ISSN 2645-8357

Nepalese Journal of Aquaculture and Fisheries

The Journal of the
Nepal Fisheries Society

Volume 3 & 4 2016 & 2017

ISSN 2645-8357

Nepalese Journal of Aquaculture and Fisheries

The Journal of the Nepal Fisheries Society

Volume 3 & 4 2016 & 2017

Editorial Board

Content

Nepalese Journal of Aquaculture and Fisheries
Vol. 3 & 4 (2016 & 2017) : 1-11

Status and development trend of aquaculture and fisheries in Nepal

Prabesh Singh Kunwar*and Baikuntha Adhikari
Directorate of Fisheries Development, Balaju, Kathmandu
*Email: prabeshkunwar@yahoo.com

Abstract
Aquaculture is one of the fastest growing agricultural subsectors in Nepal. The current total national fish production is 77,000 mt of which 28% contributes from capture fisheries while 72% is from aquaculture. Aquaculture is expanded to 55 districts of the country and has generated direct employment for 584,839 people. During the period of 1981/82 to 2015/16 annual per capita fish consumption has been significantly increased from 330 g to 2,750 g. Private sectors are encouraged for seed supply while government has confined its role in quality control. Fish is marketed by the producer themselves from production site or through agent, contractor or whole-seller. Higher fish demand is in winter while least fish consumption is found in Asadh, Shrawan and Bhadra. In fiscal year 2015/16, domestic production occupied 90% and imported fish occupied 10% of the total national fish consumption.

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 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 programmes etc. are the key factors in the development of 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 has demanded more aquaculture industries. Government of Nepal is also providing support to establish commercial farms which generate employment as well as income. Most of the newly established farms are dominated by the youths those are back from abroad employment and has 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 generation and 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 in future.

History of aquaculture

History of Nepalese 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. At present, Directorate of Fisheries Development (DoFD) and District Agriculture Development Offices (DADO) under Department of Agriculture (DoA), Ministry of Agriculture Development (MoAD) is the focal government organization for aquaculture development whereas fisheries research is being carried out by the Fisheries Research Division (FRD) under Nepal Agricultural Research Council (NARC). Education is provided mainly by Agriculture and Forestry University (AFU) and Tribhuvan University (TU) in Nepal.

Fisheries program in Nepal was initiated in 2004 BS (1947/48 AD) and aquaculture started from late 1950s by introducing Common carp (*Cyprinus carpio*) whose 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 and Yadav, 1996) which was significant achievement in aquaculture history that provided momentum to polyculture system in Nepal.

Natural water resources

Nepal is rich in natural water resources. Rivers, lakes, reservoirs, swamps and irrigated paddy fields are the major source of fresh water in Nepal (Figure 1). Among them rivers and irrigated paddy fields are the most dominant natural water resources. Besides, there are 7,900 km of irrigation canals in the country (Gurung, 2014).

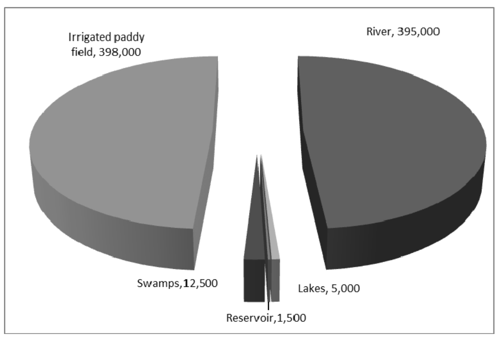


Figure.1: Natural water resources (ha) in Nepal

Capture fisheries is an important sector because of its role in fish production as well as employment generation. Capture fisheries production is 21,500 mt which seems 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 2). Lakes and reservoirs occupies less water surface area compared to other natural water resources.

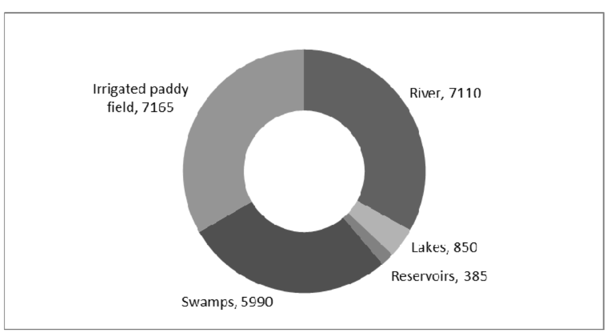


Figure 2: Fish capture (mt) from various water bodies

Status of aquaculture and fisheries

Various types of aquaculture practices are being adopted that has produced 55,500 mt fish in fiscal year 2015/16 (DoFD, 2016). Pond aquaculture is the major contributor which alone generated 87.46% (48,543 mt) of the total aquaculture production (Table 1). In pond aquaculture, Chinese carps and Indian major carps are the dominant species with average productivity of 4.89 mt/ha. These species are generally stocked under polyculture system. However, monoculture of Common carp, Tilapia and catfish have also been reported in some places. Interest in aquaculture is growing and has expanded to 55 districts out of 75 districts compared to 30 districts a decade ago.

Table 1: Status of aquaculture and fisheries in 2015/16 (DoFD, 2016)

Particulars	Pond (no)	Total Area (ha)	Fish Production (mt)	Productivity (mt/ha)
A. Fish Production from Aquaculture			55,500	
A1 Pond Fish culture	40,336	9,934	48543	4.89
A2 Other area (swamps)		3,300	5680	1.72
A3 Paddy cum fish culture		200	557	0.36
A4 Cage fish culture (m^3)		70,000	420	6 kg/m^3
A5 Trout Fish Culture in Raceway		3	300	10kg/m^2
B. Fish Production from Capture Fisheries			21,500	
B1 Rivers		395,000	7,110	18 kg/ha
B2 Lakes		5,000	850	170 kg/ha
B3 Reservoirs		1,500	385	257 kg/ha
B4 Swamps		9,200	5,990	540 kg/ha
B5 Irrigated low land Paddy Fields		398,000	7,165	18 kg/ha
Total Fish Production (mt)			77,000	

Pond aquaculture has been categorized into extensive, semi-intensive and intensive farming. Intensive farming of *Cirrhina mrigala* under single stocking and multiple harvesting to produce finger size fish, called Chhadi, is also a successful farming system in Nepal. Now it is popular in Central Terai and gaining popularity in other regions. Farmers have reported productivity of Chhadi system up to 12-15 mt/ha. Such finger sized fish are demanded in hotels and restaurants mainly on highways as snacks.

After pond aquaculture, second contributor in fish production is swamps. There are 3,300 ha area of swamps being used in aquaculture with 5,680 mt production in 2015/16. Most of these swamps are concentrated in mid-western and far-western region of Nepal. Fish culture in cage produced 420 mt fish in 2015/16. Cage technology was used for the first time in 1972 in Lake Phewa to raise brood fish of common carp and current data shows that cages occupy 70,000 m^3 with average productivity of 6 kg/m^3. This is a proven technology of income generation for land less fisher communities who rely on water resource for their livelihood. However, cage culture is confined to only few lakes of Pokhara Valley and Kulekhani reservoir which can be extended in other potential water bodies 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 species, crab and prawn production. Rice cum fish culture is successful in neighbor countries but this farming system could not get much attention in Nepal due to which only limited area of paddy fields are left for this culture system.

Rainbow trout, a cold water species, was introduced for the first time in 1969 from India and re-introduced from Japan in 1988 (Rai, 2010). Commercial farming started from Rasuwa and Nuwakot district under one village one product (OVOP) program. With the technological innovation of highly commercial rainbow trout aquaculture, today trout culture has spread to 23 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 2015/16, trout production has reached to 300 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. National Inland Fisheries and Aquaculture Development program is the focal government organization that implements and monitors trout development programs in the country.

Employment generation by fisheries subsector

Like other developing countries, employment is a serious problem in Nepal. Large number of youth emigrates 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. Nepalese economy is largely dependent on remittance. Such economy may crash anytime and is not sustainable. Therefore, expansion of aquaculture might be one of the options to overcome such outmigration problem and create jobs within the nation to attract youngsters and utilize them in national development.

Aquaculture in employment generation

Aquaculture is still a small and primitive sub-sector in Nepal, however it plays significant role in employment generation. People of different age and sex are involved in aquaculture from equipment preparation, fish husbandry to marketing of fish and fisheries items. There are about 122,772 people working directly or indirectly in this sub-sector among them male covers 67% while female occupies only 33% (Figure 3).

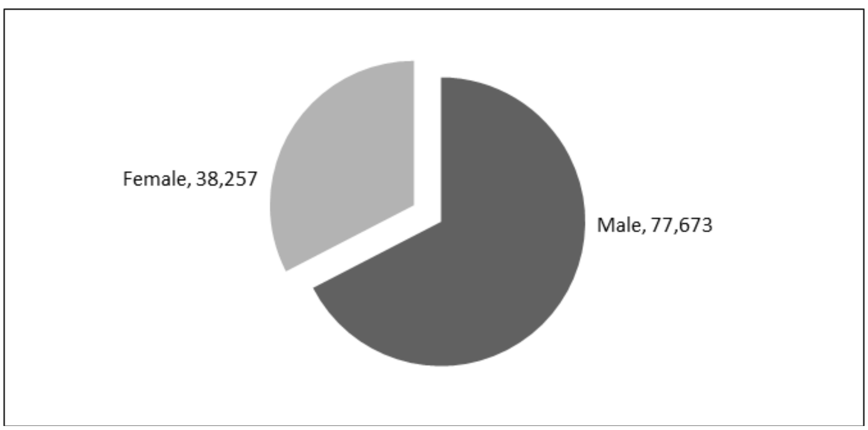


Figure 3: Employment opportunity generated by aquaculture

Capture fisheries in employment generation

Natural water especially rivers 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 in the vicinity of water resource and depending on such resource for their livelihood from generation to generation. There are 462,067 people engaged in capture fisheries among them 60% are female (Figure 4). Females are not only engaged in capturing fish but also in preparing fishing gears and equipment, as well as selling fish in the market.

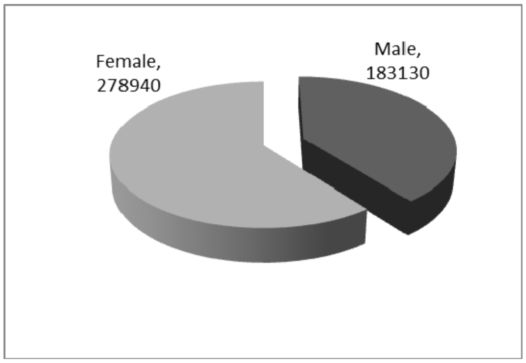


Figure 4: Employment opportunity generated by capture fisheries

Development trend

Among the various culture practices, pond culture is the dominant fish farming practice and is increasing rapidly while other aquaculture activities remained more or less standstill in last decade.

Expansion of pond area

In last two decades (from 1997/98 to 2015/16), 4550 ha ponds have been constructed for aquaculture. There was slow growth of pond construction in the beginning but it took its pace after implementation of fish mission program from the fiscal year 2007/08. The drop-in pond area in fiscal year 2008/09 is the consequence of natural disaster (flood) that damaged many fish ponds in Terai region. Again, the sluggish progress in pond expansion in 2012/13 (81 ha) was due to limited construction subsidies allocated by the then government, which significantly affected pond construction program. The highest pond construction (734 ha) was achieved in the fiscal year 2015/16 due to high priority given by the MoAD to aquaculture sub-sector and introduction of special program for area expansion from the same fiscal year (Figure 5). Pond fish culture is mainly carried out in Terai region but it is being practiced in mid-hill region as well, wherever climatic condition allows. Fish farming in earthen ponds in mid-hill districts was accelerated from fiscal year 2011/12 after implementation of pond expansion program by Nepal government. Since then, this program is being continuously implemented and significant amount of fish is produced annually to supply local demand.

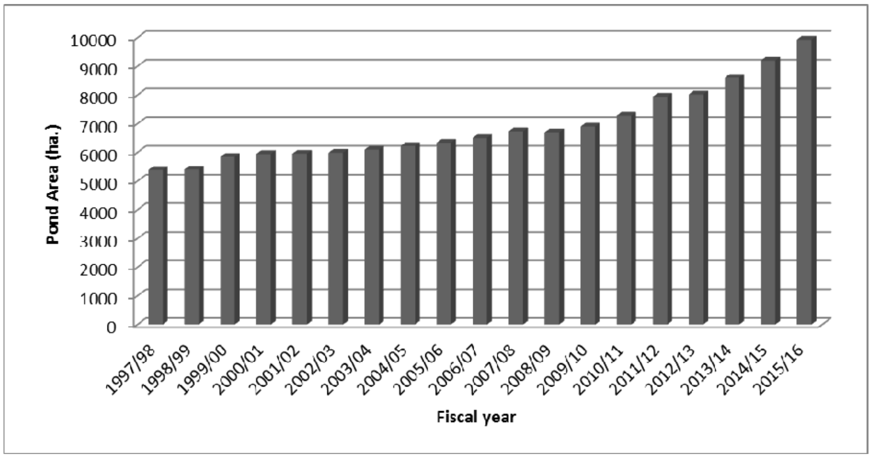


Figure 5: Expansion trend of pond area

Pond production and productivity

There was slow and steady increment of fish production annually. Significant improvement of fish production was recorded in the fiscal year 2015/16, this is obvious because at the same fiscal year highest area of pond construction was achieved (Figure 6). In last fifteen years, fish production from pond aquaculture was increased by 33,027 mt mainly due to both horizontal and vertical expansion of pond aquaculture and productivity.

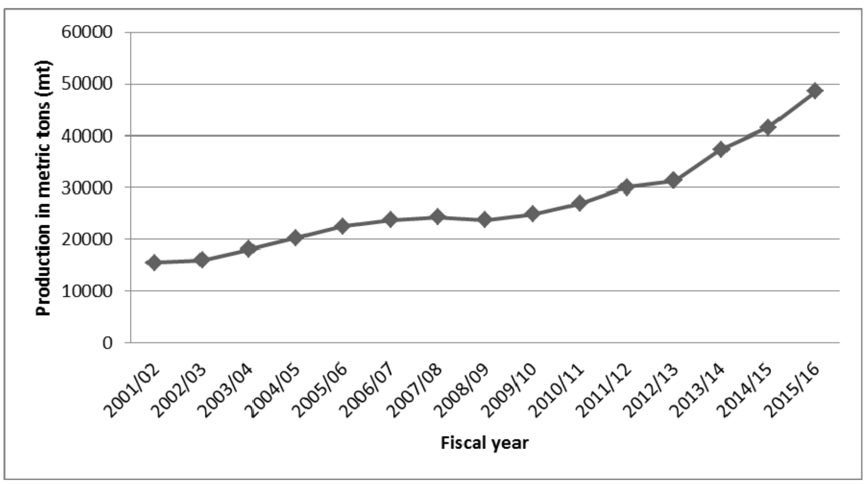


Figure 6: Production trend of pond culture

Pond productivity in 1981/82 was only 0.8 mt/ha, which is increased to 4.89 mt/ha in 2015/16 (Figure 7). This increased productivity has significant impact on national fish production. Improvement in technology, mechanization and good management practices (GMP) are the reasons for increased productivity. Government of Nepal always give emphasis to farm mechanization like using pellet machines to produce farm made cheap and quality feed and using aerators to improve water quality and enhance stocking density for higher production per unit area.

