Then total number and weight of fishes in each cage were recorded. After data collection, average weight, survival and production of fish of each treatment were calculated. Specific growth rate, SGR (%) has been calculated following the formula as given below:

$$SGR\ (\%) = \{\ln (Wt_2) - \ln (Wt_1)/t_2 - t_1\} x\ 100$$

Where, Wt_2 and Wt_1 are weights at respective time t_2 and t_1 and the difference of t_2 and t_1 are the time duration in days.

Data analysis

The collected data were subjected to different statistical analyses to find out the mean±standard deviation and the level of significance of variations of different production parameters among different stocking densities. All computations were made utilizing "STATGRAPHICS Version 7" software package.

Results

During the experimental period mean values of water temperature, transparency, pH, dissolved oxygen and unionized ammonia were 24.62±3.5°C, 62±6.21cm, 7.76±0.53, 6.20± 1.89 mg/l and 0.04±0.01mg/l, respectively. Monthly variations of water quality parameters are shown in Fig.1. Water temperature of the river varied from 20.04 to 27.50°C. Temperature was lowest in

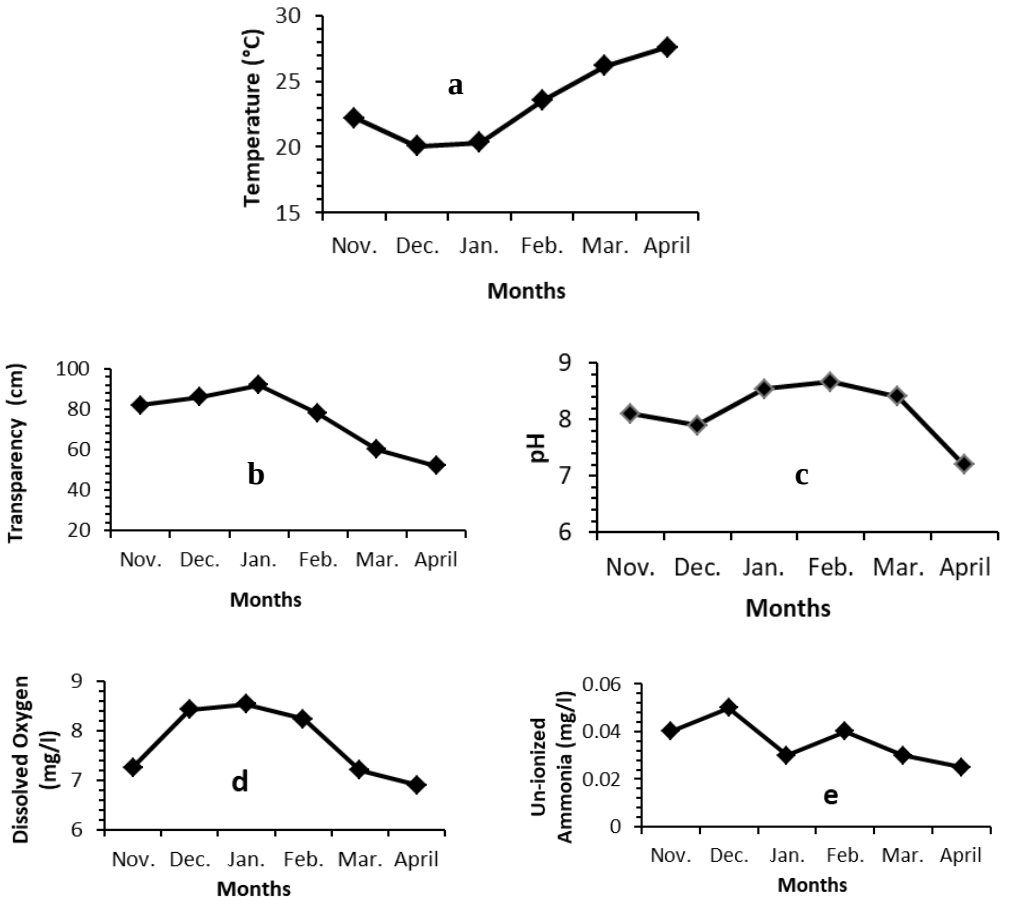

Fig. 1: Monthly variations in physicochemical characteristics of water of the experimental site of the old Brahmaputra river

December and gradually increased to highest level at the end (April) of the culture period (Fig 1a). As shown in Fig. 1b, transparency of water varied from 62 to 80 cm with the higher transparency during winter months and gradually decreased with the progress of culture period. Water of the river was alkaline throughout the culture period and pH of water was 7.13 to 8.78 (Fig. 1c). Dissolved oxygen (DO) varied from 6.91 to 8.54 mg/l. Like transparency, highest value of DO was also recorded in January and gradually decreased with the progress of culture period (Fig. 1d). As shown in Fig. 1e, unionized ammonia concentration in water was very low and varied from 0.025 to 0.05 mg/l.

The growth and production performance of walking catfish, *Clarias batracus* locally called Magur are summarized in Table 1. After six months rearing in cages, the mean wt of fish was 148±4.20, 136±3.95, 125±4.68 and 106±3.96g with the mean specific growth of 1.93±0.02%, 1.89±0.03%, 1.83±0.01% and 1.79±0.03% per day at 100 (T_1), 150 (T_2), 200 (T_3) and 250 (T_4) fishes/m^3 stocking densities, respectively indicating higher growth rate at lower stocking cages. The mean wt. of fishes at highest stocking density (T_4) was significantly lower than those of other stocking densities. Mean survival of fishes was 74±3.51%, 65±5.13%, 62±7.09% and 55±5.85% in T_1, T_2, T_3 and T_4, respectively also indicating inverse relationship ($Y = -0.12x + 85$, $R^2 = 0.967$) with density. The survival of fishes was significantly highest at lowest stocking density (T_1), whereas survival was significantly lowest at highest stocking density (T_4) than those of other stocking densities.

Table 1: Production performance of walking catfish, *Clarias batrachus* reared in cage at different stocking densities in the lotic habitat of the old Brahmaputra river from November to April

Treatments (T)	Stocking density (No/m^3)	Mean wt (g) at harvest	Specific growth rate(%)	Survival (%)	Production (kg/m^3)	Production (kg/cage)	FCR
T_1	100	148±4.20^a	1.93±0.02^a	74±4.51^a	10.95±0.22^b	32.85	1.83^a
T_2	150	136±3.95^b	1.89±0.03^a	65±5.13^b	13.26±0.44^a	39.78	1.71^a
T_3	200	125±4.68^{bc}	1.83±0.01^b	62±7.09^b	15.50±0.44^a	46.50	1.61^a
T_4	250	106±3.96^d	1.79±0.03^b	55±5.85^c	14.58±0.17^a	43.74	1.78^a

* Dissimilar superscript indicates significant difference at 5% level of probability

However, mean gross production of fish in different cages was 10.95±0.22 kg/m^3, 13.26±0.44 kg/m^3, 15.50±0.44 kg/m^3 and 14.58±0.17 kg/m^3 at the above four densities, respectively showing highest production in T_3. This production was significantly ($p<0.05$) different than that of T_1 with lowest stocking density. But the differences of production among T_2, T_3 and T_4 were insignificant. The feed conversion ratio (FCR) value at lowest density (T_1) was highest of 1.83. This value reduced to 1.71 and 1.61 in T_2 and T_3, respectively. But FCR value (1.78) in T_4 with highest stocking density of 250 fishes/m^3 was higher than those of T_2 and T_3. This might be due to significantly lowest mean wt. and survival in T_4 than those of T_2 and T_3. However, there was no significant difference among the FCR values at different stocking densities.

As opined by Muangkeow *et al*. (2007), there are several factors such as yield, sale price, feed cost, fingerling cost, system investment and operating cost affecting economic return of the system. As revealed from Table 2, cost of cage preparation and operational cost was same in all four treatments. But cost of fingerlings was different depending on the different numbers of

fingerlings stocked in cages at different stocking densities. In the same way, cost of feed was also different and higher expenditure was incurred in treatments with higher quantity of feed. But percent of feed cost over total production cost in all treatments was almost same and varied from 43.17-44.21%. Due to these facts, cost of production was higher in higher fish stocking cages. Gross return (Tk./m^3) was highest of 16275.00 in T_3, followed 14917.50 in T_2, 14434.20 in T_4 and 13140.00 in T_1. Net return (Tk./m^3) was also highest in T_3 (6775.00) followed by T_2 (6259.50), T_1 (5380.00) and T_4 (4316.20).

Table 2: Production cost and income from cage culture of *Clarias batrachus* at different stocking densities (cage size, $3m^3$ each)

Particulars	**Treatments (stocking densities)**							
	T_1 (100 fishes/m^3)		T_2 (150 fishes/m^3)		T_3 (200 fishes/m^3)		T_4 (250 fishes/m^3)	
	Qty.	Value (Tk.)	Qty.	Value (Tk.)	Qty.	Value (Tk.)	Qty.	Value (Tk.)
Production cost/cage								
Cage preparation (No)	1	3000	1	3000	1	3000	1	3000
Fish fingerling (Nos.)	300	900	450	1350	600	1800	750	2250
Feed (kg)	60	3360	68	3808	75	4200	78	4368
Operational cost		500		500		500		500
Total cost		7760		8658		9500		10118
Income/cage								
Gross (Sale price of fish, Tk. 400/ kg)	32.85 kg x Tk.400/kg	13140	39.78 kg x Tk 375/kg	14917.5	46.50 kg x Tk.350/kg	16275	43.74 kg x Tk.330/kg	14434.2
Net	-	5380[a]	-	6259.5[ab]	-	6775[b]	-	4316.2[c]

Discussion

The water quality parameters recorded during the study period from November to April was congenial for aquaculture as also recommended by Boyd and Tucker (1998). Accordingly feed intake and growth of fishes were also observed satisfactory throughout the culture period. Generally, temperature of water, which control metabolic rate of fish (Boyd, 1982) remains >20°C in pond ecosystem during winter period from November to April in the Mymensingh region. During this period, fishes don't take feed and growth becomes stunted in ponds. But in the river ecosystem, temperature of water even in the winter period was always higher than this level (Fig. 1a).

In the present study, attempt was made to optimize the stocking density of *Clarias batrachus* in cages in the flowing water of the old Brahmaputra river. In this context, among the attempted four stocking densities from 100 to 250 fishes/m^3, growth rate of fishes was observed to be decreased with the increase in stocking densities. Similar findings were also reported by Quattara *et al.* (2003), Barcellos *et al*.(2004) and Nunoo and Asase (2017) in cage culture of black-chinned tilapia (*Sarotherodon melanotheron*), American catfish (*Rhamdia queien*) and Nile tilapia (*Oreochromis niloticus*), respectively. In Bangladesh, Ahmed (1982), Haque *et al.* (1994), Kohinoor *et al*. (2013) and Kohinoor and Rahman (2013 and 2015) also obtained similar findings in cage culture of *Labeo rohita, Cycrinus carpio* and *Mystus cavasius*. Production performance of *Clarias batrachus* is much better than that of Sangma *et al.* (2017) who conducted a study on the cage culture of *Clarias batrachus* in ponds at a density of 100 fishes/m^3 during July to October period and using pellet feed with 26% crude protein. After 97 days of culture, gross production was 61.866.81 kg/ha with the mean wt of fish of 29.22±0.84 g and mean survival of 21.335.03%. Individual growth and population density are known to be closely linked. This agrees with the findings of T_1 which showed the best result in terms of growth and survival.

The efficiency of any aquaculture system depends upon the profitability of the product. The market price of fish always depends upon its size. In the present investigation, price of fish was highest of Tk. 400.00/kg in T_1 due to the highest size, whereas price of fish was lowest of Tk. 330.00 in T_4 due to the lowest size. However, considering both market price and production of fish, highest gross income was achieved from T_3, where stocking density was 200fishes/m^3. Net return was Tk.5380.00/cage at lowest stocking density of 100 fishes/m^3 (T_1). This income increased up to T_3 where net return was Tk.6775.00/cage. But lowest net return of Tk. 4116.20/cage was achieved from cage of highest stocking of 250 fishes/m^3 (T_4). This return was significantly different from those of other stocking densities. In spite of higher production than T_1 and T_2, lowest net return from T_4 was due to small size of fish which was sold at lowest market price.

Conclusion

The findings of the present study indicate that stocking density of walking catfish Magur, *Clarias batrachus* for rearing in cages in the lotic environment should not be higher than 200 fishes/m^3. However, further study is needed for proper optimization of stocking density. The study also indicate that it is possible to culture fish in cage in the river environment even in the winter season when temperature of water in closed water bodies remains uncongenial for fish culture.

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Assessing the numbers of mantle tissues in non-nuclei pearl production in freshwater mussel, *Lamellidens marginalis*

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Abstract

In order to determine the suitable number of mantle tissues for non-nuclei (rice) pearl production, a research was conducted at Freshwater station, Bangladesh Fisheries Research Institute (BFRI) from July 2014 to June 2017 by inserting the numbers of mantle tissues 2 (t_1), 4 (t_2), 6 (t_3), 8 (t_4) and 10 (t_5) in freshwater mussel *Lamellidens marginalis*. Earthen pond was used for stocking operated mussels (80 mussels/dec). Different water quality parameters *viz.*, Temperature, Dissolve oxygen, pH, Ammonia, Alkalinity, Calcium and Phytoplankton were monitored fortnightly and found within normal range. After three years of culturing, survival rate of operated mussel was found highest (77%) in t_2 followed by t_1 (76%), t_3 (73%), t_4 (72%) and t_5 (62%). Pearl production rate was highest in t_3 (34%) followed by t_4 (33%), t_5 (30%), t_2 (18%) and t_1 (10%). Highest nacre layer was observed as 4.85±0.21 mm in t_3 while, 4.7±0.27 mm in t_1, 4.6±0.32 mm in t_2, 2.3±0.28 mm in t_4 and 2.12±0.30 mm in t_5. From the current study, considering the pearl production rate, nacre layer, luster and shape of produced pearl implantation of 6 numbers of mantle tissues showed the best performance than others.

Key words: Non-nuclei pearl, *Lamellidens marginalis*, Mantle tissue, Survival, Pearl production, Pearl quality

Introduction

Pearl is the precious and wondrous gem which is biologically produced in the living animal (Rathor, 2017; Tanu *et al.*, 2019b). Pearl's jewelry is one of the most attractive objects considered as symbol of beauty, love, purity and aristocracy; it added unique levels of all style and fashion (Dirlam *et al.*, 1985; Pandey and Singh, 2015). Not only for jewelry but also it has

other uses like, raw materials of medicine, cosmetics etc. (Li and Li, 2009; Misra and Mukhapadhyay, 2008). Non-nuclei pearl is a special kind of pearl which is full of pearly layer (Li and Li, 2009). Different color and shape of pearl like oval, round, rectangular can be produced after insertion of mantle tissue which looks like the grain or rice and that`s why it is called rice pearl. A piece of mussel epithelial membrane located at the outer edge of mantle tissue (mantle tissue block) is inserted into a living mussel's mantle. The inserted mantle tissue slice starts cell division, and then forms a pearl sac and secretes nacre, layer by layer, then accumulates as rice pearl (Dan *et al.*, 2001).

Freshwater pearl culture is growing as a source of employment and income in many South-East Asian countries (Ram, 1997). Chinese freshwater cultured pearls have high demand throughout the world. Twenty percent of total pearl production in the world market comes from Chinese freshwater pearl having the price of 150 million US$ (Anon, 2006). Pearl culture has a great potentiality but due to lack of technical knowledge, it has not yet been commercially developed in Bangladesh (Sarker, 1994). The prospect of rice pearl culture is bright and promising in the country due to the warm weather which is favorable for the growth of pearl producing mussel and pearl. Pearl culture might provide more employment opportunity to the rural women and can play a vital role for women empowerment. The native mussel *Lamellidens marginalis* and *L. corrianus* are suitable for producing rice pearl which is available in the country (Hossain *et al.*, 2004, Tanu *et al.*, 2019a). Pearl producing mussel can be cultured with fish in pond, ditches, river, lake with low input and fish farmer can earn additional money from this integrated culture system (Dan *et al.*, 2001). Pearl production and its quality depend on various factors such as mussel species, operation technique, age of mussel, culture environment, water quality, natural food, sunlight penetration to the water body and management techniques (Ram, 2003). Different numbers of mantle tissues transplantation may also affect the production of pearl quality. Considering the above factors this experiment is designed to find out the implantation of suitable numbers of mantle tissue slices in freshwater mussels in order to produce highest numbers of non-nuclei quality pearls in freshwater mussel *L. marginalis*.

Materials and Methods

Mussel collection

Disease free, healthy and young mussels (*L. marginalis*) were collected from different habitats of Mymensingh region and stocked in a pond at Freshwater station, BFRI, Mymensingh. The average length, width and age of stocked mussels were 8.93±0.30 cm, 4.91±0.23 cm and 1-1.5 years, respectively.

Mussel rearing

Collected mussels were reared in pond of which soil was sandy, clean water and pollution free bottom. For plankton production, the pond was fertilized with 5 kg organic manure, 0.125 kg TSP and 0.1 kg urea /dec fortnightly. To maintain the optimum level of pH and calcium, 0.5 kg lime/dec were applied to the ponds fortnightly.

Operation equipment

Mussel cutting knife, Obtuse-headed forceps, Sponge, Glass board, Dropper, Tray, Mantle tissue separation needle, Mussel opener, Stopple, Flat-head needle, Hook-head needle, Operation shelf were used for mussel operation

Operation chemicals

Ajomin solution, 70% alcohol and distilled water were applied during different steps of mussel operation.

Pre-operative conditioning

Before operation, mussels were kept in cistern for seven days without food to remove dirt from intestine and internal organ of the body. Then mussels were brought to laboratory and put in perforated trays for 24 hours, keeping ventral side downwards to remove water.

Mantle tissue transplantation

During operation mussels were divided into two groups; the donor and the receiver mussels. Operation includes two steps; mantle tissue block making from donor and inoculation in the receiver mussel. The whole transplantation process was completed by following flowchart (Fig. 1):

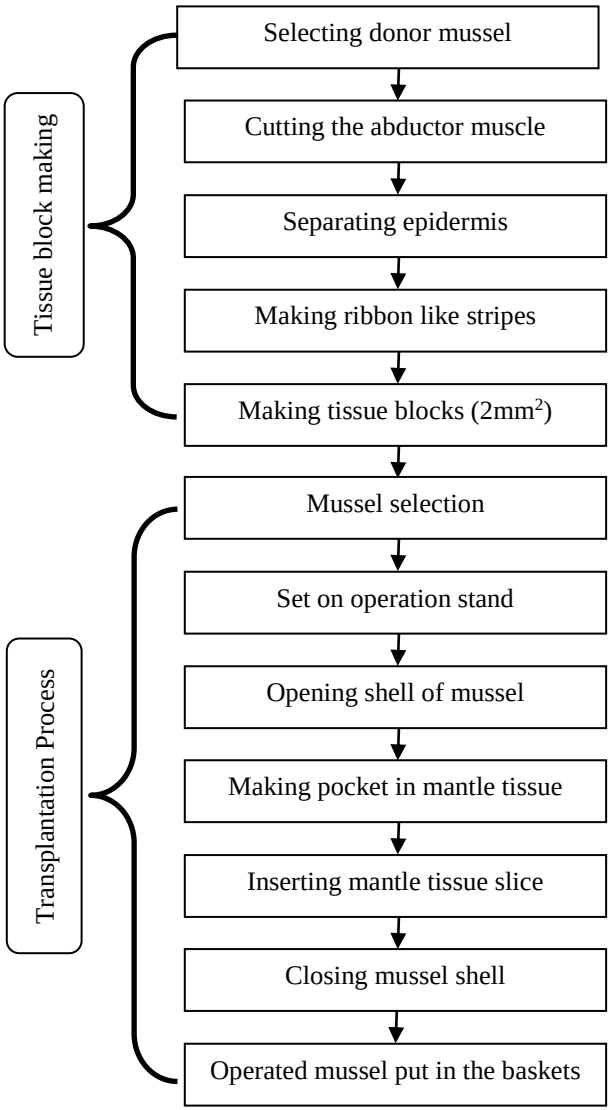

Fig. 1: Flowchart of mantle tissue transplantation

Post-operative conditioning

Post-operative care is a significant phase in pearl culture, which is required for the inoculated mussels to overcome the stressed condition. After operation, mussels were tagged and kept in

nylon bags (diameter 20cm, mesh size 1cm) at the rate of 3 mussels/net bag and put up at

0.2m depth in post-operative care units (ferro-cement cisterns of 5000 L capacity) at a stocking density of 150 mussels/cistern without food for 7 days. The mussels were subsequently fed with natural food for 3 weeks in the cistern. The operated mussels were observed daily; dead mussels were removed.

Culture Method

After post-operative treatment operated mussels were transferred to the previously prepared culture pond for three years (Fig. 2). Five treatments were done using the inserted mantle tissue slices 2,4,6,8 and 10 in t_1, t_2, t_3, t_4 and t_5, respectively. Each treatment included 200 operated mussels. A total of 1000 operated mussels were stocked with a stocking density of 80 mussels/dec. Net bag hanging culture method was practiced for mussel rearing during the experiment. The operated mussels were stocked at 3 mussels/net bag and hanged from a float attached to a rope at 0.30-0.35m depth into pond water. Rope was stretched to hang the net bag across the surface of pond water and the distance between 2 bags and 2 ropes were 0.25-0.30m and 1.5m, respectively.

Fig. 2: Pearl culture pond

Water quality management

The water quality parameters were monitored and data were recorded fortnightly throughout the culture period. Water temperature, dissolved oxygen (DO), pH, alkalinity, ammonia and calcium were measured by Celsius thermometer, digital Oxygen meter (YSI, model 58) and digital pH meter (Jenway, model 3020), Spectrophotometer (DDR-2800), Flame photometer (Buck Scientific FPF-7), Haematocyto meter, respectively. The plankton population was determined by using the following formula (Rahman, 1992)

$$N = \frac{A \times 1000 \times C}{V \times F \times L}$$

Where, N = No. of plankton cells per liter of original water, A = Total no. of plankton counted, C = Volume of final concentrated sample in ml, V = Volume of a field = 1 mm^{-3}, F = No. of fields counted, L = Volume of original water in liter. The numbers of phytoplankton and zooplankton were expressed as cells/l

Statistical analysis

All the collected data were statistically analyzed using SPSS version 17.0 with a significance level of 0.01. Pearson correlation analysis was performed to determine correlations among the treatments.

Results and Discussions

Water quality parameters

The water quality parameters of culture ponds are presented in Table 1. Mean values of temperature were 25.07±2.38°C, DO 5.17±0.85, pH 7.89±0.27, alkalinity 192 ± 24.40, ammonia 0.05±0.03, Ca^{2+}16.29±1.46 and plankton 85.14±7.9. All the water quality parameters were within the suitable ranges for pearl culture (Dan *et al.,* 2001). Temperature, pH, DO, Ca^{2+} and alkalinity ranging from 25.40 to 28.80 °C, 7.1 to 7.9, 5.3 to 6.8 mg/l, 58.90 to 71.20 mg/l and 399.00 to 594.00 mg/l were found during the study period of freshwater mussel (*L. marginalis*) in culture pond water by Natarajan and Susithira (2015). Temperature 29.99±0.20°C, DO 5.63±0.29, pH 8.16±0.12 and phytoplankton 89.817±12.4×10^3cell/l were also assessed by Yulianto *et al.,* (2016) in pearl production. Water quality parameters were also recorded as temperature 25.3±1.55 ^{0}C, pH 6.4±0.21, DO 5.63±0.17 ml/l, Alkalinity 22.44±0.34 mg/l by Rathor (2017) in pearl culture with *L. corrianus* species. The water quality parameters viz, temperature 23.5-36.0^{0}C, pH 7.5-8.5, dissolve oxygen 9.5-10.85 mg/l, alkalinity 220-275 mg/l, ammonia 0.053-0.065 mg/l were described for optimum growth of mussel by Pandey and Singh (2015). However, water quality parameters were found suitable throughout the current study period.

Table 1: Water quality parameters of trial pond water

Parameter	Average ± mean value	Suitable range for pearl culture (Dan *et al.,* 2001)
Temperature of water (°C)	25.07±2.38	15-30
pH	7.89±0.27	6.5-8.5
Dissolved oxygen (mg/l)	5.174±0.85	5-8
Total alkalinity (mg/l)	192±24.40	50-300
Ammonia (mg/l)	0.05±0.03	0.03-0.1
Ca^{2+} (mg/l)	16.29±1.46	>10

Phytoplankton (x10^3 cell/l) 85.41±7.9 50-100

Survival rate

After insertion of mantle tissue slices for rice pearl production, higher survival rate was found in t_2 (77%) followed by t_1 (76%) (Fig. 3). More or less similar result was found in t_3 (73%) and t_4 (72%). The lowest survival rate was observed in t_5 (62%). Survival rate was negatively correlated with the inserted number of mantle tissue slices (Table 2). It is noted that Miah *et al.,* (2000) observed 80% survival rate for one month rearing of *L. marginalis* having nucleus implantation. On the otherhand, Hossain *et al.,* (2004) found 100% survival rate for three months culture of *L. marginalis* with mantle tissue. In another study mortality occurred 20% in June then decreased after August in pearl culture with the species of *Parreysia Corrugata* (Suryawanshi and Kulkarni 2015), whereas Fernandez (2013) described 55-95% survival rate in freshwater mussel *Margaritifera falcate*.

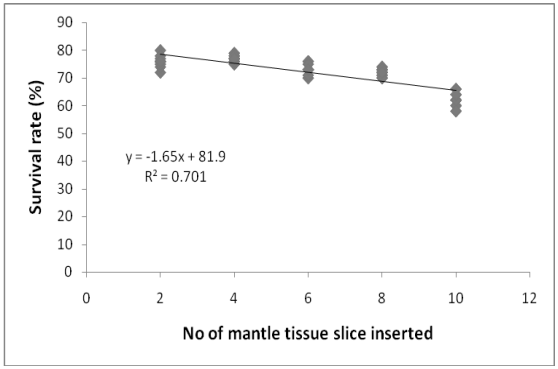

Fig. 3: Survival of operated mussels

Pearl production rate

In the current study, maximum pearl production rate was recorded in t_3 (34%) followed by t_4 (33%), t_5 (30%), t_2 (18%) and t_1 (10%) (Fig. 4). In statistical analysis pearl production was positively correlated with the inserted number of mantle tissue slices (Table 2). In a similar study Ram (1997) stated 60-70% pearl formation in *L. marginalis* and *L. corrianus* after 12 months of culture. Research conducted by Begum *et al.* (1990) on *L. marginalis* revealed 15.1% matured pearl production after inoculation of 2 mm^2 mantle tissue blocks and 1-2 mm ceramic beads.

