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# Potential substrates for periphyton enhancement in Carp-SIS polyculture

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#### Abstract

A field trial was carried out to test performance of four locally available substrates (split bamboo, whole bamboo, banana midrib and plastic bottle) for periphyton enhancement in farmer's ponds at Seri and Nandapur in Nawalparasi district for 7 months. Six carp species were stocked at 15000 fish/hectare and SIS at unrecorded densities. Carp was fed with rice bran and mustard oil cake at 1.5% BW while grass carp was fed with grass and banana leaves at 50% BW. There was no significant effect of substrates on growth and production of carp. Combined NFY was 19% higher in plastic bottle ponds than control ponds, while NFY of SIS was 50% higher in banana midrib ponds than control and other substrate ponds. FCR was significantly better (P<0.05) in split bamboo ponds than control ponds. Banana midrib decayed fast and was replaced 3-4 times during experimental period while plastic bottles performed better in terms of production and profit.

Key words: Carp, SIS, Substrate, Periphyton enhancement, Yield

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## Introduction

Carp polyculture in earthen ponds is a wellestablished aquaculture system in Nepal which contributes about 87.5% of total fish production (Kunwar and Adhikari, 2016). Pond production system is becoming greatly dependent on external resources such as feed and fertilizers for fish production. It has been shown that feed accounts for about 60% of total fish input cost in commercial fish farming (Bhujel, 2009). Also in small scale aquaculture the ratio of feed cost in total inputs is large. For small scale farmers it is not easy to bear the cost of expensive food ingredients. Providing an alternative means to reduce the feed cost has thus become essential for sustainability of the farming system. In most pond production systems, only a small proportion of nutrient input (30%) is converted into harvestable matter while the rest is lost into sediments, effluent water and atmosphere (Acosta Nassar *et al.*, 1994; Beveridge *et al.*, 1994; Olah *et al.*, 1994). Improving the conversion of nutrients into harvestable matters by enhancing the natural food production may be a suitable solution to the problem of higher cost as well as loss of nutrient inputs. Enhancing the growth of periphyton in pond production system has been proved to be a suitable method to increase the natural food production (Azim *et al.*, 2001a; Rai *et al.*, 2008; Jha *et al.*, 2018).

Many fish in nature as well as in culture relies on periphyton for its food. Indian major carp (Wahab et al., 1999; Ramesh et al., 1999; Rai et al., 2010), tilapia (Hem and Avit, 1994; Shrestha and Knud-Hansen, 1994; Milstein et al., 2009: Jiwyam, 2013), common carp (Rai and Yi, 2012) as well prawn (Udin et al., 2007) prefer periphyton as natural food. Previous study on periphyton based carp polyculture in Nepal showed a promising result with an increase of 24% in fish yield (Jha et al., 2018). Many researches in periphyton based aquaculture was carried out using different parts of bamboo as substrate in Bangladesh and Nepal (Azim et al., 2001a, b; Azim et al., 2002; Rai et al., 2008; Shirin et al., 2013; Jha et al., 2018). In previous experiment, farmers using bamboo mat as periphyton substrate for enhancement complained that it interfered with partial harvesting of fish. Considering their problem, present experiment was carried out to assess the performance of locally available alternative substrates in farmer's ponds stocked with carp and SIS.

#### Materials and methods

## Experimental design

The experiment was carried out for 210 days from  $12^{\text{th}}$  April to  $10^{\text{th}}$  November 2017 in 15 ponds of farmers involved in Mishrit Fish Farmer Cooperative at Seri and Nandapur of Nawalparasi district. The average area of experimental pond was  $502.9 \pm 68.4 \text{ m}^2$  ranging from 163.0 to 1760.0 m<sup>2</sup>. The experiment was carried out in completely randomized design (CRD). There were five treatments each with three replicates as shown in Table 1.

Table 1. Description of treatments

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Treatment	Substrate type	Fish					
T <sub>C</sub>	Control (No substrate)	Carp and SIS					
$T_{SB}$	Split Bamboo	Carp and SIS					
$T_{WB}$	Whole Bamboo	Carp and SIS					
$T_{BM}$	Banana Midrib	Carp and SIS					
$T_{PB}$	Plastic Bottle	Carp and SIS					

All ponds were drained and dried and lime was applied at the rate of 50 g/m<sup>2</sup>. Ponds were then filled with fresh water from boring. Urea and DAP were applied at the rate of 4.7 g/m<sup>2</sup> and 3.5 g/m<sup>2</sup> respectively for plankton growth. After 3 days of fertilization, substrates were installed in all ponds except control ponds.