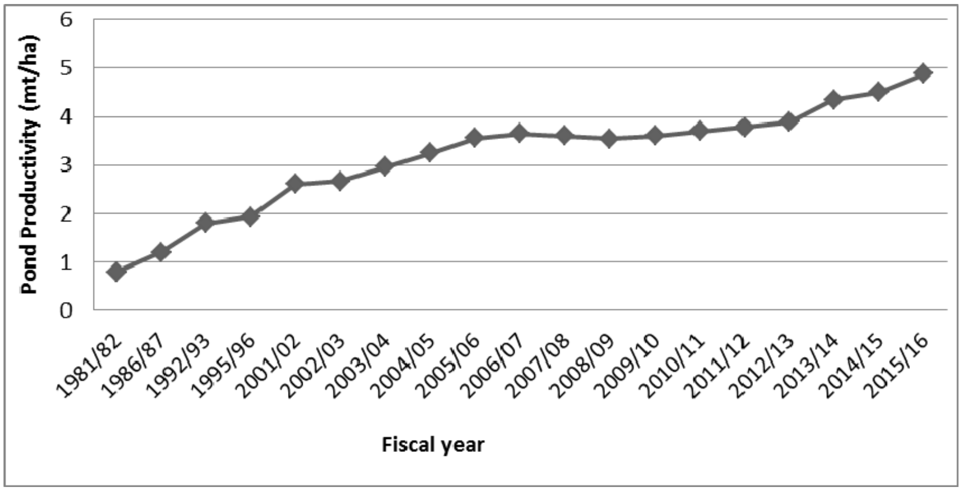


Figure 7: Pond Productivity trend

National production

FAO country profile of Nepal reports only 500 mt of national fish production in 1950. This production was entirely contributed by capture fisheries. Aquaculture production was recorded only from 1966 with total of 3 mt of fish production. Aquaculture production kept increasing slowly and steadily because of growing concern on aquaculture education, research and technology dissemination. Capture fisheries shows increasing trend in the beginning but remained constant at 21,500 mt since 2007/08. Even keeping this capture at standstill is a big challenge. Production status of fiscal year 2015/16 shows that out of 77,000 mt fish production 28% comes from capture fisheries where as 72% from aquaculture (Figure 8).

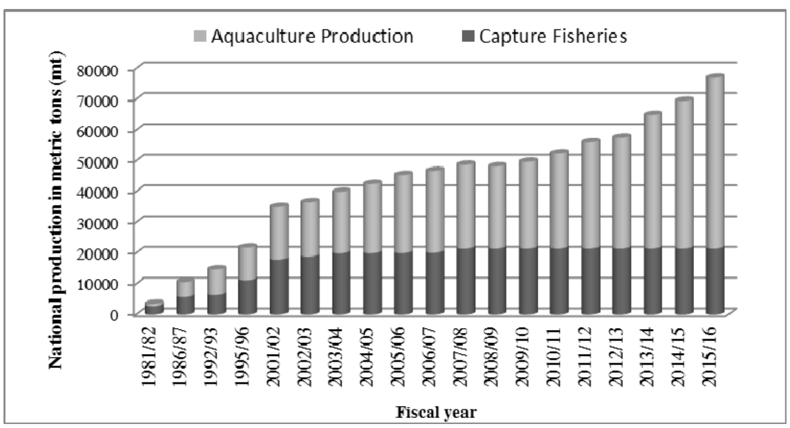


Figure 8: National fish production (Source: FAO and DoFD)

Because of increasing national fish production, per capita fish consumption is also increasing. From 1981/82 to 2015/16 it has been significantly increased from 330 g to 2,750 g (Figure 9), but this is still very low compared to global average of 16 kg per capita (Gurung, 2014).

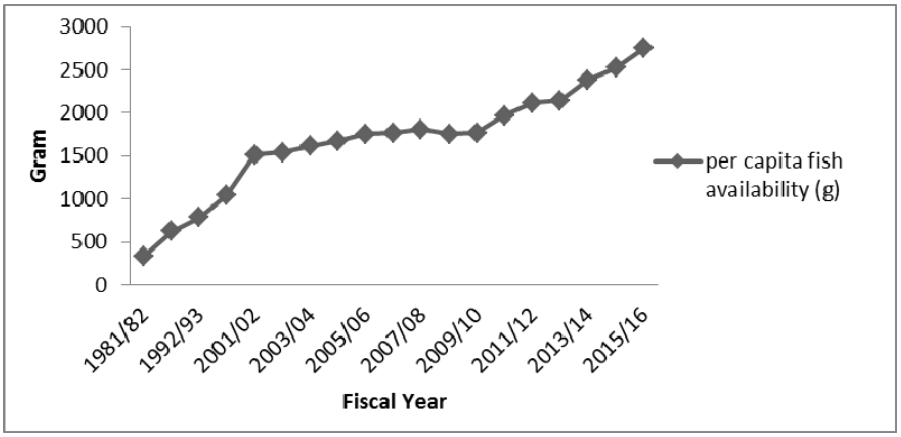


Figure 9: Trends of per capita fish consumption

Fish seed supply

Seed is one of the most important inputs in aquaculture. Quality seed is must to enhance productivity of aqua farms. In Nepal, fish seed are distributed in three forms: hatchlings: 4-5 days, fry: 2-3 cm or ~1 g and fingerlings: 5- 7 g. Both public and private sectors are contributing for seed supply. There are 14 Governments (DoFD & NARC) and 83 private hatcheries, 235 Nursery and 30 fish seed traders working in Nepal.

In last decade, seed supply by public sector didn't increase significantly while private sector jumped from 5.7 million in 2001/02 to 171 million in 2015/16 (Table 2), this is because government has given priority to private sector in seed supply confining government's role in quality control. Moreover, there is a subsidy program to establish fish seed resource centers under private ownership to empower private sector.

Table 2: Status of fish seed production in 2015/16

Fish seed (fry) Production/Distribution (No. in '000)	212,355
1. **Public Sector**	40,911
2. **Private Sector**	171444

Source: Annual report of DoFD, 2015/16

Because of increasing demand, seed supply is challenging not only in terms of quantity but also in terms of quality. Government is responsible in providing financial and technical supports as well as monitoring their activities. In 2012, Food and Agriculture Organization of the United Nations (FAO) supported national fisheries program in quality seed production and regulation through a project entitled "Improving the National Carp Seed Production System in Nepal (TCP/NEP/3303)". This project has drafted an act "Nepal Fish Seed Act and Carp Hatchery Accreditation & Seed Certification" (instead, cite as FAO, 2012) which is on the process for approval. This act will be a milestone in assuring quality seed supply within the country. Fish seed production and distribution trends of both public and private sectors are illustrated in Figure 10.

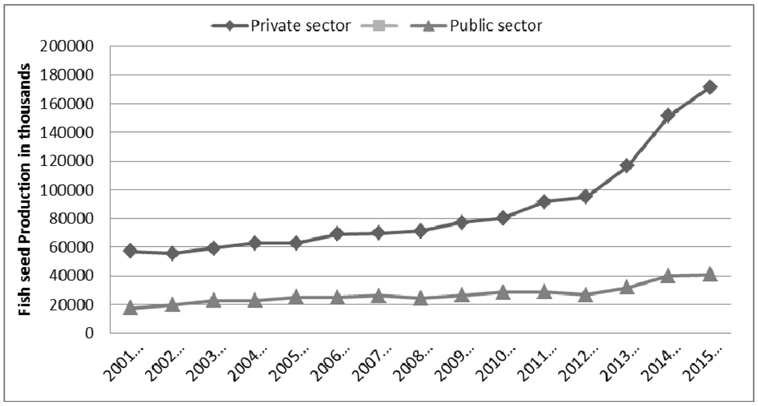


Figure 10: Fish seed (fry) production and distribution trend

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, 2067). 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 (Figure 11). In 2001/02 price of fresh fish was reported to be Rs 100 per kg which is now 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 (Cite as KBNPK, 2067).

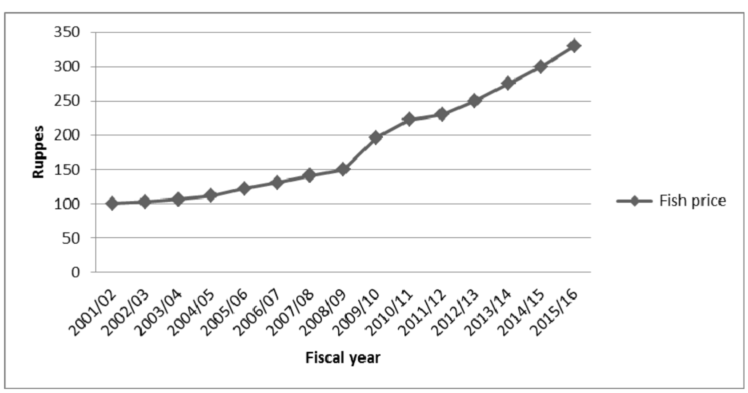


Figure11: Market Price of Fresh Fish

Table 3: Import/Export fish and fisheries products
Source: Adopted from Mishra and Kunwar (2014) and CAQO (2015)

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	

The demand of fish is not entirely full filled by national production, therefore huge amount of fish are imported. India is the major fish exporter while China, Vietnam, Bangladesh are other fish exporting nations to Nepal. According to the quarantine data, certain amount of fish is also exported from Nepal but this is negligible (Table 3). Due to long open boarder with India, all import/export dealings might have not been recorded properly in government channel. In fiscal year 2015/16, domestic production occupies 90% and import occupied 10% of the total national fish consumption. This indicates decreasing trend in import dependency which was around 14% in fiscal year 2014/15.

Conclusion and Recommendation

Aquaculture is highly blooming sub-sector in Nepal. The growth rate of aquaculture is 15.6% which is the highest among the SAARC nations which is highly commendable. Realizing its importance and potential, aquaculture is receiving attention from all sectors in recent days. The increment in budget allocation in fiscal year 2015/16 also shows this commodity with sub-sector is in government's priority.

Increased demand of fish has created market opportunity and has attracted to establish commercial fish farms. Technical support to newly established farm is necessary to make them competitive in local, regional and global market. The knowledge of our manpower is 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 interrelation 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 26.4% 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.

References

CAQO. 2015/16.Annual progress report. Central Animal Quarantine Office, Hariharbhawan, Lalitpur, Nepal.

DoFD. 2015/16. Annual Progress Report. Directorate of Fisheries Development, Balaju, Kathmandu, Nepal.

FAO. 2012. FAO Technical Cooperation Project (TCP) Mission Report. Food and Agriculture Organization of the United Nations.

Gurung T.B. 2005. Responsible introduction of alien fish and biodiversity in southern Nepal. Aquaculture Asia X (2): 13-15.

Matsya Palan Srinkhala- 2. Central Fisheries Building, Balaju, Kathmandu, Nepal.

Matsya Palan Srinkhala- 4. Central Fisheries Building, Balaju, Kathmandu, Nepal

Mishra, R.N. and P.S. Kunwar. 2014. Status of Aquaculture in Nepal. Nepalese Journal of Aquaculture and Fisheries 1: 1-10.

Rai, A.K. 2010. Rainbow trout Fish Farming Technology and practice. Nepal Agriculture Research Council, Fisheries Research Division, Godawari, Lalitpur.

Singh, D.M. and S. Yadav. 1996. Economics of Aquaculture in Nepal. In: Proceedings of the national symposium on the role of fisheries and aquaculture in the economic development of rural Nepal (15-16 August 1996), Nepal Fisheries Society, Kathmandu.

Nepalese Journal of Aquaculture and Fisheries
Vol. 3 & 4 (2016 & 2017) : 12-20

Rainbow trout (*Oncorhynchus mykiss*) based mountain aquaculture in Nepal

Tek Bahadur Gurung[1*], Suresh Kumar Wagle[2], Agni Prasad Nepal[1], and Gopal Prasad Lamsal[1]
[1] Nepal Agricultural Research Council
[2] Program for Aquatic Natural Resources Improvement (PANI)
*Email: tek_fisheries@hotmail.com

Abstract
Mountain aquaculture is fish farming in highlands cold water zone, where generally warm water aquaculture cannot be performed economically. The main purpose of the present paper is to review recent achievements and future perspectives of mountain aquaculture using mainly the cold water fish rainbow trout (*Oncorhynchus mykiss*) in Himalaya of Nepal. For the purpose we compiled data obtained from research activities carrying in mountain areas under the Nepal Agricultural Research Council and other relevant studies. It is revealed that aquaculture technology of cold water rainbow trout has expanded in several mountain districts with increasing demand in others adjacent areas after the intervention in private sector from 1995 onwards. The total number of trout farms has increased up to 110 producing >260 metric ton by employing about 1025 youths by year 2014 from 24 districts. The trout aquaculture technology could be expanded in areas having pristine, cold and clean water resources having road access for market in all Trans Himalayan countries as a meant to food and nutritional security and business opportunities in mountainous regions. We conclude that if other constraints are resolved, then from water abundance perspective Nepal highlands could be one of the globally lead destinations for rainbow trout production.

Key words: Mountain aquaculture, rainbow trout, feed, cold water fish, growth trends

Introduction

Mountain aquaculture, in general is fish farming in higher altitude area where warm water fish species may not biologically perform economical sustainable growth. Nepal is a predominantly mountainous country occupied by world's highest peak, the Mt. Everest and other world renounced eight peaks. The mountains of Nepal are also known as the 'water tower'. These water resources coming down from the Himalaya offers excellent opportunities to develop cold water mountain aquaculture as many glaciers, rivers, tributaries, lakes of mid hill and mountain could provide high quality pristine waters. To utilize such abundant water resources rainbow trout (*Oncorhynchus mykiss*), sockeye salmon (*O. nerka*) and brown trout (*Salmo trutta)* having food and sport values were introduced earlier in Nepal (Gurung 2010). However, those all attempts failed, probably due to inadequate technical knowhow, care and facilities such as raceways (flowing water fish holding system and feeds) for rearing (Swar 2008). Later, 50,000-eyed eggs of rainbow trout (*O. mykiss*) were brought from Japan in 1988 as souvenir and token of friendship between Miyazaki Prefecture, Japan and Nepal (Nakagawa 1998, Yamada et al. 1998, Gurung and Basnet 2003). The eggs were successfully hatched and reared in Nepal.

The rainbow trout farming technological packages after comprehensive research, testing, refinement, and innovation in Nepalese agro-ecological conditions were developed under Nepal Agricultural Research Council. The rainbow trout suited well in Nepal Himalaya, demonstrating that trout is a hardy fish and suitable for cultivation in cold waters. Now the trout is contributing in productivity enhancement of hill and mountain agricultural sector (Joshi and Lofvall 1997, Gurung et al. 2008, Voorhees 2011). It is likely that rainbow trout farming offers substantial opportunities of food and nutrition security in hills and mountains through job and income opportunities. At present, the success of rainbow trout farming has been distinctly visible in terms of fish production, income and increasing income flow to rural hills from urban areas in wider parts of the Himalayan highlands. Thus, the objective of present paper is to elucidate the emerging trends of rainbow trout farming adoption using information on present status for further scaling up in wider areas in Nepal Himalaya.

Rainbow trout farming technological package development

Rainbow trout attains approximately 200-300g individual body weight in a year in aquaculture practices in Nepal (Nepal et al. 2002). The trout requires clean, high dissolved oxygen containing cold water for farming in high densities about 75 or more fish/m^2 with high production rate of about 10-15 kg/m/yr highly depending on quality and quality of seed, feed and water used. Initially, Nepal Agricultural Research Council (NARC) scientists carried out research activities on rainbow trout; as a result, series of selection-based activities were performed in order to develop viable production and breeding technologies (Voorhees 2011, Gurung 2010, Rai et al. 2005). Later, a package of practices on breeding, nursing and cultivation of trout, including feed formulation using locally available feed ingredients was developed for practical and cost-effective farming in prevailing socio-economic situation.

