ISSN 2645-8357

Nepalese Journal of Aquaculture and Fisheries

The Journal of the Nepal Fisheries Society

Volume 6 & 7

2019 & 2020

ISSN 2645-8357

Nepalese Journal of Aquaculture and Fisheries

The Journal of the Nepal Fisheries Society

Volume 6 & 7 2019 & 2020

Content

Nepalese Journal of Aquaculture and Fisheries
Vol. 6 & 7 (2019 & 2020) : 1-12

STATUS AND DEVELOPMENT TREND OF AQUACULTURE AND FISHERIES IN NEPAL

Pramod Kumar Rijal and Subhash Kumar Jha*
Central Fisheries Promotion and Conservation Center, (CFPCC), Balaju, Kathmandu
*Email: jhasuvas2012@gmail.com

ABSTRACT
Aquaculture is one of the fastest growing agricultural subsectors in Nepal. The current total national fish production is 91,832 mt of which 23% is contributed by capture fisheries while, 77% is from aquaculture. Aquaculture and fisheries together have generated direct employment opportunities for about 6 lakh people. Fish consumption trend is increasing in Nepal. During the period of 1981/82 to 2018/19 annual per capita fish availability by national production has been significantly improved from 330 g to 3420 g. Timely supply of quality seed is essential for aquaculture development. Thus, private sectors have been encouraged for seed production, rearing and supply while government is playing role in quality control aspects. There are various fish marketing strategies exist in Nepal. Fish are sold either by producer themselves from production site or through agent, contractor or by the support of whole-sellers. Fish demands in market varies usually according to seasons and occurrence of festivals. Higher fish demand occurs in winter, while least fish consumption is found in rainy season during monsoon. In fiscal year 2018/19, domestic production occupied 89% and imported fish occupied 11% of the total national fish consumption whereas fish export remained negligible.

Keywords: Fish production, pond productivity, employment, per capita production

INTRODUCTION
Aquaculture is one of the fastest growing agricultural subsectors in Nepal. Having landlocked in nature, Nepal depends only on inland aquaculture with finfish farming. Climatic condition favors cultivation of both warm and cold-water species. The most common species under cultivation are indigenous and exotic Carps, Pangas, Tilapia, Magur catfish and Rainbow trout. Institutional development of aquaculture in Nepal was started almost seven decades ago but its development pace was rather slow. Nevertheless, the progress achieved by this sector in last decade is highly commendable. Government programs like fish mission, one village one product, resource center establishment, Prime Minister Agriculture Modernization Project (PMAMP) etc. are the key factors in the developmentof this sector. Fish consumption in Nepal is rather low compared to poultry, pork, buff and mutton. Increasing health awareness among people has led to rise in fish consumption demanding more aquaculture industries. Government of Nepal is also providing support to establish commercial farms, which generate employment as well as income in rural areas. Most of the newly established farms are run by the youths those are back from abroad employment and have contributed in reduction of youth migration to some extent.

This paper discusses present status of aquaculture and fisheries, its contribution in economic development and employment generationand provides information on aquaculture/fisheries sector and its development trend in Nepal. This paper will be useful to planners and policy makers in identifying intervention areas and developing appropriate fisheries and aquaculture policies, plans and programs for sustainable development of this sector.

ORGANIZATION DEVELOPMENT

History of Nepalese finfish aquaculture is very short however; catching fish from nature is being practiced since ancient time. In Nepal aquaculture development was institutionalized in 2003 BS (1946/47 AD) by establishing fisheries unit under Agriculture Council. This fisheries unit faced several phases of organizational modification time to time passing through the golden era of fisheries, in terms of organizational strength, when department of fisheries was established.

The first ever fisheries program in Nepal was initiated in 2004 BS (1947/48 AD) and aquaculture started from late 1950s by introducing Common carp (*Cyprinus carpio*) with successful breeding took place in mid 1960s. Three cultivable species of Chinese carps (*Ctenopharyngodon idella, Hypophthalmicthys molitrix, Aristichthys nobilis*) were introduced in the early 1970s followed by their successful induced breeding in mid 1970s. In the late 1970s breeding techniques of indigenous major carps (*Labeo rohita, Cirrhina mrigala and Catla catla*) were established (Singh, & Yadav, 1996) which was significant achievement in aquaculture history that provided momentum to polyculture system in Nepal.

Previously, Directorate of Fisheries Development (DoFD) and Fisheries Development Centers (FDC) was under Department of Agriculture (DoA), Ministry of Agricultural Development (MoAD) is the focal government organization for aquaculture development whereas fisheries research was being carried out by the Fisheries Research Division (FRD) under Nepal Agricultural Research Council (NARC). Aquaculture and Fisheries education is being provided mainly by Agriculture and Forestry University (AFU) and Tribhuvan University (TU) in Nepal. According to the constitution of Nepal, 2072; restructuring of the entire government organization has been carried out with different role and responsibilities under Central, Province and Local Government. The new structure of government has given the new name to the previous Directorate of Fisheries Development (DoFD) as Central Fisheries Promotion and Conservation Center by the fiscal year of 2074/75. The fisheries and aquaculture development program are one of the important commodity programs of Government of Nepal, Ministry of Agriculture and Livestock Development, Department of Livestock Services. It is carried out through the Central Fisheries Promotion and Conservation Center (CFPCC) under the Department of Livestock Services. The Center is the commodity specific national focal body. It is responsible for central level policy issues, planning, monitoring and supervision, data base, regulatory functions etc. It also coordinates with national and international fisheries and aquaculture related institutions. There are three centers under the CFPCC:

- Fisheries Human Resources Development and Technology Validation Center, Janakpur
- Natural Water Fisheries Promotion and Conservation Center, Hetauda
- Fisheries Pure Line Breed Conservation and Promotion Resource Center, Bhairahawa

There are seven provincial Directorate of Livestock and Fisheries Development (DoLFD) one each in seven provinces, responsible for carrying out Livestock and Fisheries program, regulatory functions within the province and coordination between federal and local level institutions. Under DLF, there are forty-seven Veterinary Hospital and Livestock Services Expert Centers (VHLSECs). Out of forty-seven VHLSECs, twenty-one have fisheries technician, who are responsible for carrying out aquaculture and fisheries extension program within their respective districts. Likewise, there are seven provincial Fisheries Development Centers (FDCs), mandated for fish seed production and distribution, technical support services and basic laboratory services.

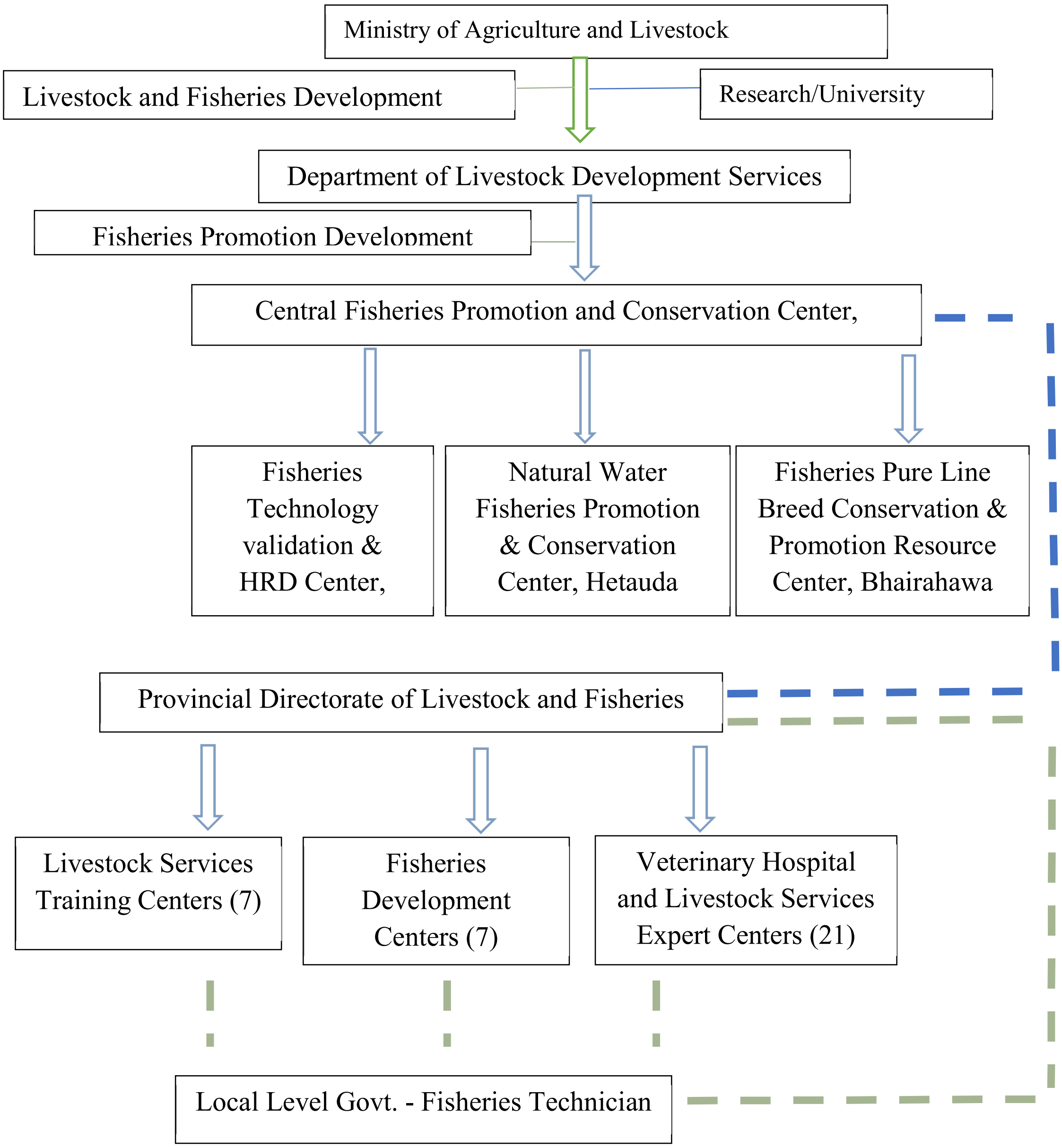

Figure 1: Institutional frameworks for fisheries and aquaculture development in Nepal

NATURAL WATER RESOURCES

Nepal is rich in natural water resources. Rivers, lakes, reservoirs, swamps and low land irrigated paddy fields are the major source of fresh water in Nepal (Figure 2). Among them rivers and low land irrigated paddy fields are the most dominant natural water resources. Besides, these natural waters, Gurung (2014) has reported 7,900 km of irrigation canals in the country.

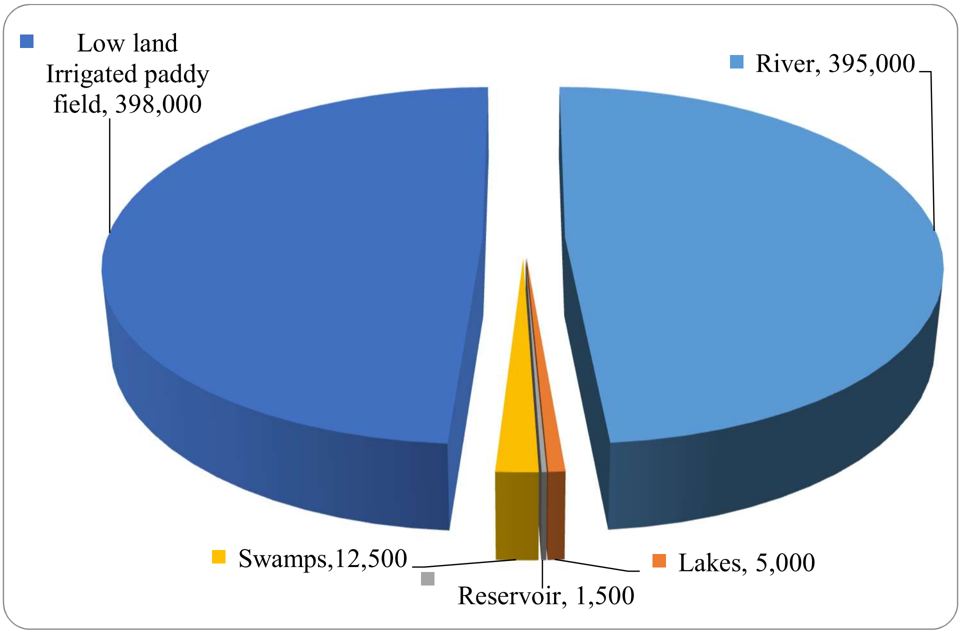

Figure 2: Natural waterresources (ha) in Nepal

Capture fisheries is an important sector because of its role in fish production as well as employment generation. Capture fisheries production is 21,000 mt which seems almost constant from last several years. Irrigated paddy fields, rivers and swamps have significant contribution in capture fish production whereas reservoirs and lakes have least contribution (Figure 3). Lakes and reservoirs occupy less water surface area compared to other natural water resources as well.

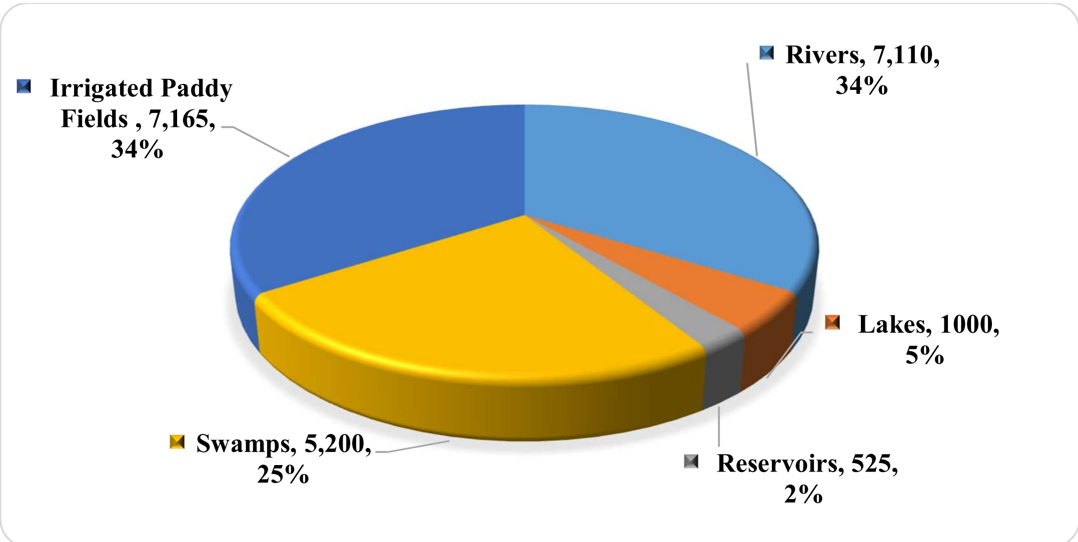

Figure 3: Fish capture (mt) from various water bodies

STATUS OF AQUACULTURE AND FISHERIES

Nepalese aquaculture is in growing stage and the amount of fish production is comparatively low to many large countries of the world, however, the progress achieved in this sector in recent years is highly encouraging. The pond aquaculture with common carps, Chinese and Indigenous Major Carps significantly dominates the overall fish production with average productivity of 4.91 mt/ha. Monoculture of common carp, tilapia and especially that of catfish are also done in few places in different of the country. Interest in aquaculture is growing fast among the youth farmers and has expanded to 55 districts out of 75 districts compared to 30 districts a decade ago (Chaudhary, & Jha, 2018) especially after the successful innovation of rainbow trout farming technologies in colder regions of hills and mountains. Presently, polyculture technology of carp fish farming in ponds have been widely disseminated in the southern plain areas and mid-hill parts of the country and become the viable and common aquaculture activity which alone generated 67.52% (70,832 mt) of the total aquaculture production in 2017/18 (CFPCC, 2018/19) (Table 1). Intensive farming of *Cirrhinus mrigala* under single stocking and multiple harvesting to produce smaller size fish, called *Chhadi*, is also a successful farming system in Nepal. The pond production system has been categorized into the extensive, semi-intensive and intensive level.

Table 1: Status of aquaculture production in 2018/19 (CFPCC, 2018/19)

Particulars	Pond (nos.)	Total Area (ha)	Fish Production (mt)	Productivity
Aquaculture production	-	-	70,832	-
Pond Fish culture	44,897	12,749	62,725	4.92 mt/ha
Other area (swamps)	-	3,816	7,289	1.91 mt/ha
Cage fish culture (m^3)	-	72,500	305	4.21 kg/m^3
Trout Fish Culture in Raceway	-	3.20	420	130 mt/ha
Others (Govt. Farm, Paddy cum fish culture and enclosure)	-	98.8	93	0.94 mt/ha
Capture Fisheries production	-	-	21,000	-
Total Fish Production (mt)	-	-	91,832	-

Total annual production of fish in the country has been increased from 3,530 mt in 1981/82 to 91,832 mt in 2018/19, while there has been a significant increase in the annual per capita fish availability or consumption from 0.33 kg in 1982 to 3.11 kg in 2018/19 (CFPCC, 2018/19).

Aquaculture is one of the fastest growing agricultural sectors in the last three decades with an annual growth of nearly 11.6 % (Wagle et al., 2011). Aquaculture in Nepal contributes about 1.13% and 4.18% of Gross Domestic Production (GDP) and Agriculture Gross Domestic Production (AGDP), respectively. Nevertheless, climate change, extreme weather events, non-climatic extremes, competition for water use, rapid urbanization, land fragmentation, overfishing or illegal exploitation of fishery, low aquaculture productivity, undermining of aquaculture in national plan and policies are compounding challenges to the sustainability of inland aquaculture. Increasing aquaculture production in the country has significantly contributed to the national food supply as well as economic development as have been shown by present level of fish availability than earlier.

Capture fisheries production in Nepal is 21000 mt (Table 2). There are around 4 lakh people engaged in capture fisheries, among them 59% are women. The women in fisheries are also are not only engaged in preparing fishing gears, equipment, fishing but also inselling fish in the market.

Table 2: Status of capture fisheries production in 2018/19 (CFPCC, 2018/19)

Particulars	Total Area (ha)	Fish production (mt)	Productivity (kg/ha)
Aquaculture production	-	91,832	-
Capture Fisheries production	-	21,000	-
Rivers	395,000	7,110	18
Lakes	5,000	1000	200
Reservoirs	1,500	525	350
Swamps	9,000	5,200	578
Low land Irrigated Paddy Fields	398,000	7,165	18
Total Fish Production (mt)	-	91,832	-

After pond aquaculture, second contributor in fish production is swamps. There are 3,500 ha area of swamps being used in aquaculture with 7,289 mt fish production in 2018/19. Most of these swamps are concentrated in mid-western and far-western Terai region of Nepal. However, to make these swamp more productive for various uses their restoration, maintenance and management may require soon for sustainability of such natural resources and marginalized communities depending on these resources for food, nutrition, livelihood and employment opportunities.

Fish production from cage culture practices in lakes and reservoir contributed about 305 mt fish in 2018/19. Cage fish culture technology in Nepal was used for the first time in 1972 in Lake Phewa to raise brood fish of common carp. The present estimate shows that fish culture coverage area has reached nearly 71,000 m^3 with average fish productivity of 4.2 kg/m^3. In cage fish cultivation, majorly plankton-feeding fish are cultivated where the fish inside the cage subsist on naturally available phytoplankton, zooplankton, detritus and some aquatic vegetation for their growth. Generally, feed from outside is not applied to raise the fish in the cage. However, it is likely this practice may be overlooked soon because the profitability of adopting the feeding practices in cages would be more attractive. Subsistence cage fish cultivation is a proven technology of income generation for landless fisher communities. However, cage culture is confined to only few lakes of Pokhara Valley and Kulekhani reservoir. This technology can be extended in other potential water bodies, especially in reservoirs in future. Vast area of reservoirs will be added when all the hydroelectric projects are accomplished, which shows great potential for cage culture in Nepal.

Rice cum fish culture is a popular farming technique in India (Asam, Meghalaya), Indonesia, China and Bangladesh. Rice field is not only used for fish but also for duck, ornamental fish, crab, and prawn production. Rice cum fish culture is successful in neighboring countries but this farming system could not get much attention in Nepal due to which only limited area of paddy fields are used for this culture system. However, to promote rice-fish farming in Nepal especially on wide and more productive areas the Terai and lower mid hill regions should be prioritized launching special long-term projects in future.

Rainbow trout, a cold-water species, was introduced for the first time in 1969 from India and second time from England and then introduced for third time from Japan in 1988 (Rai, 2010). Commercial farming started from Rasuwa and Nuwakot districts under Nepal Agricultural Research Council, Japan International Cooperative Agency (2007) and one village one product (OVOP) program joint collaboration of Directorate of Fisheries Development Program, Agriculture Enterprise Center, and Fisheries Research Division of NARC. With the technological innovation of highly commercial rainbow trout aquaculture, today trout culture has spread to 38 hill and mountainous districts of Nepal with prospects to expand in all hilly areas. Among these districts, Kaski is the leading trout producing district in Nepal. By the end of fiscal year 2018/19, trout production has reached to 320 mt. Trout is a unique and the most expensive fish species in Nepali market because of its taste and high nutritional value. Trout farm integrated with restaurant is a common and successful practice in Nepal which is necessary mainly for small-scale farmers to sustain their business.

EMPLOYMENT GENERATION BY FISHERIES SUBSECTOR

Like other developing countries, employment is a serious problem in Nepal. Large number of youths migrates annually in search of job. In this context, fisheries sub-sector can be an alternate to minimize youth migration by providing them employment opportunities in various fisheries and aquaculture related activities. Nepali economy is largely dependent on remittance. Such economyis likely to be unstable thus could jeopardize. Therefore, expansion of aquaculture might be one of the options to overcome such outmigration problem and create jobs within the nation to attract youngsters for national development.

Aquaculture contribution in employment generation

Aquaculture plays significant role in employment generation through various steps of value chain. Both men as well as women of different age are involved in aquaculture value chain. There are about 1.5 lakh people directly engaged in this sub-sector among them male represents 68%, while females only 32%.

Capture fisheries contribution in employment generation

Natural water especially rivers, swamps and lakes are the source of economy to many fisher communities. Approximately twelve different ethnic communities are involved directly or indirectly in fisheries (Gurung, 2005). These communities live near water resource depending on fisheries and aquatic resource for their livelihood from generation to generation. There are about 4.5 lakh people engaged in capture fisheries among them 60% are female. Females in capture fisheries also contribute in preparing fishing gears, nets and other equipment along with selling fish in the market. There was unrecorded amount of indigenous aquatic plants (Water chest nut, Makhana, lotus and other plants) and freshwater shellfish (gastropod, crabs, and shrimp) which are harvested from wild. The shellfish in Nepal are consumed by substantial number of populations especially snail. The other shellfish such as crabs and shrimp are low volume high value commodities collected from oxbow lakes and other aquatic sources of flood plains. Under the capture fisheries soon the contribution of these shellfish and plant products should be reflected in national fisheries plan under the research and extension activities. Further the research work on shellfish and edible aquatic plants products should be prioritized soon.

AQUACULTURE DEVELOPMENT TRENDS

Among the various fish culture practices, pond fish culture is the dominant practice and is increasing rapidly while other aquaculture activities remained may standstill in last decades. From the very beginning of the aquaculture development in Nepal major aquaculture species were finfish culture. The major part of the finfishes is occupied by major carps with common carp and Chinese carps used for carp polyculture. The carp polyculture has substantial contribution for the aquaculture production in Nepal. It looked that aquaculture production in Nepal has increased with new technologies and the species introduced.

Introduction of the Rainbow trout has brought newer dimension in cold-water aquaculture and paradigm shift in aquaculture of Nepal. The introduction of Tilapia and Pangas have showed new dimension for promotion of monoculture system with massive production potential of aquaculture in Nepal. Innovation of new technology of 'chhadi fish' pond aquaculture technology has been fruitful to give faster return of investment to fish farmers. In this technology the fry/hatchlings are stocked heavily in pond, and at least 3-4 harvest in a year are performed to harvest the fish which has attained more than 30 g size onwards. The local consumer preferred to eat the smaller but single piece of from head to tail. This smaller grown out fish is called 'chhadi' fish. The national fish production has been increased by more than five folds during last 18 years from 17100 mt in 2001/02 to 70832 mt in 2018/19 (Figure 5).