## Substrate preparation and installation

Altogether 4 different types of substrate such as split bamboo mat, whole bamboo, banana midrib and plastic bottle were installed in substrate ponds to enhance periphyton growth. Substrates covered about 2% pond surface area in each pond except control ponds. Surface area for determined different substrates was by measuring its dimensions. Split bamboo substrate was prepared by splitting a whole bamboo into slats of 2-5 cm width and 1 m length and weaving slats into a mat. Each split bamboo mat was supported with water filled (sink) and empty (float) bottles for their proper vertical position in the water column. Number of split bamboo mats per pond depended on the area of pond. For whole bamboo substrate, bamboo were installed in a manner so that all branches of bamboo lied underneath water and main stem lied about 20-30 cm below water surface. Banana midrib substrate was also prepared by weaving midribs of banana leaf into a mat similar to that of split bamboo mat. For bottle substrate, empty bottles of soft drinks were filled with water and tied in a ring. The ring was kept floating using empty water bottles on top of the ring. Number of rings per pond depended on area of pond and number of bottles per ring.

All ponds were stocked with six carp species (silver carp Hypophthalmichthys molitrix, bighead carp Aristichthys nobilis, grass carp Ctenopharyngodon idella, common carp *Cyprinus carpio*, rohu *Labeo rohita*, mrigal *Cirrhinus mrigala*) at the rate of 15,000/ha and 2 SIS viz. Pothi (*Puntius* spp.) and Dedhuwa (*Esomus danricus*) at unrecorded densities. SIS were stocked to ponds by allowing them to enter from water inlet. Stocking of fish was done after 7 days of fertilization. Stocking information of different fish species is given in Table 2.

**Table 2.** Stocking density (No./ha) of carp indifferent treatments

Species	Stocking number (No./ha)	Stocking (%)
Silver carp	3000	20
Bighead carp	750	5
Common carp	3000	20
Grass carp	3000	20
Rohu	3750	25
Mrigal	1500	10
Total Carp	15000	100

## Feeding and fertilization

Carp were fed with freshly made dough of mustard oil cake and rice bran (1:1). Feed was provided in a traditional bamboo tray placed in each pond every morning at 9-10 am. Feeding rate was 1.5% BW. Grass carp was fed with grass and banana leaves at 50% BW. Urea and DAP was applied fortnightly at rates of 9.4 g/m<sup>2</sup> and 7.2 g/m<sup>2</sup> respectively in all ponds to enhance periphyton and phytoplankton population.

### Water quality and Periphyton analysis

Temperature, DO, pH and Sechhi disk visibility of ponds were monitored at 7-9 am in situ monthly. Periphyton samples from four different types of substrate were taken from the pond and analyzed three times, in the beginning, middle and end of the trial at laboratory of Fisheries Program in AFU. Two samples were taken from each substrate; first sample was taken for periphyton biomass analysis while second sample was taken to determine periphyton abundance. Samples were collected from 1 cm<sup>2</sup> area of substrate by scrapping periphyton from the surface using a sharp scalpel.

(a) Dry matter, ash free dry matter, and ash content were determined following APHA (1980) using following formulae.

Dry Matter = 
$$W_2 - W_1$$

Ash Content (%) = 
$$\frac{W_3 - W_1}{W_2 - W_1} * 100$$
  
Ash Free Dry Matter =  $W_2 - W_3$ 

Where,

 $W_1$ = Weight of Aluminum foil and periphyton sample

W<sub>2</sub>= Weight of Aluminum foil and periphyton sample after drying

 $W_3$ = Weight after combustion

(b) Second sample was transferred into a reagent bottle containing 10% formaldehyde. Periphyton genera were identified following Prescott (1951), Guiry and Guiry (2018), Edmondson (1959) and Pennak (1978). Their abundance was estimated at genus level for each substrate.

## Gross margin analysis

Economic return was calculated using gross margin analysis. Gross margin for each treatment was determined by subtracting total variable cost of treatment from gross income.

A one-way analysis of variance (ANOVA) was used to compare effects of treatments on water quality parameters, periphyton abundance and biomass, and on fish growth and production, followed by Duncan's Multiple Range Test. All statistical analyses were performed using SPSS-v 16.0. Alpha was set at 0.05 for all comparisons. All means are reported with  $\pm$  1 standard error (SE).

# **Results and discussion**

Water quality in all ponds was within an acceptable range for carp indicating that different types of substrate did not affect water quality (Table 3). There was no significant difference between average temperature, average transparency and average dissolved oxygen (DO) among different treatments.

There were no significant differences in periphyton abundance (Table 4) among different treatments. Split bamboo had insignificantly higher abundance of periphyton among four substrates. Altogether 38 species of phytoplankton and 8 species of zooplankton were recorded from four types of substrates. Split bamboo supported higher diversity of periphyton (41genera) followed by plastic bottle (39 genera), banana midrib (38 genera) and whole bamboo (36 genera).