Earlier all selection, breeding, larval rearing and nursing practices were exclusively carried out in research farms for fry production to support private farmers. However, the demand of fry increased with the increasing private trout farm entrepreneurs. To insure the adequate supply of fry, participatory breeding and nursing activities were carried out with private farmers. Similar participatory approach for pellet mill assemblage, feed formulation and production in farmer's farm were also adopted. As a result, the dry pellet feed machine started to be assembled in local workshops using locally available mechanical tools and accessories. In addition to innovative practices of constructing the raceway ponds along the shape of hill landscape by farmers themselves after having ideas of water exchange system for trout cultivation.

Figure 1: Expansion of rainbow trout (*O. mykiss*) farming in Nepal. (shaded areas)

The trout farming has also been integrated with Pico hydropower generation, resort, livestock, floriculture (Lamsal et al. 2008). Integrated approach of rainbow trout farming has created additional attraction in mountain slopes. The trout farming demonstrated to be economically viable practices (Nepal et al. 2002, Voorhees 2011) as trout production rate ranges from 15-20 kg/m^2. Now the rainbow trout farming cultivation in private sector has been extended in 24 hill and mountain districts of Nepal Himalaya (Figure 1). Rainbow trout is one of the well-studied fish of the world (Elizabeth et al. 2014), therefore to be competitive in world market more advanced research would be desirable in future in Nepal as well.

Status and emerging trends

Rural trout hatchery and fry production

To expand the breeding program for supplying trout fry to farmers, a short-term follow-up cooperation from Japan International Cooperation Agency (JICA) was started in 2007 for 4 months. The main objective of the support was strengthening trout seed supply through participatory breeding program. To encourage the farmers in trout breeding and fry production, an innovative approach was initiated, under which mature trout broods were sold to farmers for seed production. This program gained the popularity and motivated the farmers to enter directly into the business of trout fry production. Various stakeholders associated with trout farming were also trained for better technological, managerial and marketing aspects. At the beginning 3 hatcheries were supported. Presently, there are 12 rural hatcheries for trout fry production (Figure 2).

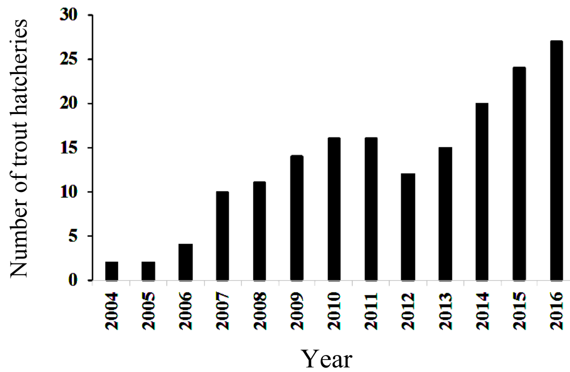


Figure 2: Temporal change in number of rainbow trout hatcheries in Nepal

In these rural hatcheries about 18 million alevin were produced in year 2012, out of which 1.1 million fries survived, suggesting that there is still need to improve fry survival (Figure 3).

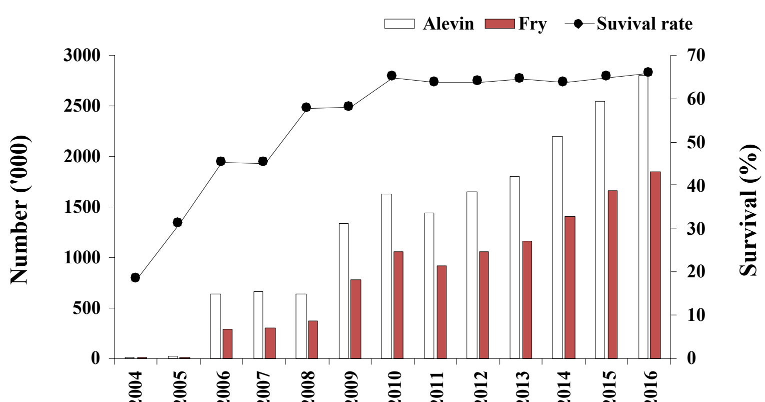


Figure 3: Survival trends of rainbow trout alevin to fry stage

Feed

The rainbow trout are carnivorous, thus requires high animal protein containing diets. In general, the protein level should be above 35%, especially for broods and fry stage trout. The amount of diets depends on the size and life stages. The feed conversion ratio (FCR) highly depends on the quality of feed. In general, the present FCR in Nepal is approximately 2.5:1. However, recent studies have claimed that the FCR in trout can be reduced to 1:1.14 by improving the quality of feed and others (Shahrzad et al. 2013). Trout (*O. mykiss*) exclusively depend on external feed from the time larvae commence first feeding. Thus, the success of trout farming enterprise highly depends on quality of the feed. To make the trout farming successful in prevailing socio-economic situation following steps were taken:

i) Local resources such as liver meat available were successfully used as an alternative at the beginning. Later, pellet mills purchased by Aquaculture Development Project (ADB funded) utilized to produce pellet feed.
ii) More comprehensive feed formulation and production was started since 1995 with the assistance of Natural Water Fisheries Development Project (NWFDP) funded by Government of Japan.
iii) The feed technology was disseminated among small scale trout farmers.
iv) Using the pellet feed technology developed by NARC; Directorate of Fisheries Development (DOFD) supported one of the private sector entrepreneurs for sale to small scale trout farmers.
v) Now, the Government has also providing Grant assistance on competition basis to trout cooperatives for commercializing production through the Project for Agricultural Commercialization and Trade (PACT) supported by World Bank.
vi) At present the total amount of trout feed produced in year 2012 has been reached approximately 427 mt produced from 12 rural small-scale feed plants operated by farmers themselves except few (Figure 4).

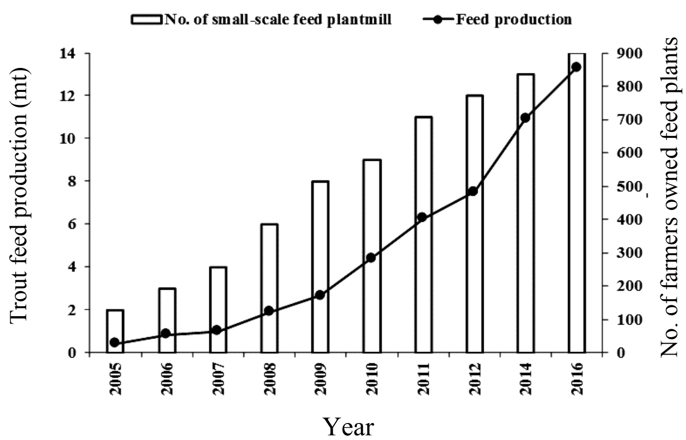


Figure 4. Temporal changes in total value of rainbow trout feed production and number of farmers owned small scale feed plants

Area coverage and present trout production

There are 85 private trout farms established in 16 districts covering an area of 13161 m^2 producing about 180 mt trout in 2012 (Figure 5). Mostly the trout raceways are constructed in sloppy land, where in general other agricultural crops are not grown. Besides, that fallow lands close to rivers and streams banks have also been useful for raceways construction for trout production. But, care should be taken to that flood and landslides would not harm fish farm.

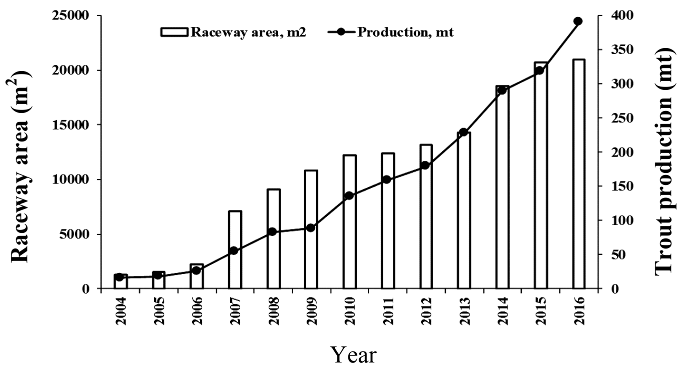


Figure 5: Raceway area and trout production in Nepal Himalaya

At present the raceways are constructed using cement due to available landscape for trout aquaculture. This has increased the capital cost investment in trout farming. Further research is needed to find out cheaper ways for trout farm infrastructure to reduce the capital cost in trout farming. Rainbow trout can be cultivated in earthen ponds; however, the only requirement of water flow should be maintained. Major parts of Nepal are hills and mountains with abundant cold water resources, therefore assumed to be highly potential for cold water aquaculture development (Rajbanshi 2002, Swar 2002). It is expected that the rainbow trout farming technology would be further expanded in other areas soon including the whole upper regions of the Himalayan countries.

Marketing and social contribution

With successful achievements of trout production market outlets were created. For the purpose market survey were carried out and efforts were made to popularize the rainbow trout dishes in hotels, restaurants, department stores targeting on general consumers. Public demonstrations of cooking and preparation of trout delicacy were also launched in various exhibitions. Recipes were published for distribution. At present rainbow trout has been one of the favorite dishes in hotels and restaurants (Shrestha 2008).

In 2006, Government of Nepal has declared two adjacent districts close to the capital city, Kathmandu, as trout growing districts under 'One village One Product' (OVOP) program where trout farming was prioritized to promote the local farmers by using cold water resources and local tourism with Public-Private-Partnership (PPP).

Under this, experts from Nepal Agricultural Research Council (NARC) and private sector Agro Enterprise Centre (AEC) of Federation of Nepalese Chamber of Commerce and Industry (FNCCI) worked together for promotion of trout. Public sector was responsible for technical backstopping and extension services required for enhancing trout production in private sector. While AEC was responsible in motivating the local entrepreneurs and communities by modern processing, packaging, distribution system, and effective marketing programs. This program was designed for 3 years from the year 2006 to 2009. Under the scaling up of the trout farming technology verification of brood management, breeding, seed transportation, rearing and nursing, feed, health and local resource management were prioritized.

Although the total trout production is far below than the demand and potentiality, however, value chain components of trout farming technology and allied activities have started to demonstrate its contribution in employment generation in various fields. A general survey showed that about 830 people mostly the youths have been employed in trout production activities (Figure 6). Majority of 63% involved in production, 21% in fish seed, 4% fish marketing, 6% restaurants, 6% in feed business. This trend is suggestive that trout production in indeed can be helpful to generate employment and retain the youth in the agricultural sector especially on hills and mountains in the country.

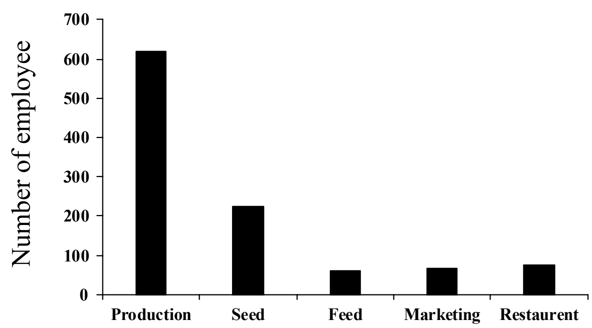


Figure 6: Number of employees engaged in trout related value chain in Nepal by 2014.

Challenges

i) Feed insufficiency, quality and quantity

Easy availability of quality feed is one of the major constraints for the rapid expansion of trout farming. Ensuring nutritionally rich and economically affordable feed supply mechanism for poor farmers have been important for sustainable trout aquaculture. Till now the feeds for trout cultivation are fulfilled from two sources: a) supply through fisheries research centers and b) trout farmers using small scale pellet plants. Both of these sources have limited capacity for production. Further expansion of trout farms might be hindered by the inadequate availability of quality trout feed.

ii) Trout seed production

Availability of suitable size of trout fry is not easy due to the ever growing demand of fish seed. Most of the new farmers need to strengthen theirs skillful in brood management, breeding and nursing technology. Moreover, limited government setups (trout hatcheries) and poor accessibility to trout farming sites (poor road network, difficult physiography) can be considered as additional barrier for enhancing commercial trout farming.

iii) Disease

Occurrence of trout diseases, especially of feed and nutrition origin have been one of the major challenges for trout farming in Nepal. Besides, that bacterial and fungal diseases (e.g. *Columnaris* and that cause tail and fin rot), gill diseases and abdominal dropsy are some of the commonly occurring ones.

iv) Inadequate skilled human resource

Sustainability of trout farming needs skilled research, extension and academic human resource networks similar to the need to other agricultural commodities. Though, a bunch of scientists, farmers, extension officers and few technicians have been trained on trout farming. More skilled human resource would be prerequisite to sustain the ever increasing trout farmers and production.

v) Weak extension mechanism
At present rainbow trout farming extension is being carried out by general agriculture extension agents without having a proper knowledge of trout farming. These extension agents mostly may not be able to provide effective technical services to trout farmers in hills. Mandatorily fisheries extension services are not available in districts having high potential for trout cultivation.

vi) Post-harvest and market linkage
Present trout marketing and post-harvest management is not sufficient to support the production enhancement of trout. There is a need to strengthen the market linkages and post harvest technological development for rainbow trout farming.

vii) Maintenance of pure line breeds
Inbreeding is a common occurrence in aquaculture species. Thus, maintenance of pure line in research stations and farms would be a challenge. It is suggestive to put considerable efforts on maintenance of the pure line of rainbow trout.

Way forward

Rainbow trout farming in Nepal Himalaya is relatively a new practice having immense potentiality to bring a 'paradigm shift' in cold water aquaculture production as approximately 70% of total areas in Nepal are suitable with abundant with pristine water resources. The present trends suggest that the farming of trout would be expanded more. However, it is likely that the current trend of commercial trout farming would not be limited to Nepal Himalaya but expanded in most of the areas of the Trans-Himalayan countries and others (Edwards 1990, Gopalkrishnan et al. 1999, Jindasa et al. 2005, Hasan et al. 2007). Several countries are planning to initiate and commercialize rainbow trout production (Petr 1999). Considering the food and nutritional security, unemployment, youth migration especially from mountainous regions, more focus to utilize the cold water resources by adopting rainbow trout production in national plans are recommended for sustainable hill and mountain development.

Conclusion

The current success further promises the wider potentiality of rainbow trout production using pristine Himalayan waters. The trout produced in the Himalayas could easily be branded as "Himalayan Rainbow trout" for domestic as well as global market. Further adoption of rainbow trout farming requires investment on quality research on feed, health management, extension network, best management practices and efficient marketing of quality products etc. On addition, if the year-round seed production technologies are developed then rainbow trout has the potentiality to be one of the leading aquaculture commodities of Trans Himalayan countries.

Acknowledgment

Thanks to all staffs at Fisheries Research Division, Godawari and Fisheries Research Center, Trishuli for providing useful information on trout farming in Nepal. Fund for this study was available from Nepal Agricultural Research Council. Thanks to Directorate of Fisheries Development for their cooperation in various studies on rainbow trout cultivation. Authorities of Government of Miyazaki Prefecture, Japan; Nepal Agricultural Research Council and Ministry of Agricultural Development are gratefully acknowledged for their support.

References

Edwards, D. 1990. Report prepared for the project Rainbow Trout Culture in Afghanistan, FI: DP/AFG/86/013, pp. 7, http://www.fao.org/docrep/field/003/U2214E/U2214E00.htm

Read, E.S., F.T. Barrows, T.G. Gaylord, J. Paterson, M.K. Petersen and W.M. Sealey 2014. Investigation of the effects of dietary protein source on copper and zinc bioavailability in fishmeal and plant-based diets for rainbow trout. Aquaculture 432: 97–105.