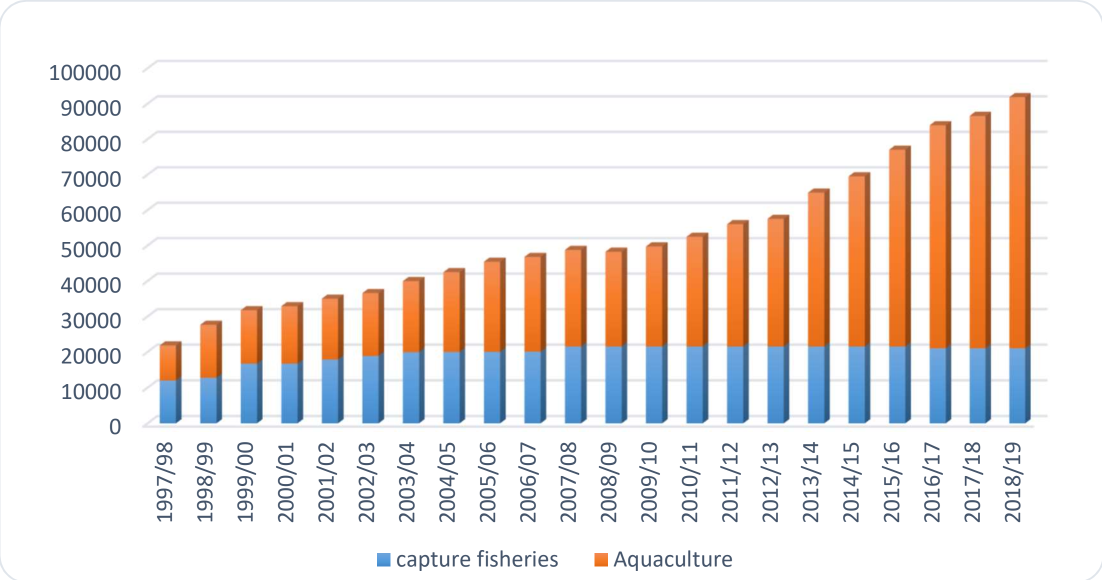

Figure 4: Fish production trend (Capture Fisheries and Aquaculture) in Nepal

Expansion of pond area

Aquaculture sector in Nepal has become good money returning sub-sector of agriculture. In Terai area of Nepal fish consumers are also increasing with the availability of the fish in market. At beginning only few farmers started pond aquaculture due to high investment in initial stage and inadequate technologies, expertise and many other infrastructural issues. Later, Government of Nepal initiated subsidy program for pond construction. The successful implementation of subsidy program for pond construction the popularity of pond fish farming increased. Presently government of Nepal has offered more subsidy program for aquaculture business those have been supportive for the popularity of pond fish aquaculture with increasing number and water surface area of fish cultivating ponds (Figure 5). The highest pond construction (734 ha) was achieved in the fiscal year 2015/16. Pond fish culture is dominant in Terai belt but its expansion in hill regions has also accelerated after implementation of pond expansion program in mid-hill districts from fiscal year 2011/12 by the government.

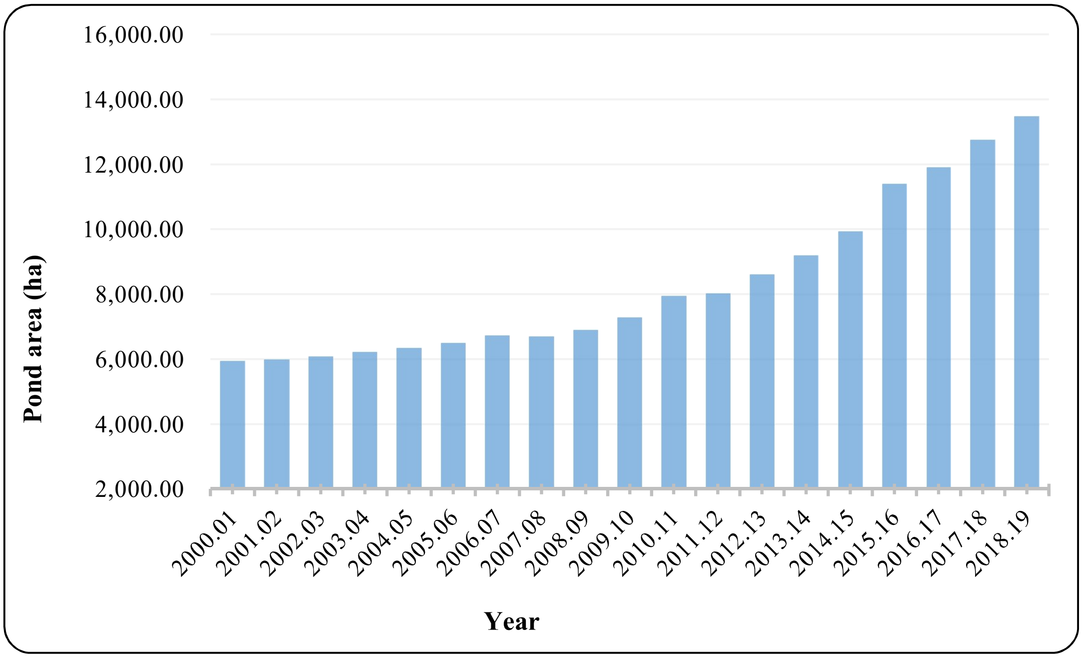

Figure 5: Aquaculture pond area (ha).

Pond fish productivity in 1981/82 was only 0.8 mt/ha, which has increased to 4.92 mt/ha in 2018/19. The pond fish productivity increase is collectively attributable to availability of fry, manuring, feeding, better management such introduction of aeration technology, control over fish diseases, training and good management practices (GMP) etc. Government of Nepal always emphasizing farm mechanization such as use of pellet machines to produce farm made cheap quality feed and use of aerators for improving water quality for achieving higher productivity.

National fish production

According to FAO country profile of Nepal only 500 mt of fish was produced in 1950. This production was probably contributed entirely by capture fisheries. Aquaculture production was started to record only from 1966 with total of 3 mt of fish production. Aquaculture production kept increasing slowly and steadily as the result of training, knowledge and education on aquaculture through extension and research. Capture fisheries yield showed increasing trend in the beginning but remained more or less constant since the year 2000 onwards. Even keeping this capture at standstill is a big challenge. Therefore, further evaluation of capture fisheries status and potential require to be revisited along with the status of freshwater snail, crab, shrimp, water chestnut and makhana collection and yield. It would also essential to estimate the communities and population that consume these products along with farming perspectives of these fisheries commodities. Production status of fiscal year 2018/19 shows that out of 91,832 mt fish production 23% comes from capture fisheries where as 77% from aquaculture.

Because of increasing national fish production, per capita fish consumption is also increasing. From 1981/82 to 2017/18 it has been significantly increased from 330 g to 3.39 kg, but this is still very low compared to global average of 16 kg per capita (Gurung, 2014).

FISH SEED SUPPLY

Seed is one of the most important inputs for aquaculture production. Quality seed is must to enhance productivity of aqua farms. In Nepal, fish seed are distributed in three forms: hatchlings: 4-5 days old, fry: 2-3 cm or ~1 g and fingerlings: 2-7 g body weight each in average. Both public and private sectors are contributing for seed supply. There are 14 Governments (CFPCC & NARC) and 83 private hatcheries, 235 nurseries and thousands of fish seed traders working in Nepal. For easy access to fish seed, especially designed fish seed markets are requiring to develop soon.

In last decade, seed supply by public sector remained more or less constant while private sector has jumped from 5.7 million in 2001/02 to 220 million in 2017/18 (Table 3.) because of government priority to encourage private sector in seed supply. To empower private sector, various supportive programs are being launched like establishing fish seed resource centers under private ownership.

Table 3: Status of fish seed production in 2018/19 (CFPCC,2018/19)

A. Fish seed (Fry) Production/Distribution (No. in '000)	**339,224**
A[1] Public Sector	66,124
a. Hatchling*	212,600
b. Fry	16,220
c. Fingerling	15,360
A[2] Private Sector (Fry)	273,100

*Hatchling of public sector is distributed for fry production in private sector

Because of increasing demand, seed supply is challenging not only in terms of quantity but also in terms of quality. Nepal government is preparing fisheries policy, which is likely to beapprove soon.This policy will be a milestone in assuring quality seed supply within the country.

FISH MARKET

There is no single fish marketing strategy in Nepal. It varies from place to place. Farmers themselves sell their fish from the production site or send it to local markets. In case of huge production, fish is generally marketed through contractors. There are also farmers' organizations that produce fish and sell them through cooperatives. Harpan Phewa Matsya Sahakari working in Kaski district is a successful example of practicing such fish marketing system. There are also such cooperatives in Nawalparasi, Rupandehi and Kanchanpur districts (KBNPK, 2010). One of the hubs of dried or cooked or ready to eat fish market on the roadside in Malekhu. On both sides of the highway of about 4 km length, the hotels and restaurants serve the cooked fish. A detail survey might require estimating the volume of fish consumed, employment opportunities offered to male and female youths and economic transaction incur in this market due to the fish marketing. It is likely that throughout Nepal, if 20-40 such markets on the highway could developed, that night contribute substantially on local economic prosperity of the local communities. Recently concept of live fish marketing system has emerged, and the number of live fish shop is increasing. Government is also providing financial support to establish fish marketing stalls and collection centers. Most of the live fish stalls are concentrated in the capital and other big cities where demand of such fish is high. At present, it is reported to have around 50 live fish stalls in Nepal.

In last ten years, price of most of the agricultural commodity including fish has hiked up substantially. In 2001/02 price of fresh fish was reported to be Rs 100 per kg which is now reached Rs 300 per kg on average, but this price is still lower than price of other animal meat products. Therefore, it is the accessible source of animal protein for lower and middle-class citizens. Fish price varies from place to place and are more expensive in metropolitan and capital cities. Fish demand also varies from month to month. Study report shows higher fish demand in winter. The least fish consumption was reported to be in Asadh, Shrawan and Bhadra (KBNPK, 2010).

The demand of fish is not entirely fulfilled by national production; therefore, huge amounts of fish is imported. India is the major fish exporter while China, Vietnam, Bangladesh are other fish exporting nations to Nepal. According to the quarantine data, certain amounts of fish is also exported from Nepal, but this is negligible (Table 4). Due to long open boarder with India, all import/export dealings might have not been recorded properly in government channel. In fiscal year 2018/19, domestic production occupies 90.8% and import occupied 9.2%of the total national fish consumption which is higher than the import recorded in fiscal year 2017/18.

CONCLUSION AND RECOMMENDATION

Aquaculture is one of the blooming food sectors in Nepal. The growth rate of aquaculture is around 13%, which is the highest among the SAARC nations. Realizing its importance and potential in Nepal, aquaculture is receiving attention from the federal as well as provincial governments. Therefore, it is likely that there would be substantial jump in fish production in the country.

Increased demand of fish has created market opportunity and has attracted to establish commercial fish farms. Technical support to newly established farms is necessary to make them competitive in local, regional and global market. The knowledge of our technical human resource still inadequate to represent aquaculture of 21st century due to limited exposure to study and training programs. Specialized hands-on trainings and studies in specific field like fish breeding, disease, nutrition, genetics and water quality is necessary which should be addressed by concerned authorities in coming days. Strong coordination mechanism is also required among development, research and educational institutions for capacity building and implementing aquaculture and fisheries program effectively and efficiently.

Pond aquaculture is the dominating and prioritized fish farming practice. However, marginal swamps occupying 12,500 ha area should not be neglected. So far only 28% of them are utilized in aquaculture, therefore proper planning and management is required for their optimal utilization in fish production, which can provide employment and income opportunities to many land less people. Availability of natural water resources like lakes, reservoirs, swamps make the nation highly potential for culture-based fisheries, which are still in underutilization for fish production. Water resource and climatic condition also favors cold water fisheries in Nepal and it is doing well specially in trout farming. To promote trout culture, it is must to minimize production cost that can attract more and more farmers in future and trout can be accessible to middle class consumers as well. To sum up, aquaculture being an important and potential agriculture sub-sectors an appropriate fisheries policy, which is currently lacking, is necessary to boost up the overall development of this commodity in Nepal.

Table 4: Import/Export fish and fisheries products

Year	Import						Export	
	Fresh Fish (mt)	Boneless fresh fish (mt)	Fish seed (no.)	Dried fish & Sidra (mt)	Fish meal (mt)	Aquarium fish (no)	Fresh Fish (mt)	Fish seed (no.)
2004/05	2547.38	-	949235	74,75	166.43		1.56	233475
2005/06	2058.11	-	1884200	246.07	1602.95		6.42	113000
2006/07	2261.23	-	849270	2510.83	30.02	549764	2.86	
2007/08	2034.77	-	172590	277.12	351.2	2611884	4.15	22300
2008/09	3469.94	-	14212	313.68	1097.75		134.65	25100
2009/10	4334.86	253.2	7493	315.23	432.2		850	
2010/11	5370.2	18	3287834	335.71	481	11158	0.36	
2011/12	7424.94	381.82	8975129	581.81	272.33	28972	0.095	
2012/13	9963.06	270.8	14564100	519.49	214.12	104548	0.2	
2013/14	12869.49	109.5		19882.79	82.86	217248		
2014/15	11176.87			825.4	376.11	256824	0.4	
2015/16	7153.48	125.6	7512360	2589.8	295.45	269825		
2016/17	11220	443.4	3781592	683.2	258.7	270979	0.115	
2017/18	10757	491				273528		
2018/19	9334	-	-	-	-	-	-	-

Source: Adopted from Chaudhary & Jha (2018) and CAQO (2018).

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Nepalese Journal of Aquaculture and Fisheries
Vol. 6 & 7 (2019 & 2020) : 13-18

COMMERCIAL AQUACULTURE ENTERPRISE IN NEPAL - A CASE STUDY

Anjala Khanal[1], Pratikshya Neupane[1], Madhav K Shrestha[2*], Suresh K Wagle[3], Deepak Rijal[3]

[1] Agriculture and Forestry University, Rampur, Chitwan
[2] Center for Aquaculture-Agriculture Research and Production, Kathar, Chitwan
[3] USAID Paani Program, Baluwatar, Kathmandu
*Email: madhavshrestha1954@gmail.com

ABSTRACT
Commercial aquaculture is recognized as one of the priority sectors by government of Nepal and expanding rapidly in recent years. There is lack of in-depth studies on commercial aquaculture relating to risks and opportunities for investment. A telephone survey with purposively sampled fifteen carp and six pangasius aqua-farms in Chitwan and Rupandehi districts was done to assess production and productivity, benefit-costs, and constraints for developing a profitable enterprise. The results showed that carp farm productivity was recorded at 6.9±1.9 mt/ha while it was 33.4±10.5 mt/ha for pangasius farms. The gross revenue secured was 1.9±0.5 million NPR/ha and 8.4±2.4 million NPR/ha from carp and pangasius farms, respectively. Gross margin and net profits recorded at 698±281 thousand NPR/ha and 496±265 thousand NPR/ha for carp farm, and 3.5±2.0 million NPR/ha and 3.3±1.9 million NPR/ha for pangasius farm. The benefit-cost ratio for carp farm and pangasius farm was 1.4 and 1.7, respectively. The costs for fish seed, fish feed, and total operation cost for pangasius farm was 10.2%, 68.9%, and 95.8% while for carp farm, these were 1.4%, 40.5%, and 85.5% of total cost, respectively. Pangasius farms were smaller in size with intensive culture, resulting in high return per unit area whereas carp farms which are larger and were possible only to adopt the semi-intensive culture and hence had lower returns compared to pangasius farms. The analysis clearly showed that both carp and pangasius farms are running in profits. We conclude that these assessment results provide minimal adequate confidence for investment in Nepal's aquaculture enterprise.

Keywords: Commercial aqua-farms, carps and pangasius, aquaculture enterprise, risk and opportunity,

INTRODUCTION
Aquaculture enterprises gained momentum in the country in the last ten years as the government emphasized and promoted this sector (Shrestha, 2020; CFPCC, 2020) (Figure 1). The dominant fish farming systems are carp-polyculture, pangasius-monoculture, African catfish-monoculture, trout race-ways, and tilapia-culture. Carp polyculture that includes 6-7 species is the primary fish production system covering more than 90% of production, followed by pangasius in the second rank (Chaudhary, & Jha, 2018). Nepal's southern Terai belt is the potential zone for farming warm water fishes. The top ten highest fish producing districts are Bara, Saptari, Dhanusha, Rupandehi, Siraha, Morang, Parsa, Rautahat, Sarlahi, and Chitwan (CFPCC, 2019). The government of Nepal has declared fish commodity super-zone districts to Bara, Dhanusa, and Rupandehi districts (CFPCC, 2020).

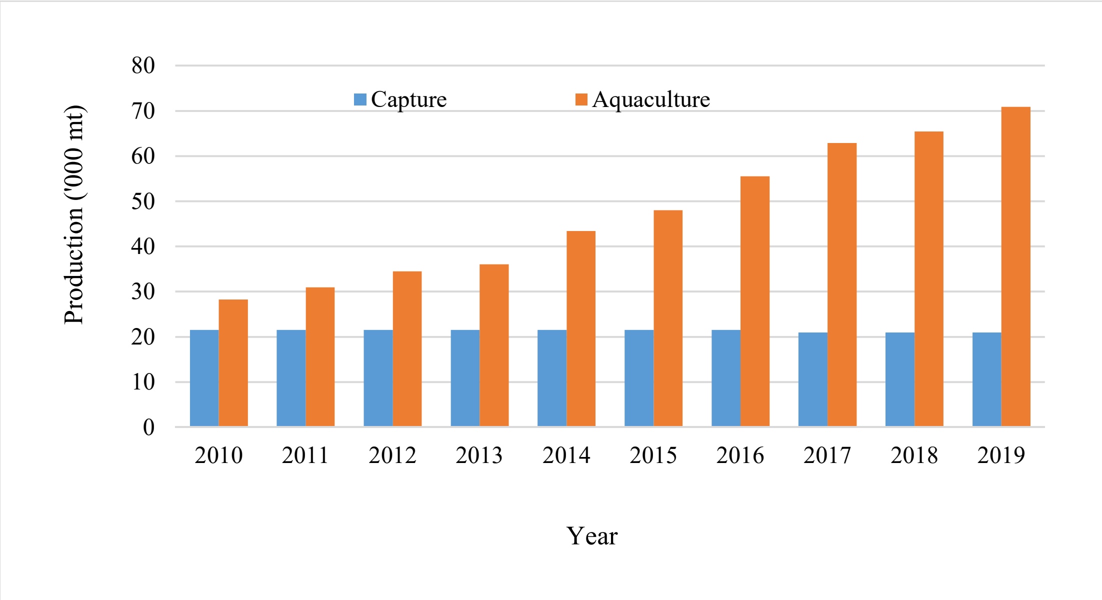

Figure 1: Fish production trend for last ten years period in Nepal (Shrestha, 2020)

Available fish production and productivity data for pond culture of the country is estimated grossly of all the species and covers all strata of culture systems, including small-scale, semi-commercial, and commercial scale. Pond production and productivity of two major aquaculture systems (carps and pangasius), which have been the major commercial systems for warm water species, do not have separate database. Thus this study aimed to assess fish production and productivity of commercial carps and pangasius aquaculture farms and their economic performance. This study also assesses the current status of carps and pangasius productivity on a commercial scale, which may provide opportunities and constraints for further private investment to Nepal's aquaculture enterprise.

METHODOLOGY

Two representative districts, Rupandehi and Chitwan, were selected for the survey (Figure 2). Selection made were based on districts of top ten highest fish production; one fish commodity super zone and district with growing commercial aquaculture; centrally located neighborhoods from east to west of Nepal, and the easy access market of major cities Kathmandu and Pokhara. Purposively selected fifteen farms from two districts were surveyed. The selection basis for aqua farms was relatively newly operated (minimum of 3 years) to a long-time operation, scale of operation with a minimum of 2 ha farm area. In consultation with the Fisheries Development Officers and District Fish Farmer's Association, 15 commercial aqua-farms (eight in Chitwan and seven in Rupandehi) were surveyed using a structured questionnaire. The telephonic survey was conducted from 27 October to 3 November 2020 during the lockdown situations.

Figure 2: Map showing location of selected districts for the survey

The collection of data and information was based on the year of 2019. The coded data on gross fish yield and gross revenue analyzed using MS excel. Production costs were calculated as total initial cost, total production cost by summing the total fixed price, and the total operating cost incurred in the production process. The cost incurred for inputs like lime, fertilizers fry/ fingerlings, feed, labor including hired and family labor, maintenance cost and electricity/ fuel, insurance, and bank interests, were considered operating expenses. The depreciation costs of fish ponds, deep & shallow tube well, feed house, including infrastructure (net shed, office, staff quarter), equipment and machinery, and rent on land tenure were considered a total fixed cost. The calculation was performed for gross margin (gross return – operating costs), net profit (gross margin - total costs), payback period (total capital investment/net profit), and benefit-cost ratio (gross return/total costs). Costs and revenues (NPR) presented as rounding values nearest 1000.

RESULTS

Farm sizes, fish yield, sale price, gross revenue, initial, fixed, operational, total cost, and economic parameters like gross margin, net profit, payback period, benefit-cost ratio, significant cost share of operating expenses, and fixed cost in total cost is presented in Table 1. Commercial farms selected for this survey ranged from 3 to 29 years under operation. Surveyed farm size ranged from 2.0 to 25.7 ha for carps and 0.1 to 4.7 ha. for Pangasius.

Mean fish yield of farms was 6.9 mt/ha/yr. for carps with a range of 3.5 to 9.4 and 33.4 mt/ha/yr. for pangasius with the range of 21.3 to 45.0 mt/ha/yr. with the mean gross revenue of 1.9 million/ha for carps and 8.4 million/ha for pangasius in Nepalese currency (NPR).

Table 1: Farm characteristics, fish productivity, costs and revenue, economic performance of commercial carps and pangasius farm.

Parameter	Carp farm (n=15)	Pangasius farm (n=6)
Farm size (ha)	2.0 -25.7	0.1 – 4.7
Gross fish yield (mt/ha/yr)	6.9±1.9 (3.8- 9.5)	33.4±10.5 (21.3 – 45.0)
Fish price (NPR/kg)	275±29 (230-325)	255±20 (220 - 280)
Gross revenue (NPR/ha)	1,899,000±580,000 (920,000 - 3,097,000)	8,431,000±2,378,000 (5,544,000– 11,250,000)
Total capital investment cost (NPR/ha)	1,401,000±727,000 (128,000–2,718,000)	1,072,000±417,000 (441,000–1595,000)
Fixed cost (NPR/ha/yr.)	203,000±133,000 (11,000–453,000)	214,000±125,000 (375,00–377,000)
Operating cost (NPR/ha/yr.)	1,200,000 ± 520,000 (317,000–2,071,000)	4,903,000±1,036,000 (4,060,000–6,410,000)
Total cost (NPR/ha/yr.)	1,403,000± 545,000 (645,000–2,524,000)	5,117,000±1,094,000 (6,694,000–4,098,000)
Gross margin (NPR/ha/yr.)	699,000±281,000 (316,000–1,260,000)	3,532,000±1,973,000 (1,336,000–6,915,000)
Net profit (NPR/ha/yr.)	496,000±265,000 (162,000–1,062,000)	3,318,000±1,888,000 (1,114,000–6,537,00)
Payback period (years)	3.7± 2.7 (0.3–8.6)	0.4± 0.4 (0.2–1.1)
Cost benefit ratio	1.4± 0.3 (1.1–2.3)	1.7± 0.4 (1.2–2.4)
Fish seed cost (%) in total cost	1.4 (0.5-2.4)	10.2 (9.4-10.6)
Feed cost (%) in total cost	40.5 (31.8-48.1)	68.9 (60.9-82.4)
Hired labor (%) in total cost	13.7 (7.6-19.6)	11.8 (1.5-17.8)
Bank loan (%) in total cost	18.4 (10.6-26.2)	0.7 (0.0-1.1)
Total operating cost (%) in total cost	85.5 (80.5-90.5)	95.8 (94.2-98-8)
Total fixed cost (%) in total cost	14.5 (9.5-19.5)	4.2 (3.2-5.8)

The mean initial costs and mean annual fixed costs were 1.4 million and 202 thousand NPR/ha for the carps' farm and 1.07 million and 214 thousand NPR/ha for pangasius farms. Mean annual operating costs were 1.2 million NPR/ha for carps and 4.9 million NPR/ha for pangasius. The mean yearly fixed values were 1.4 and 5.1 million NPR/ha for carps and pangasius farms, respectively. The mean annual gross margin and net profit for carps were 699 thousand and 496 thousand NPR/ha, whereas pangasius were 3.5 and 3.3 million NPR/ha. Pangasius farm showed better in terms of payback period and benefit-cost ratio than the carps' farm.