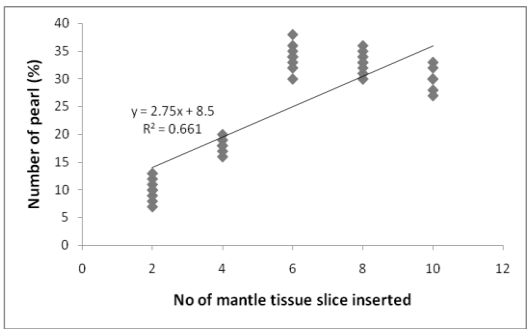

Fig. 4: Pearl producing rate of operated mussel

In the current experiment survival rate of operated mussels was found highest in t_2 (77%) but in case of pearl production t_3 showed the highest (34%) performance. The reason behind it is unknown. Further study is needed along the issue to elucidate the reason.

Pearl quality

The highest nacre layer was observed in t_3 (4.85±0.21 mm) followed by t_1 (4.7±0.27 mm), t_2 (4.6±0.32 mm), t_4 (2.3±0.28 mm) and t_5 (2.12±0.30 mm) in harvested mussels (Fig. 5). Accumulations of nacre layer in mussels were negatively correlated with the inserted number of mantle tissue slices (Table 2). Similar result was also found by Tanu *et al.,* (2019b) while nacre layer accumulated at 4.17-5.19mm with shiny and good luster. In consideration to luster and shape of pearl Rahayu (2013) showed the highest pearl nacre layer thickness of 17 µm from 9 months cultivation of freshwater mussel (*Anodonta woodiana)* after insertion of shell bead nucleus of 10mm diameter. Blay *et al.* (2013) found 0.65-1.24mm pearl nacre deposition from *Pinctata margaritifera* after 18 months of culture. According to Rathor (2017), 3-4mm nacre layer of pearl was found after 9 months of culture of freshwater mussel (*L. corrianus*). In the present study, accumulation of nacre layer was satisfactory in *L. marginalis.*

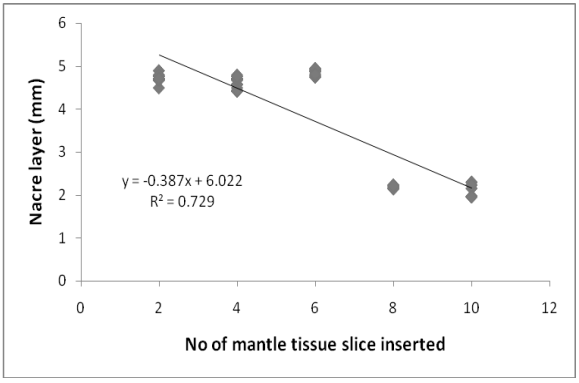

Fig. 5: Accumulation of nacre layer in harvested mussel

Table 2: Pearson's correlation among the treatments of non-nuclei pearl production in freshwater mussels, *L. marginalis*

Correlations	No. of mantle tissue slices inserted	Survival rate (%)	Pearl production rate (%)	Nacre layer (mm)
No. of mantle tissue slice inserted	1	-.838**	.813**	-.854**
Survival rate (%)	-.838**	1	-.503**	.727**
Pearl production rate (%)	.813**	-.503**	1	-.521**

Nacre layer (mm)	-.854**	.727**	-.521**	1

**. Correlation is significant at the 0.01 level (2-tailed).

However, the operated mussels *L. marginalis* having 6 numbers of inserted tissue blocks showed the highest numbers of non-nuclei pearl. Similarly these mussels also showed best performance in nacre secretion, luster, shape and size of pearl. Further research is needed along the line for the refinement of technology.

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Growth performance and estimation of single celled protein in spirulina (*Spirulina platensis*) cultured in supernatant of digested rotten apple (*Malus domestica*)

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Abstract

Spirulina platensis was cultured in supernatant of three different concentrations of digested rotten apple (*Malus domestica*) and Kosaric medium (KM) to see the growth performance and crude protein content in the cells. The three different supernatant media were 1.25, 2.50 and 5.0% digested rotten apple (DRA) added with 9.0 g/L $NaHCO_3$, 0.50 ml/L micronutrients and 0.20 g/L urea. Culture of *S. platensis* was performed in 1.0 L flasks in four treatments, three from supernatant of DRA and one KM as control, each with three replications under fluorescent light in light: dark =12 hr :12 hr condition for a period of 14 days from May 20 to June 04, 2018. Growth performances of *S. platensis* varied from one medium to another. The initial cell weight of *S. platensis* was found to be increased 0.0023 mg/L and a maximum cell weight of 12.44 mg/L was found in KM followed by 12.359 mg/L in supernatant of 2.50% DRA on the 10th day of culture. The chlorophyll a content of *S. platensis* was found to be increased from the initial value of 0.0015 mg/L to the highest content of 10.54 mg/L when cultured in KM followed by 10.468 mg/L grown in supernatant of 2.50% DRA on 10th day of culture. Similar trend was followed in the case of total biomass. A decreasing trend of cell weight was observed from 10th day of culture. The growth of *S. platensis* was significantly ($p<0.05$) better in supernatant of 2.50% DRA than other concentrations of DRA (5.0 and 1.25%). Spirulina contained high crude protein (58.52%) when grown in KM which was almost similar to those grown in 2.50% DRA medium (crude protein 57.25%). From the results obtained in the present study, it is summarized that the growth and production of crude protein (around 57%) in a single cell of spirulina found better when grown in supernatant of 2.50% DRA than other concentrations of DRA. So it is concluded that the concentration of 2.50% DRA is most cost effective and suitable for spirulina culture and production than compared with standard KM. The rotten apple is easily available, mostly free and inexpensive in the country. Therefore, supernatant of digested rotten apple can be used for commercially and economically viable mass culture of *S. platensis.*

Key words: Spirulina, Protein, Supernatant, Rotten apple

Introduction

Microalgae not only play an important role in aquaculture as feed source but together with bacteria, they also have an important role in the O_2 and CO_2 balance in water (Habib *et al*., 2008 and Sukumaran *et al*., 2018). They contain all the essential amino acids (except systine and methionine) in sufficient amount to be utilized as human, fish and animal food (Habib *et al*., 200 and Sarr *et al*. 2019). These live foods are considered to be the best food for fishes. Fish larvae grow better on living foods than non- living diets (Rosas *et al*. 2019). Microalgae are a potential source of minerals in fish diets, which can replace a mixture of minerals if incorporated in small amounts (Abouelezz *et al*., 2017). In aquatic system. fish also gets protein, lipids and minerals by feeding on rotifers, which cause bioaccumulation of nutrients by directly feeding on algae (Habib and Rahman, 1993, Habib and Kohinoor, 2018 and Khalila *et al*., 2018). The number of species of microalgae is estimated to be 22,000-26,000 out of which about 50 have been studied in detail with regard to their biochemistry and echo-physiology (Clesceri *et al*., 1989 and Habib *et al*., 2008). Most of the microalgal species are autotrophic. But microalgae represent all photosynthetic, prokaryotic and eukaryotic microorganisms. They are at the beginning of the food chain in nature and produce oxygen during photosynthesis. Algae act as an ideal waste remover in nature (Rejdalje *et al.*, 1989). Some researchers used algae to remove toxic and recalcitrant compounds from the aquatic bodies to make the environment free from hazardous materials (Rejdalje *et al*., 1989, Habib *et al*., 2008 and Habib and Kohinoor, 2018).

Among microalgae, high protein (around 60%) (Phang *et al*., 2000, Becker 2007, Habib *et al*., 2008, Jias *et al*., 2016 and Sarr *et al*., 2019) and lipids (18-20%) were found in spirulina when smartly grown in sago waste (Phang *et al*., 2000), digested poultry waste (Parvin, 2006), digested rotten potato (Habib *et al*., 2019), in fermented Thai rice noodle factory waste water (Veteyasuporn, 2004) and matters industrially produced (Muys *et al*., 2019). Spirulina contains high amount of poly-unsaturated fatty acids (PUFAs) (1.5-2.0%) of its 5-6% total lipids (Lu and Takeuchi, 2004 and Sarr *et al*., 2019); γ-linolenic acid about 36% of total PUFAs (Ayachi *et al*., 2004, Muys *et al*., 2019); and is rich in antioxidant (Anbarasan *et al*., 2011, Raji *et al*., 2018, Rosas *et al*., 2019). It also contains the essential nutrients like carotene & phycocyanin (Habib *et al*., 2008, Walter *et al*., 2011, Rosas *et al*., 2019 and Wicaksono *et al*., 2019), vitamins (Venkataraman and Beckar, 1986; Bhattacharya and Shivaprakash, 2005 and Jias *et al*., 2016). It contains all essential minerals and works as a chelatizing agent (Venkataraman and Beckar 1986; Maeda and Sakaguchi 1990 and Habib *et al*., 2008), removes chromium (Doshi *et al*., 2009) and heavy metals from the environment (Jias *et al*., 2016). According to some researchers, one gram of spirulina protein is equivalent to one kilogram of assorted vegetables. The amino acid composition of spirulina protein was ranked high among the best plant in the world, more than that of soyabean (Raji *et al*., 2018). Gamma-linolenic acid contained in this algae were reported to stimulate prostaglandin synthesis and induction of the regulation of blood pressure, cholesterol synthesis, inflammation and cell proliferation (Habib *et al*., 2008 and Borowitzka and Borowitzka, 2010). Spirulina provides all essential nutrients without excess calories and fats. It is recommended to control obesity & premenstrual stress, and chronic leukemia (Subhashini *et al*,. 2004*).* Many herbal cosmetics like face creams, bio-

lipsticks, hair lotion etc. have been formulated from phycocyanin pigment found in spirulina. The beta carotene and other carotenoids are suggested to have role in the control of cancer in human and enhancement of pigmentation of eggs and meats of hens and birds (Alvarenga *et al*,. 2011 and Zahroojian *et al.,* 2013) and coloration of ornamental fish and fish fillet (Rosas *et al*., 2019).

Spirulina is used as a potential health food for humans and other animals (Becker 1984; Alvarenga *et al*., 2011; Zahroojian *et al*., 2013 and Abed *et al*. 2016). In Mexico, spirulina is used to enrich candies. In Australia and New Zealand, beverages of this substance are marketed. In Japan, India, Singapore and South Africa, spirulina enriched appetizers are sold specially to pregnant women (Becker, 1984 and Niang *et al*., 2017), anaemic and malnourished children (Abed *et al*., 2016) and elderly people (Habib *et al*., 2008). Spirulina is a good health food (Becker, 1986) but also a natural colouring agent in Japanese chewing gums (Habib *et al*. 2008). Countries like Chile, France, Cuba, Germany, Switzerland, Spain, Portugal, Sweden, Holland, Belgium, Denmark, United Kingdom, Australia, and New Zealand market food complements which include spirulina as the main component (Habib *et al*., 2008). Internationally, skin care products, shampoos, dyes, masks, creams and tonics containing this micro-organisms are marketed. In Sweden low calorie bread enriched with spirulina is sold, and in France a vegetable paste, made of spirulina is sold as bread spread. In China, spirulina is used as a substitute of imported forage to promote the growth, immunity and viability of prawn (Habib *et al*. 2008 and Lu *et al*. 2011). Spirulina (*Spirulina platensis*) has been used as a model organism in many studies. In Biological Research Division, BCSIR, Dhaka, spirulina was cultured at pilot plant scale for over 19 years in Bangladesh (Jahan *et al*., 1994). Some media were developed in the same laboratory for domestic scale culture of spirulina in Bangladesh (Khatun *et al.,* 2006). Spirulina is used to replace fish meal in diets of fish post-larvae/fry which resulted good growth performances (Habib and Kohinoor, 2018).

In Bangladesh, a lot of waste materials and effluents of agroindustrial products are available which have nutritional value (Habib *et al*., 1998). Among these sugar mills, fertilizer factory, biscuit factory, sago factory, poultry industry etc. are important (Habib *et al*., 200; Satter, 2017; Habib and Kohinoor, 2018 and Sukumaran *et al*., 2018). Spirulina is grown well in supernatant of digested rotten potato in the laboratory (Habib *et al*., 2019), in good nitrogen regimes & temperature (Costa *et al*., 2007), ammonia & urea (Soletto *et al*., 2005), urea as source of nitrogen in real environment (Sukumaran *et al*., 2018), CO_2 & light intensity (Soletto *et al*., 2008), and pH (Ogbonda *et al*., 2007). It was produced in supernatant of digested poultry waste with high lipids (Parvin, 2006 and Habib and Kohinoor, 2018), in sea water with high pigment production (Mary *et al*., 2010), and used in diets of stinging catfish fry (Habib and Kohinoor, 2018). The quality of about 12-15% apples (*Malus domestica*) become deteriorated during storage and marketing, and are sold for human consumption producing considerable amount of waste. Apple contains high carbohydrate, protein, lipid, vitamin, mineral and phosphorus. This phosphorus might help to produce high phospho-lipids and ultimately increased the amount of total lipids (Lu and Takeuchi 2004, Habib and Kohinoor, 2018). These wastes are easily available nationwide all the time and can be collected from the market.

Therefore, the supernatant of this inexpensive waste material may be used to produce spirulina (*Spirulina platensis*). The present study was conducted to culture spirulina in supernatant of digested rotten apple (*Malus domestica*) medium, to record the growth performances of spirulina and to produce of single celled protein with the following specific objectives:

a) To evaluate the growth performances of spirulina grown in supernatant of digested rotten apple (SDRA); and
b) To analyze crude protein bioaccumulation and the proximate composition of spirulina grown in SDRA.

Materials and methods

The experiment was conducted in the laboratories of the Department of Aquaculture, Faculty of Fisheries, and Department of Animal Nutrition, Bangladesh Agricultural University (BAU), Mymensingh. The rotten apple was selected as medium for spirulina (*Spirulina platensis*) culture due to presence of high organic as well as inorganic nutrients specially carbohydrate. The rotten apple was collected from KR market, BAU campus, Mymensingh.

A. Maintenance of pure stock culture of spirulina

Pure stock culture of spirulina was maintained in the laboratory in Kosaric medium (KM) (Modified after Zarrouk, 1996). Growth of spirulina was monitored at every alternate day and was checked under a microscope to confirm its purity following the keys of Bold and Wynne (1978), Yamaguchi (1992), Vymazal (1995) and Phang and Chu (1999).

B. Preparation of supernatant of DRP and Kosaric medium (KM)

The collected rotten apple (*Malus domestica*) were cut into pieces, air and oven dried (50°C) overnight, ground, packed in polythene bag and kept in the laboratory for future use. Then 100 g/4.0 L dry rotten apple was allowed to decompose in 5.0 L glass bottle for 22 days under aerobic condition with aeration in the laboratory. Then a light reddish white coloured supernatant from bottle was screened through a net of 30 µm, mixed with 9.0 g/L sodium bicarbonate and 0.50 ml/L micronutrient from the stock solution and 0.20g/L urea (Khan *et al*), and made three concentrations at the rate of 25% (digested 2.50g dry apple/100 L), 50% (digested 2.5g dry apple/100 L) and 75% (digested 5.0 g dry apple/100 L) digested rotten apple (Table 1). The 600 ml/L supernatant of three different concentrations were taken in 1.0 L flask with three replications. Simultaneously, Kosaric medium (KM) using different laboratory grade inorganic chemicals was prepared for spirulina culture as a control (Table 2). The medium in flasks were mixed well and sterilized at 121°C for 15 minutes with moist heat by autoclave (Express Equipment, Dixon's Surgical Instrument Ltd.). After autoclaving, the media were kept for 72 hrs to make sure about any contamination before culture of microalgae.

Table 1: Experimental design for *Spirulina platensis* culture using supernatant of three different concentrations of digested rotten apple (DRA)

Types of medium	Treatments	Amount of dry rotten Apple, g/L DRA (% w/v)	Replications	Duration of culture (days)
Supernatant of DRA	1	1.25 (25%)	3	14
	2	2.50 (50%)	3	
	3	5.0 (75%)	3	
Kosaric medium	4	Different inorganic chemicals and micronutrients (Table 2)	3	14

For the preparation of Kosaric medium (KM), the mentioned amount (Table 2) of inorganic chemicals from no. 1 to 8 was weighed and took in a 1.0 L conical flask. Then 0.50 ml/L previously prepared micronutrient solution was pipetted in the flask and then distilled water was added up to the mark to make the volume 1.0 L (Zarrouk, 1996). Mixing, autoclaving at 12°C for 15 minutes and cooling were carried out pursuing the procedure used during the preparation of digested rotten apple (DRA) media.

C. Culture of spirulina (Spirulina platensis) in supernatant of DRA and KM

Four treatments, three from supernatant of digested rotten apple for three different concentrations viz. 1.25, 2.50 and 5% DRA, and one KM as control each (60 ml/L) with three replications were used to grow microalgae, *S. platensis* in 1.0 L volumetric flask. Spirulina was inoculated in each culture flask to produce a culture containing 10% spirulina suspension (Optical density, OD at 620 nm = 0.20) (Habib, 1998). Twenty ml of spirulina suspension needed for getting the required density. All the flasks were kept under two fluorescent tube lights (TFC, FL-40 SD/38 day light, Taiwan) (2000 lux/m^2/s) in light: dark (12h:12h) conditions in the laboratory.

The culture flasks were continuously aerated using electric aerator (Daivo pump). Two sub-samplings were carried out at every alternate day from each flask to record dry cell weight and chlorophyll a content of spirulina, and properties of culture media. All the glassware used in the experiment were sterilized with dry heat at 70°C overnight.

D. Estimation of cell weight (*dry weight*) *of spirulina* (*Clesceri et al., 1989*)

Sample containing 20 ml spirulina suspension was filtered through a Sartorius filter paper (mesh size 0.45 µm and diameter 47 mm) using high velocity vacuum pump. The filtered samples were washed three times to remove insoluble salts using 30% NaCl. The filter papers were dried in an oven for 24 hrs or overnight at 45°C, then put in desiccator, and weighed carefully after cooling.

E. Estimation of dry weight, chlorophyll a, total biomass and specific growth rates of spirulina

The microalgae was collected every alternate day withdrawing 20 ml sample twice from every flask and kept in two centrifuge tubes of 20 ml capacity. Filter papers were dried in oven at 50°C overnight and put in desiccator in next day. Two sets of 20 ml sample in tubes were filtered using pre-weighed Sartorius microfilter paper (mesh size 0.45µm and diameter 47mm)

Table 2: Composition of Kosaric medium (Modified after Zarrouk, 1996) for *Spirulina platensis* culture

Sl. No.	Chemicals/compounds	*Concentration in stock solution g/L*
1.	$NaHCO_3$	9.0
2.	K_2HPO_4	0.250
3.	$NaNO_3$	1.250
4.	K_2SO_4	0.50
5.	NaCl	0.50
6.	$MgSO_4.7H_2O$	0.10
7.	$CaCl_2$	0.02
8.	$FeSO_4.2H_2O$	0.005
9.	A_5 micronutrient solution[a]	0.5ml/L
	a) A_5 micronutrient solution	G/L
	i) H_3BO_4	2.86
	ii) $MnCl_2.4H_2O$	1.81
	iii) $ZnSO_4.7H_2O$	0.22
	iv) $CuSO_4.5H_2O$	0.08
	v) MoO_3	0.01
	vi) $CoCl_2.6H_2O$	0.01

using high velocity diaphragm vacuum pump (S: 2.40 m^3/h). Filter papers were carefully collected and folded (keeping algae inside). One filter paper was kept in glass petri discs and put in oven over night at 50°C. Next day, the dry filter papers were taken out and put in desiccator. Weight of spirulina with dry filter papers was weighed. Weight of spirulina was measured by subtracting weight of filter paper from dry weight of filter paper with spirulina. Then another set of 20 ml sample in tube was filtered using dry filter paper following the above procedure, cut into small pieces and put in plastic centrifuge tube (20 ml capacity). Ten ml acetone was put in plastic tube having the pieces of filter paper were broken into very minute pieces using glass rod. After breaking, microalgae were left attachment with filter paper and then acetone was put up to the mark of 20 ml of centrifuge tube. The tubes were wrapped with aluminium foil and kept at 4°C in freezer (LG Electronics Model No. GR-T312GE) overnight. On the following day, the tubes were taken out from the freezer and kept for some time to reach the samples at ambient temperature. Then the tubes were centrifuged at 3000 rpm for 10 minutes to settle down the small pieces of filter paper as ppt at the bottom of

the tubes. The supernatant from every tube was taken out using micropipette very carefully and kept in another centrifuge tube one by one. The collected supernatant was taken in cuvette, run through UV Spectrophotometer (Mittion Roy, Spectronic 1001 plus) to analyse chlorophyll-*a* of microalgae at three different OD (630, 647 and 664 nm) and data were recorded. The chlorophyll a was calculated using the formula: 11.85 (OD 664 nm) – 1.54 (OD 647 nm) – 0.08 (OD 630 nm). Total biomass was calculated using the formula given by Vonshak and Richmond (1988): Total biomass = Chlorophyll-*a* x 67.

All the microalgal samples were collected just before reaching the stationary phase. The stationary phase was recorded giving two growth trials of spirulina and then final experiment was conducted to collect spirulina before stationary phase. These samples were used to collect spirulina for the analyses of growth parameters and proximate composition of spirulina (Horwitz, 1984). All the analyses were done following Clesceri *et al*., (1989) and Habib (1998).
Specific growth rates (SGRs) on the basis of dry weight, chlorophyll a content and total biomass of spirulina were calculated using the following formulas (Clesceri *et al.,* 1989):

1. Specific growth rate (µ/day) of cultured spirulina on the basis of dry weight:
SGR (µ/day) = In $(X_1-X_2)/t_1-t_2$
Where, X_1 = Dry weight of biomass concentration of the end of selected time interval;
X_2 = Dry weight biomass concentration at beginning of selected time interval;
and t_1-t_2 =Elapsed time between selected time in day.

2. Specific growth rate (µ/day) of cultured spirulina on the basis of chlorophyll-*a*:
SGR (µ/day) = In $(X_1-X_2)/t_1-t_2$
Where, X_1 = Chlorophyll a at the end of selected time interval;
X_2 = Chlorophyll a at the beginning of selected time interval;
and t_1-t_2 = Elapsed time between selected time in day.

3. Specific growth rate (µ/day) of cultured spirulina on the basis of total biomass:
SGR (µ/day) = In $(X_1-X_2)/t_1-t_2$
Where, X_1 = Total biomass at the end of selected time interval;
X_2 = Total biomass at the beginning of selected time interval;
and t_1-t_2 = Elapsed time between selected time in day.

F. Analyses of physio-chemical properties of digested rotten apple and supernatant

The physico-chemical properties of digested rotten apple (DRA) were analyzed using different chemicals and equipments. These properties such as pH, total suspended solids, total dissolved solids, total alkalinity, nitrate-N (NO_3-N) and phosphate-P (PO_4-P) of DRA were analyzed in the laboratory of the Department of Aquaculture, BAU, Mymensingh following Clesceri *et al*. (1989).

G. Analyses of proximate compostion of rotten apple and spirulina

The proximate composition of rotten apple and spirulina such as moisture, crude protein, crude lipids, ash and nitrogen free extract (NFE) were analyzed in triplicates following the standard methods (Horwitz, 1984).

H. Statistical anatysis

Analysis of variance (ANOVA) of mean cell weight and chlorophyll- *a*, and crude protein, crude lipid and ash of *S. platensis* cultured in different media (treatments) were done and to find whether any significant difference among treatment means was done by Tukey test using statistical package following Zar (1984).

Results and discussion

A lot of fruits, vegetables, agricultural products like apple, banana, orange, potato, tomato, cauliflower, onion, garlic etc. are spoiled (rotten) in the market (Khan *et al*., 2018a and Habib *et al*., 2019). Among the fruits, apple is an important item which is spoiled in store and during marketing. These rotten apples were collected, dried, ground, and aerobically digested in glass jar in the laboratory following Habib and Kohinoor (2018). The proximate composition (Table 3) and physico-chemical properties of rotten apple (Table 4) were done. The supernatant of digested rotten apple contained carbon, nitrogen and other inorganic nutrients almost 10 times lower than raw apple (Table 5). The rotten apple contained enough carbon in collected sample and digested samples of rotten apple which worked as an important source of carbon helped for algal growth but contained low nitrogen. Usually these types of agricultural products contain high carbon and low nitrogen (Habib *et al*., 2019). To compensate nitrogen deficiency, 0.20 g /L urea was added (Khan *et al*., 2018b; Mia *et al*., 2018 and Habib *et al.* 2019). The most important algal species, *Spirulina platensis* commonly known as spirulina was cultured in supernatant of three concentrations of digested rotten apple (DRA) such as 1.25, 2.50 and 5.0 g/L on dry weight basis, and in Kosaric medium (KM) as control at ambient temperature in the laboratory. The experiment was developed to evaluate culture and growth performance of *S. platensis* in the laboratory. The initial cell weight of *S. platensis* was 0.011 mg/L in all the treatments which finally attained a maximum to 12.359 mg/L when cultured in supernatant of digested 2.50% DRA, 9.102 mg/L in supernatant of digested 5.0% rotten apple (DRA), 7.679 mg/L in supernatant of 1.25% DRA and 12.44 mg/L in Kosaric medium (KM) (Table 6, Figs. 1 & 2). The growth of cell was found to vary from one to other media. This variation in the cell weight happened most probably due to variation in composition in media and differences in nutrient concentrations (Ogbonda *et al*., 2007; Sukumaran *et al*., 2018; Habib *et al*., 2019 and Muys *et al*. 2019). The growth rate of *S. platensis* was found higher in KM than other different concentrations of supernatant of DRA. The higher cell weight and chlorophyll-*a* content of *S. platensis* was observed in supernatant of 5.0% DRA than other two concentrations of DRA. It might be happened due to suitable nutrient quantity and nutrient composition for growth of cell than other concentrations of DRA during the culture. The concentrations of 1.25 and 2.50% DRA were not suitable and favorable for growth of *S. platensis*. Habib *et al*. (2008) and

Habib and Kohinoor (2018) recorded that agro-industrial wastes such as poultry waste, biscuit factory waste effluents, other agro-industrial wastes contain organic as well as inorganic nutrients very favourable for growth of spirulina which has similarity with the present findings. Phang *et al*., (2000) recorded very good nutritional status of spirulina when cultured in sago waste effluent. Habib *et al*., (2019) cultured *Spirulina platensis* in supernatant of digested rotten potato and got very good production which has the similarity with the present results.

Table 3: Proximate composition (%) of rotten apple on moisture and dry weight basis

Composition	**Moisture basis (%)**	**Dry basis (%)**
Moisture	87.31	10.61
Crude protein	0.418	3.30
Crude lipids	0.780	6.15
Ash	0.603	4.75
Crude fiber	0.770	6.07
NFE*	10.11	69.11

*NFE (nitrogen free extract) = 100 - (moisture + crude protein + crude lipids + ash).