Gopalkrishnan, A., K.K. Lal and A.G. Ponniah. 1999. Conservation of Nilgiri Rainbow trout in India. NAGA, The ICLARM quarterly 22(3): 16-19.

Gurung, T.B. and S.R. Basnet. 2003. Introduction of rainbow trout *Oncorhynchus mykiss* in Nepal: Constraints and prospects. Aquaculture Asia 8(4): 16-18.

Gurung, T.B., N. Pradhan., D.M. Singh and T.B. Thapa. 2008. Prospects of trout (*Oncorhynchus mykiss*) farming commercialization in relation to global context in Nepal. In: Proceedings of the workshop on "Rainbow trout farming scaling-up strategies in Nepal" (ed. T.B. Gurung): 91-98.

Gurung, T.B. 2010. Rainbow trout: precious souvenir from Japan, Milan, 37th Annual Issue, pp. 15-17. JICA Alumni Association of Nepal (JAAN). www.jaan.org.np.

Hassan, A., M. Ishaq, A. Farooq and S.H. Sadozai. 2007. Economics of trout fish farming in the northern areas of Pakistan. Sarhad Journal of Agriculture 23(2): 407.

Jindasa, J., A.B. Kothlawala, H.M. and B.K. Herath. 2005. Status of rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) population after the cessation of stocking in waterways of Horton Plains, Sri Lanka. Vidyodaya Journal of Sciences 12: 9-27.

Joshi, P.L. and L.M.W. Lofvall. 1997. Production technology and prospects of trout farming in Nepal. In: Proceedings of the National symposium on the role of fisheries and aquaculture in economic development of rural Nepal (eds. D.B. Swar, G.B.N Pradhan and L.M.F. Westland), NEFIS: 27-34.

Lamsal, G.P., S.R. Basnet and P.B. Rumba. 2008. A case study on integrated trout farming in hill terraces with other developmental activities. In: Proceedings of the workshop on "Rainbow trout farming scaling-up strategies in Nepal" (ed. T.B. Gurung): 115-119.

Nakagawa, U. 1998. Rainbow trout introduction in Nepal (in Japanese). Aquaculture 6: 84-86.

Nepal, A.P., S.R. Basnet., G.P. Lamsal, Joshi, P.L. and R.M. Mulmi. 2002. Economics of rainbow trout farming in Nepal. In: Cold water fisheries in the trans-Himalayan countries (eds. T. Petr and D.B. Swar). FAO Fisheries Technical Paper, Rome.

Petr, T. 1999. Fish and Fisheries at higher altitudes: Asia. FAO Fisheries Technical Paper 385, pp. 304**.** http://www.fao.org/docrep/003/x2614e/x2614e00.html.

Rai, A.K., S.R. Basnet., R.C. Bhujel and G.P. Lamsal. 2005. Rainbow trout (*Oncorhynchus mykiss*) culture in the Himalayan Kingdom of Nepal: A success story. Asia-Pacific Association of Agricultural Research Institutions (APAARI), FAO Regional Office for Asia and the Pacific Bangkok, Thailand, 33 p

Rajbanshi, K.G. 2002. Zoogeographical distribution and the status of cold water fish in Nepal. In: Cold water fisheries in the trans-Himalayan countries (eds. T. Petr and D.B. Swar). FAO Fisheries Technical Paper No. 431, pp. 221-246, Rome.

Shahrzad, B., B.K. Masoumeh and H. Kaivan. 2013. Effects of different photoperiod regimes on growth and feed conversion rate of young Iranian and French rainbow Trout (*Oncorhynchus mykiss*). World Applied Science Journal 21(10): 1440-1444.

Shrestha, J. 2008. A study on business scope of rainbow trout farming in Nuwakot district. A Technical Report Submitted to Agro Enterprise Centre (AEC). Federation of Nepalese Chamber of Commerce and Industry (FFNCCI). 72 pp.

Swar, D.B. 2002. The status of cold water fish and fisheries in Nepal and prospects of their utilization for poverty reduction. In: Cold water fisheries in the trans-Himalayan countries (eds. T. Petr and D.B. Swar). FAO Fisheries Technical Paper No.431, Rome. P 376

Swar, D.B. 2008. History of Rainbow trout (*Oncorhynchus mykiss*) introduction in Nepal. In: Proceedings of the workshop on "Rainbow trout farming scaling-up strategies in Nepal" (ed. T.B. Gurung), pp. 21-24.

Voorhees, T.M. 2011. The potential to expand the rainbow trout (*Oncorhynchus mykiss*) commercial aquaculture industry in the mid- and high-hills of Nepal. In: Ecological Aquaculture (ed. Barry Costa-Pierce). University of Rhode Island, p 27.

Yamada, T., M.K. Karna and K.R. Bastola. 1998. Comparative studies on hatchability, abnormality and advance rate of rainbow trout, *Onchorynchus mykiss* alevins. In: Present status of fisheries research development and education in Nepal. (ed. B.R. Pradhan et al.). Natural Water Fisheries Development Project, Nepal Agriculture Research Council, Japan International Cooperation.

Nepalese Journal of Aquaculture and Fisheries
Vol. 3 & 4 (2016 & 2017) : 21-25

Assessment of dried carp testes for success on hormonal sex reversal in Nile tilapia (*Oreochromis niloticus*)

Ruchi Shrivastav[1*], Madhav Kumar Shrestha[2], Nabin Babu Khanal[2] and Narayan Prasad Pandit[2]
[1]Fisheries development Center, Bairahawa, Rupandehi, Nepal
[2]Aquaculture and Fisheries Program, Agriculture and Forestry University, Rampur, Chitwan, Nepal
*Email: ruchishrivastav362@gmail.com

Abstract
Production of monosex tilapia using 17α- methyltestosterone has been well established in commercial context for increased tilapia production. In concern to its cost of expense and residual effect on human and environment, a cost-effective natural source of androgen has been proposed. This study was conducted to assess the success of dried carp testes on sex reversal of Nile tilapia. Feeding durations for 15, 20, 25, 30 and 35 days were allotted as treatments which were triplicated in completely randomized design (CRD). Feeding with dried carp testes was done from first feeding stage in glass aquaria. After completion of treatment phase, fries were reared in outside hapa for a period of three months. Maximum male population (88.29±4.2%) was obtained with 30 days feeding duration while lowest male population (74.20±4.4%) was obtained with 15 days feeding. However, there was no significant difference in maleness percentage fed for 20, 25, 30 and 35 days. There was insignificant effect of feeding duration on survival while DWG and SGR were higher in sex reversed Nile tilapia. Dried carp testes can effectively masculinize Nile tilapia when fed for 30 days to post hatching of tilapia. However minimum feeding durations (20 days) also produces significant results.

Keywords: Nile tilapia, monosex, androgen, dried carp testes, feeding duration

Introduction

Tilapia production is increasing and most of its production is done using monosex fish, produced through hormonal sex reversal using methyltestosterone (MT) (Little, 1989). However, the cost of methyltestosterone may be prohibitive in some countries like Nepal, and using hormones, while generally accepted in many countries, may also cause concerns among the public and influence willingness to consume fish produced through hormonal sex reversal. As a result, several countries still produce Nile tilapia using mixed-sex culture, which often produced many small fish due to natural reproduction in ponds as well as competition between fry and adults (Little and Edwards, 2004). This limits economic and food value of tilapia produced in these culture systems. The most commonly used method to produce all male tilapia fry is by hormonal sex reversal using MT. MT promote hormonal sex reversal when fed for 21 days from its first feeding stage. The expense of importing MT is often viewed as a constraint to production of sex-reversed tilapia in Nepal. Recent experiments have shown that dried carp testes may be a reasonable and cost-effective substitute for MT (Khanal et al., 2014; Ranjan et al., 2015). Like the production of all-male tilapia using MT, using feed laced with dried carp testes has resulted in hormonal sex reversal (Ranjan et al., 2015), but the technology has not been fully tested and needs work on dosages, exposure times, and success rate. The purpose of this study is to assess success of hormonal sex reversal by dried carp testes.

Materials and Methods

Experiment was carried out using the facilities of Department of Aquaculture and Fisheries, Agriculture and Forestry University, Rampur, Chitwan. Well matured broods of Nile tilapia *(Oreochromis niloticus)* were collected and allowed to breed naturally in hapa with a sex ratio of 3 females to 1 male. Fish were checked for the presence of eggs in their mouth. Fertilized eggs were collected and were held in upwelling condition until hatch and swim up. Yolk sac absorbed swim up fry were transformed to treatment unit.

Treatment design

Dried carp testes was fed for 5 different feeding durations. 150 first feeding stage of fry was transferred to 60 x 30 x 40 cm^3 size aquarium and fed for different number of days.

(1) Treatment- 1: 15 days of feeding (T1)
(2) Treatment- 2: 20 days of feeding (T2)
(3) Treatment- 3: 25 days of feeding (T3)
(4) Treatment- 4: 30 days of feeding (T4)
(5) Treatment- 5: 35 days of feeding (T5)

Treatment was carried out in completely randomized design with triplicates of each feeding period. 24 hour aeration and 90% water change after every 2 days was maintained. Feeding was supplied four times a day in dried powder form. They were fed to ad-libitum with absolute dose of dried carp testes. After completion of treatment days, 100 fries were pooled and stocked in hapa for rearing phase.

Carp testes diet preparation

Carp testes feed was prepared using fresh common carp testes of more than a year old. Fresh common carp were collected from local Narayangadh and Tandi market, and from fish farm of Hetauda and Kathar. Freshly collected testes were made free from other muscle parts, blood vessels and cut into small piece. Cut pieces of testes were kept in petri-dish and oven dried at 60°C for 24 hours. Dried testes weres then powdered with grinder and sieved through 60µm mesh size sieve. Feeding of absolute dose (100%) carp testes was same for all treatments T1 to T5.

Sex determination

All fish sample were dissected thoroughly for gonad observation. Dissection was started by making a small cut near the anus and continuing forward to the base of pectoral fin. The entire gonad lying on the dorsal portion of peritoneal lining was removed and observed clearly. All fish were of good size to dissect and there was no problem in gonad identification. There was clearly visible testes and ovaries.

Statistical analysis

Data were analysed through one-way ANOVA for treatments of each androgen source. DMRT was used to compare the means of significant result. Effect of treatment days on proportion of male population, survival rate, daily weight gain (DWG) and specific growth rate (SGR) was analyzed using SPSS V 16.0. Water qualityparameters were analyzed in MS-Excel.Means were expressed as mean±SE.

Results

Feeding response and growth performance

Fish displayed an active feeding behavior throughout the treatment period. Different growth parameters of Nile tilapia fry during treatment phase is shown in the Table 1.

Table 1: Growth performance of Nile tilapia fry feeding with dried CCT powder for different durations (Mean±SE)

	Aquarium			Hapa		
Parameters	DWG (mg/fish)	SGR (%/day)	Survival rate (%)	DWG (g/fish)	SGR (%/day)	Survival rate (%)
T1	$5.62±0.45^a$	$9.84±0.35^a$	$74±0.04^a$	$1.69±0.04^a$	$36.36±0.56^a$	$73.7±6.6^a$
T2	$4.15±0.13^b$	$6.94±0.22^b$	$72±0.01^a$	$1.31±0.31^{ab}$	$27.13±1.12^b$	$66.3±9.9^a$
T3	$3.41±0.12^c$	$5.82±0.21^c$	$77±0.05^a$	$0.88±0.11^{bc}$	$21.13±0.55^c$	$76.3±7.9^a$
T4	$3.78±0.05^c$	$5.82±0.29^c$	$73±0.01^a$	$0.59±0.02^c$	$16.22±0.09^d$	$82.3±3.8^a$
T5	$5.16±0.09^a$	$6.13±0.18^{bc}$	$72±0.04^a$	$0.92±0.18^{bc}$	$14.37±0.56^d$	$70.0±3.8^a$

Note: Means with different superscripts within a column differ significantly at $p<0.05$.

Daily weight gain (DWG) and Specific growth rate (SGR) is decreasing with increasing feeding duration. DWG and SGR are significantly higher in treatment 1 both in aquarium and in hapa. Mean DWG ranged from 3.41±0.12 to 5.62±0.45 mg/fish in aquarium and 0.59±0.02 to 1.69±0.04g/fish in hapa. Mean SGR ranged from 5.82±0.21 to 9.84±0.35%/day in aquarium and 21.13±0.55 to 36.36±0.56 %/day in hapa. Mean survival ranged from 72 to 77 % in aquarium and 66 to 82 % in hapa. Mean survival rate were statistically similar in all the treatment both in aquarium and in hapa.

Sex reversal

The phenotypic male was found increasing with increasing feeding durations of dried Common carp testes (CCT) from 15 to 30 days (Table 2). CCT feeding for 30 days gave highest male population (88.3±2.5) as compared with 15 days CCT feeding (74.2±2.5). Statistically similar male population was obtained with 20, 25, 30 and 35 days CCT feeding. Male population obtained with 20, 25, 30 and 35 days were statistically different ($p<0.05$) than obtained with 15 days feeding.

Table 2: Mean percentage of male tilapia in different feeding durations of dried CCT powder (Mean±SE)

Treatments	Feeding days	Male %
T1	15	$74.2±2.5^{b}$
T2	20	$85.0±1.8^{a}$
T3	25	$87.3±1.8^{a}$
T4	30	$88.3±2.5^{a}$
T5	35	$83.5±3.1^{a}$

Note: Means with different superscripts within a column differ significantly at $p<0.05$.

Water quality

Dissolved oxygen (DO), pH and temperature measured for both indoor feeding in aquarium and outdoor rearing in hapa were found at desirable range during the culture period. Total ammonium nitrogen (TAN) was found in increasing order from day 1 to day 4 after change.

Discusssion

Common carp testes (CCT) can be used in the form of sex reversal feed. Better growth and survivability shows good acceptance of CCT as feed for tilapia fries. Better acceptability of sole CCT was also obtained in previous studies than when it was mixed with fishmeal (Khanal et al., 2014; Ranjan et al., 2015). This fact explains that carp (fish) testes can be used as strong feed for sex reversal. It is not needed to mix with fish meal for protein, due to its high protein (Ranjan et al., 2015) and androgen content during maturation stage. The specific growth rate (SGR) ranged from 5.82±0.21 to 9.84±0.35 during treatment period. The SGR was found significantly higher in Treatment 1 (15 days feeding duration) as compared to other treatments and there was no significant difference among all other treatments. Odin et al., (2011) found in a study that there was no significant difference among SGR of tilapia fry treated with natural androgen (lyophilized testes) for different durations. Daily weight gain (DWG) was in the range of 3.41 to 5.62 mg/fish/day. There was no significant difference in survival rate among the treatments, which may be due to rapid hormonal excretion in fish via faeces and gills (Cravedi et al., 1993). Also there was no significant effect of androgen (Vera-cruz and Mair, 1994) and feeding duration (Ranjan et al., 2015) on survivability.