Fish seed cost, feed cost, and total operating costs for pangasius farm (10.2%, 68.9%, and 95.8% of total cost) were far higher than carps farm (1.4%, 40.5%, and 85.5% of total cost), respectively. However, hired labor cost, bank loan cost, and total fixed cost were higher in the Carps farm than the pangasius farm (Table 1).

DISCUSSION

The approach applied for the survey is relatively new and unusual to the respondents as well to the surveyors. Long-duration telephone calls might not have comfortable and cross-questioning to validate the data confirmation too. However, this was the only choice the team had to collect data during the Covid-19 pandemic situation and travel restriction. Since the sample size was too small therefore considered to be case studies rather than a systematic survey. However, fifteen case studies for carp aquaculture and six case studies for pangasius farming representing various farm sizes assume relatively reasonable and acceptable results. Survey results on carp productivity of 6.9 mt/ha with the range of 3.5 - 9.5 mt/ha in commercial aquaculture is higher than the national average of pond productivity 4.9 mt/ha (CFPCC, 2020) with varying systems of small-scale to commercial, and species besides carps. Carp productivity in India, under a semi-intensive system is 3-6 mt/ha (Jayasankar, 2018).

Similarly, pond productivity of pangasius 33 mt/ha with a range of 21-45 mt/ha seems reasonable as pangasius productivity in India is 15-50 mt/ha (Jayasankar, 2018). Carp aquaculture is farmed with semi-intensive culture with over 5-7 species, whereas pangasius is an intensive monoculture system. Carps utilize natural foods besides feeding supplied and are stocked at 1-1.5 fish/m^2, whereas pangasius is cultured in a feedlot system with a high stocking density of 8-10 fish/m^2. Pangasius farming is relatively new, and pangasius farming's operating cost is four times higher than carp farming. Pangasius farming showed a better benefit-cost ratio (1.7) compared to carp farming (1.4). However, both the aquaculture systems are looking viable and profitable.

Carp aquaculture has a relatively long history with the readily available fish seed at a lower price and lower feed costs than the imported fish seed of higher price for pangasius with high amount feed. It makes pangasius culture expensive with a high operating cost. Moreover, it is a truly tropical species and may record a tremendous mortality rate during the cold winter when raised under natural conditions. Though pangasius farming is highly profitable, there are fewer farms due to its high operational cost, seed cost and unavailability. Thus, the risk with intensive pangasius is seed and feed availability. Seed are completely dependent to India till date. Pangasius feed are mostly supplied from India. However, based on the production and profitability, and market, there is scope for aquaculture enterprise in Nepal. Moreover, the country imports fish to fulfill market demand.

CONCLUSION

The approach used in this survey provided baseline information. It will be useful to validate with a greater number of farms through in-person interviews. Survey results reveal that commercial carp aquaculture and pangasius aquaculture enterprises in Nepal are profitable and viable. Pangasius aquaculture needs high operating costs and provides high returns too. Fish seed supply is the major challenge for pangasius aquaculture, and until recently, Nepali farms import seeds from India. The establishment of a pangasius hatchery for seed production is needed to expand commercial pangasius aquaculture. Carps can tolerate more cold temperatures meaning it grows profitably to up to mid-hills and valleys. Intensive pangasius aquaculture may have some levels of risk and uncertainties in Nepal

RECOMMENDATION

- Commercial aquaculture of carps and pangasius is profitable and viable enterprise and private investors are recommended to invest on it.
- Establishment of pangasius hatcheries are recommended to ensure in-country seed availability.
- Similar survey study is recommended for carps in mid-hill aquaculture.
- Similar study is also recommended for commercial tilapia and trout aquaculture.
- This survey conducted with fewer farms and hence considered as a case study and recommend for a survey with sufficient sample size for a valid statistical analysis.

ACKNOWLEDGEMENT

This study is supported by the United States Agency for International Development (USAID). The authors also acknowledge the participating farmers of Chitwan and Rupandehi districts for their involvement in telephone survey process. The authors declare no conflicts of interest regarding the publication of this paper.

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Nepalese Journal of Aquaculture and Fisheries
Vol. 6 & 7 (2019 & 2020) : 19-30

GROWTH PERFORMANCE OF SEX REVERSED NILE TILAPIA (*Oreochromis niloticus*) IN DIFFERENT STOCKING DENSITIES

Bhup Narayan Mandal, Sunila Rai, Dilip Kumar Jha, Narayan Prasad Pandit*
Agriculture and Forestry University, Chitwan, Nepal
*Email: panditnp@gmail.com

ABSTRACT
Nile tilapia (*Oreochromis niloticus*) is a warm water fish with greater aquaculture potential. A study was conducted to determine the optimum stocking density of sex-reversed male Nile tilapia for production. Eight days old Nile tilapia fries were fed with 17 α-methyl testosterone for 23 days for sex reversal and used for growth experiment. The growth experiment was conducted in 2 m^2 hapas fitted in concrete tanks. The experiment was set up in a completely randomized design with three treatments in triplicate. The treatments were three stocking densities: 2 (T_1), 3 (T_2) and 4 (T_3) fish/m^2. All-male Nile tilapia of average weight 4.3 g was stocked. Fish were fed with 23% CP commercial floating pellet feed daily at 4% of the body weight. The average harvest weight of Nile tilapia in T_1, T_2 and T_3 were 192.2±7.3, 176.7±2.5 and 192.7±7.9 g, respectively. The survival rate of Nile tilapia in T_1, T_2 and T_3 were 100±0, 100±0 and 95.8±4.2 percent, respectively without significant difference among treatments. The extrapolated gross yield of Nile tilapia in T_1, T_2 and T_3 were 3.84±0.15, 5.30±0.08 and 7.41±0.61ton/ha/150 days, respectively, of which T_3 was significantly higher than T_1 and T_2 ($P<0.05$). The food conversion ratio of Nile tilapia in T_1, T_2 and T_3 were 2.01±0.0, 2.17±0.09and 2.13±0.9, respectively without significant differences among treatments. The gross margin of T_1, T_2 and T_3 were 468±20, 593±16and 855±112. thousand NRs/ha/150 days, respectively. The present study demonstrates that optimum stocking density of Nile tilapia is 4 fish/m^2 among tested treatments.

Keywords: Nile tilapia, stocking density, gross fish yield, net fish yield

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) is a warm water fish with a greater aquaculture potential. It has become the second ranked fish produced in the world after carps and has reached to 5.57 million tons production per annum in 2016 (Fitzsimmons, 2016). FAO reports tilapia production from over 100 countries. China, Egypt, Indonesia, Brazil, Philippines, Thailand, Bangladesh, Mexico and Vietnam are the top tilapia producing countries. Currently, there is growing interest of tilapia farming in South-Asian countries -Bangladesh, Srilanka, India, Pakistan, Afghanistan and Nepal. It is anappropriate fish for both resource poor and resourceful farmers to grow because of its several good attributes. Aqua-farms in many tropical regions are considering tilapia as a species for large-scale production since tilapia fillets are a low-cost protein source for the human population (Shrestha and Pandit, 2017). The major attributes that make Nile tilapia so suitable for fish farming are its general hardiness, easy reproduction, rapid growth, efficient omnivorous feeding habit and high-quality flesh with good taste. The biggest constraint of tilapia farming is their early sexual maturity and highly precocious reproductive efficiency (Shrestha et al., 2011). Tilapia becomes sexually mature within 4 to 5 months. The early maturation and frequent breeding causes overpopulation of the ponds with young fish due to indiscriminate breeding which leads to excessive recruitment of fingerlings and ultimately will result to severe competition for food between the stocked tilapia and the newborn recruits (Beardmore et al., 2001). This will in turn decrease the growth rate of the originally stocked fish, resulting in high numbers of small-sized tilapia at harvest. Nile tilapia diverts energy to gametic rather than somatic growth, and, if reproduction occurs, a significant part of the harvest may be unmarketable juvenile fish (Teichert-Coddington & Green, 1997). Another disadvantage of mix-sex culture of tilapia is their possible impact on local aquatic biodiversity if they escape from culture conditions and established in new habitat. To overcome these problems, the concept and culture of mono-sex Nile tilapia is being popular. The productivity of mono-sex male tilapia is about 50-70% higher than the production of mixed-sex tilapia (Bhujel, 2012). All male Nile tilapia are commercially produced by using 17α methyl testosterone (Bhujel, 2012).

Although government of Nepal has introduced Nile tilapia in 1985, yet there is no production technology of tilapia farming available for farmers. Recently, Agriculture and Forestry Uiversity (AFU) and commercial private farm – Center for Aquaculture-Agriculture Research and Production (CAARP) have started all-male Nile tilapia seeds production in Nepal. So, there is great possibility of expanding tilapia aquaculture in Nepal. However, the culture technology of mono-sex Nile tilapia needs to be developed. For example, it is still uncleared how much stocking density, what type of feed, fertilizer and how long culture period is needed for monosex male Nile tilapia production in Nepal. The main purpose of this research is to develop technology for commercial mono-sex tilapia aquaculture and establish it to the farmer level. The specific objectives were to assess survival, growth, production and profit of sex-reversed male Nile tilapia in different stocking density.

MATERIALS AND METHODS

Experimental location

This experiment was carried out at the fish hatchery and research complex of the Aquaculture and Fisheries Program, AFU, Rampur, Chitwan, Nepal during June 5, 2018 to November 3, 2018 for 150 days. Brood fish rearing unit, egg incubation unit and aquaria were used for eggs collection, hatching and rearing of fries until complete yolk sack absorption, respectively. Aquaria were maintained with continuous aeration. This experiment was conducted in two phases. In the first phase, 8 days post hatch Nile tilapia (*Oreochromis niloticus*; GIFT Strain) fries were fed with 17α-methyltestosterone (MT) feed for 23 days for sex reversal in 1.0 m × 1.0 m × 1.0 m size hapas and reared for additional 28 days before stocking. In the second phase, growth performance, production and economics of all-male Nile tilapia in different stocking density was determined.

<u>Phase-I: Sex reversal</u>

Selection and maintenance of brood fish

About 50 well matured brood fish of 200 to 300 g were collected from AFU aquaculture farm ponds. Brood fish were maintained in 2 concrete tanks of size (4.9 m × 4.8 × 1.50 m). Both female and male fish were stocked together in the hapas at 2:1ratio. Brood fishes were fed, at the rate of 2% of its body weight, with commercial floating pellet feed (Machhapuchre Agro-Product Pvt. Ltd., Kapilvastu) containing 23% crude protein. After one week of stocking, all female fishes were checked weekly for eggs in their buccal cavity. Fertilized eggs were detected; they were removed from the mouth and placed in hatching jars in incubation tray.

Egg collection, incubation and fry rearing

Brood fish were checked weekly for eggs in their mouth. When eggs were detected, they were removed from the mouth and placed in hatchling jars in incubation tray and held in upwelling conditions until hatch and swim up. During incubation, regular supply of water was maintained in a way that eggs remained in continuous moving conditions. After being hatched when the yolk sac absorbed completely and reached to first feeding stage (7-9 days) were transferred to hapas of size 1.0 m × 1.0 m × 1.0 m.

Fish meal preparation

Small size Nile tilapia fish were collected from production ponds, freshly killed, dried in hot air oven at 105 ºC for 24-36 hours and then ground in grinder machine until it became fine powdered. Then, fine fish meal was screened with a fine mesh size sieve and stored in a plastic jar.

Preparation of MT-stock solution and MTfeed

MT-stock solution is prepared using 0.25 g of 17α-methyltestosterone was first dissolved in 50 ml 95% Ethyl alcohol and then diluted to 500 ml with 95% Ethyl alcohol (Bhujel, 2012). 100 g of finely powdered fish meal was kept in grinder machine. Then 6.0 ml of MTstock solution was kept in measuring cylinder and 6.0 ml ethanol (95%) was added, the solution was poured in fish meal kept in grinder and mixed by grinder machine. Again, 6.0 ml of MT stock solution was kept in measuring cylinder and 6.0 ml ethanol (95%) was added, the solution was poured in fish meal kept in grinder and mixed by grinder machine. The dose of MTwas 60 mg MT/kg feed. After thoroughly mixing, MT feed was dried in shade for 6 hours and stored in a plastic jar.

Feeding of MT feed

After complete yolk sack absorption, 300 swim-up frieswere kept in three hapas each of 1.0 m × 1.0 m × 1.0 m size. MT feed was started as 1st, 2nd & 3rd lots at 10th March,13th April and 17th April, respectively. They were fed with MT feed 4 times a day i.e at 7am, 10 am, 1 pm and 4 pm of a day for 23 days.

Phase-II: Producion

An experiment was conducted in outdoor concrete tanks (4.9 m ×4.8 m ×1.5 m) at AFU, Rampur, Chitwan Nepal for 150 days (June 5 to November 3, 2018). The experiment was laid out in a completely randomized design (CRD) with three treatment replicated thrice. Three stocking density of sex reversed all-male Nile tilapia were tested. The treatments were: (1) 2 fish/m^2, (2) 3 fish/m^2, (3) 4 fish/m^2.

Pond preparation and fish stocking

Three outdoor concrete tanks (4.9 m ×4.8 m ×1.5 m) were dried for 24 hours in sunlight after draining and then filled with tap water. The water depth was maintained 1.5 m in each pond. Each pond was fertilized with 82 g DAP and 40 g Urea. Three fine-meshed nylon hapas (2.0 m x 1.0 m x 1.25 m) were fixed in each pond with the help of bamboo poles. 0.25 m of the top side of hapas were kept outside water so that the water volume in hapas was 2 m^3. On the 6th day of fertilization, sex-reversed all-male Nile tilapias were stocked in hapas. Average stock size of fish in T_1, T_2 and T_3 were 4.3±0.2 g, 4.4±0.3 g, and 4.3±0.2 g, respectively.

Feeding

Fish were fed with commercial floating pellet feed (Machhapuchre Agro-Product Pvt. Ltd., Kapilvastu) containing 23.0±1.2% crude protein at the rate of 4% body weight twice a day i.e., 11.00 am and 3.00 pm for the period of 5 months, starting from June 5, to November 3, 2018. Fish were sampled monthly and fed was adjusted accordingly.

Fish sampling and growth measurement

Fish sampling was done monthly to measure the growth of all-male Nile tilapia using electronic balance. At least 50% of fish were netted for sampling and weighed to determine the growth. Batch weight of sampled fish from respective experimental ponds was recorded. Fish mortality was recorded daily throughout the experimental period.

Harvesting, fish growth and production
Final harvesting of fish was done by removing each hapa on 3rd November 2018. Harvested fish were measured using electronic balance. Fish were counted and their batch weight was recorded.

Water quality analysis
Water temperature, dissolved oxygen, pH and transparency were measured weekly at 7.00-8.00 am. Total alkalinity, Total ammonium nitrogen (TAN), Soluble reactive phosphorus (SRP) was analyzed fortnightly taking composite water samples by plastic column sampler at 7.00-8.00 am (APHA, 1985).

Proximate analysis of feed
Two batches of pellet feed used in grow out experiment, one at the first half and the other at the second half of the experiment, with three replications in each batch, were analyzed for moisture, crude protein (CP), ether extract (EE), crude fiber (CF), nitrogen free extract (NFE) and total ash (TA) following the methods used in AOAC (1980).

Sex confirmation
Sex of Nile tilapia was identified by dissecting 50% of fishes after a culture period of 5 months. Sex confirmation was done based on morphological observation of testes and ovary.

Economic analysis
Simple economic analysis was performed to determine the economic returns from each treatment (Shang and Tisdell, 1997). The economic analysis was mainly based on farm gate price for the stocked and harvested fish and current local market prices for all other inputs in Nepal. Price for sex reversed all-male Nile tilapia fingerlings was 3.0 NRs per piece. Farm gate price of harvested tilapia was 250 NRs per kg. Prices for DAP, urea and feed was 54, 28 and 55 NRs per kg, respectively. The calculation for cost of working capital was based on an annual interest rate of 10%.

Gross margin (NRs) = Gross revenue (NRs) –Total variable costs (NRs)

Data analysis
Statistical analysis of data was performed by using one-way analysis of variance (ANOVA) using SPSS (version 16.0) statistical software package (SPSS Inc., Chicago) (Gomez and Gomez, 1984). Microsoft excel computer program was used for data tabulation and figure preparation. Arcsine transformations were performed on percent data. Differences were considered significant at the 95% confidence level ($P<0.05$). All means were given with ± standard error (S.E.).

RESULTS
Sex reversal
The mean stock number, total stock weight and average stock weight of 8 days old fry in different hapas were 300, 75.3g and 0.25g/fish, respectively. Similarly, mean harvest number, total harvest weight and average harvest weight of fry after 23 days of MT treatment period in different hapas were 225, 816.5 g and 3.6 g, respectively. The mean survival rate of fry was 75%. The average male percentage was 100%.

Table1. Growth, survival and male percentage of Nile tilapia fry in different treatments during MT treatment period

Parameter	Hapa-1	Hapa-2	Hapa-3	Mean± SE
Total stock number	300	300	300	300±0
Total stock weight (g)	75	73	78	75.33±1.45
Average stock weight (g/fish)	0.25	0.24	0.26	0.25±0
Total harvest number	225	220	230	225±2.8
Total harvest weight (g)	816.53	798.38	934.67	816.53±10.48
Average harvest weight (g/fish)	3.63	3.63	3.63	3.63±0
Survival (%)	75	73.33	76.67	75.00±0.96
Male (%)	100	100	100	100±0

Proximate analysis of feed

Proximate analysis of pellet feed used in the present experiment showed that it contained 88.3±0.5% dry matter (DM), 22.9±1.2% crude protein (CP), 2.6±0. 3% ether extract (EE), 15.4±1.1% crude fiber (CF), 0.5±0.1% total ash (TA) and 58.6±0.4% nitrogen free extract (NFE) in dry matter basis (Table 2).

Table 2. Proximate composition of pellet feed used in the experiment

Parameter	Sample-1	Sample-2	Sample-3	Mean±SE
Dry matter (DM) (%)	88.2	89.2	87.6	88.3±0.5
Crude protein (CP) (%)	21.0	25.1	22.6	22.9±1.2
Ether extract (EE) (%)	4.2	6.0	7.0	5.7±0.8
Crude fiber (CF) (%)	9.0	7.4	7.4	7.9±0.5
Total ash (TA) (%)	9.3	6.2	7.7	7.7±0.9
Nitrogen free extract (NFE)* (%)	56.4	55.4	54.4	55.4±0.6

* NFE = 100 - (CP+CF+EE+TA)

Fish growth, survival and production

The average stock weight of fish in T_1, T_2 and T_3 were 4.3, 4.4 and 4.3 g, respectively. The total harvest weight in T_1, T_2 and T_3 were 768.7, 1060.0 and 1482.7 g/hapa, respectively. The total harvest weight was significantly highest in T_3, intermediate in T_2 and lowest in T_1 ($p<0.05$). The average harvest weight of fish in T_1, T_2 and T_3 were 192.2, 176.7 and 192.7 g, respectively without any significant difference among treatments ($P>0.05$; Table 3).

The daily weight gain of sex-reversed male Nile tilapia in T_1, T_2 and T_3 were 1.25, 1.15 and 1.25 g /fish/day, respectively (Table 3). The daily weight gain of Nile tilapia in T_2 was significantly lower than T_1 and T_3 ($p<0.05$). The survival rate of Nile tilapia in T_1, T_2 and T_3 were 100, 100 and 95.8 percent, respectively. There was no significant difference in survival rate of fish among treatments ($P>0.05$). Monthly growth trend of fish in each treatment during the experimental period is given in Figure 1.

Table3. Growth performance and survival of sex reversed male Nile tilapia in different treatments. Data based on 2 m^2 water area. Mean values with different superscript in the same row are significantly different (P<0.05).

Parameters	Treatments		
	T_1	T_2	T_3
Stocking			
Total count	4±0	6±0	8±0
Total weight (g)	17.3±0.7^a	26.7±1.8^b	34.7±1.8^c
Average weight (g)	4.3±0.2^a	4.4±0.3^a	4.3±0.2^a
Harvesting			
Total count	4±0^a	6±0^b	7.7±0.3^c
Total weight (g)	768.7±29.0^a	1060.0±15.0^b	1482.7±121.2^c
Average weight (g)	192.2±7.3^a	176.7±2.5^a	192.7±7.9^a
DWG (g/fish/day)	1.25±0.04^a	1.15±0.02^a	1.25±0.04^a
Survival (%)	100±0^a	100±0^a	95.8±4.2^a

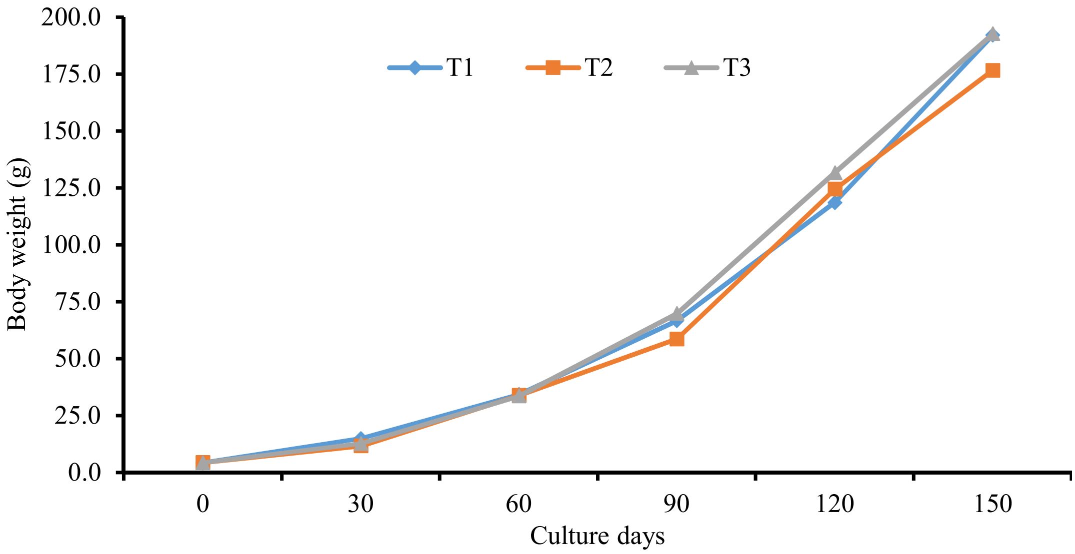

Figure1. Monthly growth trend of sex reversed male Nile tilapia in each treatment during the experimental period. T_1=2 fish/m^2, T_2=3 fish/m^2, T_3=4 fish/m^2

The gross yield of sex-reversed male Nile tilapia in T_1, T_2 and T_3 were 0.77, 1.06 and 1.48 kg/2m^2/cycle, respectively. The gross yield was significantly highest in T_3, intermediate in T_2 and lowest in T_1 (P<0.05). The extrapolated gross yield of all-male Nile tilapia in T_1, T_2 and T_3 were 3.84, 5.30 and 7.41 ton/ha/cycle which were significantly different among treatments (P<0.05; Table 4). Similarly, the extrapolated net yields were 3.76, 5.17, and 7.24 ton/ha/cycle in T_1, T_2 and T_3, respectively. The extrapolated net yield was significantly highest in T_3, intermediate in T_2 and lowest in T_1 (P<0.05; Table 4).The AFCR were 2.01, 2.17 and 2.13 in T_1, T_2, and T_3, respectively. AFCR were not significantly different among treatments (P>0.05; Table 4).

Table 4. Gross and net yield of sex reversed male Nile tilapia in different treatments during the culture period of 150 days. Mean values with different superscript in the same row are significantly different (P<0.05).