	T <sub>C</sub>	T <sub>SB</sub>	$T_{WB}$	$T_{BM}$	T <sub>PB</sub>
Temperature (°C)	29.3±0.3ª	29.8±0.7 ª	30.0±0.8 <sup>a</sup>	30.1±0.5 <sup>a</sup>	30.1±0.5 <sup>a</sup>
Transparency (cm)	24±3 <sup>a</sup>	25±4 ª	25±3 <sup>a</sup>	22±3 <sup>a</sup>	23±3 ª
DO (mg/L)	3.9±0.7 <sup>a</sup>	4.6±0.8 <sup>a</sup>	4.3±0.8 <sup>a</sup>	4.5±0.5 <sup>a</sup>	4.3±0.6 <sup>a</sup>
рН	8.0	8.2	8.0	7.3	8.1

Table 3. Water quality parameters in different treatments

Table 4. Abundance (not	o./cm <sup>2</sup> ) of periphytor	in different substrates

Treatment					
Group	T <sub>SB</sub>	$T_{WB}$	$T_{BM}$	T <sub>PB</sub>	
Phytoplankton					
Bacillariophyceae					
Coscinodiscus	3333±1735 <sup>a</sup>	3056±1547 <sup>a</sup>	2500±241ª	$1806 \pm 1410^{a}$	
Cyclotella	$6250 \pm 867^{a}$	5833±636 <sup>a</sup>	8472±2661 <sup>a</sup>	4722±1959 <sup>a</sup>	
Diatoma	6389±2074	$7778 \pm 3456^{a}$	7361±2074 <sup>a</sup>	6389±2504 <sup>a</sup>	
Fragillaria	694±367 <sup>a</sup>	$0\pm0^{b}$	$0\pm0^{b}$	$278 \pm 278^{ab}$	
Navicula	10556±4938 <sup>a</sup>	6806±3852 <sup>a</sup>	6111±2434 <sup>a</sup>	5694±2410 <sup>a</sup>	
Nitzschia	2500±1339 <sup>a</sup>	$2778 \pm 2778^{a}$	2778±2778 <sup>a</sup>	4167±2295 <sup>a</sup>	
Surirella	$0\pm 0^a$	$0\pm0^{a}$	417±417 <sup>a</sup>	$0\pm0^{a}$	
Synedra	4538±1502 <sup>a</sup>	3611±1602 <sup>a</sup>	3333±1667ª	3889±1325ª	
Total Bacillariophyceae	34306±12523 <sup>a</sup>	29861±6974 <sup>a</sup>	30972±7500 <sup>a</sup>	26944±5198 <sup>a</sup>	
Chlorophyceae					
Actinastrum	1111±1111 <sup>a</sup>	$2500 \pm 2500^{a}$	278±278 <sup>a</sup>	$0\pm0^{a}$	
Ankistrodesmus	8889±1234 <sup>a</sup>	6667±1049ª	8889±773ª	6944±1637 <sup>a</sup>	
Chlamydomonas	2361±2361ª	694±694 <sup>a</sup>	278±278 <sup>a</sup>	2222±2222ª	
Characium	$0\pm0^{a}$	556±556ª	556±556 <sup>a</sup>	278±278ª	
Chlorella	5972±2989 <sup>a</sup>	9583±4829ª	10556±3729 <sup>a</sup>	11111±3546ª	
Closterium	972±605 <sup>a</sup>	0±0 <sup>a</sup>	0±0 <sup>a</sup>	$0\pm0^{a}$	
Cosmarium	1806±1085 <sup>a</sup>	1528±972 <sup>a</sup>	556±556 <sup>a</sup>	2361±2361 <sup>a</sup>	
Crucigenia	5278±735 <sup>a</sup>	3750±1667 <sup>a</sup>	5694±3046 <sup>a</sup>	3889±2286ª	
Gonatozygon	2639±1869 <sup>a</sup>	0±0 <sup>a</sup>	1528±1528ª	2500±2500ª	
Mougeotia	1806±1085 <sup>a</sup>	1250±1250ª	833±833ª	3056±1547 <sup>a</sup>	
Oocystis	$0\pm0^{a}$	0±0 <sup>a</sup>	0±0 <sup>a</sup>	417±417 <sup>a</sup>	
Pediastrum	6528±2504 <sup>a</sup>	4861±2572 <sup>a</sup>	10139±5576 <sup>a</sup>	6667±1684 <sup>a</sup>	
Scenedesmus	2222±1137 <sup>a</sup>	1667±962 <sup>a</sup>	3750±833ª	1667±1667 <sup>a</sup>	
Selenastrum	417±417 <sup>a</sup>	694±694 <sup>a</sup>	$0\pm0^{a}$	694±694 <sup>a</sup>	
Staurastrum	556±556 <sup>a</sup>	694±694 <sup>a</sup>	833±481 <sup>a</sup>	278±278ª	
Tetreedron	833±833 <sup>a</sup>	1250±1250ª	3056±3056ª	0±0 <sup>a</sup>	
Tetraspora	0±0 <sup>a</sup>	0±0 <sup>a</sup>	1389±1389 <sup>a</sup>	694±694 <sup>a</sup>	
Ulothrix	972±972 <sup>a</sup>	$0\pm 0^a$	0±0 <sup>a</sup>	1111±1111 <sup>a</sup>	
Volvox	0±0 <sup>a</sup>	0±0 <sup>a</sup>	$0\pm0^{a}$	11111±11111ª	
Oedogonium	25972±17783 <sup>a</sup>	10139±6590 <sup>a</sup>	10417±5320 <sup>a</sup>	20556±1038ª	
Pithophora	2083±2083ª	0±0 <sup>a</sup>	0±0 <sup>a</sup>	0±0 <sup>a</sup>	
Uronema	2222±2222a	0±0 <sup>a</sup>	972±972 <sup>a</sup>	0±0 <sup>a</sup>	
Total Chlorophyceae	72638±6943 <sup>a</sup>	45833±3971ª	59722±4968 <sup>a</sup>	65556±2022ª	
Cyanophyceae		·			
Anabaena	6111±4654 <sup>a</sup>	5000±2927 <sup>a</sup>	3056±2457 <sup>a</sup>	5000±3960 <sup>a</sup>	
Chroococcus	3611±2819 <sup>a</sup>	2917±2917ª	6111±6111 <sup>a</sup>	4861±2457 <sup>a</sup>	
Merismopedia	3194±1707 <sup>ab</sup>	5694±2183 <sup>a</sup>	3750±2774 <sup>ab</sup>	2361±1450 <sup>b</sup>	
Microcystis	2222±605 <sup>a</sup>	2500±1735 <sup>a</sup>	2361±1187 <sup>a</sup>	556±556 <sup>a</sup>	
Oscillatoria	4444±1806 <sup>a</sup>	3333±1463ª	4444±1602 <sup>a</sup>	6111±1325ª	
Total Cyanophyceae	19583±8819 <sup>a</sup>	19444±9343 <sup>a</sup>	19722±11056 <sup>a</sup>	18889±4728ª	
Euglenophyceae					
Euglena	8333±1869 <sup>a</sup>	5417±636 <sup>a</sup>	8056±1869 <sup>a</sup>	7778±1325 <sup>a</sup>	
Phacus	$972 \pm 605^{a}$	$1111\pm605^{a}$	$1667 \pm 636^{a}$	1111±735 <sup>a</sup>	