Male population was increasing with increasing feeding duration of natural hormone from 15 to 30 days which was supported by similar previous studies (Khanal et al., 2014; Ranjan et al., 2015). Khanal et al., 2014) reported increase in male population with increase in feeding duration from 15 (82.5%) to 24 (91.4%) days. Ranjan et al. (2015) reported highest male population (95.8%) when 100% CCT was fed for 30-35 days treatment duration. Similar results were also obtained with synthetic androgens methyltestosterone (MT) reported by other studies (Phelps et al., 1996; Macintosh et al., 1985; Phelps et al.,1996) found difference in male population when same feed was fed for 14 and 21 days. Lower male percentage (65%) was obtained with 14 days with it was higher (91%) when fed for 21 days. Similarly, (Macinthosh et al., 1985) found increase in male percentage from 58 to 79% when tilapia was fed from 30 to 60 days feeding durations. However, there was no significant difference obtained in male population when fed for 20, 25, 30 and 35 days in present study. Increased maleness with increased feeding durations can be attributed to the fact that more amount of androgen consumed with longer durations. But because they were fed to ad libitum, after certain duration decrease in palatability was observed. It can be supported by a fact that ammonium level begins to depress food consumption at concentrations as low as 0.08mg/L when exposed for prolonged duration (Pompa and Masser, 1999). Ammonium value was significantly higher in treatment 5 (fed for 35 days) in comparison to other treatments. There was decrease in male population (83.5%) obtained with 35 days feeding which can be supported by the findings of (Orose et al., 2016) which showed that 42 days feeding of bull testes produced only 80% of male population. A peak in male population was obtained with 30 days feeding. This duration of treatment must be adequate to allow all fish to complete gonadal differentiation during the treatment period as 21 days feeding of 60mg/kg MT feed is recommended standard for maximum male population. A study conducted by (Desprez et al., 2003) on sex reversal treatment of Nile tilapia fry with duration ranging from 10 to 35 days with natural androgen 11βOHA showed a significant deviation of male obtained with 28 days feeding. On a follow up study, treatment duration of 28 days was applied to increasing stocking density of fry which produced a mean male percentage of 99.1 %. It can be concluded that a follow up study on duration must be carried out to assess the efficacy of this natural androgen on sex reversal.

Ammonium level recorded during treatment phase did not affected much on survivability of fish fry showing a range of 72 to 77%. (Benli and Koksal, 2005) showed that the acute toxicity of ammonium (48-h LC50) on Nile tilapia larvae and fingerlings were determined as 1.007-1.01 mg/l and 7.39 to 7.41 mg/l which caused 30% mortality. This result is in contrast with result of the present study which showed 26.4% mortality during treatment phase. (Pompa and Masser, (1999) reported that massive mortality occurs when fish are suddenly transferred to water with NH_3 concentration greate 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 first day of water change to the final day before water change in which fish gets better acclimatized. The DO, pH and temperature recorded during treatment and post treatment phase was in optimum range for fish growth and survival. This shows that feeding CCT has no adverse effect on water quality parameters.

Conclusion

CCT is well accepted and can be used as feed for sex reversal as one of the cost effective and environment friendly substitute for MT. Though highest male percentage was obtained with 30 days of feeding but there was no significant difference between 20, 25, 30 and 35 days feeding on male percentage. Thus, 20-30 days feeding CCT is optimum for sex reversal with further refinement in processing (temperature and duration) technique of Common carp testes.

Acknowledgement
I would like to acknowledge my gratitude to AquaFish Innovation Lab project for all the economic support. I would also like to express my sincere gratitude to Aquaculture and Fisheries Department, AFU for providing me research facilities and support during entire research period.

References

Cravedi, J.P., G. Delous, L. Debrauwer, and D. Prome. 1993. Biotransformation and branchial excretion of 17 alpha-methyltestosterone in trout. Drug metabolism and disposition 21(2): 377-385.

Khanal, N.B., M.K. Shrestha, S. Rai andR.C. Bhujel. 2014. Comparative evaluation of Carp testis as an alternative to 17α-Methyltestosterone on Tilapia sex reversal. Our Nature 12(1): 1-7.

Little, D.C. 1989. An evaluation of strategies for production of Nile tilapia (*Oreochromis niloticus* L.) fry suitable for hormonal treatment. Ph.D. Dissertation, University of Scotland, Stirling, Scotland.

Little, D.C. and P. Edwards. 2004. Impact of nutrition and season on pond culture performance of mono-sex and mixed-sex Nile tilapia (*Oreochromis niloticus*). Aquaculture 232(1): 279-292.

Ranjan, R., M.K. Shrestha, N.P. Pandit and N.B. Khanal. 2015. Efficacy of common carp (*Cyprinus carpio*) testis on sex reversal of Nile tilapia (*Oreochromis niloticus*) fry. Nepalese Journal of Aquaculture and Fisheries 2: 92-100.

Benli, A.Ç.K. and G. Köksal. 2005. The acute toxicity of ammonia on tilapia (*Oreochromis niloticus* L.) larvae and fingerlings. Turkish Journal of Veterinary and Animal Sciences 29(2): 339-344.

Desprez, D., E. Geraz and M.C. Hoareau. 2003. Production of a percentage of male offspring with a natural androgen, 11 β-hydroxyandrostenedione (11βOHA4), in Florida red tilapia. Aquaculture 216(1): 55-65.

Macintosh, D.J., T.J. Varghese and G.P. Satyanarayana. 1985. Hormonal sex reversal of wild-spawned tilapia in India. Journal of Fish Biology 26(2): 87-94.

Odin, R.Y., R.B. Bolivar, L. Liping and K. Fitzsimmons. 2011. Masculinization of Nile tilapia (*Oreochromis niloticus* L.) using lyophilized testes from carabao (*Bubalusbubaliscarabanesis* L.), bull (*Bos indicus* L.) and boar (*Sus domesticus* L.). In: Better science, better fish, better life. Proceedings of the Ninth International Symposium on Tilapia in Aquaculture, Shanghai, China (eds. L. Liping and K. Fitzsimmons), (22-24 April 2011). pp. 105-120. AQUAFISH Collaborative Research Support Program.

Orose, E., A. Wokoma and G.N. Woke. 2016. Sex Reversal of Nile Tilapia (*Oreochromis niloticus*) using 17α-Methyltestosterone, wet testes from selected animals and plant extracts. Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria 4(3): 23-29.

Phelps, R.P., L.L. Lovshin and B.W. Green. 1996. Sex reversal of tilapia: 17 α-methyltestosterone dose rate by environment and efficacy of bull testes. In:Pond dynamic and aquaculture collaborative research support program (eds. D. Burle, B. Goetec, D. Clair and H. Egna). pp. 89-91. 14^{th} annual technical report.

Pompa T. and M. Masser. 1999. Tilapia Life history and Biology. Southern Regional Aquaculture Centre. Publication No. 283.

Vera Cruz, E.M. and G.C. Mair. 1994. Conditions for effective androgen sex reversal in *Oreochromis niloticus* (L.). Aquaculture 122(2-3): 237-248.

Nepalese Journal of Aquaculture and Fisheries
Vol. 3 & 4 (2016 & 2017) : 26-33

Assessment of optimum feeding rate on growth and production of carp polyculture in Nepal

Sumitra Laudari[1*], Chaudhary Nagendra Roy Yadav[2], Arvind Kumar Singh[2] and Rama Nanda Mishra[1]
[1]Directorate of Fisheries Development, Balaju, Kathmandu, Nepal
[2]Institute of Agriculture and Animal Science, Rampur, Chitwan, Nepal
*Email: sumitra_laudari@hotmail.com

Abstract
Polyculture of carp was done in fertilized earthen ponds to identify appropriate feeding rates for optimal growth performance and production of carps. The experiment was carried out in 3 earthen ponds partitioned into 12 experimental units by nylon net with size of 129.0-138.7 m^2 for 128 days. Feeding rates of 2% (T1), 3% (T2) and 4% (T3) of fish body weight and no feeding (T4) were experimented in triplicate. Carps were stocked at the ratio of 8:5:3:3 with combined stocking density of 1.5 fish/m^2. Fish were fed daily with a locally factory made pelleted feed (22% CP) six days a week. The overall mean survival rate for all species ranged from 87% to 92.6 %. Total fish production (t/ha/yr) was 2.66, 3.69, 3.61 and 1.98 in T1, T2, T3 and T4 respectively. The rate of return was 342.1 % in T1, 368.2 % in T2, 198.1 % in T3 and 3045.2 %. Total net yield (t/ha/yr) was significantly higher ($p<0.05$) in T2 (3.69) than T1 (2.66) and T4 (1.98) whereas T2 (3.69) and T3 (3.61) are statistically similar ($p>0.05$). Feed conversion ratio was 1.18, 1.16 and 1.85 in T1, T2 and T3 respectively. Thus, among the four different feeding levels, T2 (3% feeding rate) treatment showed optimum rate for better growth and production of carps.

Keywords: Feeding rate, polyculture, carps

Introduction

Aquaculture development began in Nepal in the mid-1940s on a small scale in ponds with indigenous major carp seed imported from India. Further development began in 1950s with the introduction of the exotic species common carp (*Cyprinus carpio L*) and Chinese carp species silver carp (*Hypophthalmichthys molitrix Val*.), bighead carp (*Aristichthys nobilis*) and grass carp (*Ctenopharyngodon idella*) (FAO, 2006). Fisheries is an important sector with fastest growth rate, production reached to 57,520 mt. in 2012/13, in which aquaculture contributed nearly 63% (DoFD, 2012). Pond fertilization (applications of organic and/or inorganic fertilizers) increases production (Boyd, 1981, Olah, 1986). Additionally, artificial feed may be provided to increase fish growth and production above that possible in fertilized ponds. Although supplemental feeding in fertilized ponds resulted in significantly higher growth rates and greater yields than fertilization alone (Green, 1992).Feeding costs contributes up to 60% of the variable costs of culture systems. Therefore, it is essential to provide a proper and applicable feeding management program. In fact, an inadequate food supply directly affects production costs and water quality (Ng et al. 2000, Mihelakakis et al. 2002, Silva et al. 2007). Overfeeding mostly causes feed spillage, decreasing in feed efficiency, and polluting the environment. Similarly, underfeeding results in decreased feed efficiency as well as degraded growth (Dediu et al. 2011). Therefore, it is essential, in terms of both economy and biology, to determine the optimum feeding rate for growth (Aydin et al. 2011). It should be noted that optimal feeding rate is essential not only because of promoting best growth and minimizing feed conversion rate (FCR), but also for economic and environmental aspects, preventing water quality degradation (Yuan et al. 2010). There are no quantitative data available on optimum feeding rates for polyculture in fertilized earthen ponds. Also, to our knowledge, no work-feeding rate of polycultured fish has ever been done. When optimum feeding rate is not known or has not been adequately determined, increased inputs of supplementary food might occur. In fact, commercial fish farmers frequently use higher feeding rates than necessary, resulting in a lower economic return and water quality problems. Supplemental feeding is expensive and constitutes an appreciable portion of the total production cost, and fish farmers cannot afford any misuse of feed. Therefore, the availability of reliable data for the appropriate feeding level of fish in a polyculture system is important for rapid growth of fish and cost control of feed. Determination of feeding levels based on reliable data may help to save feed while promoting fish growth and production, thus increasing profitability. A study of this sort contributes towards a better understanding of the feed requirements of a combination of fish species that has access to natural food items. It may also contribute to improving aquaculture management practices and increasing fish farm profitability. The objective of the study was to assess the optimum feeding rates on growth and production of Carps polyculture in Nepal. Therefore, a study on better understanding of the optimum feeding rate of Carps polyculture has been tried.

Materials and Methods

The experiment was conducted in 3 outdoor earthen ponds which are partitioned by nylon net into 12 experimental units of nearly about 135.2 m^2 (129-138.7m^2) at Central Fisheries Building, Balaju, Kathmandu, starting from 28 August 2013 to 5 January 2014 for 128 days. The earthen ponds were completely drained out and dried about 1 week before the stocking of the fishes. The ponds were kept sun dried for one week, liming at the rate of 500 kg/ha and filled with boring water. The ponds were fertilized with inorganic fertilizer (Urea and DAP) at the rate of 100 kg/ha/month.

The experiment was conducted in a Complete Randomized Design (CRD) consisting of 4 treatments (T1- Feed @ 2% of fish body weight, T2- Feed @ 3% of fish body weight, T3- Feed @ 4% of fish body weight and T4- Control (no feeding) with 3 replications. Common Carp was used for main species in all treatments where stocking ratio of Common Carp, Silver Carp, Bighead Carp and Grass Carp was 40%, 30%, 15% and 15% in T1, T2, T3 and T4 treatments respectively. Stocking density was same in all 4 treatments. The stocking density of all fish species was 1.5fish/m^2 in all treatments. Fish seeds were procured from Central Fisheries Lab, Kathmandu. Large size of fingerlings of Common Carp (80-85 no.) having average weight (41.6-46.24 g) were stocked in each pond. Similarly, Silver carp (60-65 no.) having average size (108-115.8 g) were stocked in each pond. Bighead carp (30-35 no.) having average size (89.7-106.9 g) was stocked in each pond. Grass carp (30-35 no.) having average size (44.2-48.5 g) was stocked in each pond. Batch weight of each species was taken to determine the average stocking weight by using electronic balance.

Feed and feeding

Fish were fed on factory made (Himalayan Rainbow Trout feed factory, Balaju, Kathmandu) pellets contained of 22% crude protein. Feed was given at the rate of 2%, 3% and 4% of total fish body weight. The feed was given once a day i.e. at 10 am in the morning. Feeding tray was used for feeding the fish. The quantity of feed was adjusted at monthly i.e. 30 days intervals based on fish growth measurements.

Water quality

Weekly water quality parameters were measured at 6am from August 30, 2013. Transparency, dissolved oxygen, pH and temperature were measured in situ.

Fish growth and production

Initial mean weight of fingerlings was recorded and fingerlings were stocked in respective earthen ponds. Monthly fish growth was determined by taking samples at 30 days interval. Fish of each species (10-20%) was sampled periodically and feed adjustment was done accordingly. Fishes were harvested after 128 days and final weight of each species was measured with the help of weighing balance. Growth and production parameters such as daily growth rate, net fish yield (t/ha/yr), FCR and survival rate were calculated.

Economic analysis

Gross margin and rate of return were analyzed from total variable cost (operational cost) and total value of all products. The variable cost item included was pellet feed required for the production based on current market price. Labor, fertilizer and seed is not accounted in production cost analysis. Gross margin and rate of return was calculated based on product sold at farm gate price.

Statistical analysis

Statistical analysis of data was performed by using one-way analysis of variance (ANOVA) using M-Stat Microsoft computer programs for windows. The results with statistical differences at the level of 5% significance were further analyzed with LSD. All means were given at ± 1 standard error (S.E.).

Results and Discussion

Mean growth

Survival and growth of fish species under different feeding treatments are given in Table 1. The mean growth of carps was highest in T2 (88 ±0.91) followed by T3 (82.3±.91g), T1 (75.6±.91) and T4 (52.1±.91). The mean growth (g) was found highest in common carp followed by grass, silver and bighead carp in decreasing order in polyculture in different feeding rates. Growth rate of common carp was lower in T4 (48.9g) and highest in T3 (104g) treatment. Similar trend found by Pandey (2002) reported mean growth (g) of common carp was found highest in four species polyculture of the common carp, followed by silver carp, grass carp and bighead carp. The present study showed that growth rates of common and grass carp was found better than silver carp and bighead carp. Common carp is omnivorous, bottom-dwelling fish that can be raised on supplementary feed (MOAAF, 1988) and prefers more artificial feed than other carps (Schroeder, 1983). The growth rate of grass carp found better though with application of grasses in the pond. Woynarovich (1975) reported that grass carp eats the artificial feed supplied specially for common carp, but extent of such utilization is comparatively little. The growth rate of common carp and grass carp was also found better in all polyculture with feeding system. Abdelghany and Ahmad (2002) stated that supplemental feed caused increases in yields by 14.1%, 22.4%, 39.7% and 34.4% in the treatments in which fish were fed at 0.5%, 1%, 3% and 5% of their body weight per day respectively. These results suggest the efficacy of supplemental feed for promoting better growth and production. The growth of fish is temperature dependent as a cold-blooded animal (Woynarovich, 1975). Fish growth is faster in higher temperature under suitable range. In the present study, fish growth is higher in the initial month and lower after the October whereas water temperature dropped below 20°C. Water temperature below 20°C was observed from the middle of November to December.