Parameters	Treatments		
	T_1 (2 fish/m^2)	T_2 (3 fish/m^2)	T_3 (4 fish/m^2)
Gross yield (kg/2 m^2/cycle)	0.77±0.03^a	1.06±0.02^b	1.48±0.12^c
Extrapolated gross yield (ton/ha/cycle)	3.84±0.15^a	5.30±0.08^b	7.41±0.61^c
Extrapolated net yield (ton/ha/cycle)	3.76±0.14^a	5.17±0.07^b	7.24±0.60^c
AFCR	2.0±0.0^a	2.2±0.1^a	2.1±0.9^a

Water quality

Most of the water quality parameters, showed cyclic variation but were within the recommended range for the growth performance of Nile tilapia. The weekly mean of water temperature in T_1, T_2 and T_3 were 28.6, 28.8 and 28.8°C, respectively (Table 5). Similarly, weakly mean dissolved oxygen content of T_1, T_2 and T_3 ponds were 6.8, 7.2 and 6.9 mg/L, respectively. Likewise, weakly mean pH of T_1, T_2 and T_3 were 7.2,7.5 and 7.2, respectively. The weakly mean secchi disk depths were 59, 54 and 46, respectively. The fortnightly mean total alkalinities were 119.2, 98.3 and 108.5 mg/L, $CaCO_3$ in T_1, T_2 and T_3, respectively. The fortnightly mean total ammonia nitrogen (TANs) was 0.6, 0.4 and 0.5mg/L in T_1, $T_2$and T_3, respectively. Similarly, fortnightly mean soluble reactive phosphorus (SRP) was 0.5, 0.3 and 0.4 mg/L, respectively (Table 5).

Table 5. Mean and ranges of water quality parameters in each treatment

Parameters	Mean and Range		
	T_1 (2fish/m^2)	T_2 (3 fish/m^2)	T_3 (4 fish/m^2)
Temperature (°C)	28.6±0.4	28.8±0.5	28.8±0.5
	(22.8-31.0)	(22.3-31.9)	(22.8-31.1)
Dissolved oxygen (mg/L)	6.8±0.4	7.2±0.3	6.9±0.5
	(3.3-8.2)	(5.1-12)	(4-12.5)
pH	7.2	7.5	7.2
	(6.5-8.7)	(6.97-8.8)	(6.25-8.14)
Secchi disk depth (cm)	59.1±1.4	54.1±2.0	46.2±1.4
	(35-100)	(40-100)	(45-75)
Total alkalinity (mg/L $CaCO_3$)	119.2±4.7	98.3±3.4	108.5±4.2
	(97.7-146.1)	(76.7-107)	(85-128.5)
Total ammonium nitrogen (TAN, mg/L)	0.6±0.2	0.4±0.2	0.5±0.2
	(0.1-1.7)	(0-1)	(0-1.3)
Soluble reactive phosphorous (SRP, mg/L)	0.5±0.2	0.3±0.0	0.4±0.2
	(0-1.5)	(0.1-0.8)	(0-1.2)

Economic analysis

The seed costs were NRs. 60.00, 90.00 and 1, 20.00 thousand in T_1, T_2 and T_3, respectively. Similarly, feed costs were NRs. 40.89, 605.12 and 828.49 thousand in T_1, T_2 and T_3, respectively. The total variable costs were NRs. 492.7, 732.35 and 998.38 thousand in T_1, T_2 and T_3, respectively. The total variable costs were significantly different among treatments (p<0.05) with highest in T_3, intermediate in T_2 and lowest in T_1. Total fish sales were NRs. 960.83, 1325.65and 1853.33 thousand in T_1, T_2 and T_3, respectively. The total fish sale was significantly different among treatments ($p<0.05$) with highest in T_3, intermediate in T_2 and lowest in T_1. The gross margins were NRs. 468.13, 592.65 and 854.95 thousand in T_1, T_2 and T_3, respectively. The B:C ratios were 1.95, 1.81 and 1.85 in T_1, T_2 and T_3, respectively. The B:C ratios were not significantly different among treatments ($p>0.05$). The costs per kg fish production were NRs. 128.25, 138.06 and 135.69 in T_1, T_2 and T_3, respectively (Table 6).

Table 6. Comparative economic analysis in Nepalese currency (NRs. thousand) for each treatment on per ha per 5 month basis. Mean values with different superscript in the same row are significantly different ($P<0.05$).

Parameters	Treatments		
	T_1 (2 fish/m^2)	T_2 (3 fish/m^2)	T_3 (4 fish/m^2)
Variable costs			
Seed	60.00±0.00^a	90.00±0.00^b	120.00±0.00^c
Feed	406.89±15.35^a	605.12±32.2^b	828.49±38.84^c
Fertilizer	2.35±0^a	2.35±0^a	2.35±0^a
Sub Total	469.24±15.35^a	697.48±32.2^b	950.84±38.84^c
Interest (10%)	23.46±0.77^a	34.87±1.61^b	47.54±1.94^c
Total variable costs (A)	492.7±16.12^a	732.35±33.81^b	998.38±40.79^c
Return			
Fish sale (B)	960.83±36.3^a	1325±18.76^b	1853.33±151.43^c
Gross margin (B-A)	468.13±20.21^a	592.65±16.31^a	854.95±112.36^b
B/C ratio	1.95±0.01^a	1.81±0.06^a	1.85±0.08^a
Cost per kg fish production	128.25±0.69^a	138.06±4.56^a	135.69±6.20^a

DISCUSSION

Sex reversal

17-α methyl testosterone (MT) is the most commonly used synthetic androgen to produce all-male fish. It has proven to be effective in a number of different species of tilapia and under a variety of management scenarios. However, the percentage of male tilapia production using MT varied in different experiments from 90-100%. Popma and Green (1990) discussed how the presence of 3 to 5% females in tilapia production ponds can result in excessive reproduction and reduced growth. The present study examined the possibility of using 17α- methyl testosterone for commercial production of all-male Nile tilapia fry in Nepal. The results of the present experiment showed that oral treatment of MT to sexually undifferentiated fry of Nile tilapia at the dose of 60 mg/kg feed for 23 days induce 100% masculinization. The mean survival rate of fry was 75%.

The finding of the present study is similar or higher to many previous studies. Khanal et al. (2014) reported 92.9% male tilapia fry when feeding MT at the dose of 60 mg/kg feed for 21 days at Rampur, Nepal. Jiménez-Badillo and Arredondo-Figueroa (2000) reported that 17α- MT was most effective for male ratio (92%) and concluded that the dose of 40 mg/kg MT diet for 30 days was effective for tilapia sex reversal. Shepperd (1984) obtained 98% males using 17 alpha-MT at dosages 60 mg/kg diet in *O. niloticus* for 28 days. Vera-Cruz and Mair (1994) obtained 95 to 98 % males with 40 mg MT/kg of diet and 99% with 60 mg MT/kg of diet fed at 20% body weight for 25 days. Lindsay et at. (2000) and Shalaby et al. (2006) observed that 100% males were produced in Nile tilapia at a dose rate of 60 mg/kg MT feed with 40% crude protein. Smith and Phelps (2001) reported that 99-100% males were produced in Nile tilapia when it was fed with a 60 mg MT/kg. Shamsuddin et al. (2012) observed 95% males using 60 mg MT/kg feed 17 α-MT for 21 days through oral administration in GIFT strain.

Fish survival and growth

The effect of stocking density on survival, growth, production and economics of sex reversed all-male Nile tilapia in hapa culture system was assessed. The survival rate of Nile tilapia in 2 fish/m^2, 3 fish/m^2 and 4 fish/m^2 densities were 100, 100 and 95.8 percent, respectively. This experiment indicates that there is no problem in survival issue of sex-reversed Nile tilapia. Although lower stocking densities gave better result, there were no significant differences in survival among treatments. In this study, the similar survival rates of tilapia at high stocking density indicate amenability of tilapia to intensive culture. The survival rate of Nile tilapia in the present experiment was higher than those reported by Ahmed et al. (2013) who observed that the survival rate of mono-sex tilapia was varied from 79-92%. The higher survival in the present experiment could be attributed to favorable environmental conditions during the experiment.

The daily growth rate of Nile tilapia in 2 fish/m^2, 3 fish/m^2 and 4 fish/m^2 densities were 1.25, 1.15 and 1.25 g/fish/day, respectively. The daily weight gain of Nile tilapia in 3 fish/m^2 was significantly lower than 2 fish/m^2 and 4 fish/m^2 ($p<0.05$). This finding was supported by findings of Saha and Khatun (2014) who found that daily weight gain was 1.54-2.05 g by rearing for 105 days at stocking density of 5/m^2 in Nile tilapia. The growth rate of Nile tilapia in the present experiment was higher than those reported by Ahmed et al. (2013) who found daily weight gain of 0.71g for GIFT reared for a period of 180 days and fed with rice bran and similar to those reported by Ahmed et al. (2013) who found 1.56 g using prepared feed. It is well-known fact that growth rate progressively increases as the stocking density decreases and vice-versa. This is because a relatively a smaller number of fish of similar size in a cage could get more space, food, less competition and dissolved oxygen etc. reported by various authors in different fish species (Narejo et al., 2010). However, the present experiment showed that the growth rate of all-male tilapia cannot be affected up to stocking density of 4 fish/m^2.

The average harvest size of Nile tilapia in 2 fish/m^2, 3 fish/m^2 and 4 fish/m^2 densities during the 150 days culture period were 192.2, 176.7 and 192.7 g, respectively without any significant difference among treatments ($P>0.05$). Although the harvest size of Nile tilapia seems smaller than those in international markets, this size is good and easily sold in Nepalese market.

Fish production and extrapolated yields

The total fish productions in 2 fish/m^2, 3 fish/m^2 and 4 fish/m^2 densities during the 150 days culture period were 768.7, 1060.0 and 1482.7 kg/ 2 m^2 size hapa, respectively. The total fish production was significantly highest in 4 fish/m^2, intermediate in 3 fish/m^2 and lowest in 2 fish/m^2 ($p<0.05$). The present results showed that yield of Nile tilapia increases with increasing stocking density within its carrying capacity. This can be explained by the fact that at appropriate stocking density before attainment of carrying capacity, the fish grow properly, thus at this stocking density did not affect fish growth. This is supported by findings of Saha and Khatun (2014) who found that at stocking density of 5/m^2 for a culture period of 105 days, mean weight was 176.50±18.44 in Nile tilapia. The present result also agreed with the findings of Malik et al., (2014) in pangas catfish, they obtained highest production from higher stocking density. According to Shang and Tisdell (1997), farm productivity usually increases with culture intensity, but it eventually declines after a certain level of intensity due to deteriorated water quality, diseases, and thus, resulting in reduced growth and high mortality.

The extrapolated gross yields in 2 fish/m^2, 3 fish/m^2 and 4 fish/m^2 densities during the 150 days culture period were 3.84, 5.30 and 7.41 ton/ha/150 days, respectively. This means that if we do two culture cycles per year, the extrapolated yield will be 7.68, 10.6 and 14.82 ton/ha/year in 2 fish/m^2, 3 fish/m^2 and 4 fish/m^2 densities, respectively. This extrapolated fish yield in the present study is higher than the average fish productivity of Nepal which is 4.92 ton/ha/year (DOFD, 2018) and many tilapia experiments conducted in Nepal Pandit et al., (2004).

The apparent feed conversion ratios in 2 fish/m^2, 3 fish/m^2 and 4 fish/m^2 densities in the present study were 2.01, 2.17 and 2.13, respectively, which were not significantly different among treatments (P>0.05). The present result is slightly higher than the findings of Kunda et al. (2015) and Ahmed et al. (2014) found FCR of Nile tilapia 1.18-1.25 and 1.11-1.41, respectively. The present finding is higher than finding of Hossain et atl. (2004) who reported FCR was 1.71-1.77 for GIFT Nile tilapia. The present study demonstrates that 4 fish/m^2 is the best density for Nile tilapia culture.

Water quality
The DO ranges from 3.3 to 12.5 mg/L. The mean DO were 6.8±0.4 mg/L, 7.2±0.3 mg/L and 6.9±0.5 mg/L in T_1, T_2 and T_3, respectively. The concentration of dissolved oxygen in the present study is similar to findings of Dewan et al. (1991) was 2.2 to 8.8 mg/L and Sayeem (2014) was 4 to 6 (mg/L). The DO have no adversely affect growth of the fish. The mean values of pH were 7.2,7.5,and 7.2 in T_1, T_2 and T_3,respectively. The pH ranges from 6.25 to 8.8. Islam (2007) reported that the range of pH of water body suitable for fish culture would be 6.8 to 8.27. According to Alam et al. (2009) the range of pH would be 7.72 to 8.3. Total ammonium nitrogen was 0.6±0.2, 0.4±0.2 and 0.5±0.2 were in T_1, T_2 and T_3, respectively. Soluble reactive phosphorus were 0.5±0.2, 0.3±0.0 and 0.4±0.2 were in T_1, T_2 and T_3, respectively. The mean transparencies were 59.1±1.4, 54.1±2.0 and 46.2±1.4 cm in T_1, T_2 and T_3, respectively. Transparency varied from 35 to 100 cm. Dewan et al. (1991) who measured water transparency (cm) in ponds of BAU Campus, Mymensingh and found to vary from 54 to 90 cm.Total alkalinity ranged from 76.7 to 146.05 mg/L with means 119.3±4.8, 98.3±3.4 and 108.5±4.2 mg/L in T_1, T_2 and T_3, respectively, which were suitable range for culture. Banerjee (1967) found water having total alkalinity above 90 mg/L was suitable for culture. Alikunhi (1957) reported that in highly productive water, the alkalinity out to be over 100 mg/L. So, more transparent but due to commerciall pellet feeding have negligible negative effecy on growth.

Economics
Fixed costs such as ponds, cage etc. were not included in the analysis as it was intended to only compare relative differences in efficiency between the treatments and fixed costs were assumed to be similar for all the treatments. All cost estimation was based on local market prices of fingerlings, fertilizers and feed. In the present experiment experiment, the gross in 2 fish/m^2, 3 fish/m^2 and 4 fish/m^2 densities during the 150 days culture period were NRs. 468.13, 592.65 and 854.95 thousand, respectively. The gross margin of 4 fish/m^2 density was significantly higher than 2 fish/m^2 and 3 fish/m^2 densities (P<0.05).

The extrapolated gross yield in 2 fish/m^2, 3 fish/m^2 and 4 fish/m^2 densities during the 150 day culture period were 3.84, 5.30 and 7.41 ton/ha, respectively. The present findings are lower than finding of (Saha and Khatun, 2014) who found 6.33-8.09 ton/ha/105 days and similar to finding of Hossain et al. (2004) who reported 4-6 ton/ha/120-180 days in semi-intensive culture in fresh water pond.

CONCLUSION
The present experiment showed that sex-reversed male Nile tilpia stocked at 4 fish/m^2 produced 7.41 ton/ha in 150 days with average size of 192.73 g. We can produce 2 cycle in a year, with production 14.81 ton/ha/year.

ACKNOWLDEGEMENTS

The authors would like to acknowledge the Aquaculture and Fisheries Program, Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal for providing research and laboratory facilities. This work was financially suported by the Directorate of Research and Extension, AFU, Rampur, Chitwan, Nepal.

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Nepalese Journal of Aquaculture and Fisheries
Vol. 6 & 7 (2019 & 2020) : 31-38

INCLUSION OF PROBIOTICS IN MONOSEX NILE TILAPIA FEED TO ENHANCE GROWTH AND PRODUCTION

Sabita Jha[1*], Ravi Lal Sharma[1], Rahul Ranjan[2]
[1]Fisheries Human Resource Development and Technology Validation Center, Janakpurdham
[2]Agriculture and Forestry University, Chitwan
*Email: sabita.jha.iaas@gmail.com

ABSTRACT
Monosex Nile tilapia (*Oreochromis niloticus*) culture is gaining popularity in Nepal as rest of the world with the availability of seed locally. However, increased production of tilapia could not be achieved with traditional feeding practice Nepal. Thus, there is need to formulate a feed that might yield more with not much change in the feed ingredient. An experiment was carried out at the Fisheries Human Resource Development and Technology Validation Center (FHRDTVC), Janakpurdham for 120 days to compare the growth and production parameters of tilapia with two types of feed and pond management. The experiment was carried out with two treatments viz. local feed mixture (T_1) and local feed fortified with molasses and pond sprayed with probiotics, Everfresh Pro (T_2) in hapa fitted in two different ponds with an average area of 1979.7 m^2. Monosex all male tilapia were stocked at the rate of 4 fingerlings/m^2. The feed in both treatments contained mustard oil cake, rice bran and soybean flour in the ratio of 1:1:0.5. It was found that the addition of molasses in normal feed dough significantly increased the weight gain in tilapia. However, there was no significant difference in the survival and AFCR with both feed mixtures. The DWG, TWG, EGFY and ENFY were significantly higher in tilapia fed with probiotic fortified feed. Fortification of feed with probiotics substantially increased tilapia yield under same culture conditions.

Keywords: Nile tilapia, feed, probiotics, molasses, fish-growth

INTRODUCTION

Tilapia, the native fish of Africa has become a popular farmed aquaculture species in the tropics and subtropics (Pullin and Maclean, 1992) with wide temperature adaptation between 31 to 36°C. The terai region of Nepal have temperature ranging between 7 to 23°C in winter and more than 35°C in summer (NTB, 2019). The climate in terai of Nepal thus can be considered suitable for tilapia culture. Nile tilapia (*Oreochromis niloticus*) is the most common species of tilapia accounting for roughly 75% of the global farmed tilapia production (Towers, 2013). It is an omnivorous fish that feed on both animal as well as plant materials. However, the prolific breeding habit of Nile tilapia is one of the major constraints for its commercial culture (FAO, 2005). But, the monosex male production technology has solved this problem to a greater extent and thus, the monosex male tilapia culture is established system. It has been also reported that the monosex culture gives more yield than the mixed sex culture (Chowdhury et al., 2007; Shamsuddin et al., 2012) and that is why it has become the new culture technique popular among farmers.

The goal of aquaculture is to produce healthy fish to assure the maximum profit from limited resources. In commercial aquaculture, the fish feed alone accounts for about 60% of the total variable cost (Kleih et al., 2013; Shaalan et al., 2018). Majority of fish farmers in Nepal depend on farm-made feed for fish production due to lack of proper commercial fish feed at reasonable price. The farm-made feeds may not provide sufficient nutrients to fish for its proper growth thus, hindering the production potential. Therefore, there is a need to formulate a feed that can be made locally along with increase the production. Present study was carried out to test the effectiveness of inclusion of molasses in fish feed with supplementation of probiotics as water solution. Probiotics are specific microbial strains that help to improve the growth and survival of fish by boosting the immunity and improving water quality. The probiotics are used as food additives in aquaculture mainly to modify and manipulate the microbial population of the environment and to reduce or eliminate the selected pathogenic species of microorganisms leading to better growth and survival (Chang and Liu, 2002; Irianto and Austin, 2002; Gram et al., 2001; Austin et al., 1995; Austin et al., 1992). The microbial food web is an integral part of aquaculture ponds and have direct impact on productivity (Moriarty, 1996). The use of antibiotics in aquaculture to cope with the problem of fish diseases and water quality problems have caused the antibiotic resistance in pathogenic bacteria and elimination of both useful and harmful microflora in the gut. Thus, use of probiotics is also useful as it helps to establish the useful micro-floral population in fish gut and helping to improve the FCR (Feed conversion ratio), PER (Protein efficiency ratio) as well digestibility of food (Cruz et al., 2012; Allameh et al., 2017).

MATERIALS AND METHODS

The present experiment was conducted from 19th January to 18th May, 2020at the Fisheries Human Resource Development and Technology Validation Center (FHRDTVC), Janakpurdham. The trial was carried out in 6 netlon hapa each 2.5 m long, 2 m wide and 1.5 m deep (1 m water depth) installed with the support of bamboo and rope in four earthen ponds with an average 1979.7 square meter water surface area. Since, the probiotics powder was sprayed, two different ponds served for different treatments. Each treatment was replicated in three different hapa. The two treatments included T_1 (monosex tilapia fed with local feed mixture) and T_2 (monosex tilapia fed with local feed mixture fortified with molasses and pond sprayed with commercial probiotics).

Prior to starting of trial, the ponds were drained, dried and liming was done at 450 kg/ha to eradicate all the wild and predatory fish and insects. After 7 days of liming, the ponds were fertilized with inorganic fertilizer at the rate of 470 g/100 m^2 and 350 g/100 m^2, respectively of urea and DAP (Shrestha and Pandit, 2017). Fertilizer was soaked in water, mixed and then sprayed all over the pond as per the transparency recorded. Fertilization was repeated fortnightly.

The male and female Nile tilapia were separated manually using methylene blue as staining agent applied on vent and only male tilapia were selected for stocking. Each hapa was stocked with 20 male tilapias (stocking density 4 fish/m^2) with and average size of 55.3±2.9 for T_1 and 62.9±2.7 for T_2. Stocking was done one week after the fertilization. Commercial probiotic (Everfresh Pro- Product of Blue Weight, India), formulated for pond spraying, was sprayed fortnightly on pond of T_2 (after first application on day before stocking) at rate of 2 kg/ha. The Everfresh Pro contained *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Rhodococcus* spp., *Rhodobacter* spp., *Nitrosomonas* spp. and *Nitrobacter* spp., and enzymes like amylase, protease, cellulose, xylanase and lipase along with excipients and stabilizers.

Feeding was done with a mixture of locally available material viz. mustard oil cake, rice bran and soybean flour in the ratio of 1:1:0.5. The feed for T_2 also contained molasses (10% of feed). Feeding was done daily at morning (10-11 AM) at 5% of total estimated biomass for initial 2 months and 3% for next two months in dough form. Feed ration was adjusted by monthly sampling of fish. During sampling about 20% of fish from each hapa were weighed individually and estimation of total biomass was done.

Water quality parameters like DO, pH, temperature and Secchi disk transparency was recorded (in situ) at weekly interval between 7 to 10 AM. Lutron YK-22DO meter was used to monitor Dissolved Oxygen (DO) and temperature whereas the pH was measured by using Lutron pH-222 pen type meter. Different growth and production parameters of fish were calculated based on stocking and harvesting data. Students' T-test was used to compare the means among two treatments.

RESULTS AND DISCUSSION

Water quality parameters

Table 1 shows the average water quality parameters during present experiment. Since, the experiment was started in January due to time constraints, the temperature during experimental period was found to be gradually increasing. It reached highest at the end of the experiment. The lowest temperature was recorded during January as 17.3ºC while highest temperature was recorded during May as 29.9ºC. Although a great variation in temperature was recorded during experiment, the temperature was within the range of tolerance for Nile tilapia which is found to be 9.1°C (Atwood et al., 2003) to more than 35°C (Khater et al., 2017). It had been suggested that the growth of fish increases with the increase in temperature within the tolerance limit. It had also been suggested that Nile tilapia stops feeding below 16°C, do not spawn below 20°C and severe mortality may occur below 12°C (Bucur et al., 2012), so, during present study it can be assumed that fish did not feed very well during the initial fortnight of stocking after which temperature increased.

Table 1: Water quality parameters of two treatments during the experiment (Mean ±SE).

Sampled month	Temperature (°C)		Transparency (cm)		DO (mg/L)		pH	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
Jan 2020	17.8±0.2	17.8±0	32.8±1.2	34.3±1.6	4.0±0.1	3.1±0.3	8.8	8.7
Feb	19.7±0.8	19.7±0.9	32.5±0.9	30.7±0.9	3.9±0.2	3.2±0.2	8.6	8.5
Mar	24.2±0.6	24.2±0.5	31.3±1.1	33.3±0.6	3.5±0.3	3.9±0.3	8.6	8.6
Apr	26.7±0.2	26.8±0.2	33.5±0.6	31.4±0.9	2.8±0.1	3.2±0.2	8.3	8.5
May	28.0±0.8	28.0±0.9	30.1±1.1	30.3±0.8	2.8±0.1	2.7±0.1	8.4	8.4
Average	23.7±0.1	23.7±0.0	32.0±2.0	31.9±1.9	3.4±0.1	3.3±0.2	8.5	8.5

The transparency of water was found to be within suitable range for semi-intensive aquaculture (Shrestha and Pandit, 2017). The optimum DO level is foremost requirement for efficient feed utilization and growth and the DO level along with diet is found to exhibit changes in the intestinal morphology of Nile tilapia (Tran-Ngoc et al., 2016). The dissolved oxygen was also found to be within the range of tolerance for Nile tilapia. Tilapia is found to survive at very low (as low as 1.0 mg/L) for up to 11 weeks in experimental setup. However, it has lowest growth rate as compared to fish grown at 3 and 6 mg/L (Abdel-Tawwab et al., 2015). The pH was also found to be within the range of tolerance for Nile tilapia. It had been suggested that the suitable range of pH for Nile tilapia rearing is 5.5 to 9.0 with adverse effect on growth with further increase or decrease in pH (Reboucas et al., 2016; Mustapha et al., 2018). A number of studies have shown that adding probiotics in water of feed enhances the water quality of culture unit (Shichehchian et al., 2001; Dohail et al., 2009; Zhou et al., 2009; Wang et al., 2010). However, no any significant change in water quality parameters was observed due to use of probiotics in present study.