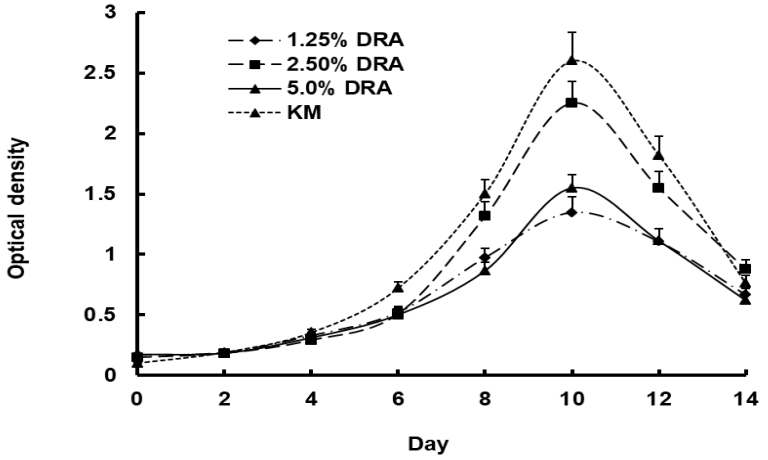

Fig. 1: Mean values of optical density of media contained *Spirulina platensis* in supernatant of three different digested rotten apple and Kosaric medium. Vertical bars represent standard errors

Table 4: Physico-chemical characteristics of ground rotten apple

Sl. No.	Characteristics of rotten apple paste	Properties
1	Colour	Reddish white
2	Odour	Little bid bad
3	Structure	Semi-solid
4	Temperature	28.30-28.60ºC
5	pH	6.30-6.45
6	Total solids (TSS + TDS)	1954-2135 mg/L
7	Alkalinity	132-142 mg/L
8	Total N	1.55-1.76 mg/L
9	Available N (NO_3-N)	1.10-1.15 mg/L
10	Available P (PO_3-P)	2.90-3.30 mg/L

Table 5: Physico-chemical properties of supernatant of digested rotten apple after digestion in aerobic condition

Sl. No.	Characteristics	
1	Temperature	28.20-29.50°C
2	pH	6.80-6.90
3	Total solid (TSS + TDS)	125-153 mg/L
4	Alkalinity	140-160 mg/L
5	Total-N	1.20-1.40 mg/L
6	Available-N (NO_3-N)	1.05-1.10 mg/L
7	Available-P (PO_3-P)	2.40-2.70 mg/L

Table 6: Comparison of cell weight, chlorophyll a and total biomass of *Spirulina platensis* grown in supernatant of three different concentrations of digested rotten apple (DRA), and Kosaric medium on the 10 day of culture before stationary phase

Parameters	T_1 (1.25% DRA)	T_2 (2.50% DRA)	T_3 (5.0% DRA)	T_4 (KM)
Optical density	1.331 ± 0.12^b	2.270 ± 0.15^c	1.568 ± 0.12^c	2.63 ± 0.20^a
Cell weight (mg/L)	7.679 ± 0.23^b	12.359 ± 0.52^c	9.102 ± 0.42^c	12.44 ± 0.21^a
Chlorophyll-*a*(mg/L)	6.919 ± 0.14^b	10.468 ± 0.32^c	7.360 ± 0.20^c	10.54 ± 0.14^a
Total biomass (mg/L)*	463.57 ± 8.13^b	701.36 ± 9.28^c	493.12 ± 8.30^c	706.18 ± 9.50^a

*Total biomass = Chlorophyll a x 67 (Vonshak and Richmond, 1988). Figures with common letters in the same row do not differ significantly at 5% level of probability.

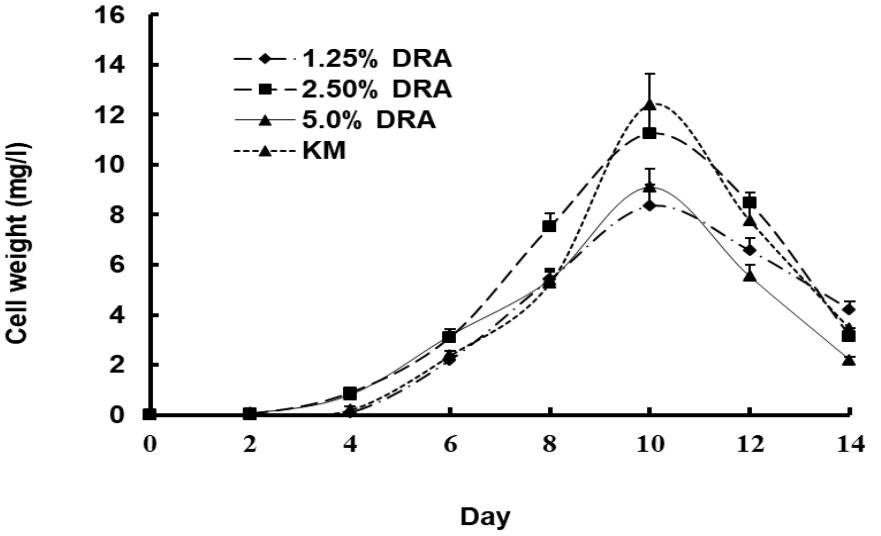

Fig. 2: Mean values of cell weight of *Spirulina platensis* grown in supernatant of three different digested rotten apple, and Kosaric medium. Vertical bars represent standard errors

During the culture period, exponential phase was found up to 10th day from the beginning and then the cell weight declined i.e. stationary phase started. The physico-chemical properties such as light intensity, aeration, temperature and pH played a significant role to the whole culture system. During the culture, the climatic condition was more or less suitable and favourable for the growth of *S. platensis*. Similar type of work was carried out by Mary *et al.* (2010) where the annual yield of biomass of *Spirulina maxima* strain 4MX grown in fertilized sea water in out door system was 7.36 mg i.e. 0.39 g/L/d which was higher than the present study. In the present study, the cell weight of *S. platensis* in supernatant of DRA, and KM were lower than the findings of Sukumaran *et al*., (2018) who grown spirulina in media enriched with urea in real environment. The variation in production probably happened because of different nutrient component of media used in culture, different culture technique and different species cultured. An experiment conducted by Becker (1984) on algal culture in a series of different horizontal ponds recorded that yield of spirulina sp. was 8 to 12 $g/m^2/d$. This yield found from the experiment was also much higher than the present findings. Sukumaran *et al*., (2018) reported that the biomass output rate in Chinese production plant was 7.0 $g/m^2/d$ which was almost similar with the results of the present study. Similarly, Tanticharoen *et al.*, (1991) reported that the addition of $NaHCO_3$ and nirogen fertilizer in waste water from the stabilization pond of topica starch factory raised the productivity up to 7-10 g/m/d which was much higher than the findings of the present study. The variation in the above results might occur due to nutrient composition of different media and physico-chemical factors involved in

the culture. Khan *et al*., (2018b) found that molasses is a good source of carbon where microalgae grew very well which has similarity with the present results. Crude lipids were bio-accumulated in spirulina about double than the same in spirulina cultured in Kosaric medium (Table 7) which might be due to presence of high inorganic carbon in media of DRA. Satter (2017) and Habib and Kohinoor (2018) stated that lipids were bioaccumulated in high amount (more than double) in spirulina (*Spirulina platensis*) when grown in supernatant of aerobically digested poultry waste than spirulina cultured in KM. This might be happened due to the formation of phospho-lipids in high amonut in this spirulina due to presence of high phosphorus in poultry waste (Satter, 2017 and Sukumaran *et al*., 2018).

Table 7: Proximate composition (% in dry matter basis) of *Spirulina platensis* cultured in supernatant of three different concentrations of digested rotten apple (DRA) and control as Kosaric medium

Treatments	T_1 (1.25% DRA)	T_2 (2.50% DRA)	T_3 (5.0% DRA)	T_4 (KM)
Moisture	8.20 ± 0.07	8.21 ± 0.07	8.21 ± 0.07	8.20 ± 0.07
Crude Protein	53.74 ± 0.38^b	57.25 ± 0.42^a	54.25 ± 0.52^b	58.52 ± 0.44^a
Crude Lipids	10.15 ± 0.28^b	14.62 ± 0.23^a	11.02 ± 0.19^b	6.31 ± 0.23^c
Ash	9.20 ± 0.16^b	10.14 ± 0.19^b	10.42 ± 0.24^b	13.52 ± 0.13^a
NFE*	17.99 ± 0.35^a	19.19 ± 0.18^a	15.35 ± 0.22^b	12.72 ± 0.28^c
Crude Fibre	0.71 ± 0.04	0.72 ± 0.03	0.74 ± 0.04	0.72 ± 0.03

*NFE (nitrogen free extract) = 100 - (moisture + crude protein + crude lipids + ash). Figures with common letters in the same row do not differ significantly at 1% level of probability.

Spirulina is a good source of single celled protein which may be produced in different media but bioaccumulation of protein and sometimes lipids may vary in respect to the inorganic nutrient concentrations of media. The contents of protein and lipids in spirulina were found higher (57.25% protein and 14.62% lipids) cultured in supernatant of DRA than standard Kosaric medium (Table 7) where Phang *et al*., (2000) recorded around 60% protein and 20% lipids in spirulina grown in supernatant of sago starch factory waste effluent. The present results has similarity with the findings of Tantichareon *et al*., (1991) when cultured spirulina in tapioca starch wastewater, Vetayasuporn (2004) grown spirulina in Thai rice noodle factory wastewater and Habib *et al*., (2019) cultured spirulina in supernatant of digested rotten potato. Cheirsilp and Louhasakul (1913) found very good growth of spirulina in different industrial wastes (palm oilmill waste, cerum latex rubber waste, mollasses and crude glycerol) and produced high lipids which directly transesterified into biodiesel production and also bioelectricity (Thong *et al*., 2019).

Cell weight of spirulina (*Spirulina platensis*) had highly significant ($P< 0.01$) direct correlation with chlorophyll-*a* ($r = 0.942$) of spirulina grown in the supernatant of different digested rotten apple and Kosaric medium during the study (Fig. 3). Similarly, total biomass of *S. platensis*

was highly (P< 0.01) and directly correlated with chlorophyll-*a* (r = 0.961) of spirulina cultured in the supernatant of various digested rotten apple and Kosaric medium (Fig. 4). Again, total biomass of spirulina was found to be highly (P < 0.01) and directly correlated with the cell weight (r = 0.889) of spirulina grown in the supernatant of different digested rotten apple and Kosaric medium (Fig. 5). These correlations meant that cell weight, chlorophyll a and total biomass of spirulina were increasing and decreasing accordingly. Habib *et al*., (2019) recorded highly significant (P<0.01) correlation among cell weight and chlorophyll-*a* of spirulina, total biomass with chlorophyll-*a* of spirulina and total biomass with cell weight of spirulina which are more or less similar with the present findings. Costa *et al*. (2007), Jais *et al*., (2016) and Muys *et al*., (2019) also found similar results.

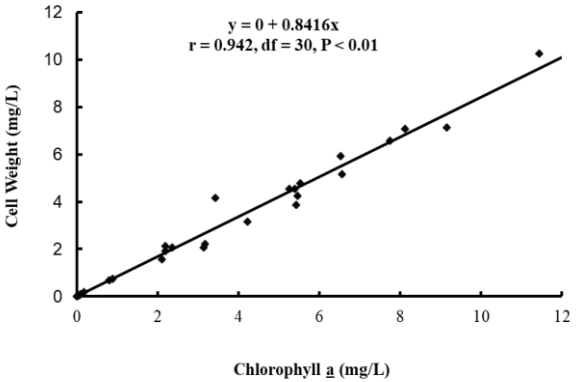

Fig. 3: Correlation coefficient (r) of cell weight (mg/L) with chlorophyll a (mg/L) of spirulina grown in supernatant of three digested rotten apple, and Kosaric medium

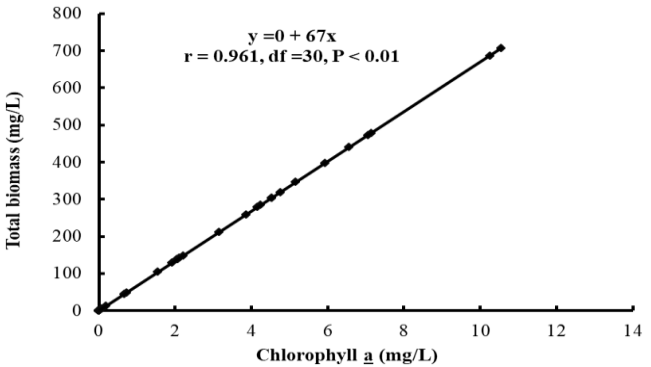

Fig. 4: Correlation coefficient (r) of total biomass (mg/L) with chlorophyll a (mg/L) of spirulina grown in supernatant of three digested rotten apple, and Kosaric medium

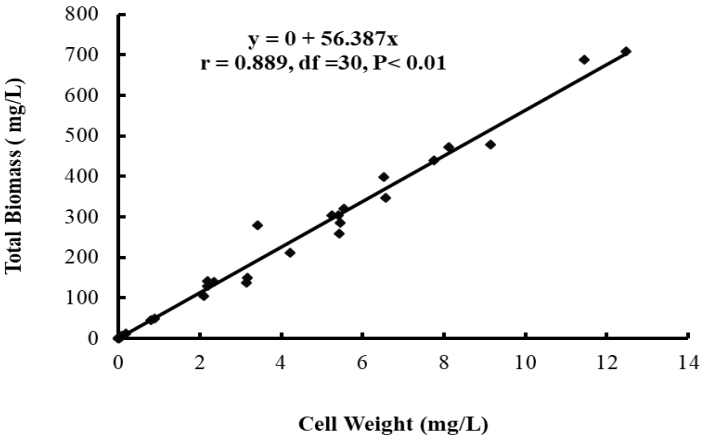

Fig. 5: Correlation coefficient (r) of total biomass (mg/L) with cell weight (mg/L) of spirulina grown in supernatant of three digested rotten apple, and Kosaric medium.

From the findings of this study, it is important to mention that spirulina may be successfully grown in agro-industrial waste materials like fruits, vegetables etc. and used in feed of different domestic animals like fishes, poultry, cattle etc. due to presence of high percentage of protein.

Conclusion

From the results obtained in the present study, it can be concluded that the growth of spirulina (*Spirulina platensis*) was better in the concentrations of 2.50% DRA than other concentrations of DRA. Thus, the concentration of 2.50% DRA was most suitable for *S. platensis* culture compared with standard Kosaric medium. These media are easily available and most inexpensive in contrast of Bangladesh. So digested rotten apple can be used for commercially and economically viable mass culture of spirulina.

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Studies on the quality of sauce prepared from SIS and a comparison with the commercial one

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Abstract
The study was conducted for the development of fish sauce from Small Indigenous Species (SIS) by using 25% salt concentration and to examine their organoleptic, biochemical and microbiological changes during fermentation period. For this purpose Gulsa (*Mystus cavasius*), Mola (*Amblypharyngodon mola*), Tengra (*Mystus vittatus*), Chanda (*Chanda nama*) and Punti (*Puntius sophore*) were used. The fishes were fermented by the combined action of microorganisms and enzymes present in fish tissue and viscera. Throughout fermentation about 90% fish became liquefied. At the initial stage of fermentation the moisture, ash, protein, lipid, total volatile base nitrogen (TVB-N), pH and aerobic plate count (APC) in fish content were found to be 75.75%, 3.76%, 14.64%, 4.89%, 1.44 mg/100g, nearly neutral (around 7) and 4.97×10^6CFU/g, respectively. With the progress of fermentation process the organoleptic, biochemical and microbial parameters changed gradually and after 210 days of fermentation the moisture, ash, protein, lipid, total volatile base nitrogen, pH and aerobic plate count were changed to 74.03%, 19.37%, 2.96%, 3.42%, 19.28 mg/100g, 6.37 and $9.71\text{x }10^7$ CFU/g, respectively. After fermentation and aging the color of the product turned to brown, flavor was light fishy, taste was salty and appearance was clear which was overall acceptable like commercial fish sauce. The proximate composition of final product in fish sauce were 68.17% moisture, 17.02% ash, 12.04% protein and 2.68% lipid and the Na content was 12.13%. The TVB-N and pH in fish sauce were within acceptable limit, 19.54 mg/100g and 6.72, respectively. Aerobic plate count (APC) was also within acceptable limit with 3.19×10^6 CFU/ml. Fish sauce prepared in laboratory was compared with one commercial fish sauce (Anchovy extract, King Bell brand). The physical properties and chemical composition of laboratory prepared sauce and commercial fish sauce were found to be almost similar.

Key words: Small Indigenous Species (SIS), Fermentation, Fish sauce, Quality parameters, Comparison.

Introduction

Traditional fish products are native to a country or culture. A large part of fish catch in Bangladesh is used in the form of a number of different processed products such as dried, salted, smoked, semi-fermented, fermented etc. Fermentation has been a popular technology for the preservation of fish in south-east Asian countries from time immemorial. In most cases the process is accelerated by the use of salt, as well as by added carbohydrate. The end product in such cases is liquid. A series of complex biochemical reactions take place during fish fermentation process. Fish enzyme such as chymotrypsin has high catalytic activity and thus it can hydrolyze more peptide bonds in protein substrates. As the major changes in fermentation process are the conversion of protein into small peptides and amino acid, the total nitrogen value become important indicator in determining the quality of fish sauces. Fish sauce is called by different names in different continents, e.g. 'budu' in Malaysia, 'patis' in Philippines, 'nampla' in Thailand, 'pissala' in France, 'yeesui' in Hong Kong, 'nuoc-nam' in South Asia (Lopetcharat *et al*., 2001). In Thailand, Kanpuchea, Malaysia, Cambodia, Philippines and Indonesia, these products are a staple part of the diet. Amano (1962) reported that in Cambodia, some 7.5% of the total dietary protein is derived from fish sauce. It is usually made from small fish that would otherwise have little value for consumption. According to Rahman (1989), there are 260 species of freshwater indigenous fishes in Bangladesh. Among them, which grow to a size of 25 cm or 9 inches at mature or adult stage in their life cycle are known as Small Indigenous Species (SIS) (Felts *et al*., 1996). He has included 45 fish species on the list of SIS including carps and minnows (18 species), catfishes (9 species), perches (9 species) and others. Ali (1997) listed 143 species of SIS in Bangladesh. SIS species contains a huge amount of vitamin A and Vitamin D which are essential for human bones, teeth, skin, and eyes. SIS also supply good amount of calcium, phosphorus, iron, iodine etc. These minerals are essential for developing body resistance against disease. Some SIS like Punti (*Puntius sp*.) contains double the amount of iron compared to many cultured carps like Silver carp (*Hypophthalmicththys molitrix*) and Rui (*Labeo rohita*); another SIS Mola (*Amblypharyngodon mola*) contains three times more calcium and fifty times vitamin A, than that of Silver carp and Rui (Villif and Jorgensen 1993).Therefore, it is necessary to utilize SIS in different form of fish products for value addition while they are abundant. Among different processes, preparing fish sauce with SIS have some advantages due to the low cost of these fishes, ease of sauce preparation, safety and improved digestibility and absorbability of the product. Fish sauce has also therapeutic effects against certain types of gastrointestinal infection. A good quality fish sauce contains 200g/l of salt and 14 to 18g/l of nitrogen. The amino acid content ranges from 40g/l for ordinary quality to 60g/l for superior quality (Truong Van Chom, 1954). The sauce has a high content of volatiles, especially volatile fatty acids and methyl ketones (Dougan and Howard, 1975). On the other hand, there is no requirement of chilling of fish sauce during processing and preservation, no need any extra storage facilities or complex transport and distribution facilities. Though some works have done on preparation of fish sauce but detailed research on quality parameters and comparison with commercial one is rare. So, the present study was done to prepare fish sauce from Small Indigenous Species (SIS) of fish using 25% salt (as Foisal *et. al*., 2015 reported fish sauce produced with 25% salt was found to be of best quality than produced with 30% and 35%) and to observe the changes in

biochemical and microbial parameters throughout the fermentation process. At the same time, study was also performed to compare the quality parameters of the laboratory prepared fish sauce with the commercial one.

Materials and Methods

Materials

The present study was conducted in the laboratory of Fisheries Technology Department, Bangladesh Agricultural University (BAU), Mymensingh. The samples which included Gulsa (*Mystus cavasius*), Mola (*Amblypharyngodon mola*), Tengra (*Mystus vittatus*), Chanda (*Chanda nama*) and Punti (*Puntius sophore*) were collected from Kamal-Ranjit (KR) market of BAU, Mymensingh in fresh condition.

Preparation of Fish Sauce

The collected fish samples were washed properly with fresh water to remove all dirt, slime and unnecessary particles. Then the samples were weighed and mixed properly with 25% of salt (Plate 1). A layer of salt was placed at the bottom of a plastic jar, and then salt mixed fish were poured in it. Finally, a layer of salt was spread to cover the surface of the fishes. Plastic jar with salted fish samples were covered with lids and kept in a dry place. After 7 days saturated brine solution was added to submerge the fish and prevent it from oxidation. Floating fishes on the surface of brine water kept in submerged condition by placing a weight. The plastic jar was closed with lids and allowed to ferment for 7 months. During fermentation the chemical and bacteriological changes of fish samples were determined at 15 days intervals. After 7 months the liquid part was extracted from the fermented fish samples, aged in the direct sunlight for 15 days, and filtered to separate the undigested portions. Thus the fish sauce becomes ready for use.

Analytical Procedure

Proximate composition (percent moisture, ash, protein, lipid content) analysis of raw fishes, fish sauce (at different stages of fermentation process) were carried out according to the methods AOAC (1990). Total Volatile Base Nitrogen (TVB-N, mg N/ 100g) was determined according to the methods given in Pearson (1976) and pH was determined by Mettler Toledo pH meter. Bacterial colonies developed were counted for the determination of standard plate count as CFU/g of sample. All the representative fish species (fresh) were mixed together and ground in a sterile mechanical grinder to make a paste. This ground sample for raw fishes/fish sauces (at different stages of fermentation process) was used for all biochemical and bacteriological analysis.

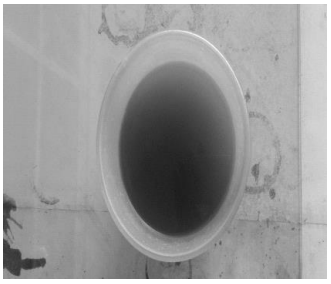
A

B

C

D

E



F

G

Plate-1: Preparation of Fish Sauce from SIS. Here, A – Small Indigenous Species (SIS)of Fishes; B- Fish and Salt in proper Ratio; C- Mixing of Salt with Fishes; D-Samples in Plastic Containers, E- Fish-Salt mixture after 2 months; F- Prepared Fish Sauces; G- Laboratory prepared fish sauces and the commercial one

Results

Biochemical Composition and Microbiological Analysis of Fresh Fish

The proximate composition and bacterial load of fresh fishes like Gulsa (*Mystus cavasius*), Mola (*Amblypharyngodon mola*), Tengra (*Mystus vittatus*), Chanda (*Chanda nama*) and Punti (*Puntius sophore*) were determined and it was found that initial average moisture content was 75.75%, ash content 3.76%, crude protein content 14.64%, lipid content 4.89%, and TVB-N content was 1.44 mg/100g. The initial aerobic plate count for bacterial load was found 4.97×10^6 CFU /g.

Biochemical and Microbiological Changes during Fermentation

Changes in moisture content

The changes in moisture content in fish during fermentation showed in Fig. 1. Initial moisture content (%) of fish was 75.75% before addition of salt. After addition of salt the moisture content dramatically decreased due to entrance of salt in fish muscle. After 24 hours the moisture content was 59.12%. With the progress of fermentation process moisture level again started to increase and after 210 days the moisture content reached at 74.03%.

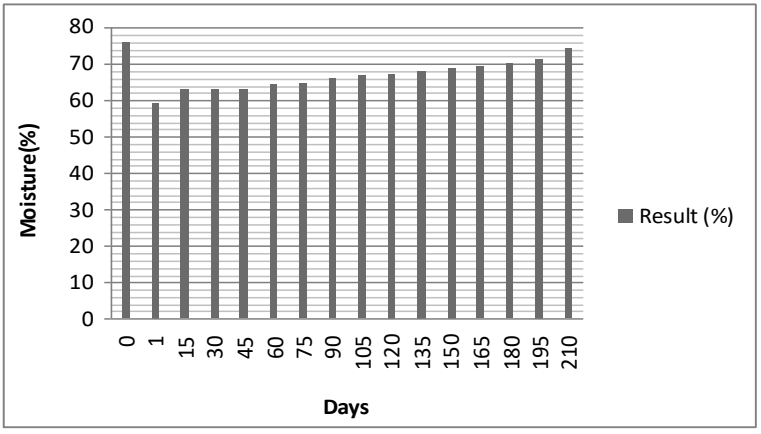


Fig. 1: Changes in moisture content (%) in salted fish during fermentation

Changes in Ash content

Fig. 2 shows that, at the beginning before addition of salt the ash content (%) in fish was 3.76%. After addition of salt the ash content increased to 10.61%. After 15 days the ash content decreased to 9.13% due to addition of saturated brine solution to prevent rancidity. The ash content again started to increase gradually as the fish muscle liquefies resulting in the increased bone content and salt. After 210 days it reached at 19.37%.

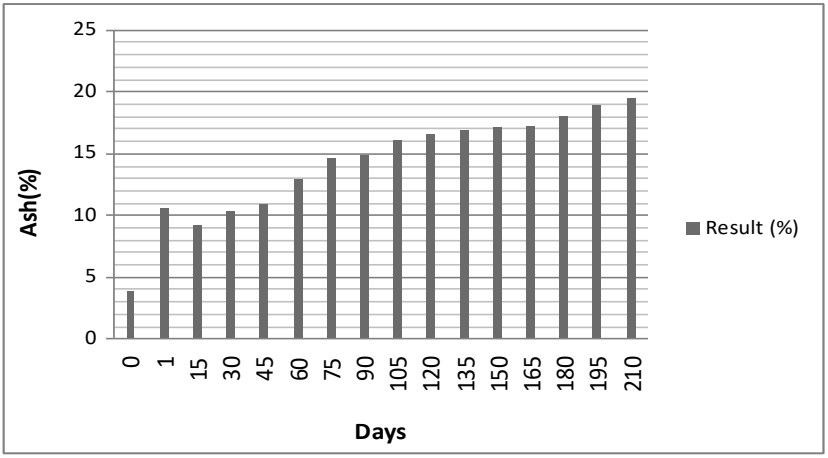


Fig. 2: Changes in Ash Content (%) in Salted Fish during Fermentation

Changes in Protein Content

Fig. 3 shows the changes in protein content (%) in fish during fermentation. Protein content in fish was initially 14.64%. After 24 hours addition of salt the protein content increased to 24.49%. As the time passed the protein in fish body started to liquefy, as a result the total protein content gradually decreased in fish. After 210 days the protein content reached at 2.96%. Protein content in fish body suddenly increased due to addition of salt, which resulted in the loss of moisture. As the fermentation process progress the protein in fish body gradually broken down by the combined effect of enzyme and microorganisms.