Total Euglenophyceae Other Total Phytoplankton	$\begin{array}{c} 14167{\pm}1502^a\\ 30278{\pm}15696^a\\ 140694{\pm}35210^a \end{array}$	12083±2546ª 10139±6590ª 107222±31753ª	17222±3266ª 11389±5700ª 127639±41629ª	14028±1187ª 20556±10348ª 125417±19731ª
Zooplankton				
Sarcodina				
Difflugia	4722±1707 <sup>a</sup>	2639±845 <sup>a</sup>	2361±1773 <sup>a</sup>	3611±1822 <sup>a</sup>
Total Sarcodina	4722±1707 <sup>a</sup>	2639±845 <sup>a</sup>	2361±1773 <sup>a</sup>	3611±1822 <sup>a</sup>
Rotifera				
Asplanchna	3056±911 <sup>a</sup>	4167±2295 <sup>a</sup>	2639±1325 <sup>a</sup>	3194±1211 <sup>a</sup>
Brachionus	6111±1773 <sup>a</sup>	3611±2312 <sup>ab</sup>	4722±2650 <sup>ab</sup>	2917±1102 <sup>b</sup>
Keratella	417±417 <sup>a</sup>	972±972 <sup>a</sup>	556±556 <sup>a</sup>	$278 \pm 278^{a}$
Lecane	556±556 <sup>a</sup>	556±556 <sup>a</sup>	694±694 <sup>a</sup>	278±278 <sup>a</sup>
Total Rotifera	10139±1002 <sup>a</sup>	9306±972 <sup>a</sup>	8611±1470 <sup>a</sup>	6667±636 <sup>a</sup>
Crustacea				
Cyclops	$556 \pm 556^{a}$	972±501 <sup>a</sup>	556±556 <sup>a</sup>	833±481 <sup>a</sup>
Daphnia	278±278 <sup>a</sup>	139±139 <sup>a</sup>	$0\pm0^{a}$	$0\pm0^{a}$
Nauplius	556±139 <sup>a</sup>	833±481 <sup>a</sup>	556±278 <sup>a</sup>	694±367 <sup>a</sup>
Total Crustacea	972±972 <sup>a</sup>	1944±972 <sup>a</sup>	$1111\pm735^{a}$	1528±845 <sup>a</sup>
Total Zooplankton	15833±1879 <sup>a</sup>	13889±911 <sup>a</sup>	12083±3014 <sup>a</sup>	11806±2650 <sup>a</sup>