Table 1: Mean stocking weight; mean harvesting weight and mean survival rate of different carp species in different treatments (Mean ± SE.) during experimental period of 128 days

Fish species	Treatments	Mean stocking weight (g)	Mean harvesting weight (g)	Mean survival rate (%)
Common carp	T1	45.3±1.46	106.6±3.93c	90.6±9.51a
		(44.1-48.2)	(105.3-107.0)	(83.7-100)
	T2	41.6±1.46	119.7±3.93b	91.6±9.55a
		(40-42.3)	(114.9-123.6)	(71.7-100)
	T3	41.6±1.46	145.6±3.93a	95.9±9.79a
		(37.6-44.7)	(134.5-158.6)	(88.2-100)
	T4	46.2±1.46	95.1±3.93c	96.3±9.81a
		(44-48.2)	(90.9-99)	(96.2-96.4)

Silver carp	T1	115.8±2.13 (110.7-118.4)	195.8±3.59b (186.8-203.7)	88.9±9.41a (76.9-100)
	T2	112±2.13 (107.2-115)	212.3±3.59a (204.6-217.2)	96.8±9.84a (95.3-100)
	T3	108±2.13 (104.6-110)	187.2±3.59b (182.9-189.3)	98.4±9.92a (96.9-100)
	T4	112.1±2.13 (109.2-115)	169.9±3.59c (165-170.1)	95.2±9.75a (90.7-100)
Bighead carp	T1	94.6±4.39 (91.4-103.3)	184.4±5.59b (176.4-195.8)	92.3±9.60a (80-100)
	T2	106.9±4.39 (88.5-126.6)	209.2±5.59a (199.4-216)	100±10a
	T3	89.7±4.39 (88.3-91.7)	172.7±5.59b (162.2-170.6)	91.9±9.58a (85.7-100)
	T4	92.7±4.39 (88.5-98.3)	147.1±5.59c (138.5-154.1)	90.4±9.50a (80-100)
Grass carp	T1	45.7±3.11 (37.3-51.4)	117±3.34ab (105-123.4)	76.5±8.73a (66.6-88.5)
	T2	47.8±3.11 (46-50.2)	119±3.34a (116.8-120.4)	82±9.02a (62.8-100)
	T3	44.2±3.11 (42.8-46.5)	106.9±3.34b (104.1-109.4)	71.1±8.37a (51.4-93.3)
	T4	48.5±3.11 (41.3-55.7)	95.5±3.34c (93.2-100)	70.7±8.34a (46.6-85.7)
Total	T1	75.3±3.20	150.9±4.11	88.2±3.60
	T2	77±3.20	165±4.11	92.9±3.92
	T3	70.8±3.20	153.1±4.11	92.1±3.90
	T4	74.8±3.20	126.9±4.11	91.2±3.88

Note: Figure in parenthesis is range values. Means with different superscripts within treatments within each group differ significantly at $p<0.05$.

Net fish yield

Extrapolated total net fish yield (NFY) of T2 (3.69 ton/ha/yr) was significantly higher ($p<0.05$) than the T1 (2.66 ton/ha/yr) and T4 (1.98 ton/ha/yr). However, the NFY of T2 (3.69 ton/ha/yr) and T3 (3.61 ton/ha/yr) were not significantly different ($p>0.05$) to each other. NFY of four species polyculture was little lower than the government (4.9 mt./ha) estimated yield (Table) (DoFD, 2017) and 4-5 ton/ha/year (Pradhan and Shrestha, 1997). Lower production in present study might be due to the low temperature and low dissolved oxygen.

Table 2: Net fish yield and FCR of different carp species in different treatments (Mean ± SE)

Treatments	Net Fish Yield(ton/ha/year)	FCR
T1	2.664±0.14b	1.18±0.07
T2	3.694±0.25a	1.16±0.10
T3	3.617±0.22a	1.85±0.08
T4	1.981±0.09c	0

Mean values with same superscript letters in the same column were not significantly different at $p< 0.05$.

Food conversion ratio (FCR)

During experimental period the feed conversion ratio (FCR) of fish was 1.18, 1.16 and 1.85 in T1, T2 and T3 respectively. These results are in complete agreement with results of Wu-Goangyum (1992), Abdel-Hakim et al. (2000) and Yasser (2005) who studied the high yield of polyculture of mullet and carps. It was stated that feed conversion decreases as the amount of feed fed decreases and increases with an increase in the amount of feed fed (Halver, 1989). Food conversion ratio depends on the oxygen concentration and temperature of the pond water, the size of the fish and its feeding habit and health condition.

Economic analysis

Mean fish production, net income, rate of return and variable costs involved in fish production in different treatments are shown in Table 3. The total variable costs involved in fish production were statistically similar (p>0.05) in all treatments, though T3 demands comparatively higher expenditure. The net income was higher in T2 (725.4±68) than T1 (515.2±37.7), T3 (601.1±49.6) and T4 (479.4±24.8). Rate of return is significantly higher in T4 (3045.2 ±0) than T3 (198.1±13). T1 (342.1±25.8) and T2 (368.2±41.6) are statistically similar (p>0.05).

Table 3: Economic analysis of Carps with different feeding rates computed in thousand NRs/ha/year

Particulars	Treatment (Mean±SE)			
	T1	T2	T3	T4
Variable cost				
Pellet Feed (Nrs/ha)	135.5±0.7	182.3±5.7	287.2±7.7	—
Fish seed (Nrs/ha)	15.7±0.1	15.7±0.1	15.7±0.1	15.7±0.1
Total variable cost	151.2	198	302.9	15.7
Revenue				
Fish production (kg/ha)*	2612±149.5	3693.7±256.3	3616.3±222.2	1980.7±99.6
Income	665.8±37.3	923.4±64.1	904±55.5	495.2±24.9
Net income	515.2±37.7	725.4±68	601.1±49.6	479.4±24.8
Rate of return (%)	342.1±25.8	368.2±41.6	198.1±13	3045.2

* Fish production in thousand

Pellet feed @NRs 43/kg, Fish seed@1Nrs/no. and Fish Farm gate price @ NRs 250/kg.

Water quality parameters

Most of the water quality parameters were within the suitable range for fish production (Table 4). Water quality parameters such as transparency, pH, dissolved oxygen concentration and temperature were fluctuated during the experimental period without any particular trend. Pond water temperature was higher in stocking period, decreased continuously and reached upto 13°C at the end of experiment. Pond water temperature started to decrease from November 1st week to December last week which resulted into retarded growth of fish.

All carps showed better growth performance at water temperature above 20°C and slower growth was observed below this temperature with very minimal growth at 13°C during this study. Azad (1996) observed the growth of all carps was better at 20 -30°C but not eat well below 13°C. Bakos (2001) reported 2% body weight daily feeding and fortnightly application of manure seems to be safe in term of water quality. In the present study the highest dissolved oxygen (4.4 mg/ L) was obtained in control ponds (T4), with natural feed, while the lowest (DO) was recorded in ponds of T1, T2 and T3, supplemented with feed. Swingle (1969) considered concentration of dissolved oxygen below 5mg/L as undesirable in ponds. Boyd (1992) postulated that excess feeding can result in an increase in organic material which increases oxidation through metabolic processes by bacteria resulting a decrease in DO. pH values were almost in suitable range (6.8 – 7.6) which are in the tolerable range for fish and for primary productivity to flourish (Boyd, 1992).

Table 4: Mean and range values of water quality parameters in different treatments during experimental period of 128 days

Parameter	T1	T2	T3	T4
Dissolved Oxygen (mg/L)	4.3±0.16 (2.9-5.3)	4.2±0.22 (2.4-5.4)	3.9±0.23 (2.2-5.2)	4.4±0.25 (2.7-5.9)
Temperature (ºc)	23±0.0 (15.1-27.6)	22.7±0.1 (15.1-27.8)	22.7±0.0 (15.3-27.4)	22.7±0.1 (15.6-27.6)
pH	7.0 (7.1-7.6)	7.3 (6.8-7.6)	7.3 (7-7.6)	7.3 (6.9-7.6)
Secchi disc visibility (cm)	21±0.6 (19-21)	20.8±1.1 (19.3-22.3)	20.5±0.5 (17.3-21.7)	22.4±0.4 (21-25)

Conclusion

Fish growth retarded after mid of November to December last week while water temperature was below 20°C. So, active growth period of fish was short. The time of active growth was April to October. Although, feed based fish species showed higher performance in growth and production, common carp played major role to increase production and grass carp has economic production. Silver carp and bighead carp has little role besides maintaining the aquatic environment. So, the fish species can be used as a bonus fish for ecological point of view for suitable pond environment management. The growth rate of common carp was higher than the silver carp and bighead carp. Grass carp was second in position for growth performance. Small size fish seed of grass carp had high mortality which decreases the production. Due to short time of fish production multiple stocking and multiple harvesting systems will be suitable to increase production. Fish production can be increased by early stocking, high density of large size of fingerling of grass carp and common carp with adequate feeding in polyculture. In the present study, it appears that the daily feed application rate of 3% body weight was near to optimum when the fish grows. The results suggest that, although a feeding rate of 4% gave the highest growth performance, carps should be fed at the rate of 3% body weight per day, considering feed conversion efficiency, survival rate, economic efficiency and growth response. This level of feeding minimized feed wastage as optimal utilization was achieved. It is therefore recommended that carps should be raised at this level of feeding rate to ensure effective usage of high cost of feed and feeding.

Acknowledgements

The author would like to acknowledge the Central Fisheries Lab, Balaju, Kathmandu, Nepal for providing necessary facilities to conduct this experiment. This work was financially supported by National Agricultural Research and Development Fund. The authors acknowledge the help of team from Institute of Agriculture and Animal Science, Chitwan for help throughout the study period.

References

Abdelghany, A.E. and M.H. Ahmad. 2002. Effects of feeding rates on growth and production of Nile tilapia, common carp and silver carp polycultured in fertilized ponds. Aquaculture Research 33(6): 415-423.

Abdel-Hakim, N.F, M.N. Bakeer and M.A. Soltan. 2000. Growth performance of eel (*Anguilla anguilla*), Nile tilapia (*Oreochromis niloticus*) and grey mullet (*Mugil cephalus*) cultured in cages under two feeding systems. In: Proceedings of 3rd All Africa Conference of Animal Agriculture and 11th Conference of the Egyptian Society of Animal Production, Alexandria, Egypt, 6-9 November 2000: 329-335.

Aydın I., E Küçük, T Şahin and L. Kolotoğlu. 2011. The effect of feeding frequency and feeding rate on growth performance of juvenile black sea turbuot (*Psetta maxima*, Linneaus, 1758). Journal of FisheriesSciences.com 5(1): 35-42.

Azad, I.S. 1996. Growth performance of grass carp fingerlings fed on *Agartum conizoids*, a common terrestrial weed of Manipur. Journal of Inland Fisheries Society of India 28(1): 44-49.

Bakos, J. 2001. Guidelines for broodstock management, fish seed production and brood stock management mission. Living Aquatic Resources Research Center, (LARREC) in Lao PDR. National Aquatic Resource Institute (NARI).

Boyd, C.E. 1992. Water quality in warm water fishponds. Alabama Agriculture Experiment station, Auburn University, Alabama. 650 pp.

Boyd, C.E. 1981. Fertilization of warm water fish ponds. Journal of Fish Soil and Water Conservation 36(3): 142–145.

Dediu L., V. Cristea, M. Mocanu, D. Dicu, A. Docan and I. Grecu. 2011. The effect of feeding frequency on growth performance of rainbow trout fingerlings reared in recirculating system. AACL Bioflux 4(2): 141-145.

DoFD. 2012. Country Profile Nepal, Fisheries Sub-sector. Department of Agriculture, Directorate of Fisheries Development, Balaju, Kathmandu, Nepal.

DoFD. 2017. Country Profile Nepal, Fisheries Sub-sector. Department of Agriculture, Directorate of Fisheries Development, Balaju, Kathmandu, Nepal.

FAO. 2006. Food and Agriculture organization of the United Nations, Helping to Build A World without Hunger, Fisheries and Aquaculture Department National Aquaculture sector Overview Nepal.

Green B.W. 1992. Substitution of organic manure for pelleted feed in tilapia production. Aquaculture 101(3-4): 213–222.

Halver, J. E. 1989. Fish Nutrition. Academic Press, San Diego, CA, USA.

Mihelakakis A., C. Tsolkas and T. Yoshimatsu. 2002. Optimization of feeding rate for hatchery-produced juvenile gilthead sea bream *Sparus aurata*. Journal of World Aquaculture Society 33(2): 169–175.

MOAAF. 1988. Fish farming in pond. Ministry of Agriculture and Fisheries, Agricultural Educational Department, The Hauge (Netherlands). 129 pp.

Ng W.K., K.S. Lu, R Hashim and A. Ali. 2000. Effects of feeding rate on growth, feed utilization and body composition of tropical bagrid catfish. Aquaculture International 8(1): 19–29.

Olah J. 1986. Carp production in manured ponds. In: Aquaculture of Cyprinids (eds. R. Billard and J. Marcel), pp. 295–303. IRNA, Paris.

Pandey, C.M. 2002. Productivity Assessment of Carp Polyculture System and Tilapia Culture System under Farmers pond condition in Chitwan, Nepal. M.Sc. Thesis, Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan, Nepal. 96 pp.

Pradhan, G.B.N. and S.B. Shrestha. 1997. Status of fisheries and aquaculture development and their potential for expansion in Nepal. In: Proceedings of the National Symposium on the Role of Fisheries and Aquaculture in the Economic Development of Rural Nepal. (eds. D.B Swar, G.B.N. Pradhan and L.M. Lofvall Westland) (15-16 August 1996). Nepal Fisheries Society (NEFIS). pp. 3-14.

Schroeder, G.L. 1983. Source of fish and prawn growth in polyculture ponds as indicated by δC analysis. Aquaculture 35:29-42.

Silva C.R., L.C. Gomes and F.R. Brandão. 2007. Effect of feeding rate and frequency on tambaqui (*Colossoma macropomum*) growth, production and feeding costs during the first growth phase in cages. Aquaculture 264(1): 135–139.

Swingle, H.S. 1969. Methods of analysis for waters, organic matter, and pond bottom soils used in fisheries research. Auburn University, Auburn, Alabama. 119 pp.

Woynarovich, E. 1975. Elementary Guide to Fish Culture in Nepal. FAO, Rome. 131 pp.

Wu-Goangyun. 1992. Experiment on high yield of polyculture of mullet and carps in the pond. Shandong-Fish-Qilu-Yuye 4: 37-39.

Yasser, T.A. 2005. Effect of fertilization on fish production in earthen ponds. Ph.D. Thesis, Animal and Fish Production Department, University of Alexandria.

Yuan Y.C., H.J. Yang, S.Y. Gong, Z. Luo, H.W. Yuan and X.K. Chen. 2010. Effects of feeding levels on growth performance, feed utilization, body composition and apparent digestibility coefficients of nutrients for juvenile Chinese sucker, *Myxocyprinus asiaticus*. Aquaculture Research 41(7): 1030-1042.