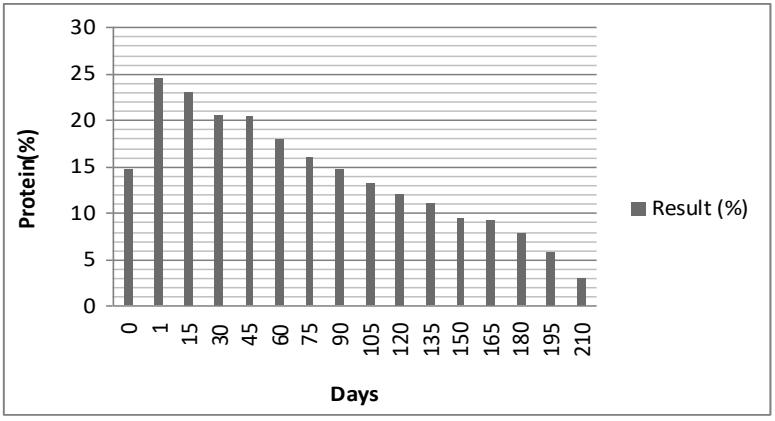


Fig. 3: Changes in Protein Content (%) in Salted Fish during Fermentation

Changes in Lipid Content

Fig. 4 shows the changes in lipid content (%) in fish during fermentation. Initial lipid content of fish body was 4.89% before addition of salt. After addition of salt the lipid content decreased. After 24 hours the lipid content was 4.82% and finally reached at 3.42%.

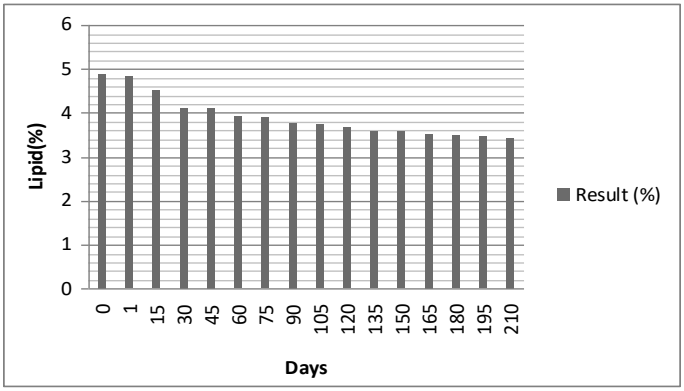


Fig. 4: Changes in Lipid Content (%) in Salted Fish during Fermentation

Changes in Total Volatile Base Nitrogen (TVB-N) Content

Fig. 5 shows that, the initial TVB-N content of fish was 1.44 mg/100g before addition of salt. After addition of salt the TVB-N content increased. After 24 hours the TVB-N content was 2.37 mg/100g. With the progress of fermentation protein in fish body gradually break down and the amount of total volatile nitrogen increased and after 210 days the total volatile base nitrogen reached the maximum level 19.28 mg/100g.

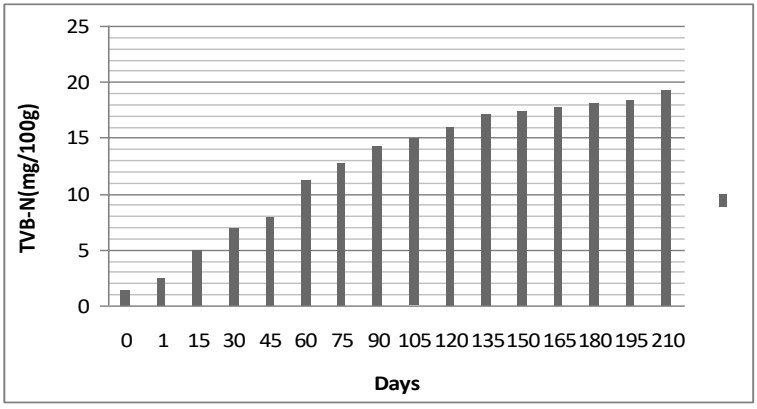


Fig. 5: Changes in Total Volatile Base Nitrogen (TVB-N) Content in Salted Fish during Fermentation

Changes in pH Value during Fermentation

Fig. 6 shows the changes in pH value in fish during fermentation. In the present study, the decrease in pH was observed on the initial stage of fermentation process, increased on the following days and again decreased after 60 days of fermentation.

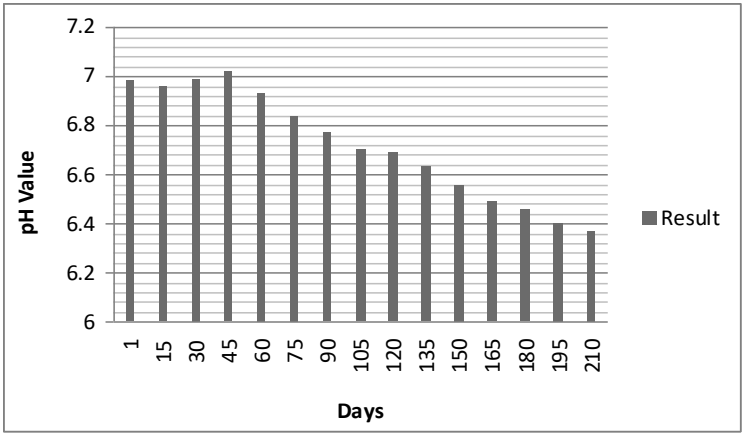


Fig. 6: Changes in pH Value in Salted Fish during Fermentation

Bacteriological Analysis during Fermentation

Fig. 7 show the total bacterial load in fish counted in ordinary medium during fermentation. APC was initially 4.97×10^6 CFU/g before addition of salt but after addition of salt total bacterial load decreased. After 90 days of addition of salt, bacterial load was 4.92×10^6 CFU/g. But after 120 days total bacterial load again increased and finally reached at $9.71\text{x } 10^7$ CFU/g.

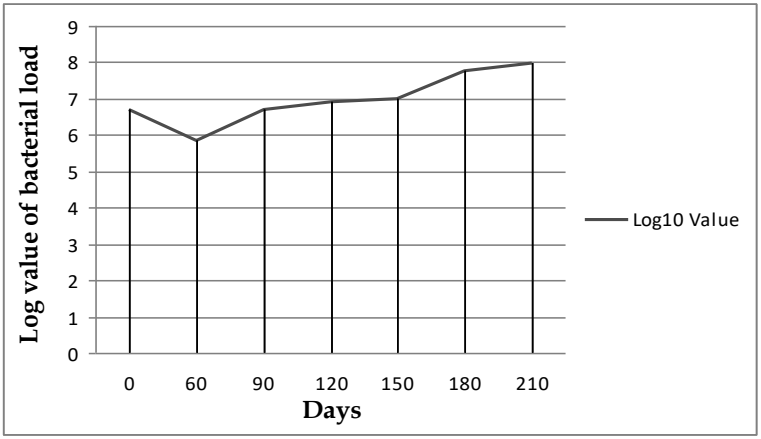


Fig. 7: Bacterial Load during Fermentation

Physical Changes during Fermentation

Before addition of salt fish sample had shiny appearance and elastic texture of fresh fish. After the addition of salt within few days fishes lost their shininess and gradually lost the scales, skin, flesh exposed, belly disappeared and finally skeleton become exposed and during this period about 90% of fish liquefied. Elastic texture of fish become soft and gradually muscles become digested due to action of enzymes. Similarity, at the beginning colors of the exudates (brine) was light yellowish and gradually it becomes light red, dark red, dark red and turbid and finally highly turbid (Table 1). In fact those physical changes are common phenomenon for the fish fermentation process.

Table 1: Physical Properties of Fish Sauce

Parameters	Results
Color	Brown colored liquid
Flavor	Light fishy flavor
Taste	Salty
Clearness	Clear
Liquidity	Liquid like water
Acceptability	Highly acceptable

Chemical composition and bacterial load of fish sauce at final stage of fermentation

Table 2 shows the chemical composition and bacterial load of the fish sauce. The moisture content of fish sauce was found 68.17%, ash content 17.02%, protein content 12.04%, and the lipid content of the final product was 2.68%. As the time passed the TVB-N value reached to 19.54 mg/100g and the Na content of the final product wad found 13.12%. The pH value of fish sauce was 6.72. The total bacterial load was determined in the ordinary medium. The bacterial load was found 3.19×10^6 CFU/ml.

Table 2: Chemical Composition and Bacterial Load of Fish Sauce

Parameters	Results
Moisture	68.17%
Ash	17.02%
Protein	12.04%
Lipid	2.68%
TVB-N	19.54 mg/100g
pH	6.72
Na	13.12%
Bacterial load	3.19×10^6 CFU/ml.

Comparative Study between Laboratory prepared and Commercial Fish Sauce

In the present study one experiment was carried out for comparison of laboratory prepared fish sauce and commercial fish sauce. Table 3 shows the comparison between fish sauces prepared in the laboratory and the commercial one. Data displayed at Table 3, clearly shows that, the physical properties and chemical composition of laboratory prepared fish sauce and commercial fish sauce almost similar. So, that experiment was conducted in correct ways.

Table 3: Comparison between Laboratory Prepared and Commercial Fish Sauce

Parameters	Laboratory Prepared Fish Sauce	Commercial Fish Sauce
Color	Brown colored liquid	Lighter brown colored liquid
Flavor	Light fishy flavor	Light fishy flavor
Taste	Salty	Slightly less salty
Clearness	Clear	Clear
Liquidity	Liquid like water	Liquid like water
Acceptability	Highly acceptable	Highly acceptable
pH	6.72	6.65
Na	13.12%	14.50%

Discussion

In the present study the moisture content gradually increased during progress of the fermentation process. These gradual increases in moisture level took place in fish-salt mixture might be due to liquefaction of muscle. The increase in moisture content could be adduced to the fact that fermentation has taken place. Kim Yong *et al*. (2004) stated that the moisture percentage of anchovies and other small fishes varies from 46 to 77%, during fermentation period. Result of the present study fall within this range. In the case of ash content, with the lapse of time of fermentation process increased from initial value of 3.76% to 19.37%. A similar result was found by Lopetcharat *et al*., (2001). They mentioned that the ash content of Pacific whiting during preparation of fish sauce reached up to 25%. On the other hand, the initial protein content in fish found 14.64%, which decreased to 2.96% after 210 days of fermentation. Similar result for decrease in protein content during fermentation was reported by Hjalmarsson *et al.,* (2007) and Xu-Wei *et a*l., (2008). In the present study lipid content did not change dramatically. Initially the lipid content for fish was found 4.89% which slightly decreased to 3.42%. Khan (2013) also reported the same result as obtained in this study. Moini and Koochekian (2003) prepared sauce from kilka fishes of the Caspian Sea (Iran) from 3 different preparations (whole kilka, cooked whole kilka and dressed kilka) where the total volatile nitrogen was found 25 mg/100g after 6 months of fermentation. Similar result was found by Xu Wei *et al.,* (2008). These findings are quite similar to the finding of the present

study for TVB-N. The pH of the fermentation mixture influences the extraction of fish proteins and the maximum extraction of protein takes place between pH 7.0 and 9.0 (Sanchez 2001). The same author stated that the decrease in pH in salt-fermented fish sauce samples was probably due to dissociation of amino acids and small peptides in the presence of salt. The drop in pH value on the first few weeks of fermentation was also observed in the study of El Hag *et al.* (2012) and Kilinc *et al.,* (2006) wherein similar pattern of decreasing pH values in their fermented fish samples during the early stages of fermentation using debs, *Labeo* sp. with 25% salt and sardines with 10% salt added, respectively. After fish dies, decomposition occurs through enzymatic digestions of fish muscle and gradually increased the pH of the flesh. The use of proper concentration of salt in the fermentation process inhibits decomposition of fish. The initial pH was 6.98 to its final value of 6.37. The pH difference may be due to protein hydrolysis which resulted from free hydrogen ions, free amino acids, and amino acid of oligopeptides (Tungkawachara *et al.,* 2003). The same authors reported the pH of fermented Pacific whiting fish sauce, fermented for 9 months (270 days) ranged between 7.05 (0 month) and 5.42 (9 months). Lopetcharat and Park (2002) reported the pH of fish sauce using the same fish species fermented for 40 days ranged between 6.1 and 6.3. These findings coincide with the decreasing pattern of pH in the present study. Faisal *et al*., (2013) prepared sauce from small freshwater fishes with three different concentrations of salts 25%, 30% and 35% where the total bacterial load initially was found 5.99×10^6 CFU/g and finally 1.19×10^7 CFU/g after 7 months of fermentation in case of 25% salt concentration in ordinary medium. This result is quite nearer to the finding of present study for aerobic plate count of bacteria.

Fish sauce is translucent, clear amber yellow or brown liquid, with a salty taste and fish flavor obtained from fermentation of a mixture of fish and salt, and the fermentation takes not less than 6 months (Codex Alimentarius, 2013). Faisal *et al.*(2013) did chemical composition and microbial analysis of fish sauce they prepared and found 67.44% moisture, 12.28% protein, 2.81% fat and 17.32% ash, TVB-N content 19.88 mg/100g, and the total bacterial load 1.53×10^6 CFU/ml. The obtained results in present study for fish sauce prepared from SIS are almost similar.

The results of chemical composition and microbial analysis of fish sauce at final stage of fermentation (Table 2) are in agreement with those reported by Cho *et al.* (2000). Park *et al.* (2005) reported the range of chemical composition of fish sauce was 61.40-79.20% moisture, 0.9-13.70% crude protein and 18.20-25.80% ash content. The value (6.72) of pH in this study is slightly high compared to those (4.66-5.91) reported by Cho *et al.* (2000) and Aquerreta *et al.,* (2001) but is in agreement with those reported by Ijong and Ohta (1995), Cho *et al*., (1999), and Park *et al*., (2001). The high pH value of sauce may reflect bacterial activity during fermentation and probably as a consequence of the accumulation of basic compounds (Aquerreta *et al*., 2001). Concerning TVB-N content was laid in range 14.1-338.6 mg TVB-N/100 ml reported by Aquerreta *et al*., (2002) and Ruiz-Capillas *et al*., (2000). The content of TVB-N might be attributable to the rate of hydrolysis of fish muscles by fish enzymes and microbial activity during fermentation under the lower salt concentration (Ijong and Ohta 1995).

The obtained results in the comparative study indicated that, the fermentation procedure followed in the present study to prepare fish sauce was rightly chose.

Conclusion

Based on obtained results and above discussion it could be concluded that, the salt concentration of 25% provides a suitable environment for the production of best quality fish sauce. The percentage of protein as well as other biochemical compositions of laboratory prepared fish sauce is similar with the commercial fish sauce available in the market. Therefore, nutrient enriched fish sauce can be prepared as a value added product from small indigenous species of fish while they are abundant.

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Potentials of plant polyphenol on growth performance of farmed pangasius (*Pangasianodon hypophthalmus*)

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Abstract
This experiment was conducted by using natural bioactive additive from sugarcane plant (*Saccharum officinarium*) named as 'polyphenol' has the potential to replace some of the functions of feed additives as it contains antioxidants, minerals, nutrients and essential amino acids. The experiment was done in cages where Pangasius (*Pangasianodon hypophthalmus*) were stocked in 16 cages at 50 fish per cage. A trade product named as Polygain extracted from *Saccharum officinarium* which contain polyphenol was provided to the fishes by mixing it with the feed ingredients at different concentration (0% polyphenol [T_0], 0.2% polyphenol [T_1], 0.4% polyphenol [T_2] and 0.6% polyphenol [T_3]). Initial average weight of the fish was 4.74g which had an average length of 5 cm. Final sampling showed that the average weight of individual treatment were T_0 (39.93±0.75 g), T_1 (53.61±7.88 g), T_2 (43.77±2.66 g) and T_3 (45.14±3.04 g). Average length of fish was increased which fed with T_1 [(18.63±0.95) cm] treated fish than the T_0 (17.1±0.08 cm), T_2 (17.8±0.36 cm) and T_3 (17.98±0.39 cm) treated fish. Among the four treatment, T_1 showed better growth [(53.61±7.88) g] than the control [(39.93±0.75) g] ($p<0.05$). T_1 had lower FCR (1.49) than that of control (2.00). The present study revealed that polyphenol containing diet can improve the growth performance of Pangasius fish.

Key words: Pangus, Polyphenol, minerals, nutrients

Introduction

Fish play a crucial role in the diet of Bangladeshi people by providing more than 60% of animal protein consumption. Fish (including shrimp and prawn) is the second most valuable agricultural crop, and its production contributes to the livelihoods and employment of millions (Belton and Little 2011). The fisheries sector of Bangladesh has been growing at a rate of 5.43

percent since the last ten years (DoF 2017). Bangladesh ranked 5th position as aquaculture producing country in the world (FAO 2016). Bangladesh is one of the most suitable countries in the world for freshwater aquaculture in rural areas, because of its' so many resources and climate that supports agriculture. *Pangasius* farming has achieved descent attention for supply of protein, increase income and employment opportunity. *Pangasius* can be stocked at a much higher density in ponds compared to other cultivable species. Disease is the main constrain to improve the aquaculture production including *Pangasius* culture where higher stocking density is maintained. To get rid of the diseases many aquaculture drugs have been used. The excess application of these antibiotics is detrimental for human consumption (Serrano 2005). But there are strict regulations on the application of antibiotics and chemotherapeutics in aqua feeds because of presence of antibiotic resistance bacteria has adversely affect both freshwater ecosystem and human health (Lim et al. 2013). Many countries have made hard and fast rules to remove aqua drugs from fish feeds. Removing antibiotic means the capabilities of antibiotic must be replaced by some natural additive. Natural bioactive additives from plants such as 'polyphenol' have the potential to replace some of the functions of these additives. Polyphenol is secondary plant metabolites from sugarcane plants (*Saccharum officinarium*) which have been shown to exert anti-oxidative and anti-inflammatory effects of poultry. Polyphenol is rich in minerals, nutrients and anti-bacterial properties. The present study aimed at assessing the effects of polyphenol on growth performance (growth, FCR, specific growth rate and condition factor) of *Pangasius*.

Materials and Methods

The present study was done to determine the effect of bioactive compound on farmed *Pangasius* .To do so, following procedures were followed:

Study area

The current experiment was conducted from August to November, 2017 at the Fish Farm named as Halda Fisheries Ltd. situated at Potenga, Chittagong.

Making of cages for '*Pangasius*' culture

The experimental *Pangasius* has been cultured for 4 months in 16 cages in a rectangular pond which covers 43 decimal areas. Four different treatments have been applied where each treatment requires 4 cages for replication. The materials such as plastic drum, net, pipe, float were collected from local market. The sizes of the cages were 26 feet × 13 feet.

Experimental diet preparation

The feed which were applied in the cages as treatment was prepared in a feed mill of "Halda Fisheries Ltd." by adding appropriate amount of Polygain from sugarcane plant (*Saccharum officinarium*). Polygain contains natural polyphenol. The polyphenol content of "Polygain" used in the study was 30.40g/kg. The experiment was conducted with four treatments where each treatment replicated four times. The treatments were:

1. 0% polygain denoted as T_0 (Control regarded as without 'Polygain')
2. 0.2% 'Polygain' denoted as T_1 (60mg polyphenol/kg feed)
3. 0.4% 'Polygain' denoted as T_2 (120mg polyphenol/kg feed)
4. 0.6% 'Polygain' denoted as T_3 (180mg Polyphenol/kg feed).

The analysis of proximate composition of experimental diet was done in "Nutrition Laboratory" of the Faculty of Fisheries, Chittagong Veterinary and Animal Sciences University according to standard methodology. The feed formulation and proximate composition of experimental diets are shown in Table 1 and Table 2.

Table 1: Feed ingredients with their inclusion level

Ingredient	Inclusion (%)
Fish Meal 40%	18.75
Fish Meal 60%	18.75
Soya bean Meal	10
Meat & Bone Meal	15.63
Rice Bran	10.63
Wheat Bran	11.25
Mustard Oil Cake	6.25
Maize	5
Wheat Flour	3.75
Total	**100**
Additives	
DCP	0.5
Pellet binder	0.5
Soybean oil	0.5

Table 2: Proximate composition of feed

Name	Percentage (%)
Moisture	14.91
Crude protein	35.41
Crude lipid	8.82
Ash	22.4

Experimental design

The statistical design used for the experiment was completely randomized design (CRD)**.** For the continuation of experiment, 16 cages were set in the pond where individual cage was stocked with 50 *Pangasius* fingerling (Table 3).

Table 3: Layout of the experiment showing the distribution of 'pangasius' in cages and the applied treatments

Dietary treatment groups	Treatment × Replication (Tn× Rn)	No. of fishes per cage	Total no. of fish per treatments
To (0% 'Polyphenol')	T_0R_1	50	200
	T_0R_2	50	
	T_0R_3	50	
	T_0R_4	50	
T_1 (60 mg 'Polyphenol'/kg feed)	T_1R_1	50	200
	T_1R_2	50	
	T_1R_3	50	
	T_1R_4	50	
T_2 (120 mg 'Polyphenol'/kg feed)	T_2R_1	50	200
	T_2R_2	50	
	T_2R_3	50	
	T_2R_4	50	
T_3 (180 mg 'Polyphenol'/kg feed)	T_3R_1	50	200
	T_3R_2	50	
	T_3R_3	50	
	T_3R_4	50	
Grand total			800

Sampling

Sampling of the experimental fish was done in regular interval of one week. Sampling acts as important tool for checking the growth performance of fish and also adjusting the feeding rate and feeding frequency to their body weight. Growth of fish in each sampling was taken by weight of fish where weight of sampling fish was taken by using a weight machine (RADAG-AS 220.R2) and length of fish was taken by using measuring scale.

Calculation of growth indices

The following parameters were calculated:

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total amount of feed given (g)}}{\text{Total weight gain (g)}}$$

$$\text{Specific growth rate (SGR)} = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{duration in days}} * 100$$

$$\text{Condition factor (CF, \%)} = \frac{\text{Weight of fish}}{(\text{Length of fish})^3} * 100 \text{ (Fulton, 1902)}$$

Proximate composition analysis

The proximate compositions of the experimental diet were done in "Nutrition Laboratory" of Faculty of Fisheries, Chittagong Veterinary and Animal Sciences University. Protein was determined by Kjeldahl apparatus (Distillation unit: VELP-UDK 129; Digestion unit: VELP-DK 20/26), lipid by digital soxhlet apparatus (FoodALYT RD 40), ash content by muffle furnace (Nabertherm-L9/13) and moisture content by hot air oven (BINDER-ED115).

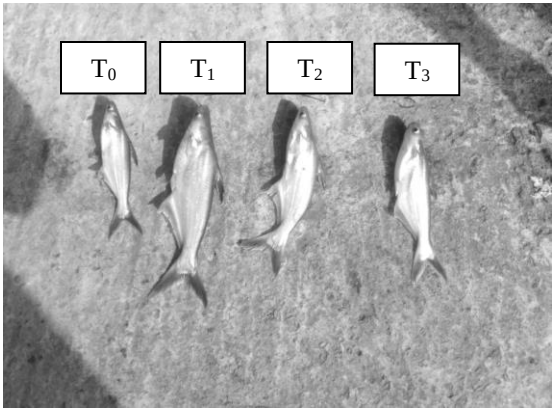
Data analysis

Data were analyzed by using Microsoft Office Excel-2007, USA and IBM SPSS Statistics 23 Version. Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to assess statistically significant differences among the control and different treated values. Statistical significance was set at $P < 0.05$.

Results

Effects of polyphenol on fish weight and length

Sampling was done in regular interval of one week and total 16 sampling were performed in four months. The initial average weight of the fish was 4.74g and average length was 5cm. Final sampling showed that the average weight of each treatment such as T_0, T_1, T_2 and T_3 were 39.93(±0.75)g, 53.61(±7.88)g, 43.77(±2.66)g and 45.14(±3.04)g, respectively and average length were 17.1(±0.08) cm, 18.63(±0.95) cm, 17.8(±0.36)g and 17.98(±0.39)cm, respectively. The above findings can be concluded as the fish provided with 'T_1' feed have higher growth performance in terms of weight (Fig. 1) and length (Fig. 2) in comparison with other treatments (Photograph 1). Among dietary treatment, significant difference between the treatments ($p<0.05$) was observed for final weight and final length. Weight and length were significantly higher in fish fed with T_1 treated feed when comparing with other treatments (Fig. 3 & 4).