Rai, Gharti, Shrestha, Ranjan, Diana and Egna / Our Nature (2018), 16 (1): 8-16

Periphyton biomass was determined in terms of dry matter, ash and ash-free dry matter (Table 5). There was no significant difference (p>0.05) in dry matter, ash content and ash free dry matter among different types of substrates. There was no significant difference (p>0.05) in growth and yield of carp and SIS among different treatments except daily weight gain in rohu (Table 6). Daily weight gain (DWG) of rohu in plastic bottle ponds was significantly higher (p<0.05) than in control ponds but the value did not differ with other substrate ponds. Higher DWG of rohu in substrate ponds compared to control ponds can be attributed to its periphyton grazing habit (NFEP, 1997, Rai et al., 2012). Similarly, net yield of carp, combined GFY and combined NFY did not differ among different treatments which can be attributed to similar abundance of periphyton found substrate in ponds. Insignificantly higher combined NFY was observed in substrate ponds compared to control ponds which may be due to insignificantly higher production of rohu and common carp in periphyton enhanced ponds. In plastic bottle ponds, combined NFY was 19% higher than control ponds. Feed conversion ratio was significantly lower (p<0.05) in split bamboo ponds than control ponds but it was similar to values in other substrate ponds. The reason might be comparatively higher population of periphyton in split bamboo among four substrates and no periphytons in control ponds.

Comparatively higher total carp yield in substrate ponds than control ponds can be attributed to the provision of additional food in terms of periphyton (Miller and Falace, 2000) and bacterial biofilm (Ramesh, 1999). All treatments with periphyton enhancement gave comparatively higher fish yield than the control. Among substrates used, ponds with plastic bottle substrate gave higher fish yield than natural substrates which differed from results obtained in previous work, where natural substrates such as bamboo produced higher yield (van Dam et al., 2002). Most likely differences in the surface area of each substrate type, the exposure to sunlight, and the attraction of algae to the substrate surface made each substrate type a unique environment for production of periphyton and the resulting difference in fish production.

Among substrates used, farmers complained about using banana midrib as it has to be replaced 3-4 times during a production cycle. Banana midrib decayed in ponds within 2-3 months which created trouble to farmers. Although banana is easily available from the farm and has multiple uses, replacement effort is important and care should be given on use of it because its decay may cause oxygen depletion in the pond.

There was no significant difference (p>0.05) in feed cost and total variable cost among different treatments (Table 7). Similarly, there was no significant difference (p>0.05) in

	T <sub>SB</sub>	$T_{WB}$	$T_{BM}$	$T_{PB}$
Dry matter	$0.0292 \pm 0.0068^{a}$	$0.0271 \pm 0.0099^{a}$	0.0432±0.0123ª	$0.0409 \pm 0.0056^{a}$
Ash content	$0.0205{\pm}0.0061^{a}$	$0.0157{\pm}0.0071^{a}$	$0.0337 {\pm} 0.0107^{a}$	$0.0313 \pm 0.0044^{a}$
Ash free dry matter	$0.0087 \pm 0.0010^{a}$	$0.0114 \pm 0.0031^{a}$	$0.0095 {\pm} 0.0025^{a}$	$0.0095 \pm 0.0012^{a}$

 Table 5. Periphyton biomass (g/cm<sup>2</sup>) in different substrates

 Table 6. Growth performance of carp and SIS in different treatment (Mean±SE)