Nepalese Journal of Aquaculture and Fisheries
Vol. 3 & 4 (2016 & 2017) : 34-38

Effect of different formulated feed on growth and survival of gardi (*Labeo dero*) fry

Surendra Prasad*, Md Akbal Husen and Ram Prasad Dhakal
Fisheries Research Station, Pokhara
*Email: g43sdp@yahoo.com

Abstract
A study was carried out to evaluate the effect of three different formulated feeds on growth and survival of gardi (*Labeo dero*) fry for farming in pond and other aquaculture systems. The experiment was conducted in 2 m^2 size hapa (2m X 1m) fixed in pond. Gardi fry with average body weight 0.01±0.01g to 0.03±0.01g were stocked at rate of 200 per/ hapa (1,000,000/ha) with two replicates per treatment. Three different formulated diets (micro feed, powdered milk with egg and soya milk) were provided ad-libitum to fry over a period of 45 days. Survival was greater than 64% in all treatments with no significant differences (p>0.05) among treatments. Similarly, there was no significant difference (p>0.05) in relative growth rate (RGR 2.10-2.23%) of the fish among treatments. Significantly highest (p<0.01) body weight gain was observed with fry fed with micro feed (0.523±0.080g) followed by powdered milk with egg (0.400±0.029g) and soya milk (0.330±0.034g). The result suggested that there was an apparent effect of different diets at the level tested on body weight gain but gardi fry prefer micro feed containing high protein preferably of more animal origin in early stage.

Keywords: Fish diet, gardi fry, growth rate, micro feed, survival

Introduction

Gardi (*Labeo dero*) is one of important indigenous species relatively rich in river systems of Nepal (Shrestha 1981, Shrestha 2003). It is the main source of animal protein for almost all low-income rural households in mid-hill region. Propagation of this species has been successful due to recent advancement in breeding technology (Prasad and Rai 2010). However, availability of gardi fry is still meager for commercial production and biodiversity conservation. The greatest problem existing with nursing of gardi fry is low survival during nursing in ponds from hatchlings to fry stage. Fry rearing is one of the important phase and aims at obtaining high growth and survival for production of fingerlings required for stocking into grow out ponds as well as rehabilitation in natural habitat. Growth responses and survival of fish depend upon several factors, feeding being one of the most important (Pillay 1993). The significance of qualitative and quantitative feeds is well recognized (Das et al. 1991) and the types of feed containing different level of protein is of fundamental importance, because it significantly influences growth, survival and yield of fish (Manivannan and Saravanan 2012).

At present there is only limited knowledge on feed, feeding and nutritional requirement of gardi. Gubhaju (2002) observed that this fish feed on detritus and algae in natural condition. Gardi is considered herbivore (Raina and Petr 1999), however in captivity they also accept supplemented feed (Prasad and Rai 2010, Prasad et al. 2011). Therefore, this study was carried out to evaluate the effect of three different formulated feeds on growth and survival of gardi fry for farming in pond and other aquaculture systems.

Material and Methods

The study was conducted at Fisheries Research Center, Pokhara over a period of 45 days from July to September, 2011. The pond were prepared as per standard pre-stocking management practices of nursery ponds for commercial carps involving drying, liming, fertilization, watering and chemical application for zooplankton regulation. The experiment was conducted in 2 m^2 size hapa (2m X 1m) fixed in pond. Four days fry (mean body weight 0.01±0.01g to 0.03±0.01g) were stocked at 100 no/m^3 (1,000,000/ha). Three different formulated diets were used to test. Diet 1 was micro feed which was prepared from soya puff, fish meal, milk powder, egg yolk, fish oil, vitamin and mineral mixture. Gelatin was used as binder. Diet 2 was prepared from 50% instant full cream milk powder mixed with 50% egg yolk. Diet 3 was made from sole source of soybean. Roasted soybean was made powder and dissolved in water. The fish were fed daily with formulated diets to ad-libitum over a period of 45 days. Each diet was supplied to two replicate hapas.

Water temperature was measured daily. Weekly fish samples were taken to monitor the weight gain. Survival was recorded every two weeks after total counting of fry. Relative growth rate ws calculated using following equation:

Relative growth rate (RGR %/day) = [(Final Weight - Initial Weight)/Experimental days] X 100

Data were analyzed using Microsoft Excel program.

Results and Discussion

In the present investigation, three different kinds of fish feed were formulated with varying feed ingredients and given to *L. dero* to assess the impact of the protein diet on its growth and survival. The results on fish growth from hatchling to fry are depicted in Figure 1. The overall growth of fish showed exponential growth in all treated diets.

The result showed that significantly ($p<0.01$) higher (0.52±0.08g) weight gain were recorded with diet 1 than diet 2 (0.40±0.03g) and diet 3 (0.33±0.03g). This result was comparable with weight gain in other species like common carp (ARS Fish 2010-2011), sahar (Bista et.al. 2006), gardi (Prasad et al. 2011) and climbing perch, *Anabas testudineus* (Alam et al. 2010) in which they found weight gain of 0.40-0.78g in nursing experiment with artificial diet. The results indicated formulated diet had positive impact on weight gain of fry. Amirkolaie et al. (2010) in an experiment also found that formulated diet had positive effects on growth performance and survival rate for grass carp juveniles. However, the variation in weight gain might be due to differences in diet composition and availability of nutritional condition in different types of feed used (Rahman et al. 2012).

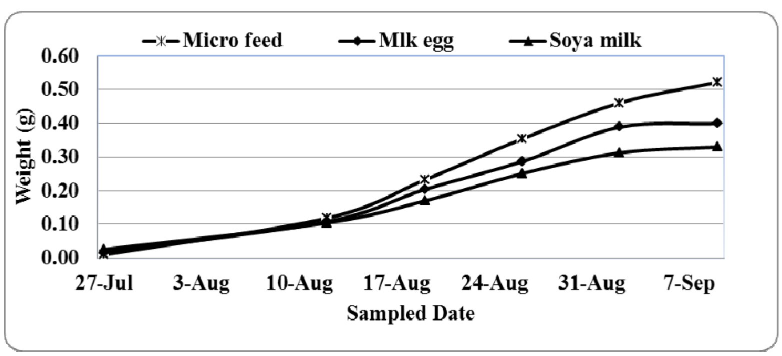


Figure 1: Body weight gain of gardi (*Labeo dero*) fry fed with different formulated diets

On other hand, there was no apparent effect of feed type on growth rate and survival of gardi fry at the level tested (Table 1). However, relative growth rate (RGR) of 2.10-2.23% obtained in the present study was similar to 2.27% reported for common carp *Cyprinus carpio* (Msiska et al. 1991), 2.30g for *Oreochromis karongae* (Nyirenda et al. 2000) and 2.12 for rabbit fish *Siganus rivulatus* (Saoud et al. 2007) suggesting growth rate of gardi fry could be similar to that of cultured carps.

Table 1: Growth characteristics of gardi (*Labeo dero*) fry fed with different formulated diets

Parameter	Micro feed	Milk egg	Soya milk
Total no fry stocked	200	200	200
Initial mean weight (g) ± SD	0.010±0.004	0.017±0.012	0.026±0.001
Final mean weight (g) ± SD	0.523±0.080^{a}	0.400±0.029^{b}	0.330±0.034^{c}
Relative growth rate (% day)	2.23^{ns}	2.18^{ns}	2.10^{ns}
Survival % ± SD	64.8^{ns}	64.3^{ns}	66.5^{ns}

The survival rate of fish fed with different feeds is presented in Figure 2. The survival rate 64-66% achieved in this study could be considered satisfactory in comparison of the results obtained in fry nursing of different cultivable species like gardi (Prasad et al. 2011), *mrigal* (Rahman et al. 2012), common carp and grass carp (ARS Fish 2010-2011). They reported variation in survival from 12.2% to 98.0%. These differences could be attributed to difference in types of feed, feeding practices and species used. Coche and Edward (1988) reported that availability of proper type of food in required quantities is one of the major factors determining the survival and growth of carp fry.

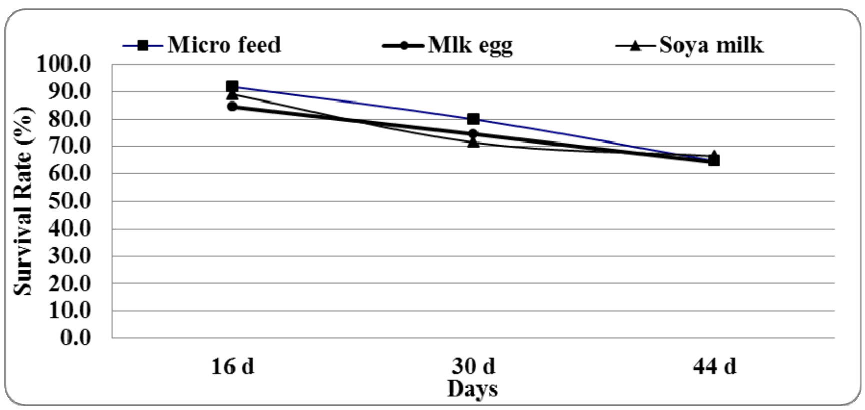


Figure 2: Survival rate of gardi (*Labeo dero*) fry fed with different formulated diets

This study was conducted in fertilized pond where natural food (plankton) was present to meet the minimum feeding requirements of fish which might be advantageous for growth and survival. Studies on larval nutrition have indicated that live food is essential for few days after the start of exogenous feeding (Verreth et. al. 1992). In similar experiment, Prasad et al. (2011) reported that young one of gardi feed both on zooplankton and artificial diet.

Water temperature is one of the important variable influencing the growth rate of fish. Water temperature of 29.0 to 30.0°C observed in this study was conducive for accelerated growth of fry. Pillay (1993) pointed out that many freshwater species survived over a wide temperature range but the range for maximum growth for warm water species might be from 25°C to 30°C.

Conclusions

This study suggested that gardi fry can easily accept and could be reared with different types of formulated feed in early stages. Though diet 1 (micro feed) resulted in high body weight gain, diet 2 (powdered milk mix with egg yolk) and diet 3 (soya milk) used in this study was equally good to increase survival and growth rate. However, soybean milk could be one of the alternative sources of feed to increase survival and growth rate of gardi fry and eliminating the extra cost of animal protein source in diet. Further study is necessitated to develop cost effective diets based on different level of protein in future.

Acknowledgement

This study was supported by Nepal Agricultural Research Council. Authors are thankful to staffs of Fisheries Research Station, Pokhara for their active involvement in this experiment.

References

Alam, M.J., M.G. Mustafa and M.M. Islam .2010. Effects of some artificial diets on the growth performance, survival rate and biomass of the fry of climbing perch, *Anabas testudineus* (Bloch, 1792). Nature and Science 8(2):.36-42.

Keramat, A.A., M.B. Lashkar and A.S. Mirzaee. 2010. Effects of pellet and grass diets on growthand morphology of gastro-intestinal tract in grass carp (*Ctenopharyngodon idella*). Iranian Journal of Natural Resources 63(3): 209-216.

ARS Fish Pokhara. 2010/11. Effect of larval stocking density on carp in government and private sector ponds survivability and growth of fry. Annual Technical Report 2010-2011. pp. 34-37.

Bista, J.D., S.K. Wagle and T.B. Gurung. 2006. Growth performance of sahar (*Tor putitora*) in earthen pond in Pokhara Valley. In: Proceedings of 6th National workshop on Livestock and Fisheries Research, NARC/NASRI. pp. 189-194.

Coche, A. and D. Edward. 1988. Selected aspects of warm water fish culture. A compilation based on lectures presented at a series of FAO/AGFUND, International Training Courses in Aquaculture hosted by Hungary in 1987 and 1988.

Das, K.M., S.N. Mohanty and S. Sarkar. 1991. Optimum dietary protein to energy ratio for *Labeo rohita* fingerlings. In: Fish nutrition research in Asia, Proceedings of 4th Asian Fish Nutrition Workshop, pp. 69-73. Asian Fisheries Society, Phillipines Special Publication 5.

Gubhaju, S.R. 2002. Impact of damming on the aquatic fauna in Nepalese rivers. In: Cold Water Fisheries in the Trans-Himalayan Countries (eds. T. Petr and D.B. Swar.), FAO Fisheries Technical Paper. 431: 129-146.

Manivannan, S. and T.S. Saravanan. 2012. Impact of formulated protein diets on growth of the Indian major carp, *Labeo rohita* (Hamilton). Fisheries and Aquaculture Journal 2012:1-7.

Msiska, O.V., B.B.A. Rashid and B.E. Kamvabingu. 1991. Growth performance of a planktivorous Clariid, *Bathyclarias loweae*, in an experimental pond. Aquabyte 4: 5.

Nyirenda, J., M. Mwabumb, E. Kaunda and J. Sales. 2000. Effect of substituting animal ptotein sources with soybean meal in diets of *Oreochromis karongae*. Naga, The ICLARM Quarterly 23(4): 13-15.

Pillay, T.V.R .1993. Aquaculture: Principles and Practices. University Press, Cambridge, UK.

Prasad, S. and A.K. Rai 2010. Captive breeding of river fish *Labeo dero* (Ham.-Buch.) for conservation. In: Sustainable Use of Biological Resources in Nepal. (eds: P.K. Jha, S.B. Karmacharya, M.K. Balla, M.K. Chettri and B.B. Shrestha), pp. 161-170. Ecological Society (ECOS), Kathmandu, Nepal.

Prasad, S., J.D. Bista, R.P. Dhakal, D.R Acharya, A. Husen and A.P. Nepal. 2011. Recovery of gardi (*Labeo dero*) fry in hapa nursing with and without supplementary feeding. In: Proceeding of the 8th National Workshop on Livestock & Fisheries Research (eds. T.B. Gurung, P.K. Pokharel, C.R. Upreti, B.R. Joshi, S.M. Pradhan and S.K. Wagle), Nepal Agricultural Research Council (NARC), Nepal. 336 pp.

Rahman, S.M., M.A.A. Shahin, M.A.H. Chisty, M.M. Rahman, A.M.H.B Tareque and M. M. Hasan. 2012. Growth performance of mirror carp (*Cyprinus carpio* var. s*pecularis*, Lacepède, 1803) fry fed with varying protein content feeds. Our Nature 10(1): 17-23.

Raina, H.S. and T. Petr. 1999. Cold water fish and fisheries in the Indian Himalayas: lakes and reservoirs. In: Fish and fisheries at higher altitudes: Asia. pp. 64-88. FAO Fisheries Technical Paper No. 385, FAO, Rome.

Saoud, I.P., J. Ghanawi and N. Lebbos. 2007. Effects of stocking density on the survival, growth, size variation and condition index of juvenile rabbitfish. *Siganus rivulatus*. Aquaculture International 16(2): 109. DOI.1007/s10499-007-9129-7.

Shrestha, J. 1981. Fishes of Nepal. Curriculum Development Center. Tribhuvan University, Kathmandu.

Shrestha, T.K. 2003. Conservation and management of fishes in the large Himalayan rivers of Nepal. LARS2-The second international symposium on the management of large rivers for fisheries: Sustaining livelihoods and biodiversity in the New Millennium, Cambodia.

Verreth, J.A., J.E. Torreelee, E. Spazier, A. Van der Sluiszen, J.H.W.M Rombout, R. Boom and H. Segner. 1992. The development of a functional digestive system in African catfish *Clarias gariepinus*(Burchell). Journal of World Aquaculture Society 23(2): 286-298.