Photograph 1: Comparison among T_0, T_1, T_2 and T_3 treatment group

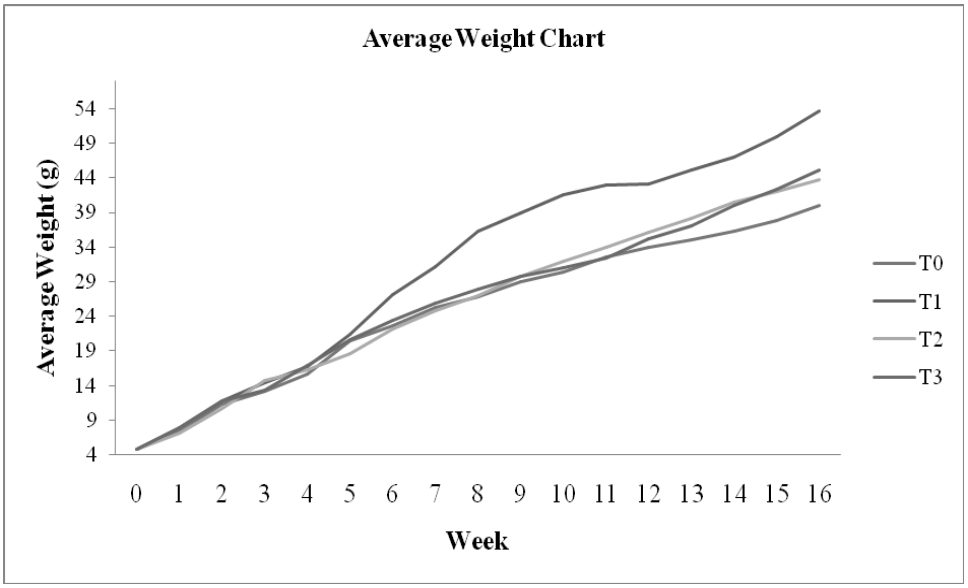


Fig. 1: Growth trend chart in g of pangasius over 16 weeks

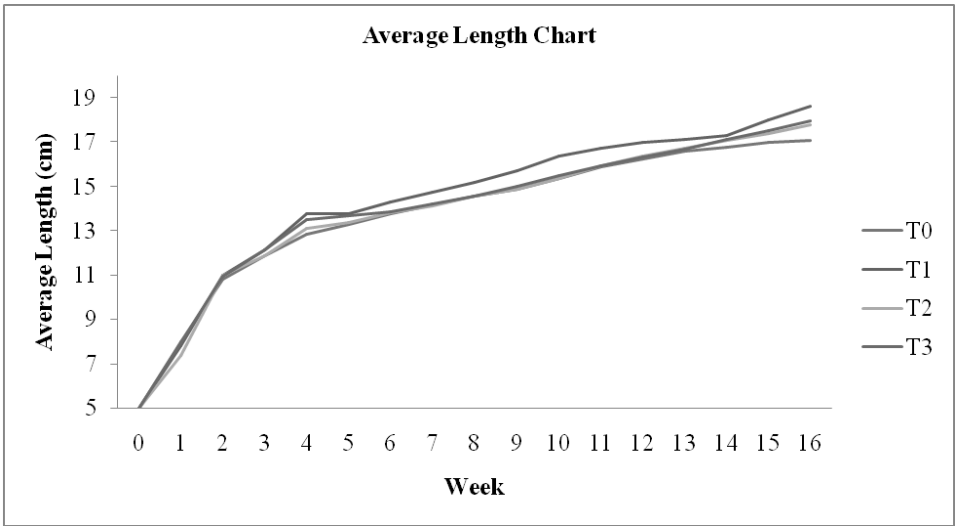


Fig. 2: Growth trend chart in cm of pangasius over 16 weeks

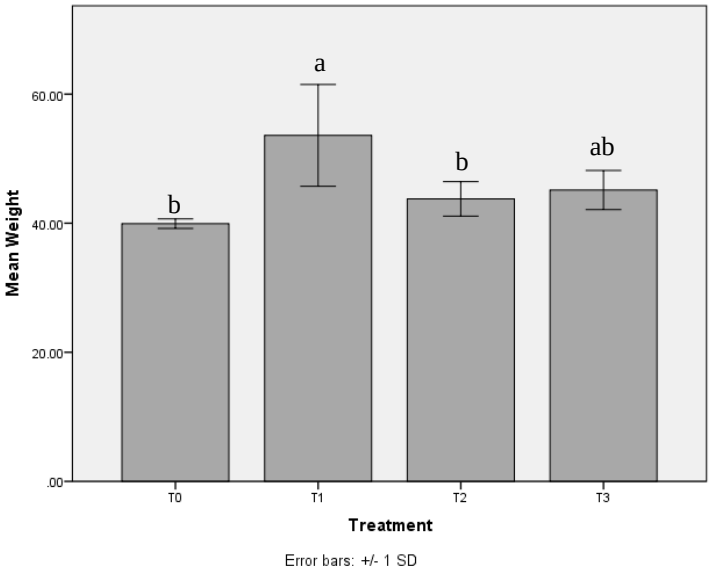


Fig. 3: Effects of polyphenols on weight of fish body (Mean ± SD) after 4 months. Values accompanied by different letters are statistically significantly different ($p < 0.05$, n=4)

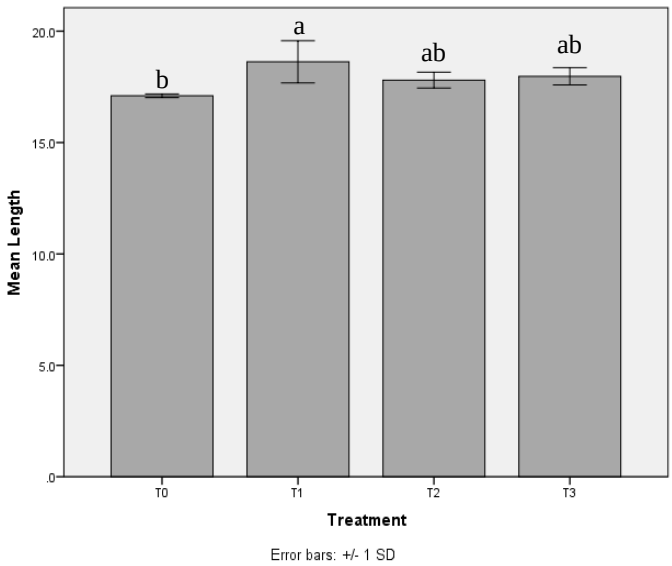


Fig. 4: Effects of polyphenols on length of fish body (Mean ± SD) after 4 months. Values accompanied by different letters are statistically significantly different ($p < 0.05$, n=4)

Condition factor

The values of condition factor found in the present study were 0.65±0.004, 0.83±0.033, 0.78±0.011 and 0.78±0.043 in T_0, T_1, T_2 and T_3, respectively. Higher K was found in T_1 than the T_0, T_2 and T_3. But no significant difference was observed among treatments (Fig. 5) ($P<0.05$).

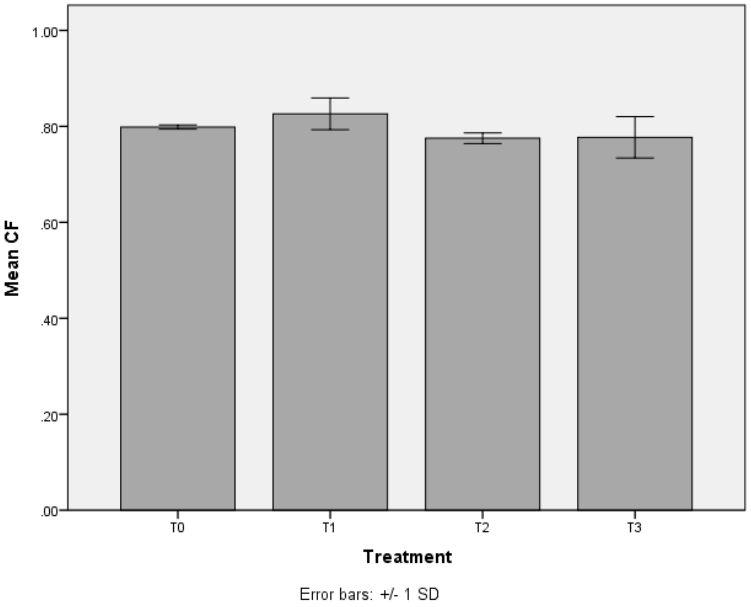


Fig. 5: Effects of polyphenols on condition factor (Mean ± SD) after 4 months. The condition factor of fishes of T_1, T_2 and T_3 were compared to the control group

Specific Growth Rate

The SGR values were 1.9±.016, 2.16±0.13, 1.98±0.05 and 2.01±0.06 for T_0, T_1, T_2 and T_3 respectively. Among dietary treatment, significant differences ($p<0.05$) were observed (Fig. 6).

Feed Conversion Ratio (FCR)

Experimental feed showed best FCR (1.49) in 0.2% polygain treated feed, whereas the FCR value of 0.4% and 0.6% polygain treated fish were 1.83 and 1.77, respectively. It reveals that polyphenol treated feed showed better FCR performance than control feed (2.00) (Table 4).

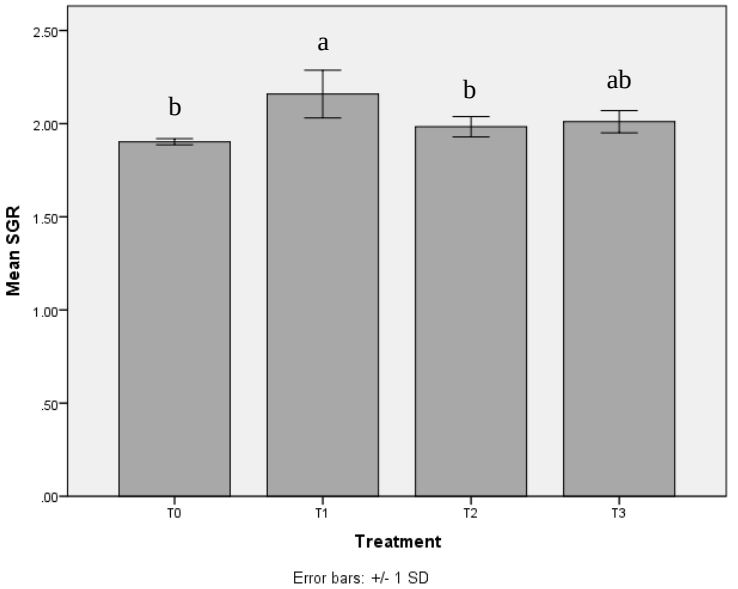


Fig. 6: Effects of polyphenols on specific growth rate (Mean ± SD) after 4 months. Values accompanied by different letters are statistically significantly different ($p < 0.05$, n=4)

Table 4: FCR value of different experimental feed

Treatment	FCR
T_0	2.00
T_1	1.49
T_2	1.83
T_3	1.77

Discussion

After completion of the experimental period, better growth performance both weight (53.61±7.88g) and length (18.63±0.95cm) was found in 0.2% polygain (60mg polyphenol/kg feed) treated fish. The research showed that the difference in weight and length of the fishes among various treatments was significant ($P<0.05$) throughout the culture period. Significantly higher ($P<0.05$) weight was observed by the fishes of T_1, T_2 and T_3 groups. Average weight of fishes of control group was lower than other three groups (T_1, T_2 and T_3). However, fishes of T_1 treated feed showed highest final weight at the end of the experiment. No feed residues were left in polyphenol treated fish than the control. Polyphenol seems to be acting as an attractant. Using polyphenol removes the fishy odor of fish meal used in formulating feed and creates a fresh smell in it. All fish were attracted to the feed, fighting and splashing to consume

the feed containing polyphenol than the control. It showed to enhance feed consumption which ultimately increase the survival rate and also responsible for reducing wastage of feed. The growth of fish is directly dependent on feed composition and quality. Significantly higher feed consumption was observed by the fishes of T_1, T_2 and T_3 treatment fishes. Feed intake by the fishes of control group was lower than other three groups (T_1, T_2 and T_3). However, fishes of T_1 treatment exerted highest feed consumption. A few studies have been made on the effect of polyphenol in fish and fish feed. But a large number of studies have been made on poultry and other animals. Gessner *et al.* (2013) observed a significantly improved feed conversion ratio and similar to Sehm *et al*. (2006) an increased villus height: crypt depth ration in the duodenum. This is in agreement with a broiler study of Viveros *et al*. (2010) who observed an increased villus height in the small intestine by feeding polyphenol-rich products of wine/grape juice processing. It is assumed that an increased villus height leads to an improvement of digestive and absorptive functions of the intestine as a result of increased absorptive surface, expression of brush border enzymes and nutrient transport systems (Caspary 1992). During the study period, no disease outbreak was observed and no mortality was observed. The weather condition was unsuitable for several periods of the culture period. The fishes struggled against the rough weather and remain alive as well as disease free. This may happen because of polyphenols as it helps to build strong immune system in animals which help them to sustain in rough weather. Magrone *et al*. (2016) attempted to administration of a polyphenol enriched feed to farmed sea bass which suggest that Polyphenol create lower levels of intestinal pro-inflammatory cytokines helping as an expression of a robust and protective adaptive immune response. Kousoulaki *et al*. (2015) performed an experiment of nutritional requirements, feed management and farming protocols of European sea bass. His study summarizes that, replacement of fish meal in formulating balanced diet by a variety of plant-based ingredients showed better growth potential. In mice receiving oral treatment with polyphenols rich extracts from date palm tree, an increment of the immunecompetent cells, including T helper 1 (Th1), natural killer (NK), macrophages and dendritic cells (DCs) in both Peyer's patches and spleen (Karasawa *et al*. 2011) was observed. Similar effects were obtained in response to treatment of aged rats with polyphenols from *Cassia auriculata*, increasing splenic T and B cells (John *et al.* 2011). Baur *et al*. 2006; Aguirre *et al*. 2014, conducted a research where they found that the polyphenols are most promising than the other secondary plant metabolites because it has anti-oxidative and gene regulatory properties. Polyphenols are act as anti-inflammatory both *in vitro* and *in vivo.* It inhibits the activation of nuclear factor kappa B (NF-κB) and also able to induce antioxidative and cytoprotective effects by inducing nuclear factor' (Tangney and Rasmussen 2013). Polyphenols supplementation has potential benefit for athletes helpful to exercise performance or oxidative damage (Kathryn *et al*. 2014).

The condition factor of a fish acts as indicator of health status of fish including physical, chemical and biological condition. The values of condition factor were .65±.004, .83±.033, .78±.011 and .78±.043 in T_0, T_1, T_2 and T_3, respectively. But among treated fish had no significant relation ($p<0.05$). According to Datta *et al*. 2013, condition factor of greater than one showed the well being of fishes fed with different experimental diets. The values of 'K' in higher in T_1 than the T_0, T_2 and T_3 suggesting that fish fed with diet containing 0.2 % Polygain

(60 mg polyphenol/kg feed) were much more cherished and healthy than the fish fed with other treated diet.

The SGR values were recorded in present study were 1.9±0.016, 2.16±0.13, 1.98±0.05 and 2.01±0.06 in T_0, T_1, T_2 and T_3, respectively. The higher specific growth rate is an indication of higher growth performance. Though Thai Pangus and rohu has different feeding habits, it has been seen that these species mostly prefer balanced diet (DoF 2000). Higher SGR were found in 0.2% Polygain (60mg polyphenol/kg feed). Among dietary treatment, significant differences ($p<0.05$) were observed.

FCR acts as an indicator, indicating how efficient a feeding strategy. Fishes of 0.2% Polygain (60mg polyphenol/kg feed) showed better feed conversion ratio (1.49) than other treatments such as 0.4% polygain (1.83) and 0.6% polygain (1.77). Polyphenol treated feed showed better FCR performance than control fish (2.00). As the FCR value is calculated to be low in T_1, it can be concluded that using 0.2% of polygain in fish feed will be cost effective for the farmers. Plant extract as phytogenic products in broiler diets named as black-cumin used in poultry feed which has significantly increased body weight and reduced FCR without any harm effects on feed intake (Khalaji *et al*. 2011).

Addition of polyphenols in fish feed is safe for the consumer. Phenolic compounds have been of increasing interest to science and food industry for their beneficial health effects. Epidemiological data have related a high intake of phenolic-rich food to a decreased rate of chronic diseases such as diabetes, cardiovascular diseases, Alzheimer's disease, Parkinson's disease, and inflammation (Bravo, 1998). So, using polyphenols in fish feed may ensure the better profit for the farmer as well as food safety for the consumers.

Conclusion

Pangasius contributes a major part in our aquaculture production. A large number of people who lives from hand to mouth depend on this fish to fulfill their protein demand as the fish is cheap compared to other species of fish. *Pangasius* fish can be stocked at higher density therefore useful for the poor farmers to benefit easily. The research showed the polyphenol has potential role in the growth performance of farmed *Pangasius*. Further research can be conducted on the effects of polyphenol in different fishes as well as effect of polyphenol in fish muscle. This type of research work will be a new dimension for improving fisheries industry in Bangladesh.

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Development of fish ball from silver carp (*Hypophthalmichthys molitrix*) and shelf life under various storage conditions

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Abstract
Fish ball was prepared from unwashed mince of silver carp (*Hypophthalmichthys molitrix*) incorporating with white potato, red potato and boiled rice. Mince was mixed with salt, sugar, spices and variable level (%) of ingredients (0%, 10%, 15%, 20% and 25%), then the mixtures were shaped into balls and dip fried in vegetable oil. The Sensory attributes, chemical and microbiological analysis were conducted in order to determine the quality and self-life of the product in the laboratory of Fisheries Technology Department, Bangladesh Agricultural University (BAU), Mymensingh. It was observed that white potato smash gave the better result than red potato smash and boiled rice. The similar quality products were found for both 20% and 25% white potato smash but from the view point of economic consideration, by the panelists, 25% white potato smash was recommended. Proximate compositions changed with the lapse of storage period. Moisture content (%) slightly increased when stored at non-sealed polythene pack but slightly decreased in sealed polythene pack storage at room temperature (28°C to 32°C). On the other hand moisture content (%) decreased at refrigeration temperature (5°C to 8°C) both in non-sealed and sealed polythene pack storage. Protein content (%) decreased at room (28°C to 32°C) and refrigeration temperature (5°C to 8°C), both at non-sealed and sealed polythene pack storage. Lipid and ash content (%) increased with the progress of storage period at room (28°C to 32°C) and refrigeration temperature (5°C to 8°C) both in non-sealed and sealed polythene pack storage. The initial TVB-N value of fish ball was 3.86 mg/100g. After 5 days storage at room temperature (28°C to 32°C) the TVB-N value reached to 30.27 and 25.28 mg/100g in non-sealed and sealed polythene pack storage, respectively. On the other hand after 9 days storage at refrigeration temperature (5°C to 8°C) the TVB-N value reached to 28.70 and 25.70 mg/100g in non-sealed and sealed polythene pack storage, respectively. In the microbial study it was observed that in fish ball the initial bacterial load was 1.45×10^4 CFU/g. After 5 days storage at room temperature (28°C to 32°C), the bacterial load increased to 4.93×10^8 CFU/g and 4.53×10^7 CFU/g in non- Sealed and sealed polythene pack storage, respectively. In case of refrigeration temperature (5°C to 8°C) the bacterial growth pattern was somewhat different. After 9 days storage at refrigeration temperature (5°C to 8°C) the bacterial loads were found 2.67×10^7 CFU/g and 1.85×10^7 CFU/g in non-sealed and sealed polythene pack storage, respectively. The present study showed that, the

bacterial growth at room temperature (28°C to 32°C) in both non-sealed and sealed polythene pack storage was very rapid and the shelf life of fish ball was short, not more than 3 days. Whereas, at refrigeration temperature (5°C to 8°C) fish ball may remain in good condition for 5 days. In this case, rate of bacterial growth was slower and during the 3 days of storage time no big change was observed in Aerobic plate count (APC), after that the bacterial growth gradually increased. Therefore, refrigeration/chill temperature is the primary preservation method for preserving fish ball.

Key words: Silver Carp (*Hypophthalmichthys molitrix*), Fish ball, Packing, Storage, Shelf life.

Introduction

Among the Chinese carps, Silver carp has got popularity due to its fast growth and unique food habit. They are mainly phytoplankton feeder and can control water body from obnoxious bloom formation. Moreover production of silver carp relatively higher than other carps (i.e. Rohu, Catla, Mrigal etc.) but the market value is less than these carps. So, if we prepare value added food from Silver carp, we can get more profit. Value added foods are the main items of fast food. In recent years the preference of the consumers was directed towards the fast food consumption since there has been a rapid urbanization and an increase in working women population. There have been many studies about the production and quality stability of the fishery fast food products including fish cake, fish balls, fish stick and fish burgers (Herborg, 1976; Sipos *et al*., 1979; Siaw *et al*., 1985; Choi *et al*., 1988; Lazos, 1996 and Hoque *et al*., 2007). Fish balls are acceptable and popular fast food product by the consumers in the world. Unfortunately, in Bangladesh fish ball not yet popular probably due to some marketing impediment and food habit of the people. Generally, tuna, mackerel, sardine and some fresh water fish species were used in the production of the fish balls in many studies. Gokoglu (1994) prepared fish balls from mackerel and studied the quality changes at 4ºC. It was noticed by the researchers that the main problem encountered was fishy taste and smell in the production of the fish balls from marine fish (Hoogenkamp, 1992 and Almerderes Martinet, 1993). Lazos (1996) also stated that fish balls were produced at first quality when fresh water fish were used as the raw material. Today the world wide growing demand for animal protein, can be meet by the fish protein especially fresh water fish protein. From our less consumed and less market price fish, we can prepare value added foods especially fish ball according to our preference. This is highly nutritional food with its high protein and low fat content, which may fulfill the demand of nutritional diet to the new generation. Fish protein digestibility rate high, so, patients and growing children can use fish as their food effectively; more over-fish protein contain important poly unsaturated fatty acid (PUFA) and W-3 fatty acid which is very helpful for health especially control heart disease. After frying the fish ball achieves excellent brown color and nice flavor which attracts consumer's acceptance worldwide, specially the new generation young people who are now more depending on the fast foods. Considering above points, the study was carried out to develop fish ball with the mince of a low priced, less

demanded fish for value addition and also shelf life study was done to supply quality products to consumers.

Materials and methods

Species selection for fish ball preparation

Silver carp was selected for the production of fish ball. Silver carp (*Hypophthalmichthys molitrix*), is a planktivorus Chinese carp species having good nutritional value but less market price fish. Silver carp are available in local market throughout the country. Silver carp was introduced in Bangladesh from China (Source: DoF, 2005). A 100g silver carp contains 20g protein 1.1g fat, 11g iron, 350mg calcium and 382 mg phosphorus (Siddique and Chowdhury, 1996).

Preparation of spices

In this study, various spices were incorporated into starch-mince mixture to prepare good quality and tasty fish ball. For this purpose various local spices like onion, garlic, ginger, cinnamon and red chilly were purchased from the local market. The spices were dried in hot air oven at 50°C for 24 hr. The dried spice were ground with a mechanical grinder to make powder and sieved by a fine mesh metallic sieve. Fine powdered spices were stored in a refrigerator (5°C to 8°C) in small plastic pots with labeling. The level of various ingredients and species used for fish ball preparation are given in Table 1 and Table 2.

Table 1: Level of ingredients used for fish ball preparation

Ingredients	**Level (%)**
Table salt (NaCl)	2.0
Sugar	1.6
All spice (Table 3)	1.5
MSG	0.1
Starch	0, 10, 15, 20 and 25
Vegetable oil	2.0
Water	Required amount

Table 2: Level of spices used for fish ball preparation

Spices	**Level (% out of 1.5% of all spices)**
Red paper powder	30
Onion	30
Garlic	20
Ginger	10
Cinnamon	10

Preparation of fish mince from Silver carp

Fresh silver carp was collected from Kamal Ronjit (K.R.) Market then transported to the laboratory of the Fisheries Faculty, BAU, Mymensingh. Fish were then beheaded, scaled, washed and skinned properly. Since the fish has a big head so, mince was collected carefully from dorsal region and upper region of head base to obtain maximum amount of mince. The skinned fish was filleted by knife and separated spines, manually. Then bone less fish was minced by a mechanical grinder (Plate 1). Connective tissue fibers, small scale, intercellular bone and minute spire were removed from fish muscle by the fine mesh sieve of grinder. Then mince was collected in a small ceramic bowl and it was kept in ice containing plastic bowl to kept mince in cooled condition (5°C to 8°C, Plate 1). This mince was used for the preparation of fish ball.

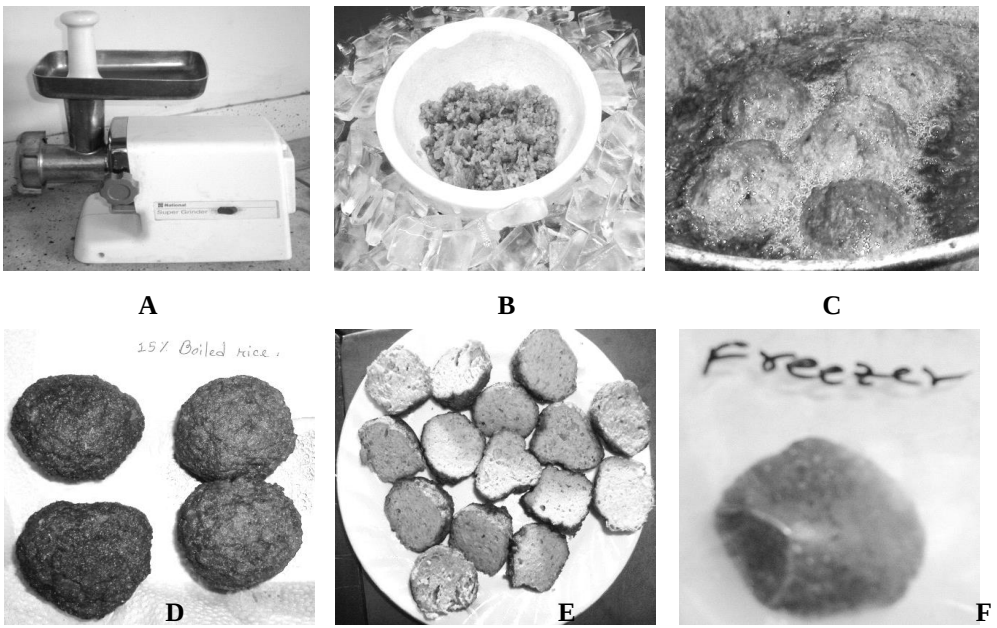

Plate 1: Preparation of Fish Ball. Here, A - Grinder to prepare fish mince; B- Fish mince; C- Frying of Fish Ball in vegetable oil; D- Cooling of Fish Ball after frying, E- Fish Ball for quality analysis; F- Fish Ball in polythene pack

Preparation of fish ball from mince

Unwashed mince was used for the preparation of fish ball. At first mince were ground with 1.6% sugar, 2.0% NaCl, 1.5% spices (onion, garlic, ginger, cinnamon and chilly powder), 0.1% MSG and 2% vegetable oil. Then mince blended with 5 levels (0, 10, 15, 20 and 25%) of

potato starch and boiled rice. Mince was blended with salt for 5 minutes; then sugar, spices and starches were added and ground for 5 minutes. Finally the whole mixtures were ground again for another 7 minutes. Then a little amount oil was taken in hand and required amount of mince-starch-species mixture was taken in hand and given round ball shape to prepare fish ball. Then the fish ball was fried into vegetable oil. (Plate 1). After frying of fish balls, they were kept at room temperature for about 1 hour to cool down for further analysis or packing.

Packing and storage of fish ball for analysis

After preparation of fish balls, they were packed in transparent non-sealed and sealed polythene pack, mechanically. Fish ball containing 25% white potato (according to panelist's suggestions) was stored in polythene pack and stored for shelf life study both at room (28°C to 32°C) and refrigeration temperature (5°C to 8°C).

Quality analysis of fish ball

Sensory evaluation

A preliminary product selection was made in order to reduce the number of samples to be submitted to the panel which was made up by untrained judges. Selection was made on the basis of an acceptance test performed by five expert assessors (ISO 8586-2, 1994) specialized in the evaluation of snack products. Selection results were given as: acceptable/not acceptable. For determination excellent, very good and good were acceptable and poor were unacceptable mentioned. Acceptability was assessed on the basis of sensory specifies defined for snack products in the producer quality manual such as folding test, color and flavor test, softness/firmness (S/F), chewiness/rubberiness (C/F) tests, which were previously selected by the experts.

Color and flavor tests

Color and flavor were evaluated organoleptically. Scores used were from 10-1; where 10 = desired color and flavor; 1 = absent of color and flavor (Table 3 and 4).