Parameters			Treatments		
	T <sub>C</sub>	T <sub>SB</sub>	$T_{WB}$	$T_{BM}$	$T_{PB}$
		Silver carp			
Initial mean weight (g/fish)	$0.7 \pm 0.0^{a}$	$0.7 \pm 0.0^{a}$	$0.7{\pm}0.0^{a}$	$0.7\pm0.0^{a}$	$0.7 \pm 0.0^{a}$
Initial total weight (g/100m <sup>2</sup> )	21.0±0.0 <sup>a</sup>	21.0±0.0 <sup>a</sup>	21.0±0.0 <sup>a</sup>	21.0±0.0 <sup>a</sup>	21.0±0.0 <sup>a</sup>
Final mean weight (g/fish)	$138.4 \pm 27.4^{a}$	$172.1 \pm 38.8^{a}$	<b>160.8</b> ±30.1 <sup>a</sup>	$198.1 \pm 32.8^{a}$	<b>183.5</b> ±42.4 <sup>a</sup>
Final total weight (kg/100m <sup>2</sup> )	<b>3.5</b> ±0.8 <sup>a</sup>	4.6±1.1 <sup>a</sup>	4.0±0.9 <sup>a</sup>	$4.1 \pm 0.7^{a}$	4.5±0.7 <sup>a</sup>
DWG (g/fish/day)	0.66±0.13 <sup>a</sup>	$0.82\pm0.18^{a}$	$0.76\pm0.14^{a}$	$0.94{\pm}0.16^{a}$	$0.87 \pm 0.20^{a}$
TWG (kg/pond)	$3.5{\pm}0.8^{a}$	4.6±1.1 <sup>a</sup>	4.0±0.9 <sup>a</sup>	$4.1\pm0.7^{a}$	4.5±0.7 <sup>a</sup>
Survival (%)	$84.5 \pm 7.2^{a}$	$88.5\pm3.6^{a}$	$81.7\pm3.6^{a}$	$74.1 \pm 17.2^{a}$	$85.5\pm7.6^{\mathrm{a}}$
Extrapolated GFY (t/ha/yr)	0.62±0.14 <sup>a</sup>	$0.80\pm0.20^{a}$	0.70±0.16ª	0.72±0.13 <sup>a</sup>	0.79±0.12ª
Extrapolated NFY (t/ha/yr)	0.61±0.14 <sup>a</sup>	0.80±0.20ª	$0.69 \pm 0.16^{a}$	$0.71 \pm 0.13^{a}$	0.78±0.12 <sup>a</sup>
		Bighead carp			
Initial mean weight (g/fish)	25.6±0.0 <sup>a</sup>	25.6±0.0 <sup>a</sup>	25.6±0.0ª	25.6±0.0 <sup>a</sup>	25.6±0.0 <sup>a</sup>
Initial total weight (g/100m <sup>2</sup> )	194.4±0.5ª	191.6±2.0 <sup>a</sup>	193.5±0.8ª	194.4±0.2 <sup>a</sup>	192.6±1.3ª
Final mean weight (g/fish)	203.8±52.3ª	165.8±20.7ª	217.6±21.9ª	224.5±32.6ª	272.7±1.6 <sup>a</sup>
Final total weight (kg/100m <sup>2</sup> )	1.2±0.4 <sup>a</sup>	$0.8{\pm}0.1^{a}$	1.2±0.2ª	1.1±0.1ª	1.7±0.2ª
DWG (g/fish/day)	$0.85 \pm 0.25^{a}$	0.67±0.10 <sup>a</sup>	$0.91 \pm 0.10^{a}$	$0.95{\pm}0.16^{a}$	1.18±0.01ª
TWG (kg/pond)	$1.0{\pm}0.4^{a}$	0.6±0.1ª	$1.0\pm0.2^{a}$	0.9±0.1ª	$1.5{\pm}0.2^{a}$
Survival (%)	73.8±4.7 <sup>a</sup>	$67.7 \pm 6.0^{a}$	$69.5\pm6.5^{a}$	$62.7 \pm 4.5^{a}$	$83.4\pm8.9^{a}$
Extrapolated GFY (t/ha/yr)	$0.21\pm0.07^{a}$	$0.14\pm0.15^{a}$	0.20±0.03ª	$0.18 \pm 0.02^{a}$	0.30±0.03ª
Extrapolated NFY (t/ha/yr)	$0.17 \pm 0.07^{a}$	0.11±0.12 <sup>a</sup>	$0.17 \pm 0.03^{a}$	$0.15{\pm}0.02^{a}$	0.26±0.03ª
		Grass carp			
Initial mean weight (g/fish)	$0.1\pm0.0^{a}$	$0.1\pm0.0^{a}$	$0.1\pm0.0^{a}$	$0.1\pm0.0^{a}$	$0.1\pm0.0^{a}$
Initial total weight (g/100m <sup>2</sup> )	$3.2 \pm 0.0^{a}$	3.2±0.0 <sup>a</sup>	$3.2\pm0.0^{a}$	3.2±0.0 <sup>a</sup>	3.2±0.0 <sup>a</sup>
Final mean weight (g/fish)	234.2±54.7ª	245.1±21.8ª	247.0±45.2ª	286.5±54.7ª	241.7±15.3ª
Final total weight (kg/pond)	3.9±1.0 <sup>a</sup>	3.3±0.7 <sup>a</sup>	$4.7{\pm}1.5^{ab}$	$4.0{\pm}0.4^{b}$	4.1±0.6 <sup>a</sup>
DWG (g/fish/day)	1.66±0.26 <sup>a</sup>	1.84±0.50 <sup>a</sup>	1.73±0.10 <sup>a</sup>	$2.01\pm0.25^{a}$	2.11±0.29 <sup>a</sup>
TWG (kg/pond)	$3.9{\pm}1.0^{a}$	3.3±0.7ª	$4.7{\pm}1.5^{a}$	4.0±0.4ª	4.1±0.6 <sup>a</sup>
Survival (%)	55.6±7.4ª	46.5±12.3ª	$60.7{\pm}10.0^{a}$	52.3±15.2ª	58.6±12.1ª
Extrapolated GFY (t/ha/yr)	0.68±0.18ª	0.57±0.13ª	$0.82 \pm 0.27^{a}$	$0.70 \pm 0.07^{a}$	0.71±0.10 <sup>a</sup>
Extrapolated NFY (t/ha/yr)	0.68±0.18 <sup>a</sup>	0.57±0.13ª	$0.82 \pm 0.27^{a}$	$0.70{\pm}0.07^{a}$	0.71±0.10 <sup>a</sup>