Growth and yield of Tilapia

Table 2 shows different growth and production parameters of Nile tilapia under two treatments. Figure 1 shows the growth trend of Nile tilapia in two treatments. There was significant difference between final average weight in tilapia fed with probiotic mixed feed than without probiotic as T_2 being significantly higher than T_1. Similarly, final total weight in T_2 was significantly higher than T_1. However, there was no significant difference in the survival rate between the treatments. The daily weight gain, total weight gain, extrapolated gross fish yield and extrapolated net fish yield were significantly lower in T_1 than in T_2. However, there was no any significant difference in the apparent food conversion ratio between the treatments.

Table 2: Growth and production parameters of Nile tilapia between two treatment.

Parameters	T1 (No probiotic)	T2 (Probiotic)
Initial average weight (g/fish)	55.3±2.9	62.9±2.7
Initial total weight (g/hapa)	1105±57.3	1258±53.5
Final average weight (g/fish)	250.3±3.2^b	436.1±3.6^a
Final total weight (g/hapa)	4884.8±140.4^b	8357.5±185.1^a
Survival rate (%)	97.5±1.7^a	95.8±2.0^a
Daily weight gain (g/fish/day)	1.6±0.0^b	3.1±0.0^a
Total weight gain (kg/m^2)	0.8±0.0^b	1.4±0.0^a
Extrapolated GFY (mt/ha/yr)	29.7±0.9^b	50.8±1.1^a
Extrapolated NFY (mt/ha/yr)	23.0±1.0^b	43.2±1.2^a
Apparent food conversion ratio (AFCR)	2.0±0.1^a	2.0±0.1^a

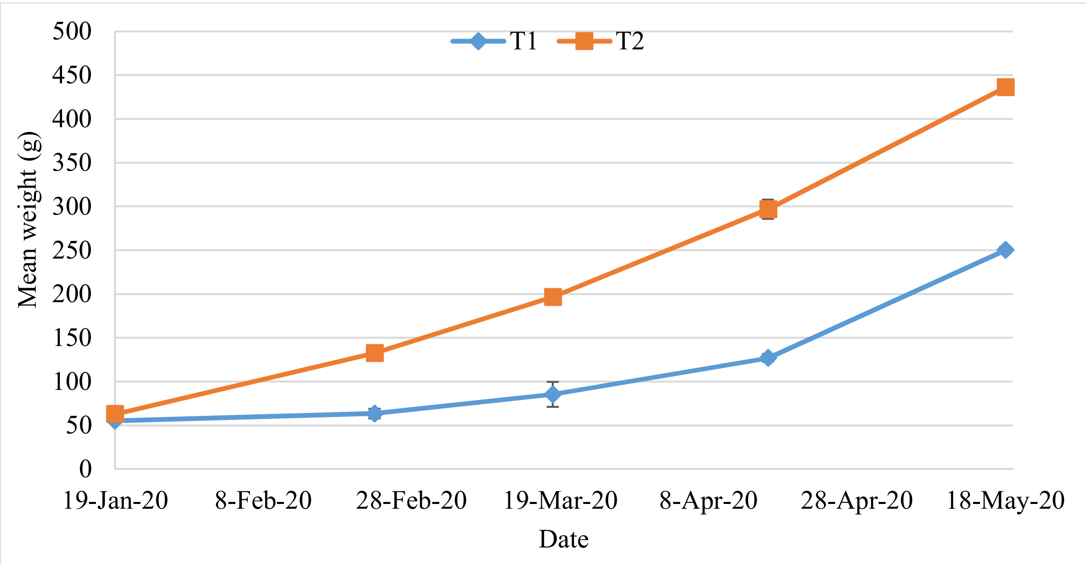

Figure 1: Growth trend of Nile tilapia in without probiotic (T1) and with probiotic (T2) treatments.

From growth trend it can be seen that fish growth for T_2 was higher from the beginning of experiment till the end. These can be attributed to the addition of molasses in feed and probiotics in pond water of T_2. A number of studies have been carried out in different aquaculture (finfish and shellfish) species with probiotics. These studies have suggested numerous advantages of probiotics in terms of growth and survival of fish. The probiotics have seen to serve as growth promoter in many fish species (Rengpipat et al., 1998; Queiroz and Boyd, 1998; Lin et al., 2012; Gatesoupe, 1999; Gildberg et al., 1997). It had also shown to inhibit the pathogens (Chang and Liu, 2002; Irianto and Austin, 2002; Gram et al., 2001; Austin et al., 1995; Austin et al., 1992), increase the digestibility of nutrients (Dohail et al., 2009; Rahiman et al., 2010; Tapia-Paniagua et al., 2012), increase the stress tolerance (Carnevali et al., 2006;

Hernandez et al., 2010; Tapia-Paniagua et al., 2012) and improve the reproduction (Ghosh et al., 2007; Gioacchini et al., 2010; Abasali et al., 2010). The high extrapolated GFY and NFY could be due to smaller experimental area which led to better survival rate due to regular monitoring. However, it may differ in normal scenario as there could be predation and other factors that may affect the survival.

The higher growth rate of tilapia even in colder environment during present study can be attributed to the building of stress tolerance capacity due to the use of probiotics. It had been shown that inclusion of different probiotics in Nile tilapia feed can increase the growth rate and feed intake by the increased levels of amylase, protease and lipase (Essa et al., 2010). The probiotics are also found to improve the yields of Nile tilapia by improving the physiological condition (measured as cholesterol level) (Apun-Molina et al., 2015). Similarly, the inclusion of probiotics in feed of Nile tilapia has also found to increase the growth of Nile tilapia by improvement in the morphology of the intestinal microvilli (Nakandakare et al., 2013). Sweetmen et al. (2008) proved that the improvement of intestinal microflora, morphology of the intestine, immune system and absorption of nutrients influences the health and performance of fishes. Similarly, it has also been shown that, supplementary diet with yeast as probiotics can give better growth performance and feed efficiency due to its growth- stimulating activity in Nile tilapia (Flores et al., 2003). Thus, the increased growth and production parameters of Nile tilapia provided with molasses in feed and probiotics as pond spray is obvious. The use of probiotics may had multi-dimensional positive impact on fish growth, thus increasing the growth and production of tilapia supplemented with probiotics.

CONCLUSION

From present study it can be concluded that the efficacy of locally made feed can be easily increased by addition of molasses and probiotics. During present study, the probiotics used was designed for pond spraying which is easily available in Nepalese market. Thus, inclusion of spraying as well as feed mixing probiotics can help to increase the growth of Nile tilapia which is an emerging sector in aquaculture. Adding probiotic in local feed improves the growth of fish making no any impact on the water quality. The growth from per unit area can be increased with the same number of fish stocked if there is fortification of feed with probiotic. Moreover, this technology can better benefit farmers with just a small intervention in the feeding practice with negligible rise in the input cost.

ACKNOWLEDGEMENTS

This study was funded by Government of Nepal under regular technology testing program conducted at FHRDTVC, Janakpurdham. The authors express their gratitude to CFPCC for the mobilization of fund and providing necessary help during this experiment. The staffs of FHRDTVC, Janakpurdham are equally acknowledged for their help during the experiment.

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Nepalese Journal of Aquaculture and Fisheries
Vol. 6 & 7 (2019 & 2020) : 39-48

USE OF PAPAYA (*Carica papaya*) SEED TO CONTROL REPRODUCTION IN NILE TILAPIA (*Oreochromis niloticus*)

Charitra Narayan Yadav*, Narayan Prasad Pandit, Dilip Kumar Jha, Kamala Gharti
Agriculture and Forestry University, Rampur, Chitwan, Nepal
*E-mail: charitranyadav@gmail.com

ABSTRACT
This study was conducted to examine the ability of papaya (*Carica papaya*) seeds in reducing reproductive performance of Nile tilapia through gonadal sterilization. In the first phase, 9 days fry of Nile tilapia were reared in 50-L size aquaria and fed with normal feed (T_1) and papaya seed powder mixed feed at 50 (T_2), 100 (T_3) and 150 (T_4) g/kg diet at the rate of 5% of body weight for 30 days. After 30 days of treatment, 50 fish from each group were reared in outdoor hapa for 6 months with normal feed, and gonadal status was observed. In the second phase, matured fish from both control and papaya seed treated group (T_4; 150 g/kg diet) were reared in two separate hapa for next 3 months to observe their reproductive performance. Results showed that papaya seed feeding had no adverse effect on growth, survival and water quality during treatment period. The gonadosomatic index of both males and females were significantly lower in papaya seed treated group (0.1±0.0%) than control (0.2±0.01%). The number of fish spawned per week was significantly lower in papaya seed treated group (1.2±0.2) compared to control group (3.6±0.3). The number of eggs per g female was significantly lower in papaya seed treated group compared to control group. The fertilization rate was significantly lower in papaya seed treated group (93.6±1.1%) compared to control group (98.2±1.3%) ($P<0.05$). This study demonstrated that feeding papaya seeds at the dose of 150 g/kg diet (15%) can reduce reproductive performance of Nile tilapia.

Keywords: Papaya seed powder, non-steroid aromatase inhibitor, masculinization

INTRODUCTION
Nile tilapia (*Oreochromis niloticus*; Linnaeus, 1758) is a warm water fish with a greater aquaculture potential (Fitzsimmons, 2016). Some negative aspects of mixed-sex tilapia farming are a risk associated with their uncontrolled reproduction such as overcrowding, stunting and possible escape from the fish-farm and genetic contamination with wild fish (Shrestha et al., 2011; Pandit et al., 2015). Under aquaculture conditions, tilapia reaches sexual maturity early and starts reproducing with multiple annual spawning before they reach marketable size. Thus, there is an increasing demand for a reliable method to control reproduction in tilapia especially from aquaculture industries (Baroiller et al., 2009). For profitable culture, various methods were conducted for the control of prolific breeding in tilapia and variation in the size of harvested fish. Various techniques to control unwanted reproduction in tilapia farming has been developed including stock manipulation (Phelps and Pompa, 2000), sterilization using chemicals (Ekanem and Okoronkow, 2003), polyploidy (Pradeep et al., 2012), heat shock (Pandit et al., 2015). However, each of these methods has its own advantage and disadvantages. A reliable, non-chemical, consumer, and eco-friendly strategy for controlling reproduction in Nile tilapia seems absolutely necessary. Induction of sterility in Nile tilapia might be a good approach to control reproduction and increase fish productivity (Pandit et al., 2015). Similarly, Ekanem and Okoronkwo (2003) and Abdelhak et al. (2013) reported success in using papaya (*Carica papaya*) seed powder in inducing sterility in adult male Nile tilapia when administered through feed. Papaya seeds contain active ingredients such as caricacin, an enzyme carpasemine, a plant growth inhibitor, and oleanolic glycoside, the last of which had been found to cause sterility in male rats (Das, 1980). If we can apply this sterilization technique in smaller tilapia fry, it will be a great achievement to control reproduction in

tilapia aquaculture. This method of reproduction control could be easier to adopt by poor fish farmers since papaya seeds are easily available all year round in the subtropical areas. The current investigation aimed to evaluate the optimum feeding dose of papaya seed in reducing reproductive performance of Nile tilapia and also to assess the effect of papaya seed on water quality.

MATERIALS AND METHODS

This experiment was carried out at Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal during August 2016 to September 2017 and was conducted in two phases. In the first phase, 9 dAH (day after hatching) Nile tilapia (*Oreochromis niloticus*; GIFT Strain) fry was fed with papaya seeds for 30 days and grown to maturity stage for 6 months. In the second phase, the reproductive performance of papaya treated fish was studied throughout the breeding season for 3 months.

Brood fish management

About 50 well matured brood fish of 200 to 300 g were collected from the AFU aquaculture farm ponds. Brood fish was maintained in 4 m x 4 m x 1.5 m nylon hapa in cemented tank (5 m x 5 m x 1.5 m size) for breeding purpose. Both female and male fish were stocked together in the hapa at 2:1 ratio. Brood fishes were fed with commercial pellet feed at 1% body weight ("Pusti" feed manufactured by Machhapuchchhre Feed Industry, Kapilvastu, Nepal, 28% CP). After being hatched when the yolk sac absorbed and reached to first feeding stage 8-9 dAH were transferred to 50-L aquarium and fed with treatment feed.

Experiment phase I

There were four treatments with three replications for each experiment. 9 dAH tilapia larvae were stocked in 50-L size aquaria (2 ft x 1 ft x 1.5 ft size) at 100 fry/aquarium for papaya seed treatments. All aquaria were maintained in good aeration condition and 80% water exchanged at each alternate day. The experiment was conducted in a completely randomized design with four treatments replicated thrice. The treatments were: T_1: Normal feed (rice bran and mustard oil cake 1: 1 ratio with 10% fish meal), T_2: Normal feed + 5% PSP (papaya seed powder), T_3: Normal feed + 10% PSP and T_4: Normal feed + 15% PSP.

Papaya seed (*Carica papaya*) local cultivar was collected and dried papaya seeds were then powdered with grinder and sieved through 60 µm mesh size sieve. Feed containing 35% CP based on each treatment was formulated. The fishes were fed at 5% of their body weight daily, in two instalments at 1000 and 1500 hours. Feeding rate was adjusted based on weekly sampling weight of fishes. Water quality parameters like dissolved oxygen (DO), pH and temperature of the aquarium water was measured by DO meter and pH meter daily. Similarly, total ammonium nitrogen (TAN) and nitrite nitrogen (NO_2-N) were analyzed in each alternate day just before and after water exchange.

Grow out or fish rearing phase was carried out in outdoor hapa maintained in cemented tanks. After 30 days of treatment in aquarium, 50 fishes from each aquarium were stocked randomly into 12 nylon hapa (2 m x 1 m x 1.5 m) of 1 mm mesh size suspended in 5 m x 5 m x 1.5 m cemented tank. Three cemented tanks were used for this purpose and each tank holding four hapa. Pellet feed ("Pusti") was fed for all treatments. The fish were fed at the rate of 2% of their body weight once a day. Fish were sampled monthly and feeding rate was adjusted. Partial water exchange of three tanks was done fortnightly. Water quality parameters like DO, temperature, pH and Secchi disc visibility were measured. All fishes in the hapa were reared for 6 months until maturation. After 6 months of culture, i.e., at the end of this phase, half of the fish from each hapa were dissected and gonadal observation was made morphologically and histologically.

Experiment phase II

Matured fish (70-150 g) from control and papaya seed treated group were reared in two separate hapa (3 m x 3 m x 1.5 m) to observe their reproductive performance. In one hapa, 12 females and 18 males from control group were stocked together. In another hapa, 12 females and 18 males from treatment group (T_4) were stocked together. Low dose treatments groups (T2 and T3) were discarded for reproduction study because of low proportion of abnormal gonad in these groups. Similar rearing conditions were maintained for both hapa. Broods were fed with commercial pellet feed ("Pusti", 4.0 mm size) at the rate of 2% body weight on daily basis. Water quality parameters such as DO, temperature, pH and Secchi disc visibility were measured weekly. Breeding performance of brood fish were checked weekly by collecting eggs from mouth and weighing and counting of collected eggs was done. The collected eggs were incubated in jars. During incubation, regular supply of water was maintained in a way that eggs remained in continuous moving condition. Fry survival rate was calculated at 7 dAH. The parameters were observed are number of fish spawned weekly, total number of eggs per unit body weight (BW) of fish, size (total length, TL) and weight of eggs and hatching rate.

Data were statistically analyzed by using the one-way Analysis of Variance (ANOVA) and t-test. The statistical analysis was performed by using the computer software SPSS (Version 21.0). DMRT was used to evaluate the differences between means for treatments at the 5% level of significance. All means are presented with ± standard error.

RESULTS

Experiment phase I

The average stock weight of juveniles in T_1, T_2, T_3 and T_4 were 0.26±0.11, 0.29±0.03, 0.24±0.03 and 0.16±0.07 g, respectively (Table 1). The mean weight of fry at the end of treatment period in T_1, T_2, T_3 and T_4 were 1.43±0.32, 1.33±0.06, 1.31±0.27 and 1.27±0.51 g, respectively without any significant different among treatments ($P<0.05$). Similarly, the mean survival rate of fry at the end of treatment period in T_1, T_2, T_3 and T_4 were 63.3±8.9, 70.0±3.2, 73.0±10.6 and 73.0±13.2%, respectively without any significant different among treatments ($P<0.05$). The specific growth rate of fry at the end of treatment period in T_1, T_2, T_3 and T_4 were 2.34±0.49, 1.93±0.17, 2.16±0.23 and 2.80±0.84% BW per day, respectively without any significant different among treatments (Table 1).

Table 1. Growth and survival of fry in different treatments during papaya seed treatment period.

Parameters	Treatments			
	T_1 (Control)	T_2 (5% PSP)	T_3 (10% PSP)	T_4 (15% PSP)
Total stock number	100±0.0	100±0.0	100±0.0	100±0.0
Total stock weight (g)	25.93±11.31	28.97±3.07	24.43±2.91	16.47±7.37
Average stock weight (g/fish)	0.26±0.11	0.29±0.03	0.24±0.03	0.16±0.07
Total final number	63.33±8.89	70.0±3.15	73.0±10.58	73.0±13.15
Total final weight (g)	86.19±9.46	93.35±5.37	89.9±4.52	86.89±10.05
Average final weight (g/fish)	1.43±0.32	1.33±0.06	1.31±0.27	1.27±0.51
Specific growth rate (% BW/day)	2.34±0.49	1.93±0.17	2.16±0.23	2.80±0.84
Survival rate (%)	63.33±8.89	70.0±3.15	73.0±10.58	73.0±13.15

Daily mean and range of water temperature, DO, pH, total ammonium nitrogen and nitrite nitrogen in each treatment during the treatment period are given in Table 2. The temperature, DO and pH varied between 27.6 to 27.8 °C, 5.2 to 5.3 mg/L and 7.4 to 7.5, respectively in different treatments. There was no significant difference in temperature and DO concentration among treatments. Similarly, the total ammonium nitrogen and nitrite nitrogen varied between 0.2 to 2.9 and 0.06 to 1.32 mg/L, before and after water exchange, respectively in all papaya seed treated groups which is not significantly differ with control. There was no significant difference in total ammonium nitrogen and nitrite nitrogen among treatments.

Table 2. Mean and range of water quality parameters in different treatments during papaya seed treatment period

Parameters	Treatments			
	T_1 (Control)	T_2 (5%PSP)	T_3 (10%PSP)	T_4 (15%PSP)
Water temperature (°C)	27.6±0.3 (25.5-31.3)	27.8±0.3 (25.5-31.2)	27.8±0.3 (25.3-31.6)	27.8±0.3 (25.4-31.5)
Dissolved oxygen (mg/L)	5.2±0.2 (3.9-7.3)	5.3±0.2 (4.1-7.2)	5.2±0.2 (3.9-8.0)	5.2±0.2 (4.0-7.1)
pH	7.5 (6.8-7.8)	7.4 (6.8-7.7)	7.5 (6.8-7.9)	7.4 (6.8-7.7)
Total ammonium nitrogen before water exchange (mg/L)	0.72±0.10 (0.16-1.66)	0.65±0.07 (0.07-1.63)	0.77±0.10 (0.14-2.37)	0.75±0.03 (0.19-1.53)
Total ammonium nitrogen after water exchange (mg/L)	1.0±0.56 (0.17-2.9)	1.1±0.53 (0.20-2.92)	1.0±0.32 (0.20-2.92)	1.1±0.43 (0.26-2.41)
Nitrite nitrogen (mg/L) before water exchange	0.01±0.00 (0.01-0.02)	0.02±0.00 (0.01-0.03)	0.02±0.00 (0.01-0.04)	0.02±0.00 (0.01-0.03)
Nitrite nitrogen (mg/L) after water exchange	0.02±0.02 (0.01-0.06)	0.02±0.01 (0.01-0.06)	0.02±0.02 (0.01-0.02)	0.01±0.00 (0.01-0.01)

Mean total feed used, amount of papaya seed used and feed conversion ratio (FCR) in each treatment during the treatment period are presented in Table 3. The amount of papaya seed used in T_1, T_2, T_3 and T_4 were 0.0±0.0, 4.3±0.3, 8.0±0.3 and 10.7±0.4 g, respectively. The amount of papaya seed used was significantly highest in T4, intermediate in T3 and lowest in T_2($P<0.05$). The overall FCR in T_1, T_2, T_3 and T_4were 1.7±0.3, 1.5±0.1, 1.4±0.0 and 1.2±0.1 g, respectively. The FCR was significantly higher in T4 compared to other treatments ($P<0.05$), whereas there was no significant difference among T_1, T_2 & T_3.

Table 3. Mean total feed used, total papaya seed used and FCR in different treatments during papaya seed treatment period.

Parameters	Treatments			
	T_1 (Control)	T_2 (5%PSP)	T_3 (10%PSP)	T_4 (15%PSP)
Total feed consumed (g)	76.8±6.6	86.0±3.2	79.7±2.2	71.6±2.9
Net fish yield (g)	50.5±7.4^a	56.8±6.6^a	57.4±3.0^a	62.6±6.2^a
FCR	1.7±0.3^a	1.5±0.1^a	1.4±0.0^a	1.2±0.1^b
Amount of papaya seed used (g)	0.0±0.0	4.3±0.3^a	8.0±0.3^b	10.7±0.4^c

Mean values with different superscript in the same row are significantly different ($P<0.05$).

Experiment phase II

The average stock weight of fry in T_1, T_2, T_3 and T_4 were 1.43±0.32, 1.33±0.06, 1.31±0.27 and 1.27±0.51 g, respectively (Table 4). The average weight of fish at the end of rearing period in T_1, T_2, T_3 and T_4 were 38.3±3.6, 35.2±7.0, 37.5±2.6 and 40.9±5.6 g, respectively without any significant different among treatments ($P<0.05$). The survival of fry at the end of hapa rearing phase in T_1, T_2, T_3 and T_4 were 80.7±5.2, 81.3±5.8, 80.0±3.1 and 76.0±9.1%, respectively without any significant different among treatments. The daily growth rate of fish in T_1, T_2, T_3 and T_4 were 0.21±0.02, 0.20±0.04, 0.21±0.01 and 0.23±0.03 g/fish/day, respectively without any significant different among treatments ($P<0.05$) (Table 4).

Table 4. Growth and survival of papaya seed treated fish in different treatments during hapa rearing phase.

Parameters	Treatments			
	T_1 (Control)	T_2 (5%PSP)	T_3 (10%PSP)	T_4 (15%PSP)
Total stock number	50±0	50±0	50±0	50±0
Total stock weight (g)	62.98±12.24	61.17±2.87	58.99±10.35	57.29±19.36
Average weight (g/fish)	1.43±0.32	1.33±0.06	1.31±0.27	1.27±0.51
Total final weight (kg)	1.53±0.05	1.30±0.18	1.49±0.05	1.51±0.07
Average final weight (g/fish)	38.3±3.6	35.2±7.0	37.5±2.6	40.9±5.6
Daily growth rate (g/fish/day)	0.21±0.02	0.20±0.04	0.21±0.01	0.23±0.03
Survival rate (%)	80.7±5.2	81.3±5.8	80.0±3.1	76.0±9.1

Fortnightly mean and range of water temperature, DO, pH and Secchi disk depth in each treatment during the experimental period are given in Table 5. The temperature, DO, pH and Secchi disc depth varied between 14.8 to 32.1 °C, 4.5 to 8.3 mg/L, 6.9 to 8.9 and 26.0 to 35.5 cm, respectively. There was no significant difference in temperature, DO and Secchi disc depth among treatments ($P<0.05$).

Table 5. Mean and range of water quality parameters in different treatments during hapa rearing phase.