Table 3: Scores used in the grading of color of fish ball

Color Score	Description	Comment on Color Quality
8 to 10	Contents appropriately colored (bright brown)	Excellent
6 to 7	Contents generally acceptable colored (brown/white)	Very Good
4 to 5	Contents moderately colored (grayish)	Good
1 to 3	Contents considerably discolored (dark gray)	Poor

Table 4: Scores used in the grading of flavor of fish ball

Flavor Score	Description	Comment on Flavor Quality
8 to 10	Contents have no abnormal odor and have a very good characteristics flavor and seasoning	Excellent
6 to 7	Contents have no abnormal odor, flavor and seasoning: satisfactory flavor	Very Good
4 to 5	Contents have slightly raw or slightly scorched odor: flavor and seasoning seems to be somewhat inadequate	Good
1 to 3	Contents have a strong abnormal odor and a markedly poor flavor	Poor

Chewiness/rubberiness (C/R) and softness/firmness (S/F) tests

A nine-person trained panel of students, teachers and staffs provided the sensory assessments of the products. Prior to testing, panelists were familiarized with the properties of muscle-based gel of fish ball and the instruction relating to the scoring of the samples. Pretests were undertaken with selected samples to familiarize panelists with the measurement procedure as described by Bertak and Karahadian (1995). Three pieces of each product were supplied to each panelist to recognize every attribute. Softness/firmness (S/F) was defined as the amount of force required to cut the sample with incisors and chewiness/rubberiness (C/R) was defined as the amount of effort the panelist had to exert in chewing to prepare the sample for swallowing (Szezesniak *et al*., 1963). The quality was evaluated by the numerical scores up to 10, where for S/F, 1 = very soft; 10 = extremely firm and for C/R, 1 = not chewy/rubbery; 10 = extremely chewy/rubbery (Table 5).

Table 5: Scores for S/F and C/R

Score	Variables				Comments on quality
	Fish ball with no binder (Control)	Fish ball with potato starch	Fish ball with mashed potato	Fish ball with boiled rice	
S/F score (1-10)					
C/R score (1-10)					

[1] The average of the 7 scores used as S/F and C/R

Folding test of fish ball

The folding test of fish ball was conducted by folding a spherical shaped sample specially cut from the interior part of fish ball (2 mm thickness) placed on the index and middle finger of the

right hand. The disc was folded first into halves and then quarters by the help of thumb and forefinger. The scale was A to 10 = no crack when folded into quarters, 5 to 7 = no crack when folded into half but crack when folded into quarter, 4 to 5 = crack when folded into half and 1 to 3 = broke and split into halves. The ball quality was graded using the scores as described by Poon et al. (1981) and is presented in Table 6.

Analytical procedure

Proximate composition (percent moisture, ash, protein, lipid content) analysis of raw fishes, fish ball (at different stages of storage) were carried out according to the methods AOAC (1990). Total Volatile Base Nitrogen (TVB-N, mg N/ 100g) was determined according to the methods given in Pearson (1976) and pH values of samples were measured by using digital pH meter (Ebro). Bacterial colonies developed were counted for the determination of standard plate count as CFU/g of sample.

Table 6: Score used in the Folding Test of Fish Ball

Score	Result of folding the disc	Fish ball quality
8 to 10	No crack visible when disc is folded into quarter	Excellent: Soft but very elastic
5 to 7	No crack when disc is folded into half but one or more cracks when folded into quarter	Good: Moderate elasticity prevails, resistance loses
4 to 5	One or more cracks are visible when disc is folded into half	Poor: Poor elasticity prevails loss of elasticity
1 to 3	Broke and split into halves	Very poor/ Fragile: Complete loss of elasticity

Results

Proximate composition and pH of fish mince and fish ball (on "0" day)

The proximate composition and pH of mince (unwashed) of silver carp and fish ball are presented in Table 7. The moisture content in fish mince was obtained 80.07% and in fish ball 78.63%; protein content of fish mince was 18.18% and in fish ball it was 16.27%. On the other hand, lipid content in fish mince was found 1.28% and in fish ball it was 3.12%; ash content in fish mince was obtained 0.97% whereas in fish ball it was 2.05%. The pH of mince was 6.8 and 6.6 for fish ball.

Table 7: Proximate composition (% and pH of fish mince and fish ball)[1]

Criteria	Product	
	Mince (unwashed)	**Fish ball**
Moisture (%)	80.07±1.06	78.63±0.71
Protein (%)	18.18± 0.75	16.27±0.32
Lipid (%)	1.28±0.33	3.12±0.25
Ash (%)	0.97±0.20	2.05±0.12
pH	6.8±0.1	6.6±0.1

[1] Results were the mean S.E. of three individual measurements.

Quality of fish ball

Sensory evaluation

A final sensory evaluation was made by a group of 12 untrained judges (22-46 year old students and teachers), who were invited to evaluate the product on the basis of hedonic ratings. Three types of ingredients (white potato, red potato, boiled rice) were used to developed fish ball from silver carp which were attractive looking, very good in texture, excellent colored, nice flavored and tasty. Different sensory quality attributes of these fish balls are presented in Table-8, 9 and 10. Three filling ingredients such as white potato, red potato and boiled rice were tested for acquiring an acceptable texture and mouth feel of fish ball. Hardness increased with the increase of ingredients content of the fish ball. Both S/F and C/R were higher in the fish ball at 25% level of starches compared to other starch level (0, 10, 15 and 20%). Both white potato and red potato gave similar sensory scores of the product in respect of folding test, color and flavor, C/R, S/F. However, the general appearance of the product produced from white potato and red potato were better than that of boiled rice. The fish ball produced from boiled rice was inferior in S/F and C/R. The result indicates the lesser ability of boiled rice in binding a protein matrix compared to white potato and red potato. However, products obtained adopting the intermediate and high ingredients content showed a higher hedonic rating of the folding test, color and flavor. The softness/firmness; chewiness/ rubberiness test result showed that the product with higher boiled rice content gave slightly low hedonic rating of the folding test, color and floor although C/R and S/F were same or slightly high. Among the five levels of white potato, red potato and boiled rice starch (0, 10, 15, 20 and 25%) 25% starch level showed better sensory attributes and mouth-feel in minces. However, the quality of 25% starch level was almost identical to 20% starch level. Considering the profitability factor of the process, a 25% white and red potato in mince-mix for fish ball can be recommended. And fish ball prepared from white and red potato were better then fish ball prepared from boiled rice in respect of folding test, color and flavor, C/R, S/F at various levels (0, 10, 15, 20 and 25%) of starch.

Table 8: Effect of white potato on sensory attributes (Sensory test* of fish ball prepared from Silver carp)

Criteria	Level (%)				
	0	10	15	20	25
Folding Test	7.7±0.2	8.3±0.2	8.6±0.1	8.8±0.3	8.6±0.1
Color out/in	8.3±0.1	8.5±0.1	8.6±0.1	8.7±0.1	8.9±0.1
Flavor	8.4±0.2	8.5±0.1	8.7±0.3	8.8±0.2	8.9±0.1
C/R	6.5±0.4	6.9±0.1	7.3±0.2	8.0±0.3	8.10±0.1
S/F	6.5±0.5	6.9±0.3	7.7±0.4	8.0±0.3	8.6±0.1

[1] C/R = Chewiness/Rubberiness; S/F = Softness/Firmness; color: out/in = color outside and inside. Results were the mean S.E. of three individual measurements.

Table 9: Effect of red potato on sensory attributes (Sensory test[1] of fish ball prepared from Silver carp)

Criteria	Level (%				
	0	10	15	20	25
Folding Test	7.7±0.2	8.2±0.2	8.5±0.1	8.7±0.3	8.6±0.1
Color out/in	8.2±0.1	8.3±0.1	8.5±0.1	8.7±0.1	8.9±0.2
Flavor	8.3±0.4	8.4±0.3	8.6±0.1	8.9±0.5	8.9±0.9
C/R	6.6±0.3	6.8±0.2	7.5±0.3	8.0±0.1	8.0±0.2
S/F	6.6±0.1	6.9±0.2	7.6±0.4	8.1±0.2	8.7±0.1

[1] C/R = Chewiness/Rubberiness; S/F = Softness/Firmness; color: out/in = color out side and inside. Results were the mean S.E. of three individual measurements.

Table 10: Effect of boiled rice on sensory attributes (Sensory test[1] of fish ball prepared from Silver carp)

Criteria	Level (%				
	0	10	15	20	25
Folding Test	7.3±0.2	7.4±0.2	7.7±0.1	6.7±0.3	6.5±0.1
Color out/in	8.2±0.1	8.3±0.1	8.0±0.1	7.8±0.1	7.6±0.1
Flavor	8.2±0.4	8.0±0.3	8.0±0.3	8.0±0.5	7.8±0.3
C/R	6.5±0.5	6.7±0.3	6.8±0.1	7.0±0.2	7.0±0.2
S/F	5.2±0.2	5.5±0.3	5.6±0.4	5.7±0.2	5.9±0.1

[1] C/R = Chewiness/Rubberiness; S/F = Softness/Firmness; color: out/in = color outside and inside. Results were the mean S.E. of three individual measurements.

Shelf life study of fish ball

In this experiment the determination of bio-chemical parameter and microbial load count were done for the shelf life study of fish ball. In order to shelf life study fish balls containing 25% white potato were kept at refrigeration (5ºC to 8°C) temperature and room (28ºC to 32°C) temperature for a certain period. In biochemical test proximate composition, TVB-N values were determined and in microbial load count total bacterial load CFU/g in fish ball at different storage condition was examined.

Changes in proximate composition at room (28°C to 32°C) and refrigeration temperature (5°C to 8°C)

Changes in the proximate composition of fish balls both at room (28°C to 32°C) and refrigeration temperature (5°C to 8°C) was studied. Fish balls were stored both in non-sealed and sealed polythene pack. In order to assess the degree of freshness of the samples some biochemical parameters were checked during 5 days storage at room temperature (28°C to 32°C) and refrigeration storage (5°C to 8°C) during 9 days of storage.

Changes in moisture

In fish ball the initial moisture content (after 2 hrs. of fish ball preparation) was obtained 78.63%. This moisture content slightly increased at room temperature (28°C to 32°C) while stored at non-sealed polythene pack but no remarkable change could be found for the samples stored at sealed pack even after 6 days of storage at this temperature. On the other hand, the moisture content decreased gradually with the progress in storage period and at the end of 11 days of storage at refrigeration temperature (5°C to 8°C) the value decreased from 78.63% to 73.50% for sample stored in on sealed polythene pack and decreased to 74.62% for the samples stored at sealed polythene pack (Table 11).

Table 11: Changes in moisture content (%) at room (28° to 32°C) and refrigeration temperature (5°C to 8°C) during storage

Storage Period (days)	Room Temperature (28° to 32°C)		Refrigeration Temperature (5°C to 8°C)	
	Non-Sealed Pack (moisture %)	Sealed Polythene Pack (moisture %)	Non-Sealed Pack (moisture %)	Sealed Polythene Pack (moisture %)
0	78.63±0.71	78.63±.71	78.63±0.71	78.63±0.71
2	78.68±0.47	78.51±0.66	77.49±0.58	78.08±0.56
4	78.80±0.32	78.38±0.49	76.39±0.70	77.35±0.37
6	78.82±.58	78.20±0.66	75.80±0.72	76.80±0.93
8	-	-	74.14±0.95	75.70±0.80
11	-	-	73.50±0.86	74.62±1.20

Results were the mean S.E. of three individual measurements

Changes in protein

At the initial stage (after 2 hrs. of fish ball preparation) the protein content was 16.27%. With the lapse of storage period, the protein content decreased gradually with the storage irrespective of storage temperature. At room temperature (28°C to 32°C) at the end of 6 days of storage protein content decrease from 16.27% to 14.05% for the samples stored in non-sealed polythene pack and from 16.27% to 14.52% for the samples stored in sealed polythene pack (Table-12). In case of samples stored at refrigeration temperature (5°C to 8°C) also moisture content decreased gradually with the progress in storage period and at the end of 11 days of storage at this temperature the value decreased from 16.27% to 14.79% in the fish balls stored at non-sealed polythene pack and from 16.27% to 15.02% in the fish balls stored at sealed polythene pack (Table-12). However, fish balls stored in sealed polythene pack remained in good condition than the fish balls stored in non sealed polythene packs.

Table 12: Changes in protein content (%) at room (28° to 32°C) and refrigeration temperature (5°C to 8°C) during Storage

Storage Period (days)	Room Temperature (28° to 32°C)		Refrigeration Temperature (5°C to 8°C)	
	Non-Sealed Pack (protein %)	Sealed Polythene Pack (protein %)	Non-Sealed Pack (protein %)	Sealed Polythene Pack (protein %)
0	16.27±0.32	16.27±0.32	16.27±0.32	16.27±0.32
2	15.97±0.07	16.01±0.15	16.03±0.15	16.14±0.22
4	14.70±0.71	15.28±0.37	15.96±0.17	16.03±0.17
6	14.05±0.21	14.52±0.38	15.68±0.28	15.81±0.14
8	-	-	15.37±0.40	15.74±0.15
11	-	-	14.79±0.48	15.02±0.20

Results were the mean S.E. of three individual measurements

Changes in lipid

In fish ball lipid content was obtained 3.12% at the initial stage (after 2 hrs. of fish ball preparation). With the lapse of storage period, the lipid content increased gradually in all samples irrespective of storage temperature. At room temperature (28°C to 32°C) at the end of 6 days of storage lipid content increased from 3.12% to 3.38% for the samples stored in non-sealed polythene pack and from 3.12% to 3.86% for the samples stored in sealed polythene pack (Table 13). In case of samples stored at refrigeration temperature (5°C to 8°C) also lipid content increased gradually with the progress in storage period and at the end of 11 days of storage at this temperature the value increased from 3.12% to 5.75% in the fish balls stored at non-sealed polythene pack and from 3.12% to 5.92% in the fish balls stored at sealed polythene pack (Table 13).

Table 13: Changes in lipid content (%) at room (28° to 32°C) and refrigeration temperature (5°C to 8°C) during Storage

Storage Period (days)	Room Temperature (28° to 32°C)		Refrigeration Temperature (5°C to 8°C)	
	Non-Sealed Pack (lipid %)	Sealed Polythene Pack (lipid %)	Non-Sealed Pack (lipid %)	Sealed Polythene Pack (lipid %)
0	3.12±0.25	3.12±0.25	3.12±0.25	3.12±0.25
2	3.15±0.26	3.29±0.24	3.77±0.40	3.93±0.11
4	3.27±0.46	3.79±0.34	4.10±0.19	4.22±0.13
6	3.38±0.47	3.86±0.27	4.97±0.25	5.05±0.79
8			5.41±0.24	5.57±0.18
11	-	-	5.75±0.20	5.92±0.44

Results were the mean S.E. of three individual measurements

Changes in ash

The ash content in fish ball gradually increased with the progress of storage period. The initial ash content was obtained 2.05%. At room temperature (28°C to 32°C) ash content showed little or no change during 6 days storage for both samples stored at non-sealed and sealed polythene pack (Table-14). In case of samples stored at refrigeration temperature (5°C to 8°C) ash content increased gradually with the lapse of storage period and at the end of 11 days of storage at this temperature the value increased from 2.05% to 4.48% in the fish balls stored at non-sealed polythene pack and from 2.05% to 4.15% in the fish balls stored at sealed polythene pack (Table 14).

Table 14: Changes in ash content (%)at room (28° to 32°C) and refrigeration temperature (5°C to 8°C) during storage

Storage Period (days)	Room Temperature (28° to 32°C)		Refrigeration Temperature (5°C to 8°C)	
	Non-Sealed Pack (ash %)	Sealed Polythene Pack (ash %)	Non-Sealed Pack (ash %)	Sealed Polythene Pack (ash %)
0	2.05±0.12	2.05±0.12	2.05±0.12	2.05±0.12
2	2.62±0.11	2.41±0.26	2.56±0.21	2.40±0.25
4	2.72±0.20	2.64±0.30	3.15±0.29	3.03±0.37
6	2.88±0.14	2.66±0.25	3.97±0.52	3.54±0.21
8	-	-	4.19±0.17	4.00±0.22
11	-	-	4.48±0.35	4.15±0.16

Results were the mean S.E. of three individual measurements

Changes in TVB-N value

Total volatile base nitrogen (TVB-N) is an important compound provides a measure of the progress of spoilage that is independent of sensory assessment. The results obtained for TVB-N is presented in Table 15. The initial TVB-N value of fish ball was obtained 3.86 mg/100g which increased with the progress in storage period. At room temperature (28°C to 32°C) at the end of 6 days of storage TVB-N value increased from 3.86 mg/100g to 30.27 mg/100g for the samples stored in non-sealed polythene pack and from 3.86 to 25.28 mg/100g for the samples stored in sealed polythene pack. In case of samples stored at refrigeration temperature (5°C to 8°C) also TVB-N value increased gradually with the lapse storage period and at the end of 11 days of storage at this temperature the value increased from 3.86 mg/100g to 28.75 mg/100g in the fish balls stored at non-sealed polythene pack and from 3.86 mg/100g to 25.85 mg/100g in the fish balls stored at sealed polythene pack (Table 15).

Changes in microbial load

Bacterial load in fish ball was determined every 2 days interval in order to monitor the bacterial growth in the product. The results are presented in Table 16. The initial bacterial load in fish ball was obtained $1.45x10^4$ CFU/g. Bacterial load in fish ball stored at room temperature (28°C to 32°C) steadily increased with the progress of storage time and within 3rd of storage bacterial load increased to 3.81 x 10^5 CFU/g. In fish ball on 3rd day of storage bad smell produced and fungal growth appeared on 5th day of storage at room temperature (28°C to 32°C). On the other hand bacterial growth pattern in fish ball stored at refrigeration (5°C to 8°C) temperature was some what different. At this temperature, the rate of bacterial growth was slower and during the 3 days of storage no big change (1.85 x 10^4 CFU/g) was observed in APC. After that bacterial load gradually increased and on 5th, 7th and 9thday of storage APC reached to $2.13x10^5$ CFU/g, 2.26 x 10^6 CFU/g and 2.67 x 10^7 CFU/g, respectively. Up to 5th day of storage at this temperature neither bad smell nor fungal growths were apparent.

Table 15: Changes in TVB-N value (mg/100g) at room (28° to 32°C) and refrigeration temperature (5°C to 8°C) during storage

Storage Period (days)	Room Temperature (28° to 32°C)		Refrigeration Temperature (5°C to 8°C)	
	Non-Sealed Pack (TVB-N Value mg/100g)	Sealed Polythene Pack (TVB-N Value mg/100g)	Non-Sealed Pack (TVB-N Value mg/100g)	Sealed Polythene Pack (TVB-N Value mg/100g)
0	3.86±0.79	3.86±0.79	3.86±0.79	3.86±0.79
2	6.95±0.46	6.21±0.59	5.13±0.76	4.67±0.51
4	10.23±0.62	9.41±0.55	7.53±0.43	6.45±0.55
6	15.67±1.04	14.46±0.59	10.67±1.34	10.37±0.53
8	30.27±0.42**	25.28±0.67*	15.38±0.43	14.65±0.99
11	-	-	20.24±0.73*	18.12±0.87*

* bad odor ** bad odor and spoiled. Results were the mean ± S.E. of three individual measurements

Table 16: Changes in microbial load (CFU/g) at room (28° to 32°C) and refrigeration temperature (5°C to 8°C) during storage

Storage Period (days)	Room Temperature (28° to 32°C)				Refrigeration Temperature (5°C to 8°C)			
	Non-Sealed Pack (ash %)		Sealed Polythene Pack (ash %)		Non-Sealed Pack (ash %)		Sealed Polythene Pack (ash %)	
	CFU/g	Log $_{10}$	CFU/g	Log $_{10}$	CFU/g	Log $_{10}$	CFU/g	Log $_{10}$
0	1.45×10^4	4.16	1.45×10^4	4.16	1.45×10^4	4.16	1.45×10^4	4.16
3	3.81×10^5	5.58	3.05×10^5	5.48	1.85×10^4	4.26	1.26×10^4	4.10
5	$^{**}4.93\times10^8$	8.69	$^{*}4.53\times10^7$	7.65	2.13×10^5	5.32	1.15×10^5	5.06
7					2.26×10^6	6.35	1.05×10^6	6.02
9					$^{*}2.67\times10^7$	7.43	1.85×10^7	7.26

Results were the mean S.E. of three individual measurements

Discussion

In this study, the moisture content decreased in fish ball than that was for fish mince might be due to release of water from fish ball during cooking. Protein content in fish ball also reduced might be due to excessive heat generated during cooking that denaturized the protein and burned into ash. Latif Taskaya *et al*., (2003) reported moisture and protein content 71.92% and 21.67%, respectively for fresh rainbow trout, but in fish burger moister content found 63.61% and crude protein 17.50%. These results are in good agreement with the results of the present study. On the other hand, in the present experiment, lipid and ash content increased in fish ball than those values obtained for fish mince. This increase in lipid in fish ball might be due to ingestion of vegetable oil during frying and increase in ash content might be due to addition of species and other ingredients (i.e. NaCl, Potato, Boiled rice). Some ash might produced during frying. Ihm *et al.,* (1992) reported similar trend for lipid and ash in sardine burgers. In this study, pHs of fish ball found near neutral as fresh fishes were used in the experiment. In this experiment for sensory evaluation, it was observed that hardness of product increased with the increase of ingredients used during prepare of fish ball. Hardness positively relates with ingredient's content (Case *et al*., 1992; Mereier and Feillet, 1975). The local ingredients like white potato, red potato and boiled rice could be used as a functional ingredient in the formulation of snack products like fish ball, in fact, when added to cereal-based mixtures, it could improve nutritional value (high content in fiber, carbohydrate, vitamin E and B etc) as well as the sensory characteristic (flavor, color, taste) of extruded products. These sensory characteristics were also shown to fit well with other extruded products such as RTE breakfast

cereals, (Sacchetti and Pinnavaia, 1999). The shelf life study of fish ball showed that during storage at refrigeration temperature (5°C to 8°C) moisture reduced might be due to the evaporation of moisture from the surface (Zaitser, 1965). But at room temperature (28°C to 32°C) moisture content slightly increased at the end of 6 days for the fish balls stored in non-sealed polythene pack might be due to moisture absorption from atmosphere which was very less in amount. In case of protein content, with the lapses of storage period the value gradually decreased in fish ball at both storage temperatures might be because of decomposition of protein by the combined action of bacterial and enzymatic action. It was reported that protein content in fish and fishery products decrease during storage due to the denaturation of fish protein and leaching out of extractable water soluble protein fraction (Arannilewa *et al*., 2005; Daramola *et al*., 2007; Siddique *et al*., 2011 and Gandotra *et al*., 2012), which supports the present finding. Unlike moisture and protein content lipid content increased in fish ball throughout the storage period. The findings of the present study indicates that the frying produced higher water loss and lipid gain mainly due to the absorption of fat by fish muscle (Kocatepe *et al*., 2011; Turkkan *et al*., 2008; Weber *et al*., 2008 and Hassab Alla *et al*., 2009). Result of the present experiment also showed that, ash content increased in fish ball irrespective of storage condition. This character of ash is related to reduction in moisture content during storage (Pawar *et al*., 2013).The TVB-N values found in fish ball stored at different storage conditions were within the range of recommended value of 25 to 30 mg/100g which is considered for fin fish acceptability (Connell, 1975). During the storage period bacterial action may also takes place, therefore, the increase in TVB-N with the lapse of storage period may be attributed to bacterial action. However, the available information indicates that TVB-N, mainly accumulated in the fish ball during the later phase of spoilage after the bacterial population has grown. Thus the TVB-N value is low during the initial storage period and only when the fish ball is in near rejection level the TVB-N value found 30 mg/100g. TVB-N values of the product packed in polythene and the products stored in non-sealed polythene pack either at room (28°C to 32°C) or at refrigeration temperature (5°C to 8°C) showed linearly increasing pattern throughout storage period neither of the value exceeded the recommended value set for fish regarded as acceptable condition. The results of microbial study of the present experiment showed a slow increase in no of bacteria at low temperature. Sarker (2000) found that fish sausage prepared with potato-starch, potato-smash and boiled rice paste had an acceptable limit of bacterial load up to 8^{th} day of refrigeration storage (5°C) and 3^{rd} day of room temperature (28°C) storage. In addition, he noted that the coliform bacteria was absent in all the products till 8^{th} day of storage. Bashar (2004) observed that the bacterial growth in fish sticks prepared from washed Queen fish mince kept at room temperature steadily increased with the progress of storage time. He also noted that bad smell was started to come out from the products after 24 hours and fungal growth was visible after 60 hours of storage. In addition he observed that in refrigeration temperature bacterial growth was slower and even after 24 hours no appreciable change in APC was observed. However, the present result in shelf life study was more or less similar to the above findings except some dissimilarity that occurred due to differences in checking time interval.

Conclusion

Based on obtained results and above discussion it could be concluded that, 25% white potato mix gave better result from both quality and economic consideration in the development of fish ball with silver carp mince. For longer storage sealed polythene pack was better than non-sealed polythene pack and shelf life of fish ball could be extended using low temperature storage.

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Use of potato tuber powder as replacement of wheat flour in the diet of GIFT (*Oreochromis niloticus*) fry

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Abstract
The experiment was carried out to evaluate the use of potato tuber powder (PTP) as replacement of carbohydrate (wheat flour) in formulated diets on the growth, survival rate and production of GIFT (*Oreochromis niloticus*) fry reared for a period of 28 days in 15 recirculatory aquaria. Fry were released at the stocking density of 20 per aquarium and allowed to feed on five different diets as treatments such as T_1, T_2, T_3, T_4 and T_5 (control), each with three replications. Diets having 30% protein were formulated with different levels of PTP *viz.* T_1 (16% PTP), T_2 (20% PTP), T_3 (24% PTP), T_4 (28% PTP) and T_5 (0% PTP) as control. Feeds were supplied at 5% body weight twice daily at 9:00 am and 5:00 pm. Samplings were done at seven days interval to see growth and to adjust feed. The study revealed that the mean weight gain (g), percent weight gain (%), specific growth rate (%/day), average daily weight gain (g), FCR and PER of tilapia fed five different diets varied significantly ($p<0.05$) with each other. Mean weight gain (17.51±0.12g), percent weight gain (281.06±2.01%), SGR (2.88±0.02%/day) and PER (1.91±0.06) of fry fed diets in T_4 (28% PTP) and T_5 (0% PTP) as control were found significantly ($p < 0.05$) higher than those of fry fed diet in T_3 followed by T_1 and T_2. In contrast, the significantly ($p < 0.05$) lowest FCR (1.66±0.07) of fry was obtained in T_4 and the highest FCR (1.92±0.09) of fry was obtained in T_2. The highest survival rate (100%) was found in T_1, T_3, T_4 followed by T_5 as control (88.80 ± 0.60% and T_2 (87.25±0.43%). Water quality parameters were maintained within the expectable ranges during the experimental period. The results also showed that the significantly highest crude protein (57.20±0.30%) and crude lipid content (12.45±0.05%) was found in the body of fishes fed the cheapest diet (T_4) (Tk. 45/kg). Therefore, the lower-priced potato tuber powder compared to relatively higher-priced wheat flour can be used as a cheaper carbohydrate source in developing low cost and nutritionally balanced diet for GIFT.