		Common carp			
	$T_1$	$T_2$	<b>T</b> <sub>3</sub>	$T_4$	
Initial mean weight (g/fish)	4.5±0.0 <sup>a</sup>	$4.5 \pm 0.0^{a}$	$4.5\pm0.0^{a}$	$4.5\pm0.0^{a}$	$4.5\pm0.0^{a}$
Initial total weight (g/100m <sup>2</sup> )	135.1±0.1ª	135.1±0.1ª	134.8±0.1ª	135.3±0.2ª	135.0±0.2ª
Final mean weight (g/fish)	353.1±55.6 <sup>a</sup>	391.5±105.3ª	368.7±21.6 <sup>a</sup>	426.4±51.9 <sup>a</sup>	447.1±60.6 <sup>a</sup>
Final total weight (kg/100m <sup>2</sup> )	5.0±0.9 <sup>a</sup>	5.0±1.0 <sup>a</sup>	5.6±1.0 <sup>a</sup>	6.5±0.3 <sup>a</sup>	6.3±1.0 <sup>a</sup>
DWG (g/fish/day)	1.11±0.26 <sup>a</sup>	1.17±0.10 <sup>a</sup>	1.18±0.22 <sup>a</sup>	1.36±0.26 <sup>a</sup>	1.15±0.07 <sup>a</sup>
TWG (kg/pond)	4.8±0.9 <sup>a</sup>	4.9±1.0 <sup>a</sup>	5.5±1.0 <sup>a</sup>	6.4±0.3 <sup>a</sup>	$6.2{\pm}1.0^{a}$
Survival (%)	46.5±4.0 <sup>a</sup>	45.8±10.1 <sup>a</sup>	50.2±6.5 <sup>a</sup>	52.4±6.1 <sup>a</sup>	49.2±11.1 <sup>a</sup>
Extrapolated GFY (t/ha/yr)	0.86±0.16 <sup>a</sup>	0.88±0.17ª	0.98±0.17ª	1.14±0.06 <sup>a</sup>	1.10±0.17 <sup>a</sup>
Extrapolated NFY (t/ha/yr)	0.84±0.16 <sup>a</sup>	0.85±0.17 <sup>a</sup>	0.96±0.17 <sup>a</sup>	1.11±0.06 <sup>a</sup>	$1.08\pm0.17^{a}$
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Parameters		Treatm	nents		
Initial mean weight (g/fish)	4.3±0.0 <sup>a</sup>	4.3±0.0 <sup>a</sup>	4.3±0.0 <sup>a</sup>	4.3±0.0 <sup>a</sup>	4.3±0.0 <sup>a</sup>
Initial total weight $(g/100m^2)$	161.3±0.1ª	160.9±0.3ª	160.9±0.1ª	160.8±0.2ª	161.3±0.1ª
Final mean weight (g/fish)	209.5±15.4ª	198.9±18.1ª	225.2±13.7ª	207.1±13.9ª	316.4±55.8
Final total weight (kg/100m <sup>2</sup> )	4.9±0.5 <sup>a</sup>	6.4±0.9 <sup>a</sup>	5.7±1.1ª	6.6±0.9ª	5.8±0.8 <sup>a</sup>
DWG (g/fish/day)	0.85±0.01 <sup>b</sup>	0.96±0.05 <sup>ab</sup>	0.93±0.05 <sup>ab</sup>	0.97±0.04 <sup>ab</sup>	1.01±0.03 <sup>a</sup>
TWG (kg/pond)	4.7±0.5 <sup>a</sup>	6.2±0.9 <sup>a</sup>	6.5±0.9 <sup>a</sup>	9.6±0.6 <sup>a</sup>	5.7±0.8 <sup>a</sup>
Survival (%)	71.3±0.9 <sup>a</sup>	$82.2 \pm 7.8^{a}$	74.2±11.0 <sup>a</sup>	84.2±9.7 <sup>a</sup>	752.1±9.7ª
Extrapolated GFY (t/ha/yr)	$0.85 \pm 0.09^{a}$	1.11±0.06 <sup>a</sup>	0.98±0.19 <sup>a</sup>	1.15±0.16 <sup>a</sup>	1.01±0.14 <sup>a</sup>
Extrapolated NFY (t/ha/yr)	0.83±0.09 <sup>a</sup>	1.08±0.16 <sup>a</sup>	0.96±0.19ª	1.12±0.16 <sup>a</sup>	0.98±0.14 <sup>a</sup>
		Mrigal			
Initial mean weight (g/fish)	4.5±0.0 <sup>a</sup>	4.5±0.0 <sup>a</sup>	4.5±0.0 <sup>a</sup>	4.5±0.0 <sup>a</sup>	4.5±0.0 <sup>a</sup>
Initial total weight (g/100m <sup>2</sup> )	67.4±0.3 <sup>a</sup>	66.9±0.4 <sup>a</sup>	$67.5 \pm 0.2^{a}$	67.1±0.1ª	67.6±0.1ª
Final mean weight (g/fish)	195.2±35.0 <sup>a</sup>	191.7±25.8ª	172.3.8±23.6 <sup>a</sup>	171.8±36.6ª	197.0±20.4
Final total weight (kg/100m <sup>2</sup> )	$2.4\pm0.4^{a}$	2.1±0.4 <sup>a</sup>	2.1±0.5 <sup>a</sup>	2.3±0.5ª	2.4±0.5 <sup>a</sup>
DWG (g/fish/day)	$0.91 \pm 0.17^{a}$	0.89±0.12ª	0.80±0.11ª	0.80±0.17ª	0.92±0.10 <sup>a</sup>
TWG (kg/pond)	2.3±0.4 <sup>a</sup>	$2.0\pm0.4^{a}$	2.1±0.5 <sup>a</sup>	2.2±0.5ª	2.4±0.5 <sup>a</sup>
Survival (%)	81.6±3.6 <sup>a</sup>	$71.9 \pm 6.6^{a}$	80.3±9.4 <sup>a</sup>	87.8±4.2ª	80.3±9.6 <sup>a</sup>
Extrapolated GFY (t/ha/yr)	$0.41 \pm 0.06^{a}$	$0.36 \pm 0.08^{a}$	$0.37{\pm}0.08^{a}$	0.39±0.09ª	0.42±0.09 <sup>a</sup>
Extrapolated NFY (t/ha/yr)	$0.40\pm 0.06^{a}$	0.35±0.08 <sup>a</sup>	0.36±0.08ª	0.38±0.09ª	0.41±0.09 <sup>a</sup>
NFY carp only (t/ha/yr)	3.63±0.49 <sup>a</sup>	$3.87{\pm}2.78^{a}$	4.05±0.74 <sup>a</sup>	4.28±0.97 <sup>a</sup>	4.34±3.75 <sup>a</sup>
NFY of SIS only (t/ha/yr)	$0.07\pm0.47^{a}$	$0.07 \pm 2.78^{a}$	0.1±0.02 <sup>a</sup>	$0.1\pm0.97^{ab}$	0.08±3.75 <sup>a</sup>
Combined GFY (t/ha/yr)	3.70±0.4 <sup>a</sup>	3.93±0.28 <sup>a</sup>	4.15±0.74 <sup>a</sup>	4.38±0.10 <sup>a</sup>	4.41±0.37 <sup>a</sup>
Combined NFY (t/ha/yr)	3.59±0.49ª	3.82±0.28 <sup>a</sup>	4.03±0.74 <sup>a</sup>	4.27±0.10 <sup>a</sup>	4.30±0.37 <sup>a</sup>
Feed conversion ratio (FCR)	2.0±0.0 <sup>b</sup>	1.5±0.1ª	1.7±0.2 <sup>ab</sup>	1.8±0.1 <sup>ab</sup>	1.9±0.2 <sup>ab</sup>
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Rai, Gharti, Shrestha, Ranjan, Diana and Egna / Our Nature (2018), 16 (1): 8-16