Parameters	Treatments			
	T_1 (Control)	T_2 (5%PSP)	T_3 (10%PSP)	T_4 (15%PSP)
Water temperature (°C)	21.0±0.0 (14.8-31.9)	21.0±0.0 (14.8-31.9)	21.0±0.0 (14.8-31.9)	21.0±0.0 (14.8-32.1)
Dissolved oxygen (mg/L)	6.5±0.1 (4.5-8.3)	6.5±0.1 (4.5-8.3)	6.5±0.1 (4.5-8.3)	6.6±0.1 (4.5-8.3)
pH	8.1 (6.9-8.9)	8.1 (6.9-8.9)	8.1 (6.9-8.9)	8.1 (6.8-8.9)
Secchi disc depth (cm)	31.2±2.9 (26.5-34.4)	30.9±2.8 (26.5-35.0)	31.2±2.9 (26.0-34.5)	31.0±2.8 (26.5-35.5)

Mean final weight of fish, GSI and suspected sterility (abnormal gonad morphology) percent of papaya seed treated fish in different treatments at the end of hapa rearing phase is provided in Table 6. The GSI of fish in T_1, T_2, T_3 and T_4 were 0.02±0.01, 0.01±0.00, 0.01±0.00 and 0.01±0.00 %, respectively. The GSI of fish in T_1 was significantly higher compared to other treatments ($P<0.05$), whereas there was no significant difference among T_2, T_3 and T_4. The suspected sterility rate of fish in T_1, T_2, T_3 and T_4 were 0.0±0.0, 10.5±1.2, 15.5±1.8and 20.0±2.1 %, respectively. Among treatments, the sterility was significantly highest in T_4, intermediate in T_3 ad lowest in T_2($P<0.05$).

Table 6. Mean final weight, gonadosomatic index and sterility rate of papaya seed treated fish in different treatments at the end of hapa rearing phase.

Parameters	Treatments			
	T_1 (Control)	T_2 (5%PSP)	T_3 (10%PSP)	T_4 (15%PSP)
Mean final weight of fish (g)	38.3±3.6	35.2±7.0	37.5±2.6	40.9±5.6
Mean length (cm)	11.4±1.7	11.1±3.2	11.3±1.4	11.9±2.6
Gonadosomatic index (GSI, %)	0.2±0.01^a	0.1±0.00^b	0.1±0.00^b	0.1±0.00^b
Suspected sterility rate (%)	0.0±0.0^d	10.5±1.2^c	15.5±1.8^b	20.0±2.1^a

Mean values with different superscript in the same row are significantly different ($P<0.05$).

The reproductive performance of normal (control) and papaya seed treated fish is shown in Table 7. The number of fish spawned per week was significantly higher in control group (3.6±0.3) than papaya seed treated group (1.2±0.2) ($P<0.05$). The mean egg number per g female in control and treatment group were 2.1±0.1 and 1.9±0.2, respectively. Similarly, the mean egg weight in control and treatment group were 6.6±1.1 mg and 5.3±0.6 mg, respectively. There were no significant difference in egg number per g female and egg weight between control and treatment group. The fertilization rate was significantly higher in control group (98.2±1.3%) than papaya seed treated group (93.6±1.1%) ($P<0.05$). The incubation period of eggs in control and treatment group were 65.2±1.1 and 64.0±1.0 hours, respectively without any significant difference between two groups ($P<0.05$). The hatching rate was significantly higher in control group (81.5±2.6%) than papaya seed treated group (73.2±2.1%) ($P<0.05$). The fry survival rate at 7dAH in control and treatment group were 92.4±3.7 and 90.5±2.4%, respectively without any significant difference between two groups ($P<0.05$).

Table 7. Reproductive performance of control and papaya seed treated fish

Parameters	Control	Treatment
Number of fish spawned per week	3.6±0.3^a	1.2±0.2^b
Egg number per gram female	2.1±0.1^a	1.7±0.1^a
Average egg weight (mg)	6.6±1.1^a	5.3±0.6^a
Fertility rate (%)	98.2±1.3^a	93.6±1.1^b
Incubation period (hr)	65.2±1.1^a	64.0±1.0^a
Hatching rate (%)	81.5±2.6^a	73.2±2.1^b
Fry survival at 7 dAH (%)	92.4±3.7^a	90.5±2.4^a

Mean values with different superscript in the same row are significantly different ($P<0.05$).

Mean final weight and Gonadosomatic index (GSI) of control and papaya seed treated fish at the end of experiment is provided in Table 8. The GSI of female fish was significantly higher in control group (3.9±0.5%) than papaya seed treated group (2.7±0.3%) ($P<0.05$). Similarly, the GSI of male fish was significantly higher in control group (2.1±0.2%) than papaya seed treated group (1.5±0.1%) ($P<0.05$).

Table 8. Mean gonad weight and gonadosomatic index of control and papaya seed treated fish at the end of experiment.

Parameters	Control	Treatment
Female		
Mean weight (g)	113.3±17.0	75.7±12.6
Mean length (cm)	18.1±0.8	15.8±0.7
Gonad weight (g)	4.3±0.8^a	2.1±0.6^b
Gonadosomatic index (GSI, %)	3.9±0.5^a	2.7±0.3^b
Male		
Mean weight (g)	116.5±35.7	92.7±3.8
Mean length (cm)	17.7±1.1	17.3±0.3
Gonad weight (g)	2.0±0.2^a	1.4±0.1^b
Gonadosomatic index (GSI, %)	2.1±0.2^a	1.5±0.1^b

Mean values with different superscript in the same row are significantly different ($P<0.05$).

DISCUSSION

The present study aimed to control reproduction in Nile tilapia by inducing gonadal sterilization in sexually undifferentiated fry feeding with papaya seed. Active feeding on papaya seed mixed diet and good growth of fishes in all treatments during treatment showed that papaya seed powder is well accepted by Nile tilapia fries. This study also shows that feeding papaya seed up to 15% of the total feed has no adverse effect on growth and survival rate of Nile tilapia fry. Weight gained, feed conversion ratio, specific growth rate and survival rate were optimal in both control and papaya seed treatment groups. The survival rate of fry during treatment period in the present experiment (70-73%) is comparable with the result of Shrivastav et al. (2016&2017; 72-77%) and higher than the result of Ranjan et al. (2015; 58-65%) feeding with common carp testis. Similarly, the specific growth rate of fry during treatment period in the present experiment (1.9-2.8% BW/day) is lower than those reported by Ranjan et al. (2015; 9.9-10.8% BW/day) and Shrivastav et al. (2016&2017; 5.8-6.9 % BW/day). Similar findings were also reported by Lakshman et al. (2014) and Thompson et al. (2003) in papaya seed treatment, where they reported that the administration of papaya seeds extract does not show much influence on the structural composition of the intestine and suggested that the papaya seeds extract acts as antioxidant effect, as well as improve the lipid profile.

In the present study, values of all recorded parameters of water during papaya seed treatment were within the acceptable limits for growth and reproduction of Nile tilapia (Pillay and Kutty, 2005). The survival and growth of fish during papaya seed treatment period were well. Fish looked active, healthy and good color. This shows that feeding papaya seed has no adverse effect on water quality parameters. Milstein amd Svirsky (1996) also reported that feeding papaya seed powder has no adverse influence on water quality. In contrast to the present study, Ekanem and Okoronkwo (2003) reported some discoloration and damage of the liver of fish from the high dose treatment of papaya seed. In the present study, the total ammonium nitrogen ranged from 0.2 to 2.9 and 0.06 to 1.32 mg/L before and after water exchange, respectively in papaya seed treated groups which is not significantly different with control group. Pompa and Masser, (1999) reported that massive mortality occurs when fish are suddenly transferred to water with NH_3 concentration greater than 2 mg/L; however, mortality will be reduced to half or less when they are gradually acclimatized to a level as high as 3 mg/L for 3 or 4 days. In the present study, after water change total ammonium nitrogen level gradually increased from day 1 to day 3 in which fish gets better acclimatized. The temperature, DO and pH recorded during hapa phase was also in optimum range for tilapia growth and survival. This shows that feeding papaya seed powder has no adverse effect on water quality parameters during or after treatment phase.

In this experiment, the gonad weights were recorded for both the control and papaya seed treated groups. The result showed that the mean gonad weight of (T_1) control fish (4.3±0.8g) was significantly higher than the (T4) papaya seed treated fish (2.1±0.6 g). Similarly, the mean GSI of (T1) control fish (3.9±0.5%) was significantly higher than the (T4) papaya seed treated fish (2.7±0.3%). While there is not significant difference between body weight in control (113.3±17.0 g) and treatment (75.7±12.6 g). Similarly, there is not significantly difference in case of body length of both control (18.1±0.8cm) and treatment group (75.7±12.6 cm). The GSI of both males and females were significantly lower in papaya seed treated group compared to control. Significant differences obtained in males GSI was in contrast with previous studies conducted on using papaya seeds as reproductive inhibitor for an experimental animal such as albino rats (Maniyannan et al., 2009) and rabbits (Lohiya et al., 1999). They recorded insignificant differences in the testis weight after administration of papaya seeds with that of negative control. On the other hand, significant decrease occurred in GSI of female agreed with the finding of Jegede and Fagbenro (2008) and Temitope (2010) who reported significant decrease in GSI of Nile tilapia females treated with other medicinal plants such as neem (*Azadirachta indica*) and Hibiscus (*Hibiscus rosasinensis*) leaf. Abdelhak et al. (2013) also reported that GSI of males was not significantly different among treatments fed with high dose of papaya seed treated feeds which induced permanent sterility in Nile tilapia. Khalil et al. (2014) reported that the fish fed with 6 g papaya seed powder per kg diet for 30 days and 2 g papaya seed powder per kg diet for 60 days recorded the highest values of GSI of males and females, respectively. While, the fish fed with 2 g papaya seed powder per kg diet for 30 day gave the lowest values of GSI of males and females among all treatments.

Very low spawning in terms of frequency and number of eggs occurred in papaya seed treated group compared to control group. Out of 12 females stocked, the number of fish spawned per week in papaya seed treated group was 1.2 which is three times lower than the number of fish spawned in the control group (3.6 fish/week). Similarly, the number of eggs spawned per g female fish was 1.7 in papaya seed treated group can equal to control (2.1), indicating that the papaya seed treatment significantly reduces the fecundity of fish. This result is similar to that of Ekanem and Okoronkwo (2003) in Nile tilapia and Udoh and Kehinde (1999) in rat. The fertilization and hatching rates were also significantly lower in papaya seed treated group compared to control group. However, papaya seed treatment has no adverse effect on egg weight, incubation period and fry survival rate. In a similar anti-fertility study by Verma and Chinoy (2002), papaya seed extract was administered intramuscularly on male albino rats at 5 mg/kg/day for 7 days and this resulted in severe decrease in the contractile response of epididymal tubules when compared with the control experiment. Akin-Obasola and Jegede (2016) also reported that milt volume and sperm count of Nile tilapia was higher in control group and decreased with increasing concentration of *Gossypium herbaceum* in the diet.

In the present experiment, few fish were spawned in papaya seed treated group. This might be due to low dose or reversible effect of papaya seeds. After treatment fish were kept under normal feeding condition for about six months until maturation. Ekanem and Okoronkwo (2003) also reported the reversible effect of papaya seeds in the low dose treatments which was due to the fact that damage done to the testes was minimal and could be repaired within a few weeks. After recovery month, sections of both ovaries and testis showed possibility of reversible effects in low and medium doses treatments, while permanent sterility occurred in high dose treatment. Ekanem and Okoronkwo (2003) reported the absence of spawning in aquaria received the high dose treatment of papaya seed after stopping the treatment for 30 days. Maniyannan et al. (2009) recorded restoration of proper spermatogenesis in male albino rat after 120 days of recovery of papaya seeds treatment. Lohiya et al. (1999) recorded complete reverse in male rabbits administrated papaya seeds after withdrawal of the treatment. Pandit et al. (2015) reported that the germ cells in the gonads have ability to proliferate and recover in the original position after rearing the fish in normal condition after short duration high temperature treatment. They explained that to achieve complete gonadal sterilization, 100% germ cells should be degenerated. Based on these findings, we can say that feed papaya seed up to 15% of total diet cannot induce 100% sterility in Nile tilapia juveniles and the remaining germ cells recovered after transferring the fish in normal feeding condition. In overall, the present results demonstrate that feeding papaya seed during juvenile stage suppress the reproduction performance of Nile tilapia either by partial sterilization or any other physiological action.

CONCLUSION

The present study comes to introduce papaya seeds, which are cheap and easily available, as a natural agent to control the reproduction of Nile tilapia and overcome the problem of early maturation, instead of expensive chemical hormones. Reproductive parameters such as gonadosomatic index, fecundity, spawning frequency and hatching rate in Nile tilapia treated with papaya seeds at doses of 150 g/kg (15%) feed revealed sterility. This makes papaya seeds at the dose of 150 g/kg (15%) diet or higher dose are recommendable for use as sterility-inducing agents in sexually undifferentiated Nile tilapia fry.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Aquaculture and Fisheries Program, Agriculture and Forestry University, Rampur, Chitwan, Nepal for providing research and laboratory facilities. This work was funded by Directorate of Research and Extension, AFU, Chitwan, Nepal.

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Nepalese Journal of Aquaculture and Fisheries
Vol. 6 & 7 (2019 & 2020) : 49-54

PROFUSION OF GASTROINTESTINAL HELMINTH PARASITES IN *Channa* SPECIES FROM PROVINCE-1, NEPAL

Gayatri Shah[1] and Shyam Narayan Labh[1]*
[1]Department of Zoology, Amrit Campus, Tribhuvan University, Nepal
* Email: snlabh@gmail.com

ABSTRACT
The present study was conducted to investigate the prevalence of different helminth parasites in the gastro-intestinal tract of *Channa* species (*C. orientalis* and *C. striatus*). The parasites showed 100% prevalence in *C. orientalis*. Among these, *Capillaria pterophylli* was detected from 68 fishes showing the prevalence of 56.67% and the two Digenean trematodes, namely *Gonocerca phycidis* and *Genarchopsis goppo* exhibited the prevalence of 100% and 12.5%. Among 130 *C. striatus* examined, 111 of them were found to be infected with helminth parasites (p=85.38%). A nematode species, *Camallanus intestinalis* was detected from 32 specimens (24.61%), a cestode, *Bothriocephalus spp.* from 38 *specimens* (29.23%) and an acanthocephalan (*Pallisentis ophiocephali*). The study confirmed the abundance of helminth parasites in gastro-intestinal tract of *Channa* spp. Effective control measures and hygienic culinary practices can reduce the burden of helminth infection in fishes.

Keywords: *Channa*, prevalence, gastro-intestinal, helminth parasites

INTRODUCTION

Fish is a vital source of human food and regarded as the cheapest source of animal protein for human and livestock. With growing demand for fish, practice of pisciculture has been intensified. Consequently, piscicultures have been facing various hazards; one among such hazards is the diseases resulting from parasitic infections. Although, fishes have resistance to parasitic infections but under certain circumstances like bad drug treatment, unsuitable food, lack of oxygen, too high or too low temperature, or other adverse conditions, they become susceptible to parasitic infection. Helminthes are a major cause of diminished productivity in fishes, characterized by devastating effects on fish health in terms of mortality and morbidity. The effect of helminth infections on particular fish depends mostly on host species, age of the fish, immunological status, genotype, parasite species involved and the intensity of helminths. The climate in a particular locality is also important factors that determine the type and severity of parasitic infections in fishes. The health of fish is affected by parasites which make them susceptible to secondary infection by other agents such as bacteria, fungi and viruses

Snakehead fishes of the family Channidae are predatory freshwater teleosts which are important food fishes (Conte-Grand et al., 2017). *Channa* species subsists on a variety of living creature including small fishes, frogs, insects, earthworms, tadpole, etc. *Channa* species are found in Koshi, Trishuli, Gandaki, Karnali and some other rivers of Nepal. These species add notably to freshwater fishery and is in terrible demand because of their delectable flesh, high protein content and presence of fewer bones.

Bhuiyan (1964) reported that these fishes may be infected by different species of helminth parasites. Luque & Poulin (2004) reported that predatory fish species harbor a greater diversity and abundance of larval helminths than herbivorous and planktivorous species. These fishes are exposed to more infective helminth in their diet, thereby making more susceptible to higher parasite colonization. The major parasitic groups found in freshwater fishes are Trematodes (Monogeneas and Digeneans), cestodes, nematodes and acanthocephalans that complete their life cycles through intermediate hosts (Kundu and Bhuiyan, 2016).

A very middling works have been compassed regarding the helminth parasites of fishes in Nepal. In other countries, study on helminth parasites of *Channa* species have been carried out by Ahmed (2007), Chaiyapo et al. (2007), Puinyabati et al. (2010), Reddy and Benarjee (2011), Chowdhury and Hossain (2015), Singha et al. (2015), Ningthoukhongjam et al. (2015), Kundu and Bhuiyan (2016) and Mangolsana et al. (2016).

Fishes infected with helminth parasites pose serious threats to pisciculture and the possibility of multiple and concurrent infections of different species of the parasites invite zoonotic transmission to consumers. Parasitic diseases not only affect the normal health condition but also disrupt the overall metabolic activities of fishes, and may also even emanate mass mortality. And in this backdrop, the present study was carried out to investigate the common helminth parasites occurring in the gastro-intestinal tract of *C. orientalis* (Schneider, 1801) and *C. striatus* (Bloch, 1793) at Biratnagar, Province 1, Nepal.

MATERIALS AND METHODS

Fish samples were procured in between May 2017 to October 2017 from the local fish markets and the surrounding rivers and ponds of Biratnagar (26°28'60"N 87°16'60"E), Province No. 1, Nepal. The samples were then brought to the laboratory where they were examined for the occurrence of helminth parasites.

The fishes were dissected, and the intestinal portion was slit opened and examined for the emergence of adult parasites. The gut content was further observed under microscope by simple wet mount and iodine mount preparation. For this, about one gram of the gut content and a drop of normal saline or iodine solution were taken on a clean, dry glass slide and mixed to make smear and covered with a cover slip. The specimens were then observed under light microscope (10X and 40X magnifications). Taxonomical identification of helminth parasites was done by adopting the works of Yamaguti (1959), Gibson (2001) and Bhattacharya (2007).

Data were recorded and analyzed using *statistical package* for the social science (SPSS) version 16.0 and interpreted according to frequency distribution and percentage. The prevalence of helminth parasites was calculated according to Margolis et al. (1982).

Prevalence (p) = Total No. of Hosts infected/Total No. of Hosts examined *100

RESULTS

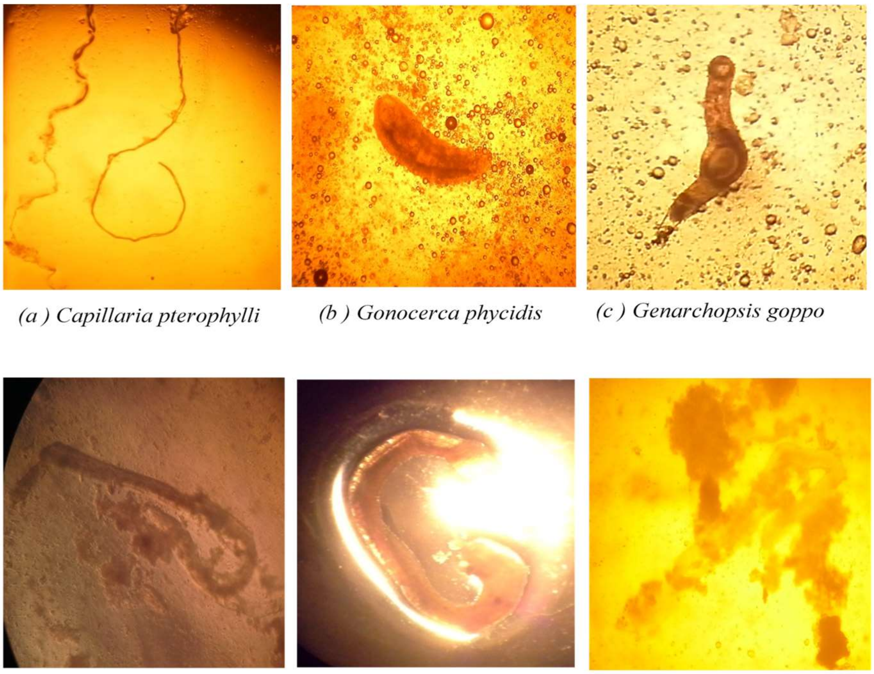
A total of 250 specimens of *Channa* (*C. orientalis*; n=120 and *C. striatus*; n=130) were examined during the study. Among the two, *C. orientalis* was found to be infected with nematode and trematode parasites, while *C. striatus* was found to be infected with nematodes, cestodes and acanthocephalan (Table 1).

Table 1: Infection of *Channa* species with gastro-intestinal helminth parasites.

Fish hosts	Helminth parasites			
	Nematodes	Cestodes	Trematodes	Acanthocephalans
Channa orientalis	+	-	+	-
Channa striatus	+	+	-	+

+ (present); - (absent)

Figure 1 showed the different parasites detected from the *Channa orientalis* and *Channa striatus,* The parasites showed 100% prevalence in *C. orientalis*. Among these, *Capillaria pterophylli* was detected from 68 fishes showing the prevalence of 56.7% and the two digenean trematodes, namely *Gonocerca phycidis* and *Genarchopsis goppo* exhibited the prevalence of 100% and 12.5% (Table 2). *Gonocerca phycidis* and *Genarchopsis goppo* were detected from 68 (56.67%) fish hosts, while *Gonocerca phycidis* and *Capillaria pterophylli* were detected from 15 (12.5%) hosts. Among 130 *C. striatus* investigated, 111 of them were found to be infected with helminth parasites (p=85.38%).



(a) Capillaria pterophylli *(b) Gonocerca phycidis* *(c) Genarchopsis goppo*

(d) Camallanus intestinalis *(e) Bothriocephalus cuspidatus* *(f) Pallisentis ophiocephali*

Figure 1: Helminths found in *Channa orientalis*

Table 2: Prevalence and distribution of helminth parasites in *Channa* species

Fish hosts	Total number	Prevalence of parasites	Helminth parasites observed		Frequency (%)
C. orientalis	120	100%	Nematode	*Capillaria pterophylli*	68 (56.7%)
			Trematodes	*Gonocerca phycidis*	120 (100%)
				Genarchopsis goppo	15 (12.5%)
C. striatus	130	85.38%	Nematode	*Camallanus intestinalis*	32 (24.6%)
			Cestode	*Bothriocephalus* species	38 (29.2%)
			Acanthocephalan	*Pallisentis ophiocephali*	41 (31.5%)

A nematode species, *Camallanus intestinalis* (Fig 1d) was detected from 32 specimens (24.61%), a cestode, *Bothriocephalus cuspidatus* (Fig 1e) from 38 *specimens* (29.23%) and an acanthocephalan, *Pallisentis ophiocephali* (Fig 1f) was detected from 41 specimens (p=31.54%).

DISCUSSION

In the present study, concurrent infections by two helminth parasites were detected which were in full agreement with the findings of Amin (1987) and Gupta et al. (2012). Concurrent infection results niche segregation and reduces the number of helminth parasites in fish (Kaur et al., 2012).

The present study depicted the varying proportions of prevalence of helminth infections in *Channa* species. Similar findings were also reported by Mangolsana et al. (2016) and Puinyabati et al. (2010). Out of 24 specimens of *C. orientalis* examined, Mangolsana et al (2016) found that 19 (p=79.17%) specimens were infected with trematode parasites (*Allocreadium fasciatusi* and *Metaclinostomum srivastavai*). Similarly, Puinyabati *et al* (2010) detected two trematodes (*A. fasciatusi* and *A. handia*) from *C. orientalis.*

The highest prevalence of an acanthocephalan (*Pallisentis ophiocephali* ; p=31.45%) in *C. striatus* agreed with the finding of Gautam et al. 2018. He reported the highest prevalence rate of 59.11% for an Acanthocephalan (*Pallisentis* sp.), followed by a nematode species (*Neocamallanus;* p= 17.18%) and then by a cestode species (*Senga sp*.; p=14.57%). Kundu and Bhuiyan (2016) and Mangolsana *et al.* (2016) also reported similar results in *C. striatus* with slight variation in the prevalence for each parasite. Mangolsana *et al.* (2016) reported 86.67% prevalence of the parasite, and Ningthoukhongjam *et al.* (2015) found that 100% *C. striatus* were infected by acanthocephalans.