Key words: Potato tuber powder, Wheat flour, GIFT

Introduction

The success of intensive and semi-intensive fish culture depends to a large extent on the application of suitable feeds. The commercialization of aquaculture is growing, thereby increasing the demand for aquaculture feeds. Traditionally these feeds have been based on animal protein, rice bran, wheat bran, rice flour, heat flour, corn starch, cassava flour, soybean meal, peanut meal etc. (Fadl *et al*. 2017; Habib and Kohinoor 2018; Sarr *et al*. 2019). For the development of aquaculture in the country, feed cost is one of the major constrains (Habib and Kohinoor 2018). Due to cost and availability considerations there is the need to utilize cheaper energy sources to replace expensive cereals in fish feed formulation (Fadl *et al*. 2017, Habib and Kohinoor 2018, Sarr *et al*. 2019). Especially with the present economic depression, cost of fish diets is increasing, not just because of inflation but most of the feedstuffs used in preparing fish diets face serious competition with human as food. To minimize the price of a well-balanced feed, locally available ingredients can be included in the feed. However, a significant number of works on the use of commonly attainable ingredients in fish feed has been carried out. Nile tilapia fed diets containing rice bran (30%), wheat bran (30%) and fish meal (40%) show better performances in cages (Hasan *et al*. 1985). The replacement of fish meal by fly larvae meal gave good growth of fish fry (Rana *et al*. 2015; Gasco *et al*. 2016; Li *et al*. 2016; Su *et al*. 2017; Habib and Kohinoor 2018; Roncarati *et al*. 2019). Good growth of fish was achieved when fish meal was replaced by spirulina meal (Habib and Kohinoor 2018, Khalila 2018, Rosas *et al*. 2019a & b). The GIFT strain of tilapia is 35.74% superior to red tilapia strain in terms of growth (Hussain *et al.* 2000). Olurin *et al.* (2006) reported that 50% replacement of maize with cassava meal in *Clarias gariepinus* diet exhibited no depression in growth or unfavorable feed conversion ratio. Sydur (2014) recorded that diet formulated by cassava tuber powder as replacement of carbohydrate could be used to prepare quality feed to enhance the growth, production and survivality of GIFT (*O. niloticus*) and to reduce pressure on wheat and rice flour (Fadl *et al*. 2017, Khalila *et al*. 2018). Similar work was done on growth performances of stinging catfish (*Heteropneustes fossilis*) by Habib and Kohinoor (2018) where 20% wheat flour replacement using cassava tuber flour gave better growth than other diets. Nevertheless, the major source of metabolisable energy in most formulated diets for fish is rice bran, wheat bran and oil cake, which are also used as the source of dietary carbohydrate for fishery elsewhere in the world. Therefore it is necessary to find out alternate, cheaper and locally available carbohydrate sources of fish feed. Potato (*Solanum tuberosum* L.) is the fourth most important food crop after corn, rice and wheat which grows throughout the world (Ballearin and Hallar 1982). Potatoes are good sources of energy, easily digestible as well as comparable with rice and wheat (Chakraborty *et al*. 2000). On the other hand, GIFT is the most widely farmed variety and performs 60% better in growth and survival than commercially available strains of tilapia (Fadl *et al*. 2017; Khalila *et al*. 2018; Sarr *et al*. 2019). It performs well on cheap feed and fertilizer and can be raised in large tracts of water and cages (Eknath *et al.* 1993). It is important to note that huge quantity of potato is producing every year in the country. Considering the above facts, the present research was carried out to evaluate the feasibility of potato tuber powder as replacement of wheat flour (carbohydrate) in the diet of GIFT post-larvae so that production cost can be reduced and fish production can be increased.

Materials and methods

Experimental site and design

The experiment was carried out in an existing recirculatory water system situated at the wet laboratory, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh for a period of four weeks (28 days). Fifteen rectangular uniform sized glass aquaria (45cm x 40cm x 35cm) containing about 40 L of water in each were used for the research work. Five different treatments such as T_1, T_2, T_3, T_4 and T_5 (control), each with three replications were used. About 300 post-larvae (initial weight 5.92±0.005 to 6.44±0.020mg) of GIFT (*Oreochromis niloticus*) were stocked at a rate of 20 per aquarium after proper treatment and acclimatization. Each aquaria was equipped with artificial aeration as well as regular water supply and exchange. Water level was controlled by an overflow drain line at the middle of the aquarium with backup system confirmed at six hours intervals to avoid the mechanical or electrical failure. Net screens and gravels were used as bio-filter.

Experimental diet preparation

Fish meal, soybean oil, potato tuber powder (PTP), vitamin and mineral premix, α-cellulose (crude fibre) and wheat flour were used to prepare the experimental diet. Attempts were made to formulate diet by progressively replacing PTP as carbohydrate source. Diet (treatment) 1(T_1), 2(T_2), 3(T_3) and 4(T_4) were prepared by replacing 16, 20, 24 and 28% carbohydrate with PTP, respectively. Diet (T_5) 5 containing 0% PTP was used as control (Tables 1 and 2). For formulation of diets, all the dietary ingredients were ground finely and sieved thoroughly to a particle size of 0.5 mm mesh and mixing thoroughly to obtain a homogenous mixture before weighing. Sufficient amount of water was added to make the mixture moisten before pelleting. This resultant dough was then passed through a 1.0 mm diameter die of a hand pellet machine. The pellets were then sun dried, and stored in an airtight plastic container for proximate analysis and used to feed fish (Table 3).

Table 1: Proximate composition (%) analysis of different feed ingredients (% dry weight basis)

Name of ingredients	Moisture (%)	Crude protein (%)	Crude lipids (%)	Crude fibre (%)	Ash (%)	NFE* (%)
Fish meal	10.75	56.00	11.26	4.80	13.85	3.34
Potato tuber flour	9.59	10.80	8.0	19.0	16.15	36.45
Wheat flour	10.45	10.20	2.10	1.78	2.40	73.06

*NFE (nitrogen free extract) = 100 – (moisture + crude protein + crude lipids + crude fibre + ash)

Table 2: Formulation of five different diets (Treatments) containing 30% crude protein using different levels of potato tuber powder (PTP) as source of carbohydrate

Ingredients	Diet (%)				
	1 (T_1 16% PTP)	**2 (T_2 20% PTP)**	**3 (T_3 24% PTP)**	**4 (T_4 28% PTP)**	**5 (T_5 0% PTP) Control**
Fish meal	50.50	50.06	49.83	49.21	53.57
Potato tuber powder	16	20	24	28	-
Wheat flour (binder)	12	10	7	6	25
Soybean meal	5	5	5	5	5
Mineral premix	3	3	3	3	3
Vitamin premix	2	2	2	2	2
Chromic oxide	1	1	1	1	1
Alpha cellulose (crude fibre)	10.50	8.94	8.17	5.79	10.43
Total	100	100	100	100	100

Table 3: Proximate composition (%) of formulated diets (% dry weight basis) used in five treatments

Components	Treatments				
	T_1	**T_2**	**T_3**	**T_4**	**T_5**
Moisture	12.15	12.66	12.45	12.65	12.56
Crude protein	29.92	29.90	30.10	29.80	29.66
Crude lipids	8.50	9.76	9.88	8.90	9.34
Crude fibre	6.10	6.18	6.29	5.88	6.12
Ash	12.17	12.26	12.38	12.39	12.12
Nitrogen free extract (NFE)	31.12	29.23	29.85	30.32	29.15

*NFE =100 – (Crude protein + Crude lipids + Ash + Crude fibre).

Fish feeding and sampling

The fishes were fed with the formulated diets twice daily in the morning at 9.00 am and afternoon at 5.00 pm at the feeding rate of 5% of their total body weight throughout the study period. The fishes were sampled at seven days interval to monitor their growth in terms of length and weight, and to adjust feed.

Determination of water quality parameters and carcass composition

Various water quality parameters *viz.* temperature (°C), dissolved oxygen (mg /l) and pH were measured at weekly intervals. At the beginning of the experiment, 100 fish from the stock was randomly collected and sacrificed, then chopped and ground for proximate composition analysis of the experimental fish. This was considered as initial carcass composition of fish. At the end of the experiment, 10 fish per replication including the control was sampled randomly and sacrificed for proximate composition analysis and this was considered as final carcass composition of fish. Proximate composition of fish included moisture, crude protein, crude lipids, ash and NFE (nitrogen free extract) following Horwitz (1984).

Economic analysis

An economic analysis was performed to estimate the cost of feed to raise a unit biomass. The cost of feed was used as single economic criteria on the assumption that all other operating costs for commercial fish production would remain the same for all the diets. The whole sale market price 2018 of all the ingredients in Mymensingh was collected and then the approximate cost of each diet was calculated. An additional 7.5% on the top of the total raw material cost was included towards the manufacturing costs, marketing expenses and operating margin. The costs of crude fiber and chromic oxide were not included in the estimations.

Statistical analysis

Recorded data were statistically analyzed using software SPSS version 11.5 (Chicabo, USA) and MS Excel 2007 (Microsoft Corporation, Redmond, USA). One way ANOVA was used to determine the effect of formulated diets on the growth of GIFT tilapia in different treatments and Tukey test was applied to identify the level of significance of variance among the treatments following Zar (1984).

Results and Discussion

Growth performances of GIFT (Oreochromis niloticus)

After feeding different diets such as Diet -1 (16% PTP), Diet-2 (20% PTP), Diet-3 (24% PTP), Diet-4 (28% PTP) and Diet-5 (0% PTP), the growth performances of *O. niloticus* in terms of initial weight (g), final weight (g), weight gain (g), percent weight gain (%), feed conversion ratio, Protein efficiency ratio, specific growth rate (%/day) and survival rate (%) were calculated at the end of experiment (Table 4).

The mean initial weight of GIFT in different treatments varied from 5.92±0.005 to 6.44±0.020 mg and mean final weight varied from 18.32±0.15 to 23.74±0.17 mg throughout the study (Fig. 1). There was significant differences (p<0.05) in mean weight gain and percent weight gain among the different treatments. The significantly highest mean weight gain (17.51±0.12 mg) and the significantly highest % weight gain (281.06±2.01) was found from T_4 whereas the lowest mean weight gain (12.02±0.09 mg) and the lowest % weight gain (190.79±1.17) was found from T_2. Similar results were found by Fadl *et al*. (2017) used to feed diets prepared

from *Spirulina platensis* and *Chlorella vulgaris*, and Khalila *et al*. (2018) and Sarr *et al*. (2019) when they gave diets prepared from wheat flour, soya bean meal, rice flour, corn flour and peanut meal to tilapia. The results indicated that the growth rates varied in different treatments which coincide with the findings of Khan *et al.* (2002). The highest weight gain in the present study was found from treatment T_4 due to utilization of 28% potato in the formulated feed. Habib and Kohinoor (2018) found good growth of stinging catfish when fed diets prepared replacing fish meal protein with cassava tuber powder.

Table 4: Growth performances (Mean±SE) and feed utilization of GIFT (*Oreochromis niloticus*) fed five different diets in aquarium

Variables / Parameters	Treatments				
	T_1	T_2	T_3	T_4	T_5 (Control)
Initial weight (mg)	6.17±0.014	6.30±0.023	6.44±0.020	6.23±0.014	5.92±0.005
Final weight (mg)	18.48±0.14	18.32±0.14	20.42±0.13	23.74±0.17	22.56±0.051
Weight gain (mg)	12.31±0.11^c	12.02±0.09^c	13.98±0.08^b	17.51±0.12^a	16.64 ±0.04^a
Percent weight gain (%)	199.51±1.22^b	190.79±1.17^b	217.08±1.14^b	281.06±2.01^a	218.58±1.15^a
Specific growth rate (%/day)	2.59±0.05^c	2.56±0.00^c	2.66±0.02^b	2.88±0.02^a	2.81±0.00^a
Average daily weight gain (mg)	0.054±0.002^c	0.051±0.00^c	0.062±0.005^b	0.090±0.003^a	0.083±0.003^a
Food conversion ratio (FCR)	1.90±0.10^c	1.92±0.09^c	1.83±0.04^b	1.66±0.07^a	1.70±0.05^a
Protein efficiency ratio (PER)	1.89±0.010^b	1.72±0.20^c	1.85±0.002^b	1.91±0.06^a	1.88±0.12^a
Survival rate (%)	100.00	87.25±0.43	100.00	100	88.80±0.60

Figures in different letters in superscripts of each row are significant at 5% level of probability.

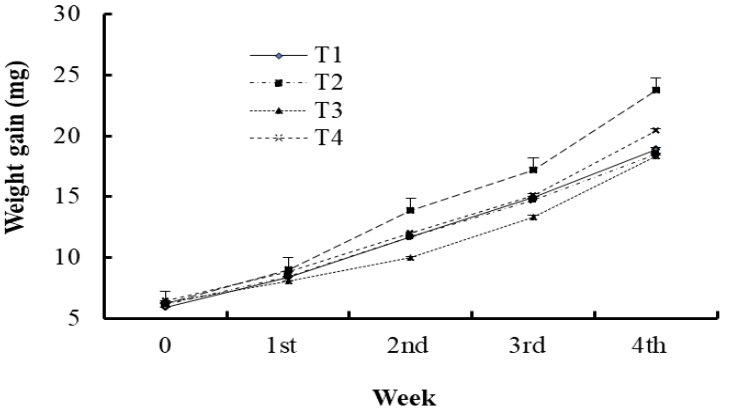

Fig. 1: Weekly growth response of GIFT (*Oreochromis niloticus*) fed five different diets. Vertical bars represent standard error

The values of specific growth rate of GIFT in different treatments ranged from 2.56±0.00 to 2.88±0.02, those were significantly varied. The significantly highest SGR value was in the treatment T_4 (28% potato) and the lowest SGR value in the treatment T_2 (20% potato). The values are more or less similar with the findings of Azad *et al.* (2004); Abara *et al*. (2017) and Sarr *et al*. (2019) but slightly lower than the findings of Hossain *et al.* (2007) who achieved SGR values ranged between 3.14 and 3.32. This might be due to the temperature differences between waters and natural productivity of the ponds, those were different from the aquarium water. And the differences in SGR values among various treatments of the present study might be due to the initial size differences of *O. niloticus* used for the experiment and also due to the differences of feeding system (Khalila *et al*. 2018; Sarr *et al*. 2019).

Feed utilization

In the present study, mean food conversion ratio (FCR) in different treatments ranged from 1.66±0.07 to 1.92±0.09, which were significantly different (Table 4). The significantly highest FCR was obtained in treatments T_2, where fishes took highest amount of food and gave lowest growth. On the other hand, the significantly lowest FCR was obtained in treatment T_4, where the lowest amount of food was taken by fish and highest growth was obtained. The differences in FCR might be due to differences in percentages of potato in formulated feed. More or less similar FCR values were found in the findings of Diana *et al.* (2004) as well as Hasan *et al*. (1993). The survival rate were observed as 100, 87.25, 100, 100 and 88.80% in treatments T_1, T_2, T_3, T_4 and T_5, respectively (Table 4). Mortality might be largely caused by injuries due to aggressive behavior. Dan and Little (2000) also reported more or less similar level of mortality when they conducted growth study using hybrid tilapia under laboratory condition. Sarr *et al*. 2019) also reported similar results when fish meal was replaced by corn flour, wheat flour, soya bean meal enriched with spirulina.

Carcass composition

Proximate composition such as moisture, crude protein, crude lipid, ash and nitrogen free extract (NFE) of fish were analyzed for carcass composition determination. There were significant differences among the five treatments (diets fed to fish) in case of crude protein, lipid and ash except moisture and NFE in fish body. Crude protein ranged from 52.30±0.35% to 57.20±0.30%, where the significantly highest protein content was found in fish fed diet-4 and the significantly lowest protein content was found in fish fed diet-1. On the other hand, the ranges of crude lipid percentage were 10.45±0.04 to 12.45±0.05, where the treatments also showed the similar trend of performances as crude protein (Table 5). The highest crude protein and lipid content found in fishes fed with diet 4 might be due to variation of proximate compositions among different formulated diets (Rosas *et al*. 2019a & b). Khalila *et al*. (2018) and Sarr *et al*. (2019) found almost similar results when they used to feed diets to tilapia prepared with cassava tuber powder and wheat flour replacing fish meal. More or less similar observation was reported by Habib *et al.* (2001) who found the highest protein and lipid content as well as lowest moisture in fishes fed higher percentages of silkworm pupae as partial replacement of fish meal in the diet.

Table 5: Carcass composition of fishes fed five different diets

Components (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5 (Control)
Moisture	10.25± 0.04	10.30± 0.04	10.20± 0.03	10.15± 0.03	10.15± 0.03
Crude protein	52.30± 0.35^{b}	53.15± 0.40^{a}	55.10± 0.40^{b}	57.20± 0.30^{a}	57.15± 0.42^{b}
Crude lipids	10.45± 0.04^{b}	10.60± 0.03^{b}	11.60± 0.04^{a}	12.45± 0.05^{a}	11.06± 0.02^{a}
Ash	15.35± 0.04^{a}	15.80± 0.05^{a}	14.20± 0.03^{a}	14.50± 0.05^{a}	14.54± 0.03^{a}
Nitrogen free extract (NFE)*	11.65 ± 0.04	10.10 ± 0.03	9.84 ± 0.04	5.63± 0.03	7.05± 0.04

*NFE = 100 – (Crude protein + Crude lipids + Ash + Crude fibre)

Water quality parameters determination

Water temperature, dissolved oxygen and pH are the most important environmental parameters. Water temperature is an important water quality parameter for healthy living of fish in the water body. Water temperature was more or less similar in different treatments which ranged from 30.7-31.60°C in both control and treatment aquaria. Hossain (2009) and Alam (2009) found the water temperature from 26.9 to 32.5°C in ponds of Mymensingh. Moreover, Hossain *et al.* (2004) and Hossain *et al.* (2007) measured the water temperature ranged from 29.4 to 33.0°C and 26.0 to 32.8°C, respectively in ponds of BAU campus, Mymensingh. Therefore, water temperature in aquaria was similar as pond temperature.

The dissolved oxygen content from present experiment ranged from 5.10 to 6.15 mg/l (Table 6). Similar findings were reported by Hossain (2009) and Alam (2009) who found dissolved oxygen of 5.5 to 6.5 mg/I in ponds of Mymensingh. DoF (1996) reported that the range of suitable dissolved oxygen for fish culture should be 5-8 mg/l. So, the oxygen content found in the present experiment lied within productive range.

During the study period, the pH value was found within the range of 6.90-8.10 (Table 6). Alam (2009) recorded pH value from 7.50 to 8.02 in fish ponds of Bangladesh Agricultural University Campus, Mymensingh during August to October, 1997 which are almost similar with the findings of present experiment. reported that the suitable pH range for fish production is 6.5 to 8.5 (DoF, 1996). Therefore, the pH observed in the present experiment remained within suitable ranges.

Economic analysis

The costs of different dietary ingredients were recorded and thereby the costs (per kg) of five different formulated diets were estimated (Table 6). The economic analysis indicated that the cost of diet 4 (28% potato tuber flour) was the lowest among all the diets. Similar finding was also reported by Habib *et al.* (2001) who found the lowest cost of the experimental diet prepared with higher percentages of silkworm pupae as partial replacement of fish meal. Sydur

(2014) In addition, percent weight gain and specific growth rate of fishes fed with the diet 4 was also excellent compared to other diets in the present study. Therefore, the low cost potato tuber flour (Tk 20/kg) compared to wheat flour (Tk 35/kg) can be used as a cheap a ingredient in developing low cost and nutritionally balanced diet for GIFT.

Table 6: Costs (Tk/kg) of different dietary ingredients and formulated diets in the experiment.

Ingredients	Cost	Feed	Cost
Fish meal	90	Diet-1 (16% potato tuber flour, PTF)	47
Potato tuber powder	20	Diet-1 (16% potato tuber flour, PTF)	47
Soybean oil	90	Diet-2 (20% PTF)	48
Wheat flour (binder)	35	Diet-3 (24% PTF)	46
Alpha cellulose (crude fibre)	50	Diet-4 (28% PTF)	45
Mineral premix and vitamin premix	440	Diet-5 (0% PTF) (control)	48

Conclusion

The best growth performances, feed utilization, survival rate and production of GIFT were obtained by feeding diet containing 28% potato tuber powder. Moreover, the diet having 28% potato tuber powder produced quality fish by reducing production cost. Therefore, potato tuber powder can be used as a good and inexpensive source of carbohydrate other than wheat flour in fish diet preparation, which is also economically stable and environmentally friendly ingredient.

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Effects of shrimp waste in supplementary diet on growth performance of Indian major carps

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Abstract

A comprehensive trial was undertaken to assess the effect of feed, formulated with shrimp waste product on the growth of Indian major carp (*Labeo rohita*, *Gibelion catla* and *Cirrhinus cirrhosus*) fingerlings. The experiment was conducted in a 60-day feeding trial in aquarium system from 1st April to 30th May, 2015. Three types of feed namely Diet-1 (feed with shrimp waste), Diet-2 (feed with plant protein source) and Diet-3 (Commercial feed) were applied in three different treatments. Protein percentage of three diets were 28.42%, 28.13% and 27.32%, respectively. The study showed highest weight gain with *Labeo rohita* (7.71 ± 0.72 g), *Gibelion catla* (9.95 ± 0.99g) and *Cirrhinus cirrhosus* (3.87 ± 0.10 g) in Treatment3 (T_3) for Diet-3 and comparable with the growth of *Labeo rohita* (7.6 ± 0.22 g), *Gibelion catla* (8.32 ± 0.48 g) and *Cirrhinus cirrhosus* (3.27 ± 0.08 g) in Treatment1 (T_1) for Diet-1. The apparently lower SGR value was obtained with *Labeo rohita* (0.92%/day), *Gibelion catla* (0.76%/day) and *Cirrhinus cirrhosus* (0.49%/day) in T_1 than that of T3; whereas T_2 showed minimum value. The best FCR value was found in T_3 (2.80) where T_1 (3.31) showed lower value than T_2 (4.01). Protein efficiency ratio (PER) in T_1 (1.06) was lower than T_3 (1.30) and T_2 (0.88) showed lowest value. Water quality parameters were in the acceptable level for carp culture. The highest survival rate (%) was recorded in T_3 with *Labeo rohita* (76.19%), *Gibelion catla* (61.90%) and *Cirrhinus cirrhosus* (80.95%) and the lowest in T_2 (61.90%, 42.86% and 66.67%). The per kilogram cost of Diet-1, Diet-2 and Diet-3 were 30.19 BDT, 37.79 BDT and 40.80 BDT, respectively. From the result of this study, it might be suggested that shrimp waste could be used as a replacement of finfish diets which would be cost effective.

Key Words: Shrimp industry waste, supplementary feed, Indian major carp, aquaria, growth performance.

Introduction

Shrimp is a high value aquaculture product and is processed for the meat, leaving the carapace and head as waste products (Omum, 1992 and Knorr, 1991). The amount of shrimp waste (40–48%) contains head and body carapace (Sachindra *et al*., 2005). The shrimp waste composed mainly of protein (40%), minerals (35%) and chitin (14-30%) (Synowiecki and Al-Khateeb 2000) and is very rich in carotenoid pigments mainly astaxanthin (Britton, 1997 and Gimeno *et al*., 2007). The shrimp wastes mostly discarded in the municipal dumping yard in coastal regions. Shrimp waste meal has higher mineral, protein and calcium content than fish meal, as well as some amino acids such as aspartic acid, glutamic acid, leucine, lysine and argentine (Ariyani, 1989). Shrimp waste could be processed in drying, fermentation, for recycle use such as shrimp meal, fish meal, poultry meal, a natural source of carotenoids and human foods. Usually, shrimp waste is dried on the beaches which encourage environmental problems (Mathew and Nair, 2006). Among commonly used feed ingredients, fish meal is considered to be the best ingredients, due to its compatibility with the protein requirement of fish (Alam *et al*., 1996). Replacement of fish meal with cheaper ingredients in fish feed is necessary because of rising cost and uncertain availability of fish meal (Higgs *et al*., 1995). Excessive shrimp waste which discarded from processing industry could be easily used as feed ingredients due to its high protein content. However, the use of shrimp waste in the formulation of fish feed is not recommended due to its high fiber and ash contents, which results in the formation of weak pellets (Meyers, 1986) with poor stability in water. Therefore, an economically viable and socially feasible simple technology is essential for the people involved with waste utilization and mitigation of environmental problems as well.

Materials and Methods

Feed ingredients collection, formulation and preparation

Different types of feed ingredients such as rice bran, maize, soyabean, wheat bran, molasses, minerals and vitamins premix were purchased from local market of Mymensingh (Table-1). The main ingredient, the shrimp waste (shrimp head and shell) was collected from different shrimp processing plants situated in Chittagong. For performing this experiment, two types of diets (Diet-1: feed with shrimp waste and Diet-2: feed with plant ingredients) were formulated for T_1 and T_2 (Table-1). The third one (Diet–3: commercial feed) was purchased from the market. The selected ingredients were milled and mixed thoroughly. Mixing of ingredients were performed by hand before adding water with stirring to form dough which finally, made into pellets using a pellet machine. The pellets were sundried in solar tent drier. The pellets were packed in air-tight water impermeable bags and stored in dry and cool place. The flow diagram of the Diets preparation procedure is shown in Fig. 1. Diet-3 which was used in T_3 was purchased from the distributor of Spectra Hexa Feeds Limited (it is particularly known as Mega feed Limited).