	T <sub>C</sub>	T <sub>SB</sub>	$T_{WB}$	$T_{BM}$	$T_{PB}$
Cost					
Carp fingerlings	488±0	487±1	487±0	488±0	488±0
Lime	72±0	72±0	72±0	72±0	72±0
Urea	164±1	166±0	166±0	165±1	166±0
DAP	329±1	333±2	330±1	331±1	245±89
Feed	1368±192 <sup>a</sup>	$1066 \pm 75^{a}$	1306±288 <sup>a</sup>	1452±117 <sup>a</sup>	1489±25 <sup>a</sup>
Total Variable Cost	2422±193ª	2124±74 <sup>a</sup>	2362±287 <sup>a</sup>	2509±115 <sup>a</sup>	2460±111ª
Return					
Carp	$6258 \pm 846^{a}$	6678±461 <sup>a</sup>	6993±1281ª	7386±206 <sup>a</sup>	7485±636 <sup>a</sup>
SIS	83±0 <sup>a</sup>	75±17 <sup>a</sup>	109±26 <sup>a</sup>	115±27 <sup>a</sup>	87±13 <sup>a</sup>
Gross Return	6342±846 <sup>a</sup>	6753±473 <sup>a</sup>	7102±1283 <sup>a</sup>	7501±180 <sup>a</sup>	7572±643 <sup>a</sup>
Gross Margin	3920±655ª	4630±440 <sup>a</sup>	4741±1062 <sup>a</sup>	4992±164 <sup>a</sup>	$5111 \pm 749^{a}$

**Table 7.** Gross margin (Rs/100 m<sup>2</sup> pond) analysis for each treatment after 210 days

return from carp and SIS, gross return and gross margin among different treatments. Equal yield of carp and SIS in control and treatment ponds resulted same return and gross margin among ponds. Although considerably higher gross return and gross margin was found in substrate ponds compared to control ponds but values were statistically similar.

#### Conclusion

Farming carp in earthen ponds with small indigenous species (SIS) is a sustainable fish production system for small scale farmers in Nepal. It provides both family nutrition and income from consuming more micro-nutrient rich SIS and selling surplus carp, respectively. Adding locally available substrates to the carp-SIS ponds enhances periphyton production which in turn increases growth and yield of carp and reduces feed cost. Adding substrates to the ponds also discourage poaching which is a common problem among small scale farms where ponds are not guarded. Among substrates used in the present trial split bamboo, whole bamboo and plastic bottles are more durable and have potential for periphyton enhancement.

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