The difference in prevalence and types of parasites in this study may be attributed due to the geographical variation and the difference in sample size. The presence of these helminth parasites in fish may be attributed to the poor water quality, crowding and other problems that give suitable habitats for the parasites and intermediate hosts. stated that the characteristic of any water body can influence and determine its parasitic fauna and when environmental conditions such as water, food and temperature become favorable for mass reproduction of parasites, the disease may spread very quickly.

CONCLUSION

The prevalence of helminth parasites was found to be higher in the gastro-intestinal tract of both the *Channa* spp. with concurrent infections of two or more helminths. With the efficient control measures and through good culinary practices the burden of helminth infection to fishes can be reduced.

ACKNOWLEDGEMENTS

The generous technical support and meticulous supervision provided by Prof. Dr. Damodar Thapa Chhetry, Dr. Bharat Raj Subba, Mr. Kul Prasad Limbu, Mrs. Pushpa Mishra and Mr. Shushil Kumar Jha, Mr. Prakash Kumar Yadav, Mr. Saligram Adhikari and other staffs of the Department of Zoology, Post Graduate Campus, Biratnagar are highly acknowledged.

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Nepalese Journal of Aquaculture and Fisheries
Vol. 6 & 7 (2019 & 2020) : 55-75

AN INVENTORY OF FISH BIODIVERSITY, SPAWNING AND FORAGING SITES OF KARNALI RIVER BASIN

Suresh Kumar Wagle*, Deepak Rijal, Arun Paudyal
USAID Paani Program, Baluwatar, Kathmandu
*Email: Suresh_Wagle@dai.com

ABSTRACT
This report describes the fish species diversity, and conservation status of fishes, along with the location of fishing, foraging, and spawning hotspots in Karnali River Basin. The assessment employed three approaches: i] review of secondary information, ii] multi-stakeholder consultation, and iii] field assessment involving local champions. The assessment recorded 196 fish species, including native (188) and exotic (8) species that belong to 91 genera and 32 families in the Karnali River Basin. The assessment also identified and recorded 48 species as migratory, 24 species that need conservation attention and five endemic fish fpecies in the basin. The diversity of fish species declined and fish composition changed with the increase in altitutional gradient and decrease in water temperature in Karnali River Basin. Stakeholders' consultation identified 70 spawning and 72 nursing sites in stretches of Karnali Basin that overlaps with 116 fishing hotspots. The study identified Karnali-Thuligad and Karnali-Ramgad's confluences and the surrounding area as suitable habitat for the spawning of flagship species, most prominently for Mahseer (*Tor putitora, Tor tor*) and Asala (*Schizothorax richardsonii*). A general conclusion drawn is to foster researches to validate the current assessment, a policy backed by robust legal instruments urgently needed to ensure sustainable capture fisheries, and protection of flagship species that remains at higher trophic order need immediate conservation attention.

Keywords: Conservation, fishing hotspots, fish species, Karnli River, spawning sites,

INTRODUCTION
Freshwater fish species not only are the most diverse group of vertebrates but also are the greatest proportion of threatened species (Leidy and Moyle, 1998). Freshwater fish assemblages can be a good bioindicator of ecosystem status owing to their vulnerability to environmental stressors and human disturbances (Dudgeon, 2010). Information on the the species composition of fish species, i.e., the presence or absence of particular species and their distributions, can provides strategic guidance for the protection of endangered species and vulnerable habitats (Arponen et al., 2005). This can also help in the identification of invasive species (Didham et al., 2007). In addition, fish species are important sources of of the economy for many communities as they have been a staple to the diet of many people. Over the past few decades, diversity of freshwater fish resources has decreased dramatically, and endemic species have been facing continuous threat in river systems of Nepal. Dam construction, overfishing, destructive fishing, water pollution and other human activities are considered as the main threats to fish biodiversity (Arthington et al., 2016). Therefore, the conservation of fish biodiversity and maintaining river health have become key action points. .

The Karnali River Basin lies between the mountain ranges of Dhaulagiri in Nepal and Nanda Devi in Uttarakhand in India. The river basin has a catchment area of 127,950 square kilometres, of which 55 percent area lies in Nepal. It is a perennial trans-boundary river originating on the Tibetan Plateau near Lake Mansarovar, with the extent of 507 km as being the longest river in Nepal, eventually joining the Ganges as one of its major tributaries (Jain et al, 2007). Karnali River with its major tributaries including Limi, Chuwa, Loti, Mugu, Kuwodi, Tila, Lohore, Seti, Thuli Gad and Bheri provides habitats for diversified aquatic flora and faunal species (NRCT, 2019). Several lLakes in lower reaches of the basin and lake systems in high altitude draining into the Karnali basin harbor flagship and endemic fish species (FAN, 2019). Various studies have recorded different number of fish species from a part of Karnali River. Smith et al. (1996) have studied aquatic biodiversity in the lower reaches of Karnali River basins and have recorded 121 fish species. However, an Environment Impact Assessment Study of the Upper-Karnali Hydropower Project has reported 48 fish species from the project area (Shrestha, 1997). Despite several studies have investigated fish resources in some areas of the Karnali River, no comprehensive study has been conducted on fish diversity and conservation status, nor these studies have assessed species diversity and the ecological characteristics of the habitat. Initial findings have shown that declining yield from capture fisheries over time and space is an indication that various human, as well as climate-induced factors, are affecting the fish population in Karnali River and its major tributaries (Paani, 2019a). Overfishing, pollution, sand extraction, flow modification and other human activities have seriously destroyed fish habitats and led to a decline in fish diversity in Karnali River (Paani, 2019b). Identification of fish spawning ground and fishing hotspots provide a basis for spawning closure which could make a contribution to the sustainable management of fish (van Overzee and Rijnsdorp, 2014). Information is scant on biodiversity hotspots in Karnali River that could hinder in formulating effective management and conservation planning of fisheries resources. The objective of this study was to comprehensively document the extent and distribution of fish species assessed across stretches of Karnali River Basin, and explore the fish biodiversity hot spots to inform the conservation strategy.

MATERIAL AND METHODS

USAID Paani Program (Program for Aquatic Natural Resource Improvement) conducted six multi stakeholder workshops during May 2018 to February 2019 one each in Middle Karnali, Lower Karnali, Rara Khatyad, Tila Karnali, Thuligad, Bogatan, and West Seti Watersheds of Karnali River Basin to assess the fish biodiversity (Figure 1). A total of 229 representatives from fishers' communities, governmental organizations, resource users' federations, and community-based organizations participated in these workshops.

The workshop proceeded with breakout sessions followed by group discussion, consolidating findings, and reporting back to the panel. In each breakout session, issues discussed include: listing of the fish species, identification of flagship species, biodiversity hotspots including spawning, nursing of fish species and fishing sites.

The participants listed fish species with local names found in natural waters across watersheds, and identified three major fish species according to their abundance, economic and ecological values. The participants located river stretches known as spawning, breeding, and fishing grounds for the top three major species on the base map of the river in the respective watersheds.

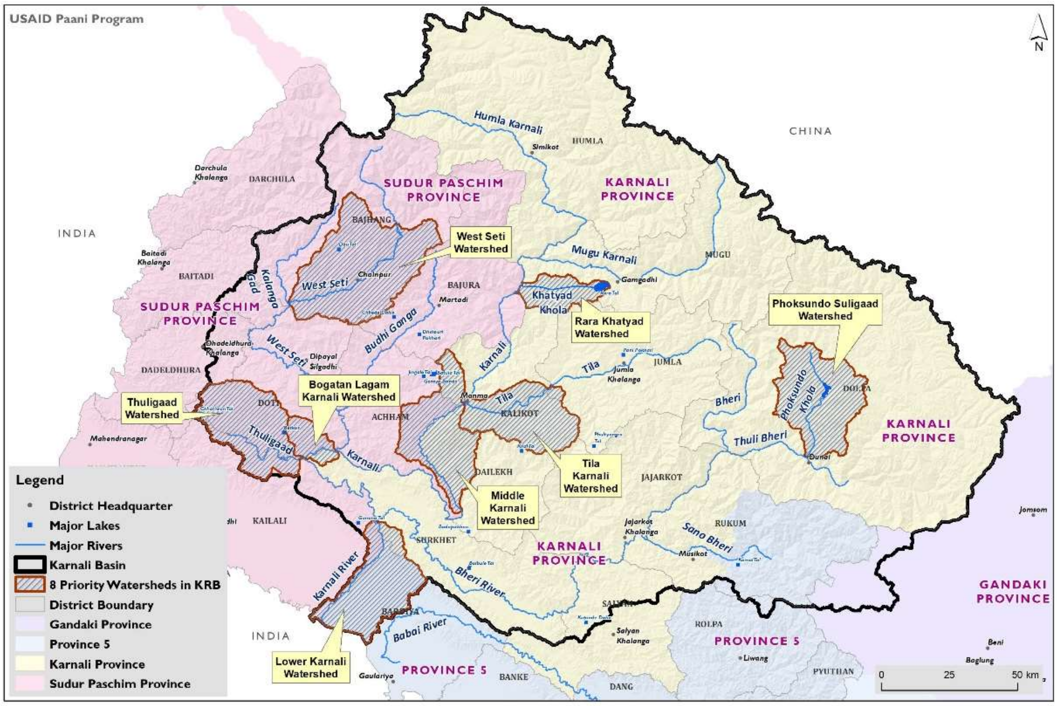

Figure 1: Map of Karnali River Basin with major watersheds (Source: USAID Paani, 2018)

Participants representing local government (e.g., mayors, deputy mayors, ward chairs of municipalities and rural municipalities), local level government offices and NGOs discussed the basin level issues and factors that connect upstream and downstream communities. The group discussed socio-cultural importance of rivers, conservation threats, community relationships, and benefit-sharing mechanisms between upstream and downstream communities and prioritized factors that affect freshwater biodiversity conservation. The discussion points upon consensus were noted and analyzed.

Paani developed knowledge products including profiles and health reports of the watershed, and Paani conducted research reports (AEC, 2019; CMDN, 2019, FAN, 2019; RHF, 2019) were reviewed for enriching and consolidating the inverntory for documenting fish biodiversity of Karnali River Basin. The Environment Impact Assessment (EIA) reports of Upper Karnali Hydropower, and Tila I and Tila II hydropower projects were reviewed to enrich the fisheries inventory. Data and information obtained fron workshops were processed using Microsoft Excel and analyzed by using tabular and descriptive methods.

RESULTS AND DISCUSSION

Fish diversity

Freshwater including rivers, lakes and wetlands of Karnali River Basin harbors 197 fish species that belongs to 32 family and 92 genera (Table 1). The number of fish species in Karnali River System currently reported is higher than the 178 fish species in a recent compilation by WWF-Nepal (Shrestha and Thapa, 2020). Among the fish species recorded from Karnali, eight exotic species dwells in the freshwater system of this basin (Annex I). Five endemic species including critically endangered *Schizothorax nepalensis* and *Schizothorax raraensis* recorded from high altitude (2990 masl) Rara Lake. The basin is also habitat for 49 migratory and 24 species of fish in IUCN Red List that needs conservation attention.

Table 1. Total number including migratory, endemic and conservation status of fish species of Karnali River Basin

S.N.	Family	Number of fish species	Number of genera	Number of migratory species	Number of species in IUCN Red List	Number of endemic Species
1	Ailiidae	2	2	2	1	
2	Ambassidae	5	2	1	1	
3	Amblycipitidae	1	1			
4	Anabantidae	1	1	1		
5	Anguillidae	1	1	1	1	
6	Badidae	1	1			
7	Bagridae	10	5			
8	Balitoridae	3	2			1
9	Belonidae	1	1	1		
10	Botiidae	4	1	1	1	
11	Channidae	7	1		1	
12	Cichlidae	2	2			
13	Clariidae	2	1	1		
14	Clupeidae	2	1	1		
15	Cobitidae	2	2			
16	Cyprinidae	90	36	27	11	3
17	Cyprinodontidae	1	1		1	
18	Erethistidae	3	3			
19	Gobiidae	1	1			

20	Heteropneustidae	1	1			
21	Mastacembelidae	4	2		1	
22	Mugilidae	2	2			
23	Nandidae	1	1			
24	Nemacheilidae	15	4		1	
25	Notopteridae	2	1	2	1	
26	Osphronemidae	5	3			
27	Percoidae	1	1			
28	Psilorhynchidae	4	1	2		1
29	Schilbeidae	4	3	1		
30	Siluridae	2	2	2	2	
31	Sisoridae	14	5	4	2	
32	Synbranchidae	1	1	1		
	Total	**196**	**91**	**48**	**24**	**5**

Lower Karnali Watershed with an area of 875 km^2 stretches in parts of Karnali, Sudur Pashchim and Lumbini provinces. The watershed contains 57 rivers and streams, and forms the downstream plain of the Karnali River Basin. Paani researches (AEC, 2019; FAN, 2019) and workshops compiled a list of 136 fish species belonging to 32 familiy from Lower Karnali (LK) Watershed. A study of IUCN recorded 121 fish species belongs to 24 family within a short range of Karnali River Stretch, Solta to Kothiyaghat (Smith et al., 1996). The majority of the fish species belong to the family Cyprinidae (57) followed by Bagridae (10), Channidae (6), each of Ambassidae, Nemacheilidae and Osphronemidae Cobitidae have five fish species (Table 2).

Table 2: Family wise number of fish species in rivers and wetlands of different watersheds of Karnali River basin

	Family	LK	TH+BG	MK	TK+RK	WS
1	Ailiidae	2	1	1		1
2	Ambassidae	5				
3	Amblycipitidae	1			1	
4	Anabantidae	1				
5	Anguillidae	1	1			
6	Badidae	1				
7	Bagridae	10				

8	Balitoridae	1	2		2	
9	Belonidae	1	1			
10	Botiidae	2	2	1	2	
11	Channidae	6	2	4		
12	Cichlidae	1		1		
13	Clariidae	2		1		
14	Clupeidae	2				
15	Cobitidae	2				
16	Cyprinidae	57	26	41	28	8
17	Cyprinodontidae	1			1	
18	Erethistidae	3				
19	Gobiidae	1				
20	Heteropneustidae	1				
21	Mastacembelidae	3		1	1	
22	Mugilidae	2				
23	Nandidae	1				
24	Nemacheilidae	5	3	9	3	1
25	Notopteridae	2				
26	Osphronemidae	5				
27	Percoidae	1				
28	Psilorhynchidae	4			2	
29	Schilbeidae	4				
30	Siluridae	2	1	1		
31	Sisoridae	4	3	8	7	3
32	Synbranchidae	1	1			
		135	43	68	47	13

LK-Lower Karnali, TH+BG-Thuligad and Bogatan Lagam, MK-Middle Karnali, TK+RK-Tila Karnali and Rara Khadyad, WS-West Seti Watershed

Thuligaad and Bogatan Lagam watershed with the total area of 1055 km² are located within the Karnali River Basin that belongs to parts of Doti, Kailali and Surkhet district. Altogether 53 streams and 156 tributaries in these watershed flow into the Karnali River. The river reaches in Thuligaad and Bogatan Lagam Watersheds provide important habitat for rich fish diversity of native origin. Paani researches

(AEC, 2019; CMDN, 2019) and workshops have recorded 44 fish species belonging to 11 family (Table 2). Of this total, 22 species are migratory and 12 species listed in IUCN Red List requires conservation attention. Family Cyprinidae consist of 26 fish species dominated by *Barilius, Schizothorax* and *Puntius* genera.

Middle Karnali watershed with an area of 903 km^2 falls within the Karnali River Basin and includes parts of Dailekh, Achham and Kalikot district of western Nepal. The Middle Karnali has numerous small rivers and tributaries scattered throughout the watershed totaling 659 km in waterways. Paani researches (AEC, 2019; CMDN, 2019; RHF, 2019) and the workshop recorded that Middle Karnali watershed provides habitats for 68 species of fish belonging to 10 families (Table 2). Fish family Cyprinidae comprised of 41 species followed by Nemacheilidae (9) and Sisoridae (8). Karnali River and tributaries of the watershed provides cruising way for 19 species of migratory fish including *Tor putitora, Anguilla bengalensis* and *Bagarius* spp. The watershed also provides habitat for thirteen fish spcies that are classified under the threat category (IUCN Red List). E-DNA study (CMDN, 2019) revealed that occurrence of five exotic fish species, viz. *Cyprinus carpio*, *Hypopthalmicthys molitrix*, *Clarias gariepinus, Carassius auratus* and hybrid of *Carassius auratus x Cyprinus carpio* in main strem of Karnali River in this watershed. The appearance of *Cyprinus carpio* and other exotic fishes is signaling biological invasion. The watershed stance for possible threats to the indigenous fish fauna, as a result of the invasion and proliferation of these exotics.

Tila Karnali watershed extends 767.48 km^2 across parts of Kalikot and Jumla districts. Tila River, with tributaries including Hima, Padamgaad, Baligaad, Bhartagaad, Dhandkhola, Kathina (Ghatte Khola), Khallagaad, Banchugaad, and Narmagaad, is the primary waterway of the watershed and flows southwesterly 45 kilometers across the watershed before joining the Karnali River (Paani, 2019c). The Rara Khatyad watershed stretches over 308 km^2 within the Karnali River Basin and includes parts of the Mugu district in western Nepal. The primary natural resource in the watershed is Rara Lake (2990 masl). Twenty streams flow into the lake, but there is only one outlet at the western end, which becomes Khatyad Khola, and twenty-five streams flow into the Khatyad Khola, which provides the important habitat for coldwater hill stream fishes (Paani, 2019d). Paani study (AEC, 2019; Paani, 2019cd) and the workshops recorded that 47 fish species with 24 genera belongs to 16 family from the river and lake of these watersheds (Table 2). Rivers in Tila Karnali watershed harbor 29 fish species and Rara Lake with its feeding and drainage streams support the life cycle of 23 fish species in Rara Khadyad Watershed. EIA report of Tila-I and Tila-II hydroelectricity project presents some 27 fish species belonging to three different families. Cyprinids with 28 fish species followed by Sisorids with seven species dominate the freshwater habitat of these watersheds (Table 3). A highest diversity (seven species) of genera *Schizothorax* from the glacier-fed and snow-fed rivers of these watersheds have been recoreded compared to other watersheds described in this report. These watersheds provide gateway for 16 migratory fish species including *Tor putitora, Accrossocheilus hehexagonolepis, Schizothorax labiatus* and others. Likewise, 10 species of immediate conservation importance (IUCN Red List) persist in the watersheds.

The West Seti watershed, with an area of 1,488 km^2 ranging in altitude from 3,400 m to just 750 m in the southern reaches, remains almost entirely inside Bajhang district, with small parts extending into Doti and Bajura. The watershed contains 151 rivers including Kalanga Khola, Bauligaad, Tarugaad, Sunigaad, Talkotgaad, Thalairgaad, Jadarigaad, Bhayagutegaad, and Listigaad. The workshop participants in West Seti Watershed recorded 13 fish species belongs to eight genera and four family (Table 8). The high diversity of migratory fish species relative to the total diversity of fish species recorded in stretches within the watershed area. The major migratory species include *Tor putitora, Tor tor, Clupisoma garua, Accrossocheilus hehexagonolepis* and *Schizothorax richardsonii.* Rivers and streams of the watershed host six spcies including *Accrossocheilus hehexagonolepis, Tor putitora, Tor chelynoides, Schizothorax richardsonii* of high conservation importance (IUCN Red Listed).

The current assessment has shown a high fish diversity in terms of family genera and species richness and accounted for 76.6% of the 256 species recorded for Nepal's freshwater system (Shrestha and Thapa, 2020). Physical structure of habitats is of greatest importance in determining both the abundance and species composition of river and stream fishes (Finger, 1982). Important aspects of habitat structure include water depth, water velocity and flow, cover, and substratum composition (Tesfay et al., 2019). The existence of good fish habitat is dependent on a number of factors, such as water flow, water quality, the presence of sufficient food, and the lack of excessive numbers of predators and competitors (Thompson and Larsen 2004). Large number of tributaries with different bio-chemical composition, food availability and wide array of bottom substrate have contributed for large number of fish species in Karnali River System.

Complex physiography and large temporal variations in water discharge, temperature, and turbidity provide an assortment of ecological niches that satisfy the environmental requirements of a large number of fish species in Lower Karnali watershed. The section of Karlali River and tributaries in this watershed have large floodplain, low velocity and substratum with stone, boulders and sandy bars covered with algae. These features of the river and streams have provided suitable habitat for many residential species. Total species richness in pool and step pool in this section of river might have played an important role in the breeding and growth for the fish community, and it is a refuge for fish assemblages in dry season, accounted for 70% of total native species recorded for Karnali River Basin. This rich fish species recorded in Lower Karnali Watershed related to their location in a transitional zone, where cool torrential waters of Himalayan hill streams are transformed into the warm and slow flowing waters with large flood plains. Species normally associated with mountain streams (e.g., *Schizothorax* spp, *Barilius* spp) inhibit the same waters as species normally associated with alluvial corridors of the tropical lowlands (e.g., *Labeo spp*, *Channa spp*) of Lower Karnali Watershed.

The confluence area of Thuligad and Karnali River provides large habitat for Mahseer (*Tor putitora*). Cool and clear water with shallow depth in large span of Thuligad River serves as foraging areas for Mahseer and the deep large pools in down stream of the Karnali River provides suitable habitats during non-migration period. Mahseer generally known to prefer cold, clear and swift flowing waters with stony, pebbly or rocky bottoms and intermittent deep pools (Dinesh et al. 2010). Participatory assessment of fish diversity held with the community at Thuligad Watershed and Paani transsect walk in Thuligad-Karnali confluence further confirmed the habitat suitability for Mahseer.

The section of the Karnali River including its warmer tributaries, e.g., Ramgad and Lohore Khola, considered important for aquatic species that thrive both in warm and cold water in middle Karnali Watershed. Workshop participants responded that the many large-river fishes such as *T. putitora, B. yarelii, Schizothorax* spp use large tributaries as well as mainstem of Karnali River for spawning. Participants also informed that tributary discharge has a strong influence on species richness and assemblage structure, reflecting the habitat requirements of individual species. Thus, tributary discharge can be an informative initial criterion for assessing which tributaries offer the most promise for conservation efforts in the absence of detailed population and habitat information. Unaltered large tributaries are important to fulfill habitat and life-history requirements for large-river fishes after the mainstem has been altered by dams or other development projects (Neely *et al*. 2009; Ziv *et al*. 2012).

The fast-flow habitat (i.e., riffle and cascade) with high velocity is most prevalent in mountain streams such as observed in Tila Karnali and Rara Khatyad watersheds, where the species richness and abundance accounted collectively for over 18% of the species richness of Karnali River System. Many fish species species of Balitoridae, Cyprinidae and Sisoridae prefer this types of habitat. Meanwhile, many fishes are well adapted to high-gradient, rapid flowing habitats and exhibit numerous morphological modifications in mouth, body shape and other associated structures and color patterns (Neely et al., 2007). For instance, the pectoral fins of those fish in Balitoridae into a sucker-shaped form, allowing the fish to adhere to the substrate and thus avoid being washed away by rapid flow (Haung et al., 2019).

Of the four endemic species recorded in water bodies of Tila Karnali and Rara Khatyad watersheds, Rara Lake alone hosts three endemic fish spcecies, *Schizothorax nepalensis, Schizothorax raraensis* and *Schizothoraichthys macrophthalmus*. Habitat characteristics such as availability of nutrient, optical property and substrate condition are factors that have determined persistence of endemic species in the Rara Lake. Rara Lake contains a good habitat of microscopic phytoplankton, zoo-planktons and myriads of aquatic insects serving as natural fish food for endemic species Shrestha (2017). Nasution (2015) established the relation between water quality components (phosphorous, organic matter), vegetation covers and distribution of endemic species in Towuti Lake in Indonesia. Analysis of relationship between the endemic fishery resources and the ecological parameters lays foundation for regulating ecological function and determine the population dynamics including reproduction and distribution of endemic species in Rara Lake and its feeding and drainage streams for the effective conservation management of high-altitude fishery resources including endemic fishes.

Fish breeding and nursing spots

Access to and quality of spawning habitats are critical to the success and productivity of a fish population (Rosenfeld and Hatfield, 2006), especially for substrate-spawning fish (Balon, 1981). Many freshwater organisms such as fishes use multiple habitats to gain access to different resources required at the various individual life stages; therefore, they are vulnerable to changes in the availability and quality of any or all of the required habitats (Lucas et al., 2009). Because the disturbances in connectivity between spawning and nonspawning habitats (including feeding habitats), and the loss or decreased suitable spawning habitats can adversely affect population structure and persistence, characterising and prioritising suitable spawning and nursery habitats are essential to conservation planning in a degraded and fragmented landscape.