Table 1: Formulation of Diet-1 and Diet-2 for T_1 and T_2

Diet-1 (with shrimp waste) for T_1		**Diet-2 (without shrimp waste) for T_2**	
Feed ingredients	**Amount of ingredients (%)**	**Feed ingredients**	**Amount of ingredients (%)**
Shrimp waste	40.00		
Soya bean meal	24.00	Soya bean	48.00
Wheat bran	10.00	Wheat bran	12.00
Maize	13.00	Maize	25.00
Rice bran	10.00	Rice bran	12.00
Molasses	2.00	Molasses	2.00
Vitamin & mineral mix	0.50	Vitamin and mineral	0.50
Salt	0.50	Salt	0.50
Total	**100.00**	**Total**	**100.00**

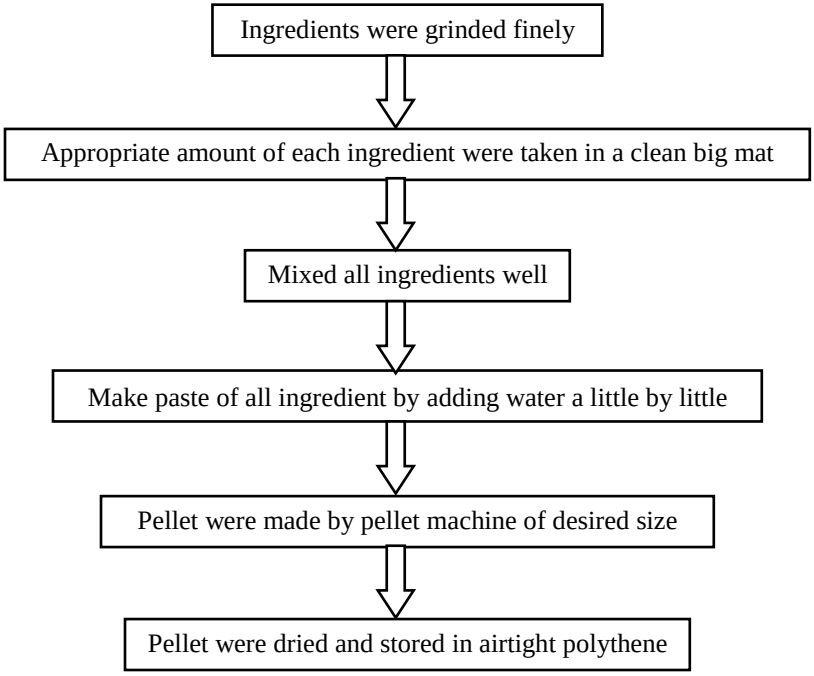

Fig. 1: Protocol for preparation of diets

Analytical methods

Proximate composition of all individual feed ingredients and prepared feeds from those ingredients were analyzed in the Fish Processing Laboratory of the Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh, Bangladesh following the methods of the Association of Official Analytical Chemists (1990) with slight modification.

Experimental species and system

The experiment was carried out in nine glass aquaria for a period of two months; from 1st April to 30th May, 2015. The experiment was carried out in the Laboratory of Fish Handling and Harvesting Laboratory at the Department of Fisheries Technology, BAU, Mymensingh, Bangladesh. Three species combination of Indian major carps fingerlings were selected for this experiment viz. *Labeo rohita*, *Gibelion catla* and *Cirrhinus cirrhosus*. Fingerlings were collected from the local fish traders and hatcheries. All the stocking and rearing activities were conducted in one experimental system, aquarium. Well aerated nine glass aquaria were used for this purpose.

All the aquaria were placed and kept on 1.0 m high cemented table for better observation and accessibility. For preparing aquaria, first any leakage of each aquarium were checked up and blocked by the glass glue. The aquaria were then cleaned with detergent and sponged thoroughly. These were washed with water and scooped out from the aquaria. The aquaria were rewashed and filled with water almost up to the top level and kept for a day. The aquaria were again washed with sodium chloride (NaCl) to destroy the harmful microorganisms. Again the aquaria were sponged and filled with tap water. On the following day, water was scooped out and then dried. For convenience the aquaria were numbered as 1, 2, 3 up to 9.There were three experimental groups, each with three replicates, in nine (09) uniform glass aquaria (30 L capacity) which followed a completely randomized design. To observe the growth performance of *Labeo rohita*, *Gibelion catla* and *Cirrhinus cirrhosus* and to measure water quality parameters, three experimental diets (Diet-1, Diet-2 and Diet-3) were assigned to three different experimental treatments. T_1, T_2 and T_3 respectively.

The fingerlings were kept in the aquaria in the laboratory for two weeks to acclimatize with the new environment. The fingerlings were fed with Diet-1, Diet-2 (formulated feed with plant ingredients) and Diet-3. Seven individuals of rui (*Labeo rohita*), catla (*Gibelion catla*) and mrigal (*Cirrhinus cirrhosus*) were stocked in each aquarium. Before releasing, initial weight of fingerlings were measured.

Artificial aeration system with the help of aerator (Aquarium air pump, Model no: SB 348A) were introduced to facilitate an adequate level of dissolved oxygen in each aquarium during the experimental period. The leftover feed and fecal matters in tanks were cleaned everyday by siphoning from tanks and the tanks were cleaned manually. Removed water was replaced by fresh deep tube well water.

The feed was supplied daily at the rate of 5% of their body weight for the first month and then the feeding rate gradually reduced to 4.5% in the next two weeks and 4% in last two weeks. Feeding rates were adjusted on the basis of fish weight gain at 10 days interval. The daily ration was divided into two parts. About half of the ration applied in the morning between 8.30 to 9.30 am and another half in the afternoon between 4:30 to 5:30 pm.

Sampling of fish and water quality parameters

Sampling of fish was done during ten days interval to observe the growth of fish and to adjust the feeding rate. Weight of fish was measured by using a digital electronic balance. Some important water quality parameters such as dissolved oxygen (mg/l), water temperature (ºC) and pH were measured during every sampling day. The sample collection was done between 8.30 to 9.30 am.

Weight gain (g)

Weight gain was calculated as:
Weight gain = Mean final fish weight —Mean initial fish weight

Percent weight gain

Percent weight gain was calculated as:
% weight gain=(Mean final weight-Mean initial weight)/(Mean initial weight)×100

Specific growth rate (SGR %/day)

Specific growth rate (SGR) was estimated as:

$$\text{SGR (\% per day)} = \frac{(\text{Loge } W_2 - \text{Loge } W_1)}{T_2 - T_1} \times \frac{100}{1}$$

Where:
W_2 = Weight of fish at time T_2 (final)
W_1 = Weight of fish at time T_1 (initial)

Feed conversion ratio (FCR)

The feed conversion ratio was expressed by the amount of food consumed to gain per kilogram weight by using following formula
FCR = (Amount of feed (kg) fed/(Live weight gain (kg)

Protein efficiency ratio (PER)

Protein efficiency ratio (PER) was measured by following formula
PER = (Live weight gain (kg))/(Amount of protein (kg) fed

Survival rate (%)

Survival rate (%) = (Total number of fish harvested)/(Total number of fish stocked)

Data Analysis

Data obtained from the present experiment were analyzed statistically to measure growth performances of different fish species in different treatments. Data were entered into the MS Excel to done simple statistics and XLSTAT for analysis of variances (ANOVA). The mean values were compared by Duncan Multiple Range Test at 5% level of significance.

Results

Proximate Composition of Experimental Diets

During experimental period, proximate composition of different experimental diets including the control (commercial feed) were analyzed which is shown in Table 2. Diet-1 contained the highest protein content (28.42%). The lipid content varied between 5.45% to 6.53% while the highest lipid value (6.53%) found for Diet-1in T_1.The highest ash content (12.35%) also was found in T_1 for Diet-1. The moisture content varied from 10.42% to 11.13%. In this experiment, the mean crude protein content of in Treatments for Diet-1, Diet-2 and Diet-3 was found 28.42%, 28.13% and 27.32% respectively whereas the mean crude lipid content for Diet-1, Diet-2 and Diet-3 found 6.53%, 5.84% and 5.45%, respectively.

Table 2: Proximate composition of the experimental Diets for different treatments

Content (%)	**Diets**		
	Diet 1 (T_1)	**Diet 2 (T_2)**	**Diet 3 (T_3)**
Moisture	10.74	10.42	11.13
Protein	28.42	28.13	27.32
Lipid	6.53	5.84	5.45
Ash	12.35	11.63	9.87

Water quality parameters

The water quality parameter such as dissolved oxygen, pH and temperature of the experimental ponds water in all the treatments were monitored in every 10 days interval during the experimental period. Monthly variation in the ranges and the mean value of different parameters of different treatment are shown in Table 3.

Table 3: Monthly variation in the ranges and mean values of water temperature, dissolved oxygen (DO) and pH in different treatments

Parameter	Months	Treatments		
		T_1 (Mean ± SD)	**T_2 (Mean ± SD)**	**T_3 (Mean ± SD)**
Temperature (^oC)	April	24.7- 25.9 (25.2 ± 0.35)	24.7-25.8 (25.2 ± 0.38)	24.6-25.8 (25.2 ± 0.37)
	May	25.5-26.2 (25.8 ± 0.26)	25.6-26.3 (25.9 ± 0.37)	25.5-26.3 (25.8 ± 0.31)
Dissolved Oxygen (mg/l)	April	6.26-6.88 (6.47 ± 0.17)	6.23-6.88 (6.50 ± 0.19)	6.19-6.89 (6.49 ± 0.20)
	May	5.97-6.41 (6.26 ± 0.15)	5.83-6.44 (6.16 ± 0.23)	5.81-6.49 (6.15 ± 0.25)
pH	April	7.74-8.23 (7.92 ± 0.13)	7.73-8.17 (7.91 ± 0.13)	7.76-8.21 (7.91 ± 0.14)
	May	7.75-8.19 (7.89 ± 0.13)	7.76-7.98 (7.87 ± 0.08)	7.72-8.21 (7.89 ± 0.15)

The mean value of the temperature measured in different treatments ranged from 24.6ºC to 26.3ºC (Table 3) during the experimental period. The minimum temperature (24.6ºC) and the maximum temperature (26.3ºC) was recorded in April and May in T_3 and T_2 respectively.

The mean value of the dissolved oxygen (DO, mg/l) of the sub-surface water in the different treatments ranged from 5.81 mg/l to 6.89 mg/l (Table 3). The highest DO concentration (6.89 mg/l) and the lowest DO concentration (5.81 mg/l) were observed in T_3 on April and May, respectively.

The mean value of the hydrogen ion concentration of the sub-surface water in the different experimental treatments during the study periods varied from 7.72 - 8.23 (Table 3). The highest pH value 8.23 was recorded in T_1 on April and the lowest value 7.72 was observed in T_3 on May.

Growth performance of experimental fishes (*Labeo rohita, Gibelion catla* and *Cirrhinus cirrhonus*)

The mean weight gain of *Labeo rohita*, *Gibelion catla* and *Cirrhinus cirrhonus* in different treatments between 2.73g to 9.85g. The highest weight gain of *Labeo rohita* (7.71g), *Gibelion catla* (9.85g) and *Cirrhinus cirrhonus* (3.87g) was found for Diet-3 in T_3. The lowest weight gain of these three treatments was found for Diet-2 in T_2. Significant variation was observed in case of *Gibelion catla* and *Cirrhinus cirrhonus* for Diet-3 and Diet-2, respectively. The mean weight gain is shown in Table 4.

Table 4: Mean weight gain (g) of *Labeo rohita*, *Gibelion catla* and *Cirrhinus cirrhonus* in different treatments

Species	**Treatments**		
	Diet 1 (T_1)	**Diet 2 (T_2)**	**Diet 3 (T_3)**
Labeo rohita	7.6 ± 0.22^{a}	6.99 ± 0.42^{a}	7.71 ± 0.72^{a}
Gibelion catla	8.32 ± 0.48^{b}	7.65 ± 0.25^{b}	9.85 ± 0.99^{a}
Cirrhinus cirrhonus	3.28 ± 0.08^{ab}	2.73 ± 0.62^{b}	3.87 ± 0.11^{a}

The highest weight increment was found for Diet-3 in T_1 and the lowest increment was found for Diet-2 in T_2. The weight increment every 10 days interval during experiment period of *Labeo rohita*, *Gibelion catla* and *Cirrhinus cirrhonus* in aquara is shown in the Table 5.

Table 5: Weight increment of *Labeo rohita*, *Gibelion catla* and *Cirrhinus cirrhonus* in different treatments during the experimental period

Weight increment of *Labeo rohita*

Treatments	Time (Days)					
	10 days	20 days	30 days	40 days	50 days	60 days
Diet 1 (T_1)	0.95	1.06	1.27	1.26	1.44	1.5
Diet 2 (T_2)	0.89	0.94	1.12	1.24	1.37	1.43
Diet 3 (T_3)	1.03	1.16	1.36	1.43	1.47	1.57

Weight increment of *Gibelion catla*

Treatments	Time (Days)					
	10 days	20 days	30 days	40 days	50 days	60 days
Diet 1 (T_1)	1.18	1.25	1.36	1.48	1.57	1.79
Diet 2 (T_2)	0.97	1.08	1.21	1.34	1.43	1.61
Diet 3 (T_3)	1.32	1.43	1.50	1.60	1.67	1.93

Weight increment of *Cirrhinus cirrhonus*

Treatments	Time (Days)					
	10 days	20 days	30 days	40 days	50 days	60 days
Diet 1 (T_1)	0.29	0.38	0.52	0.62	0.69	0.79
Diet 2 (T_2)	0.23	0.29	0.44	0.52	0.56	0.68
Diet 3 (T_3)	0.47	0.51	0.61	0.67	0.74	0.83

The percent weight gain of *Labeo rohita*, *Gibelion catla* and *Cirrhinus cirrhonu*s in different treatments ranged from 49.37- 142.43%. The higher percent weight gain was found in *Labeo rohita* (130.57%), *Gibelion catla* (100.74%) and *Cirrhinus cirrhonus* (56.78%) for Diet-1 than Diet-2. The highest percent weight gain was found in *Labeo rohita* (136.37%), *Gibelion catla* (142.43%) and *Cirrhinus cirrhonus* (67.61%) for Diet-3 in T_3 (Table 6). Significant difference was found only with *Gibelion catla* among three treatments.

The specific growth rate (SGR, %/day) of *Labeo rohita*, *Gibelion catla* and *Cirrhinus cirrhonus* in different treatments ranged from 0.44%/day to 0.97%/ day (Table 6). The higher SGR value was obtained in *Labeo rohita* (0.92%/day), *Gibelion catla* (0.76%/day) and *Cirrhinus cirrhonus* (0.49%/day) for Diet-1 in T_1. The highest SGR value was obtained for Diet-3 (controlled diet) in T_3.

Table 6: Percent weight gain and Specific growth rate (SGR %/day) of *Labeo rohita*, *Gibelion catla*, and *Cirrhinus cirrhonus* in different treatments during experimental period

Species of Fish	**Percent weight gain**		
	Treatments		
	Diet 1 (T_1)	**Diet 2 (T_2)**	**Diet 3 (T_3)**
Labeo rohita	130.57 ± 3.02^a	121.21 ± 14.54^a	136.37 ± 30.70^a
Gibelion catla	100.74 ± 9.37^b	95.77 ± 7.95^b	142.43 ± 33.61^a
Cirrhinus cirrhonus	56.78 ± 2.99^a	49.37 ± 17.91^a	67.61 ± 2.83^a
Species of Fish	**Specific growth rate (SGR %/day)**		
	Treatments		
Labeo rohita	0.92 ± 0.01^a	0.87 ± 0.07^a	0.94 ± 0.14^a
Gibelion catla	0.76 ± 0.05^b	0.74 ± 0.04^b	0.97 ± 0.16^a
Cirrhinus cirrhonus	0.49 ± 0.02^a	0.44 ± 0.13^a	0.57 ± 0.02^a

Mean feed conversion ratio (FCR) and protein efficiency ratio (PER) in different treatments ranged from 2.80 to 4.01 and 0.88 to 1.30, respectively (Table 7). The best FCR value was found for Diet-3 (2.80) in T_3. There was significant variation in FCR value among the treatments. In the case of protein efficiency ratio the highest value was found for Diet-3 in T_3 whereas in T_1 the value found lowest for Diet-1 (0.88). Here also significant variations were observed among the treatments.

Table 7: Mean FCR (Food conversion ratio) and PER (Protein efficiency ration) of diets in different treatments during the experimental period

Growth parameters	Treatments		
	Diet 1 (T_1)	**Diet 2 (T_2)**	**Diet 3 (T_3)**
FCR	3.31 ± 0.80b	4.01 ± 0.75a	2.80 ± 0.94c
PCR	1.06 ± 0.26b	0.88 ± 0.18c	1.30 ± 0.24a

Survival rate (%)

The survival rate (%) of fish in different treatments was estimated after total harvest by draining out of the aquaria. The survival rate (%) of three species of carp ranged from 42.86% to 80.95%. The highest survival rate (%) was recorded for diet 3 in T_3 and the lowest for Diet-2 in T_2. A comparison of survival rate (%) of *Labeo rohita*, *Gibelion catla* and *Cirrhinus cirrhonus* is shown in Table 8.

Table 8: Comparison of survival rate (%) of *Labeo rohita, Gibelion catla* and *Cirrhinus cirrhonus* in different treatments during the experimental period

Species	Treatments		
	(T_1)	**(T_2)**	**(T_3)**
Labeo rohita	66.67 ± 8.24	61.90 ± 16.49	76.19 ± 8.18
Gibelion catla	47.62 ± 8.24	42.86 ± 14.28	61.90 ± 14.87
Cirrhinus cirrhonus	80.95 ± 6.68	66.67 ± 8.24	80.95 ± 6.68

Cost analysis of formulated diets

Shrimp waste is a good source of protein, lipid, crude fiber and minerals. Shrimp waste along with other ingredients in fin fish diet bears a great significance to make a feed cost effective. Price of feed ingredients per unit value used in Diet-1 and Diet-2 is shown in the Table 9. Diet-3 (control diet) the commercial feed which is used widely in Bangladesh for finfish polyculture. This feed was brought direct from the market whole seller (Spectra hexa feed limited). The cost of feed sack (25kg) was 970 BDT. The calculated price including carrying cost was 40.80 BDT/kg.

Table 9: Cost estimation of experimental Diet-1(shrimp shell along with other ingredients) and Diet-2 (ingredients from plant source)

Feed ingredients	Unit price (BDT/Kg)		Amount of ingredient (g)		Cost (BDT)	
	Diet 1	Diet 2	Diet 1	Diet 2	Diet 1	Diet 2
Shrimp shell	16.66	-	400		6.32	
Soya bean meal	42	42	240	480	10.08	20.16
Wheat bran	30	30	100	120	3.00	3.60
Maize	23	23	130	250	2.99	5.75
Rice bran	24	24	100	120	2.04	2.88
Molasses	60	60	20	20	0.80	0.80
Vitamin premix	500	500	5	5	2.50	2.50
Salt	25	25	5	5	0.10	0.10
Other charge					2.00	2.00
Total					**30.19**	**37.79**

Discussion

Proximate composition of experimental diets

Protein is the major growth promoting factor in feed. The protein requirement of fish is influenced by various factors such as water temperature, feeding rate, availability and quality of natural foods. Nandeesha (1993) reported that the proximate composition of factory made feeds is reported to be 20-30 percent protein, 2-4 percent lipid, 10-15 percent fiber, 30-40 percent carbohydrate and 8-10 percent ash and often are claimed to have been enriched with lysine, methionine, vitamins and minerals. Ahmad *et al*., (2012) reported that diet containing 40% protein, 9.31% lipid and 10.08% carbohydrate is the best one for a more profitable and successful culture of Common carp. Those former studies agreed with the present study. Singh *et al*., (2006) reported that in terms of growth, food conversion ratio, protein efficiency ratio, survival and ratios of protein and lipid deposition in muscle, diet containing 30% protein level revealed a significantly ($p<0.01$) better performance for the *Labeo rohita* in comparison with other diets containing lower or higher protein levels. Nandeesha *et al*., (1994) improved the growth performance of Rohu and Catla by alternating the feeding schedules between high and low protein diets. Rahman *et al*., (2006) and Tareque *et al*., (2009) reported 30% incorporated protein in diet resulted better results with respect of growth and SGR for *Cyprinus carpio* var. *Nudus* and *Puntius gonionotus*, respectively which is similar with present study.

The protein content of three different diets matched with Wilson (2002) suggestion. They suggested that herbivorous and omnivorous fish require a diet with 25-35% crude protein. Mookerjee and Mazumdar (1946) reported that growth and production in fish culture are generally dependent on the daily feed consumption, qualities of feed and feeding frequency. According to Chakraborty *et al*., (1999) the growth of carp (*Cyprinus carpio*) increases with protein levels, and there was an approximately linear increase of growth with feeding level for any given diet.

Water quality parameters

Temperature plays a major important role in fish physiology. Water temperature affects the different factors related to growth of fish directly. Cho and Slinger (1980) observed that food intake increases with the increase in temperature up to optimum levels as the energy requirement for maintenance increases. Ideal temperature range for different cold water and warm water species are 14 to18ºC and 24 to 30ºC respectively. Jhingranand Pullin (1985) reported that Indian major carps can tolerate temperature ranging from 10 to 37.8ºC. Present experimental data was with in the tolerance level of Indian major carps as found in other studies.

In culture systems dissolved oxygen (DO) is the most important growth increasing factor which influencing the condition of fish. Oxygen consumption varies with different factor such as species, size, activity, season and temperature. According to Banerjee (1967), cyprinids require 6-7 mg/l oxygen for good growth, but the tolerance levels as low as 3 mg/l for concise periods. Cheng *et al.,* (2003) stated that DO values higher than 5 mg/l have often been recommended for intensive culture practices. Banerjee (1967) also reported that oxygen concentration above 5 mg/l is indicative of productivity. DO value of present experiment ranged from 5.81-6.89 mg/l in different treatments was in the acceptable limit.

The pH of water is a measure of hydrogen ion concentration and indicator of the water quality is either acidic or basic. Das *et al*. (1995) reported that the water having a pH range of 6.5-9.0 is more suitable for fish culture and values above 9.5 are unsuitable. Das *et al*. (1995) also suggested that a pH range of 6.12-8.6 is most suitable for survival of the Indian major carp fry. The pH in the present study ranged between 7.72 and 8.23, which is in the desirable limits for the optimum growth of three species of Indian major carps (*Labeo rohita*, *Gibelion catla* and *Cirrhinus cirrhonus*).

Growth performance of experimental fishes (*Labeo rohita, Gibelion catla* and *Cirrhinus cirrhonus*)

Abid and Ahmed (2009) conducted an experiment to determine the efficacy of varying dietary protein regimes on growth of *Labeo rohita* fingerlings under intensive rearing for a period of six months where the mean body weight gain ranged from 5.99-21.97 g in seven different treatments. The result of the present study showed that the mean body weight gain of Indian major carps *Labeo rohita*, *Gibelion catla* and *Cirrhinus cirrhonus* ranged from 6.99-7.11g,

7.66-9.95g, 2.73-8.87g respectively which is slightly lower than those of previous record. Sinha and Ramachandran (1985) reported that under crowded condition and higher stocking density fish suffer stress due to aggressive feeding interaction, eat less and grow slowly. Indian major carps are sensitive to environmental conditions and do not attain maximum growth in a confined environment compared with other hardy species such as tilapia and common carp (Benkappa and Verghese, 2003). Due to the static and controlled experimental condition, growth of Indian major carp in different treatments was less because of the unavailability of plankton and other growth promoting factors compared to that of pond system. Saha *et al*., (1997) found that the daily weight gain of Catla, Rui and Mrigal were 0.53g to 0.70g, 0.38g to 0.57g and 0.39g 0.93g which is not agreed with the present study due to location, time and culture system. In the present study, weight gain (%) is similar with Patnaik *et al*. (2005) who reported that the weight gain (%) of Indian major carp (*Gibelion catla*) fry in five different treatments varied from 82.67%-220.43% in 70 days experiment in fiberglass tank (2.5 x 1 x 1 m). Weight gain (%) is higher in *Gibelion catla* in different treatment which is similar with Abid and Ahmad (2009).

Abid and Ahmad (2009) reported that the SGR (%/day) value of *Labeo rohita* fingerlings ranged 3.11-12.21%/day in different treatments. Patnaik *et al*. (2005) observed the SGR (%/day) of Indian major carps (*Gibelion catla*) reared in fiberglass tank varied from 0.86-1.67%/day. Mustafa *et al*. (2010) conducted an experiment (60 days) on culture of climbing perch (*Anabas testudineus*) in cement tanks (12×6×1.5feet) using different protein level diets where SGR (%/day) value ranged from 0.9-1.26%/day in four different treatments. Present study showed that, the SGR (%/day) values of Indian major carps (*Labeo rohita*, *Gibelion catla*, and *Cirrhinus cirrhonus*) in different treatments ranged from 0.44%/day to 0.97%/day which are similar to the findings of Patnaik *et al*., (2005). Pillay (1992) reported that, prolonged exposure of dissolved oxygen bellow optimum level in water and too much excretory products of the fish suppress its own growth rate. In static water condition (aquarium) the growth rate also might be suppressed due to small surface area.

Savita *et al*., (2010) conducted an experiment on the growth performance of Indian major carp (*Catla catla*, Ham.) over a period of six months through formulated feeds consisting of three seaweeds, namely *Chlorodesmis fastigiata*, *Padinatetra stomatica* and *Stoechospermumm arginatum* where the average food conversion ratio (FCR) ranged from 0.67-2.54 in three treatment. Abid and Ahmed (2009) monitored the FCR value of *Labeo rohita* ranged from 0.99-1.79 in 7 different diet treatments. The present study showed that the FCR value ranged from 2.80 to 4.01 in three different treatments which were higher than the authors mentioned above.

Tiamiyu *et al*., (2014) conducted an experiment where *Cyprinus carpio* were treated with various levels of raw watermelon seed meal found PER value ranged from 1.09-1.43. Present study showed that the PER value ranged from 0.88 to 1.30. The PER value found in different treatments was similar with the findings of Patnaik *et al*., (2005) who observed the PER value of Indian major carps (*Gibelion catla*) reared in fiberglass tank and the values varied from 0.88 to 1.66.

Tiamiyu *et al*., (2014) and Rahman *et al*., (2012) found that the survival rate (%) of *Cyprinus carpio* fingerlings varied from 93.30 to 100% and from 70 to 93.33%, respectively in different treatments. In present study the survival rate (%) ranged from 42.86 to 80.95%. The survival rate of *Gibelion catla* was reported lowest (42.86% to 61.90%) whereas the survival rate (%) of *Gibelion catla* in 70 days experiment ranged from 83.33 to 100% reported by Patnaik *et al*., (2005).

Cost analysis of formulated diets

The high protein content in the naturally available feeds provides an inexpensive feed supplement during the initial grow-out period suggested by Edwards (2009) agreed with the present result. Dorsa *et al*., (1982), Ofojekwu and Ejike (1984) reported that feeds from plant origin have been reported to be effective and less expensive ingredients to fish diets. In Diet-1 (T_1) and Diet-2 (T_2), shrimp waste and plant based protein especially soybean meal was used as protein supplement respectively. Patnaik and Das (1979) observed that in the recent years, feeds from plant origin have been accepted for Indian major carps as the growth in fishes has been reported to be as good as the traditional feed.

Conclusions

The result showed that the highest weight gain as well the best growth performance of the fingerlings of Indian major carps obtained in T_3 with Diet-3 (commercial feed) and lowest T_2 with Diet-2 (feed without shrimp waste). The weight increment of the fingerlings with Diet-1 (with shrimp waste) was quite near to the values obtained in T_3 for Diet-3. The cost analysis for feed preparation indicated that, the addition of shrimp waste along with other available feed ingredients in finfish diet not only provides better protein percentage in the diet, it also minimized the feed production cost in a significant margin.

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