The workshops attempted to identify fish breeding locations to support for habitat conservation programs and inform researchers to verify and validate the experience of local community. The fisher participants of the workshops, based on their experience and close observation of spawning behavior of fish, eggs laid on variety of substrate and school of juvenile fish in riparian areas of the river and streams during their fishing trips, have identified spawning and nursing locations in respective watersheds. The participants also mapped the major spawning and nursing spots of the fish on the river base map. All the spawning and nursing hotspots digitized to guide stakeholders for easy monitoring and initiate conservation activities.

The fisher participants' idenfied 70 locations of fish spawning and 72 locations of fish fry nursing across the Karnali River Basin. The number of tributaries and stearms with gentle water flow, diverse vegetative substrates and beds composed of pabbles and sandy bars, and flows into main river with large pools immediately after the confluence at downstream were the major determinant of spawning habitats. Fisher's mapping indicated that the most of the spawning habitats are overlapped with the nursing locations (Figure 2).

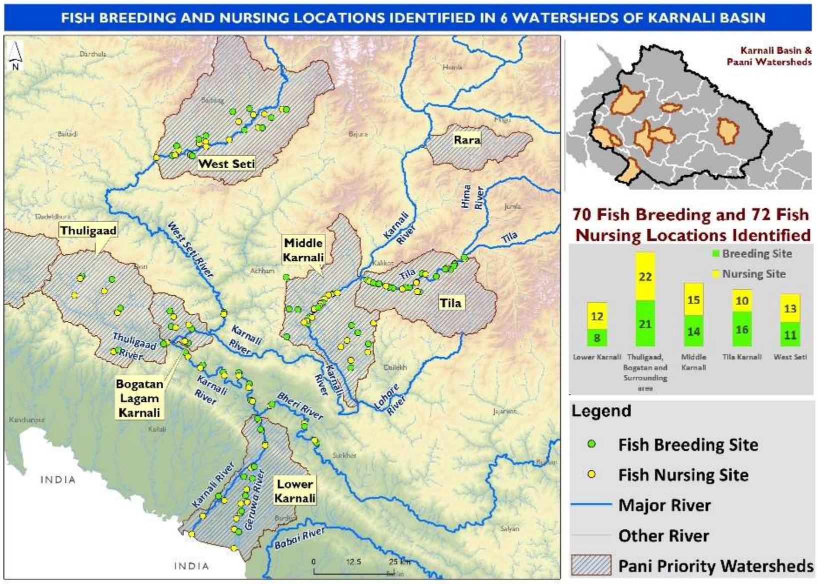

Figure 2: Fish breeding and nursing sites in Karnali River identified and mapped by workshop participants (Paani FVA, 2018, 2019)

Lower Karnali watershed has the least number of suitable habitats (8 locations) followed by Tila Karnali with a few numbers of tributaries providing habitats for spawning. The map showed that Thuligad and

Middle Karnali watersheds have the high number of breeding ground compared to that of other watersheds in Karnali River Basin (Figure 16). Water temperature of several tributaries such as Thuligad Khola in Thuligad watershed and Ramgad Khola in Middle Karnali watershed is relatively high (>20 to 26 ºC) in most of period of a year, that attract many warmwater fishes including Mahseer (*T. putitora, T. tor*) and several species of *Labeo cuvier* for spawning. Shrestha (1986) and Nautiyal (1989) recorded that springfed streams in Trishuli, Nepal and Garawal, India are important spawning grounds for Mahseer (*T. putitora*). They described the waters of such streams flow with low velocity, and held higher thermal profile as well as greater diurnal and annual rthythms when compared with the glacierfed Rivers.

Riverbed of Thuligad and Ramgad tributaries of Karnali River composed of small pabbles and boulders compounded with warmwater environment stimulates Mahseer to spawn. High vegetative cover in riparian area of these streams' favorable environment for the growth of natural food to feed upon by juvenile fishes. These features of the streams provide the spawning niche and feeding area for larval stages of fish (Shrestha, 1986). Large pools formed downstream of the Thuligad-Karnali confluence serve as hiding habitat for Mahseer during winter and ripe parents migrate from there to thuligaad stream for spawning when the flood recedes. The morphology, hydraulic and limnetic charateristics of Thuligad-Karnali and Ramgad-Karnali confluence makes the area most suitable for spawning of endangered Mahseer group of fishes. With these features of confluences, NRCT (2019) has proposed for the establishment of fish sanctary in area from Rakam Karnali to 16 km below the Thuligad confluence and 10 km up the Seti-Karnali confluence by which Mahseer and alike aquatic meghafauna could be conserved and protected (Figure 3).

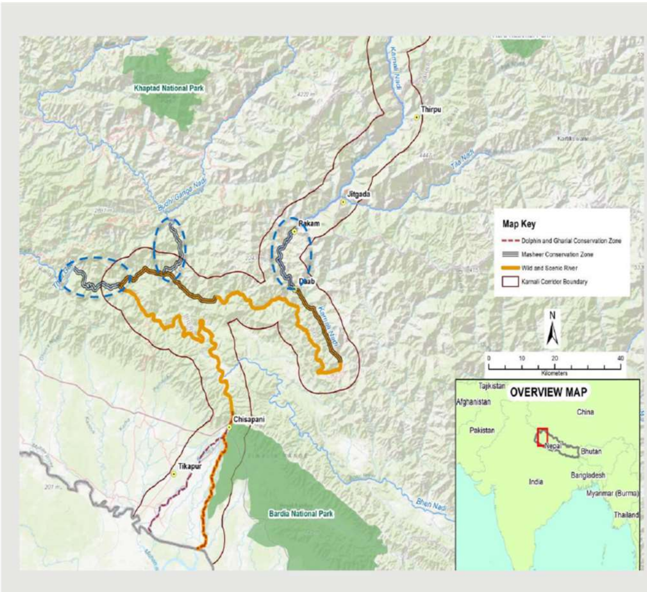

Figure 3: Location map of proposed conservation area (fish sanctuary) in area between Rakam to Thuligad-Karnali and Seti-Karnali confluence (Source: Adapted from NRCT, 2019)

Fish spawning is generally limited to specific areas and times (Cushing, 1990). Therefore, fishing in sites during spawning period may target different components of the population than during the non-spawning period. The chance of catching the larger and ripe parents may be higher during the spawning period as they gather on the spawning grounds, which are often more confined in space than the feeding grounds. Spawning aggregations have been reported in a wide variety of fish and invertebrate species in fresh water and marine ecosystems, such as fresh water cyprinids including Mahseer (de Graaf et al. 2006). Consequently, the selection pattern, i.e., the relative mortality imposed by fishing on the different age groups, sexes or maturity stages, may differ between the spawning period and the non-spawning period. Closed areas and spawning closure may promote sustainable exploitation through the protection of spawning or nursery areas (Sadovy de Mitcheson and Colin 2012). Spawning closures have been established successfully to reduce the fishing mortality in fisheries targeting large spawning aggregations that are particularly vulnerable to over-exploitation (Sadovy de Mitcheson et al., 2013). Spawning closures have also been put into place to protect spawning populations in order to enhance the reproductive output of spawning fish and hence improve the number of recruits in the exploited stock (van Overzee and Rijnsdorp, 2014). Area closure for localized species mostly littoral that breeds several times in a year and spawning closure for species that migrates for breeding could be instrumental to protect the spawning population and their early offspring from being overexploited through overfishing and destructive fishing practices.

CONCLUSION

The Assessment conducted in selected watersheds across Karnali river basin covered broad areas for generating information on fish and associated threats, and upstream and downstream connectivity issues. The Karnali River plays a significant role in maintaining and replenishing the fish resources in its tributaries and wetlands withing the river basin. The assessment reveals Karnali river basin provides habitats for over 77% of the total fish diversity recorded in Nepal. It provides suitable habitats for significant number of endemic fish species that needs immediate conservation attention including mitigation measures. This assessment urge for researches to periodic update of fish biodiversity, understand the ecology of fish migration, and identification of conservation hotspots for flagship fishes that inhibit in coldwater and warmwater within the Karnali River Basin.

ACKNOWLEDGEMENTS

This study is made possible by the support of the United States Agency for International Development (USAID). The content of this study is the sole responsibility of the authors and does not necessarily reflect the views of USAID or the United States Government. Authors wish to recognize and express our gratitude for the many local government offices in the study watersheds who generously offered time and action to help us identify the numerous key issues related to fish conservation. This report reflects their valuable contributions and assistance. We also wish to thank the many community residents – and especially fisherfolk – who shared their reflections with us to help establish baselines and this inventory of species.

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Annex I. List of fish species recorded from Karnali River Basin through Paani researches and assessment

S. No.	Family	Species	Local names					Karnali River Basin	
				WS	TH+ BL	Tila+ RK	MK	LK	KRB
1	Ailiidae	*Ailia coila*	Sutara, Patanga, Patasi, Patangu, Patsi					√	√
2		*Clupisoma garua*	Jalkapoor, Baikha	√	√			√	√
3	Ambassidae	*Chanda nama*	Nata channa					√	√
4		*Chanda Ranga*	Bhitte, Chanri					√	√
5		*Parambassis ranga*						√	√
6		*Parambassis baculis*	Himalayan glassy perchlet					√	√
7		*Parambassis lala*	Highfin glassy perchlet, Cahnerbijuwa					√	√
8	Amblycipitidae	*Amblyceps mangois*	Bindhar, Pichhi, Baljung, Luthe			√		√	√
9	Anabantidae	*Anabas testudinus*	Kabai, Kerkhi					√	√
10	Anguillidae	*Anguilla Bengalensis*	Rajbam, Lamtari		√		√	√	√
11	Badidae	*Badis badis*	Khesalei, Khesaki					√	√
12	Bagridae	*Aorichthys aor*	Kanti					√	√
13		*Hemibagrus menoda*	Satta					√	√
14		*Mystus bleekeri*	Tengra, Katena					√	√
15		*Mystus horai*	Indus catfish					√	√
16		*Mystus Seenghala*	Kanti, Sujnha					√	√
17		*Mystus Tengara*	Tengri					√	√
18		*Mystus Vittatus*	Tengra					√	√
19		Mystus gulio	Satto					√	√
20		*Rita tita*	Rita, Belunda, Capree					√	√
21		*Sperata seenghala*						√	√
22	Balitoridae	*Acanthobotis botia*	Botia		√	√			√
23		*Balitora brucei*	Titae, Patherchatti		√	√			√
24		*Balitora eddsi*	Patharchata, River Loach					√	√
25	Belonidae	*Xenentodon cancila*	Chuchche Bam, Kauwar, Dangawa, Sui		√			√	√
26	Botiidae	*Botia lohachata*	Getu, Baghe		√	√	√		√
27		*Botia dayi*	Getu					√	√
28		*Botia derio*	Baghe					√	√
29		*Botia geto*			√	√			√
30	Channidae	*Channa amphibia*	Snakehead					√	√
31		*Channa gachua*	Hilae, Charangi, Chenga		√		√	√	√
32		*Channa marulius*	Sauri, Bhaura					√	√
33		*Channa orientalis*	Chanrangi, Bhoti, Garahi, Ghau nya , Chenga				√	√	√

34		*Channa punctatus*	Hilae, Charangi, Bhote, Gauri		√		√	√	√
35		*Channa stewartii*	Charinga					√	√
36		*Channa striatus*	Charangi, Sauri				√		√
37	Cichlidae	*Pterophyllum scalare*					√		√
38		*Tilapia mossambica*	Kotre, Khesri					√	√
39	Clariidae	*Clarias batrachus*	Mungri, Mangur					√	√
40		Clarias gariepinus	Pugijhajha, African catfish				√	√	√
41	Clupeidae	*Gudusia Chopra*	Suiya, Darahai, Fulia					√	√
42		*Gudusia godanahiai*	Suiya, Darahai, Fuliya					√	√
43	Cobitidae	*Somileptus gongota*	Latani, Goira					√	√
44		*Lepidocephalichthys guntea*	Lata, Goira					√	√
45	Cyprinidae	*Accrossocheilus hehexagonolepis*	Copper Mahseer, Katle, Dhumuch, Panp, Vadalke	√	√	√	√	√	√
46		*Amblypharyngodon mola*	Mola carplet, Piruwa, Dhawaii					√	√
47		*Aspidoparia jaya*	Mara, Karanga, Bhegna					√	√
48		*Aspidoparia Morar*	Harda, Bhegna, Karangi, Karhawa, Papidopari, Chakale				√	√	√
49		*Amblypharyngodon microlepis*	Asala, Mada, Dhawai			√		√	√
50		*Barilius barila*	Chahale, Faketo		√		√	√	√
51		*Barilius barna*	Faketa, Poti, Chele, Titer kane fageta		√	√	√		√
52		*Barilius bendelisis*	Feketa, Gudari, Jhojha		√	√	√	√	√
53		*Barilius bola*	Bola				√	√	√
54		*Balitora brucei*	Pare Maachhaa					√	√
55		*Bangana dero*	Kalabans			√	√		√
56		*Barilius jalkapoorei*	Jalkapoor					√	√
57		*Barilius shacra*	Fageta, Jhilke, Baril, Chalkane				√		√
58		*Barilius tilea*	Tikahinia, Fageta		√	√			√
59		*Barilius vagra*	Tilkhina, Lam fageta		√	√	√	√	√
60		*Bengala elanga*	Bengala barb, Dedhura, Karange					√	√
61		*Brachydanio rario*	Zebra danio, Chittaripothi					√	√
62		*Cabdio morar*						√	√
63		*Carassius auratus*	Goldfish				√		√
64		*Carassius auratus x Cyprinus carpio*	Hybrid of Goldfish & Common Carp				√		√
65		*Carassius gibelio*	Prussian carp				√		√
66		*Catla catla*	Bhakur, Catlogi					√	√

67		*Changunius changuniyo*	Patherchatti, Gorahi, Kubre				√	√
68		*Cirrhinus mrigala*	Rewa, Naini, Seri, Mrigal				√	√
69		*Cirrhinus reba*	Rewa				√	√
70		*Cirrhinus fulungee*	Deccan White Carp				√	√
71		*Cirrhinus cirrhosus*	White Carp			√		√
72		*Crossoheilus latius*	Kachara, Lohori, Mata buduna		√	√		√
73		*Cyprinus carpio*	Common Carp			√		√
74		*Cyprinus latius*	Budhuna	√		√		√
75		*Chela cacius*	Neon hatchet Fish, Kachhi				√	√
76		*Chela laubuca*	Deduwa, Indian glass barb				√	√
77		*Ctenopharyngodon idella*	Grass carp, Papo				√	√
78		*Danio aequipinnatua*	Giant danio, Chittaripothi				√	√
79		*Danio dangila*	Deduwa, Dangila danio, Pothi				√	√
80		*Danio devario*	Chitharipothi, Dera				√	√
81		*Danio rerio*	Zebra				√	√
82		*Diptychus maculatus*	Scaly osman, Budune asala		√			√
83		*Esomus danricus*	Darai, Deduwa		√		√	√
84		*Garra annanndalei*	Buduna, Chuchche buduna	√	√	√		√
85		*Garra lamta*	Patharchati		√	√		√
86		*Garra gotyla*	Budhuna, Chepe, Nakate Buduna, Rock Carp	√	√	√		√
87		*Garra rupecola*	Buduna	√		√	√	√
88		*Garra mullya*	Sucker fish	√		√		√
89		*Garra sp*				√	√	√
90		*Hypophthalmichthys molitrix*				√		√
91		*Labeo angra*	Thed, Thunde, Klanch, Kalmunda	√		√	√	√
92		*Labeo boggut*	Boggut Labeo				√	√
93		*Labeo baga*	Thilke				√	√
94		*Labeo calbasu*	Klanch, Basarahill, Ghorat	√			√	√
95		*Labeo dero*	Gurdi, Rohu, Kathalegi, Gardi, Tite	√			√	√
96		*Labeo fibriatus*	Boi				√	√
97		*Labeo pangusia*	Gardi, Pangusia labeo, Grahan, Kalaacha, Termassa			√	√	√
98		*Labeo bata*	Bata, Rohu		√	√		√
99		*Labeo gonius*	Kuria labeo				√	√
100		*Labeo rohita*	Rohu, Roha, Ghorath, Kaltauke				√	√
101		*Osteobrama cotio*	Chanawro, Gurda				√	√

102	*Oxygraster argentea*	Namsehara					√	√
103	*Oxygraster bacaila*	Chelwa					√	√
104	*Oxygraster gora*						√	√
105	*Oxygraster phulo*						√	√
106	*Puntius Chilinoides*	Sidhra				√	√	√
107	*Puntius conchonius*	Rosy Barb, Sidhara, Sidre, Sidhra			√	√		√
108	*Puntius gelius*	Sidhra					√	√
109	*Puntius sarana*	Kunde, Bada, Pothi, Sidhara		√			√	√
110	*Puntius sophore*	Pothi, Sidhara, Chandapothi, Pate sidra		√	√	√		√
111	*Puntigrus tetrazona*	Tiger Barb, Sumatra Barb		√		√		√
112	*Puntius terio*	Onespot Barb					√	√
113	*Puntius ticto*	Sidhara, Pothi, Potina, Darahi, Tite pothi, Jibulbam Sedri, Ratapakhe		√	√		√	√
114	*Puntius jerdoni*	Jerdon's carp				√		√
115	*Pterophyllum scalare*						√	√
116	*Raimas bola*	Trout, Goha					√	√
117	*Raimas guttatus*	Burmese trout, Suiree faketo					√	√
118	*Rosbora daniconius*	Dedhawa					√	√
119	*Schizothoraichthys annandalei*	Thude, Asala		√				√
120	*Schizothoraichthys progastus*	Point nosed snow-trout, Chuche Asala			√	√		√
121	*Schizothorax plagioustomus*	Spotted snow-trout, Asala, Thople Asala, Suun Asala	√	√	√	√		√
122	*Schizothorax richardsonii*	Blunt nosed snow-trout, Thumree Asala, Buchche Asala, Dhumke asala	√	√	√	√	√	√
123	*Schizothorax molesworthi*	Blunt-nosed snowtrout, Buchche Asala		√	√	√		√
124	*Schizothorax labiatus*	Chuchche asala	√	√	√	√		√
125	*Schizothorax sp*	Asala	√			√		√
126	*Schizothorax nepalensis*	Tikhe Asla, Asala			√			√
127	*Schizothorax raraensis*	Rara Asla			√			√
128	*Schizothoraichthys macrophthalmus*	Asala, Tilke Asala			√			√
129	*Semiplotos semiplotus*	Padhani, Chepti					√	√

130		*Tor putitora*	Chawar, Sahar, Mahseer, Chuchche sahar, Kachala	√	√	√	√	√	√
131		*Tor tor*	Sahar, Bhaisae sahar, Choke Maachha	√	√	√	√	√	√
132		*Tor chelynoides*	Karange, Halude	√	√	√	√		√
133		*Tariqilabeo latius*	Gangetic latia				√		√
134		*Physoschistura elongata*	Stone loach, Gerudo					√	√
135	Cyprinodontidae	*Cyprinodon semiplotum*	Assamese Kingfish, Khurpe, Chepti			√		√	√
136	Erethistidae	*Pseudolaguvia kapuri*						√	√
137		*Erethistes pussilus*						√	√
138		*Hara hara*	Kosi hara					√	√
139	Gobiidae	*Glossogobius giuris*	Bulla					√	√
140	Heteropneustidae	*Hetetopneustes fossilis*	Stinking Asian Catfish, Singhi					√	√
141	Mastacembelidae	*Macrognathus aral*	Baam			√			√
142		*Macrognathus aculeatus*	Gainchi, Bamali, Dhunge Bam, Bamsemti					√	√
143		*Mastacembelus armatus*	Chusi Bam				√	√	√
144		*Mastacembelus pancalus*	Kath Gainchi, Gainchi, Bamali, Dhunge Bam					√	√
145	Mugilidae	*Siscamugil cascasia*	Rewa					√	√
146		*Rhinomugil corsula*	Dudhe/Salle satto					√	√
147	Nandidae	*Nandus nandus*	Dhala, Dewan					√	√
148	Nemacheilidae	*Nemacheilus beavani*	Stone loach, Gadero, Gadela, Pate Goira, Kanelani, Gadira					√	√
149		*Nemacheilus botia*	Gadela, Pate gadela	√				√	√
150		*Nemacheilus corica*	Kholumachha, Gadela, Gadi			√		√	√
151		*Nemacheilus rupecola var inglishi*	Kholumachha					√	√
152		*Nemacheilus savona*						√	√
153		*Physoshistura elongate*	Siyae, Suiree				√		√
154		*Schistura rupecula*	Radi, Gindula		√	√	√		√
155		*Schistura multifaciatus*			√	√	√		√
156		*Schistura sovana*					√		√
157		*Schistura scatugirina*			√				√
158		*Schistura corica*					√		√
159		*Schistura sp*					√		√
160		*Triplophysa dorsalis*					√		√
161		*Triplophysa sp.*					√		√

162		*Triplophysa tibetana*					√		√
163	Notopteridae	*Notopterus notopterus*	Golhai, Darahai, Fulia					√	√
164		*Notopterus chitala*	Mohi, Patara, Golhai, Chitala					√	√
165	Osphronemidae	*Colisa fasciatus*	Banded Gourami, Kauwa Maachha, Katara, Khesra					√	√
166		*Colisa lalia*						√	√
167		*Trichogaster lalius*						√	√
168		*Trichogaster chuna*						√	√
169		*Trichogaster labiosus*						√	√
170	Percoidae	*Pseudambasis baculis*	Chanari					√	√
171	Psilorhynchidae	*Physilorhynchus balitora*	Balitora minnow					√	√
172		*Physilorhynchus sucatio*	Sucatio minnow, Pathachatti, Tite			√		√	√
173		*Psilorynchus homaloptera*	Pathachatti					√	√
174		*Psilorynchus pseudecheneis*	Pathachatti, Titaila			√		√	√
175	Schilbeidae	*Uutropichthys vacha*	Baikha, Bachawa, Jalkapur					√	√
176		*Pseudeutropius atherinoides*	Jalkapoor, Patasi, Dudhe					√	√
177		*Pseudeutropius mirius*	Baikha, Jalkapur					√	√
178		*Silonia silondia*						√	√
179	Siluridae	*Ompok bimaculatus*	Voktam, Chottari, Pabata, Lalmuha, Chachara		√		√	√	√
180		*Wallago attu* (Schn)	Buhari, Padani, Ghoptari, Ghugunes					√	√
181	Sisoridae	*Bagarius yarelii*	Gonch, Thend, Gochara, Baghai		√			√	√
182		*Bagarius bagarius*	Catfish, Gonch		√			√	√
183		*Pseudecheneis sulcatus*	Kavre, Marcha, Dhami	√		√	√		√
184		*Pseudecheneis serracula*	Dhami			√			√
185		*Parachiloglanis hodgarti*				√	√		√
186		*Glyptothorax cavia*	River catfish, Bhitte, Capree, Vendro, Kalejunga, River Catfish, Bhitte		√	√	√		√
187		*Glyptothorax gharwali*				√			√
188		*Glyptothorax horai*	Kotel, Kathel	√			√		√
189		*Glyptothorax pectinopterus*	Karasingha, Capree, Dupmachha, Stone Cat, Vadal, Katuse Kabre			√	√		√

190		*Glyptothorax telchitta*	Kotel, River Catfish, Kabre	√				√	√
191		*Glyptothorax trilineatus*	Kavre, Torent Catfish, Junge Kabre, Stream Catfish				√		√
192		*Glyptothorax alaknandi*	Kalejunga			√	√		√
193		*Glyptothorax gracilis*					√		√
194		*Laguvia ribeiroi*						√	√
195		*Nangra viridescens*	Katenga					√	√
196	Synbranchidae	*Monopterus cuchia*	Gangetic swamp eel		√			√	√
	TOTAL			**13**	**43**	**47**	**68**	**136**	**196**

LK-Lower Karnali, TH+BG-Thuligad and Bogatan Lagam, MK-Middle Karnali, TK+RK-Tila Karnali and Rara Khadyad, WS-West Seti Watershed