Nepalese Journal of Aquaculture and Fisheries
Vol. 3 & 4 (2016 & 2017) : 39-46

The efficacy of clove oil as an anesthetic for use in handling and transportation of common carp, *Cyprinus carpio* fingerling

Anita Gautam, Prakash Kunwor and Suresh Kumar Wagle*
Fisheries Research Division, Godawari, Lalitpur, Nepal
*Email: waglesk@yahoo.com

Abstract
The use of anaesthetics becomes essential in the transportation medium for mitigating physiological stress and reducing metabolic rates of fish. Clove oil is now emerging as safe, eco-friendly, effective, and economic fish anaesthetic. Efficacy of clove oil as an anaesthetic was evaluated for the handling and transportation of common carp, *Cyprinus carpio* fingerling. The lowest concentration of clove oil that produced induction (≤5 min) and recovery (≤10 min) found was 250-300 µl/L at temperature between 18.2 to 19.3°C. Induction and recovery times were dose-dependent. An inversely proportional relationship was observed between concentrations of anaesthetic and induction time. The effective sedative dose at 21.2±2.4 µl/L of clove oil (18.02 µl/L eugenol) was found suitable for transportation in plastic bags with pure oxygen for 24h. The mortality rate (%) of fry was significantly higher (5.4±0.44%) in the control (without sedative) than sedative doses of clove oil ($p<0.05$). Post transportation mortality of fish after 7 days of treatment in control was also higher (3.8±0.91) compared to the fry treated with sedatives ($p>0.05$). The present findings revealed that clove oil is promising to be used as anaesthetic and sedative for handling and transportation of common carp fingerling.

Keywords: Anaesthetic, clove oil, common carp, sedative, transportation

Introduction

Aquaculture practices frequently expose fish to a variety of acute stressors that have the potential to negatively affect fish performance and survival (Barton 2000). Anaesthetics are used to aid in the handling of fish during practices that include enumeration, pathological analyses, hormone induction, stripping, hauling and transportation (Brown 1993). Anaesthetizing fish prior to transport can reduce metabolic rate and hence oxygen demand, reduce general activity, increase ease of handling, and mitigate the stress response. Significance mortality during and post transportation of fish seed (up to 70%) is experienced by farmers in Nepal (Husen and Sharma 2015a, b). Handling and transport are found to be important stressor for common carp and the stress causes physiological responses and mortality (Dobšíková et al. 2006; Dobšíková et al. 2009). The use of anaesthesia become important to minimize the fish losses due to increased physiological stresses and metabolic rates imposed by transportation.

The choice of anesthetic generally depends on availability, cost effectiveness, ease of use, nature of the study, and user safety (Cho and Heath 2000). Anaesthesia in fish may be produced by different agents, mainly, quinaldine sulphate, benzocaine and phenoxyethanol, which are hazardous, expensive and not very effective, and although tricaine methanesulphonate (MS-222) is safe and effective but expensive to use (Erdmann 1999). Clove oil (eugenol) is considered to be a safe, eco-friendly, effective, and economic fish anaesthetic (Woody et al. 2001, Matin et al. 2009, Husen and Sharma 2014).

Clove oil is a dark brown liquid resulting from the distillation of flowers, flower stalks, and leaves of clove trees *(Eugenia aromatica)* and used throughout the world for applications ranging from food flavouring to local anesthesia in the dentistry profession (Nagababu and Lakshmaiah 1992) It consists primarily of phenol eugenol (70-90%), eugenol acetate (>17%) and kariofilen 5 (12%). It is considered non-carcinogenic and non-mutagenic (Hernani 1988). Clove oil's properties and its status as a "generally recognized as safe" (GRAS) substance make it an ideal candidate as an anesthetic to use in the field of fisheries. Clove oil at various doses has been reported as safe sedation agent for transportation of prawn (Vartak and Singh 2006), *Barbus grypus* (Öğretmen et al. 2016), rohu (Husen and Sharma 2015a), *Oreochromis aureus* (Akar 2011) and rainbow trout *O. mykiss* (Anderson et al. 1997). The efficacy of clove oil as anesthetic in common carp (*Cyprinus carpio*) and its potential metabolism reducing capacity was studied in tropical climate (Kamble et al. 2014). Husen and Sharma (2015a) reported that doses at 0.005 and 0.0075 ml/l (3.8 and 5.6 mg/l eugnol) of clove oil for the transport of common carp fry at water temperature 28.9±0.36°C was effective. The response of clove oil as an anesthetic to the common carp reported are mostly based at high water temperature (≥25°C) (Kamble et al. 2014, Husen and Sharma 2015b). Ross and Ross (2008) reported that increase or decrease in water temperature may have a significant effect on the induction and recovery time. The information relating to the use of clove oil (eugenol) for transportation of carp in relatively low water temperature appears scanty. Hence, the present study was carried out to study the efficacy of clove oil as an anaesthetic for common carp fry (*Cyprinus carpio)* under sub-tropical condition.

Materials and Methods

Experiments on anaesthetic and sedation impact of clove oil on fingerling of common carp, *Cyprinus carpio* were carried out at Fisheries Research Division (FRD), Godawari during September to November 2015 and June to July 2016 in wet laboratory facilities, respectively. The fingerling of common carp collected from nursery ponds were kept in circular cemented pool for at least seven days with regular supply of fresh water and maintenance ration. Experimental fish were starved for 24 h prior to the experiments. After 24 h of starvation the fish were transferred to plastic bucket for one hour prior to the experiment performance.

For anaesthetic experiment, glass aquaria of (32cm×22cm×40cm) 20L equipped with aeration stone was used in entire experiment. The water in these aquaria was from fresh water supply of FRD, Godawari. The anaesthetic selected for this study was clove oil (Hi-Media P. Ltd.) which contained 85.0% eugenol. The clove oil stock solution was prepared by dissolving clove oil with 95% ethanol (1:10 ratio of clove oil-ethanol) to facilitate mixing. Nine concentrations of clove oil starting from 100 µl/L to 500 µl/L at 50 µl/L intervals was selected to determine the anaesthetic. Twenty-four hours starved 12 fingerlings with a mean weight of 4.4±0.7g were individually exposed to each clove oil concentration. Measurement of induction and recovery times was based on the stages described by Ross and Ross (2008) (Table 1). After reaching each stage of anaesthesia, (Stages AI-AIV) time was noted. At that time, the fingerlings were weighed before transferred to the resuscitation tank containing aerated freshwater only. An induction time of less than or equal to five minutes, and a complete recovery in ten minutes was used as a basis to record the anaesthesia stages for different doses. After the desirable exposure time of the chosen clove oil concentration, the fingerlings were transferred to the resuscitation aquarium for recovery. The time to intrigue in the recovery stage (BIII, Table 1) was recorded for each individual fingerling. Time for induction and recovery was recorded in seconds using electronic stopwatch. Recovered fish grouped according to dose were transferred into 1000 L tank and were monitored for survival and abnormal behaviour and no fish mortality was observed during experiment and next 7 days.

Table 1: Stages of anaesthetic induction and recovery (Ross and Ross 2008)

Stages	Descriptor	General Behaviour response of fish
A. Stages of induction		
0	Normal	Reactive to external stimuli; opercular rate and muscle tone normal
I	Light sedation	Slight loss of reactivity to external stimuli; opercular rate slightly decreased
II	Deep sedation	Total loss of reactivity to all but strong external stimuli; Slight decrease in opercular rate; equilibrium normal
III	Partial loss of equilibrium	Partial loss of muscle tone; swimming erratic; increased opercular rate
IV	Total loss of equilibrium	Total loss of muscle tone and equilibrium; loss of spinal reflexes
V	Medullary collapse	Respiratory movement ceases
B. Stages of Recovery		
I		Body immobilized but opercular movements just starting
II		Regular opercular movements and gross body movements beginning
III		Equilibrium regained and preanesthetic appearance

In transportation experiment, the treatments groups were doses of clove oil (0 µl/L, 15 µl/L, 20 µl/L, 25 µl/L and 30 µl/L) with 24h transportation time. Twenty-four-hour conditioned fry of common carp (4.51±0.6 cm, 1.33±0.4 g) was introduced at 75 fish/L in plastic bags containing clove oil treated 4 L water. Air from the plastic bag was squeezed out and 8 L pure oxygen was filled in. The plastic bags were then sealed with rope. Simulation trials were conducted in outdoor condition at temperature (23.5±1.1°C). After 24h of confinement the plastic bags were opened and dead fry were counted. The survived fry from each bag were transferred to 400 L circular tanks with constant water circulation and monitored for three days to evaluate post transportation mortality.

Water quality parameters include temperature, pH, dissolved oxygen, turbidity and conductivity were measured at the beginning and end of the experiments using Vernier analogue instrument (Model LABQUEST2). One-way Analysis of Variances were performed to test effect of doses and Univariate analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) were performed to test for significant differences in recovery times due to the duration exposure time, dose, and the interaction between those two variables using Stat Graphics 3.0 for Windows. Differences in mean value were considered statistically significant when $p<0.05$.

Results

Water quality was within acceptable ranges in all experiment (Table 2). Water turbidity increased significantly ($p<0.05$) at higher concentration of clove oil. The differences in values of all parameters of water quality were not significantly different ($p>0.05$) between time interval and among sedation treatments, except dissolved oxygen which decreased significantly ($p<0.05$) in all treatments at 24 h of transportation.

Table 2: Range and mean of water quality parameters in anaesthetic (clove oil) and resuscitation aquaria and transportation bag treated with clove oil as sedative

	Temp (°C)	Dissolved oxygen (mg/L)	pH	Turbidity (NTNU)	Conductivity (µs/s)
I. Anaesthetic aquaria (clove oil: 0-500 µL/L)					
Range	18.2-19.0	8.5-9.1	7.1-7.6	30.2-42.3	84.0-95.0
Mean	18.6	8.8		32.7	88.1
% CV	2.5	3.0	4.6	15.7	5.9
Significant (α0.05)	NS	NS	NS	*	NS
II. Resuscitation aquaria (clove oil: 0 µL/L)					
Range	18.8-19.3	8.5-8.6	7.4-7.7	31.7-44.4	93.0-97.0
Mean	19.0	8.6		36.7	94.0
% CV	1.4	0.7	1.8	18.5	2.8
Significant (α0.05)	NS	NS	NS	*	NS
III. Transportation bag (clove oil: 0-30 µL/L)					
Range	23.1-23.7	8.3-10.9	7.3-7.5	38.6-52.3	
Mean	23.5	9.16	6.42	45.82	
% CV	1.3	12.8	1.3	12.0	
Significant (α0.05)	NS	*	NS	NS	

An induction time of less than or equal to five minutes, and a complete recovery in ten minutes was used a basis to record the anaesthesia stages for different doses. Figure 1 show induction time of the fish for each dose of clove oil. Induction and recovery times were dose-dependent. There was a clear linear pattern of decreasing induction time with increasing concentration of the anaesthetic. An inversely proportional relationship was observed between concentrations of anaesthetic and induction time. The largest total induction time (937 sec) was detected in 150 µl/L dose of clove oil. However, shortest time of total induction (160 sec) was observed in 500 µl/L. The shortest (38 sec) and longest (266 sec) time to reach light sedation (stage I) was noticed in dose 500 µl/L and 150 µl/L, respectively. Similarly, the highest and lowest time taken to reach stage III (partial loss of equilibrium) and stage IV (total loss of equilibrium) was also recorded in 500 µl/L and 150 µl/L dose of clove oil. When all the four stages were examined, transition to stages I, II, III and IV took shortest time at highest (500 µl/L) dose. However, the time differences to reach in each stages at concentration 350 µl/L and at higher doses were not significantly different (p>0.05).

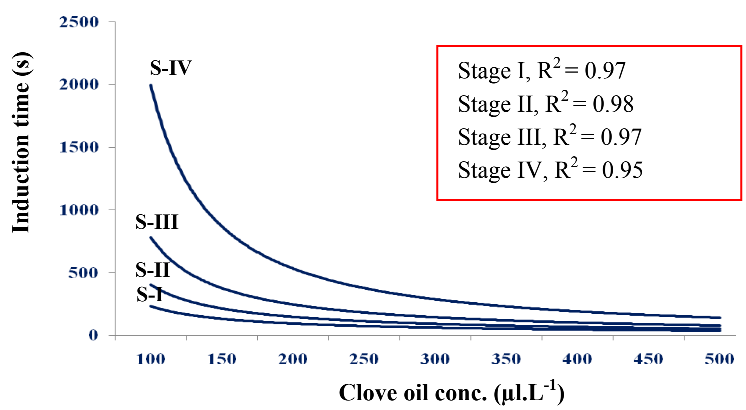


Figure 1: Induction time in relation to clove oil concentrations (µl/L) in common carp fingerling ($p<0.05$, n=12)

Figure 2 shows recovery time of the fish, indicating the lowest time for recovery at each stage in low concentration of clove oil and vice versa. Longest time (491 sec) to recovery was noticed at 500 µl/L dose of clove oil. The shorter recovery time (56 sec) was observed in 100 µl/L dose. The times of recovery in other doses were between 261 to 440 second in 150 µl/L to 450 µl/L at increasing order. The recovery time was statistically significant ($p < 0.05$) at stage III up to 250 µl/L dose of clove oil and thereafter the recovery time was not significantly different ($P > 0.05$). Concentration of 250-300 µl/L (induction 277±80 seconds and recovery time 315±66 seconds) at temperature between 18.2 to 19.3°C was determined as the lowest concentration that induces anaesthesia in common carp in less than five minutes (Figure 1 & 2). The recovered, common carp that were observed in the post-treatment period of seven days did not show any abnormal behaviour and/or mortality.

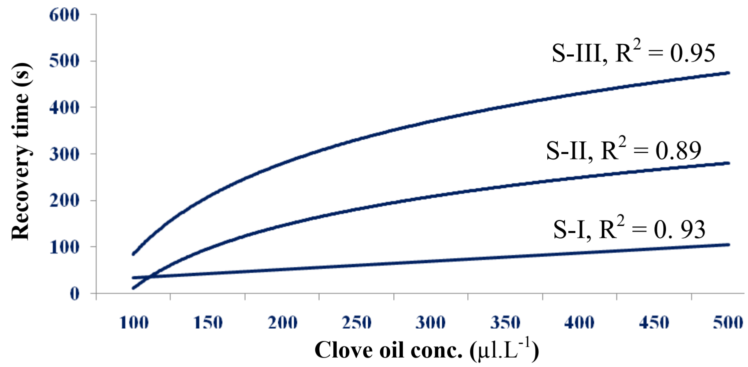


Figure 2: Recovery time in relation to clove oil concentrations (µl/L) in common carp ($p<0.05$, $n=12$)

The temperature of source water used in transportation was 23.1±0.4°C. The mortality rate (%) of common carp fingerling was found significantly higher (5.4±0.4) in the control (without sedative) than sedative doses of clove oil ($p<0.05$). Mortality rates were significantly lower ($p<0.05$) in the doses 20.0 and 25.0 µl/L of clove oil compared to other doses of clove oil employed in the experiment (Table 3). Post transportation mortality of fish after 3 day of treatment in control was also higher (3.8±0.91) compared to the fry treated with sedatives ($p>0.05$). Mean concentration of clove oil for confinement derived from regression analysis across 24 h in transportation bag and 3 days of post transportation was 21.2±2.4 µl/L (18.02 µl/L eugenol) at temperature between 23.1 to 23.7°C.

Table 3: Mortality rate (%) of common carp *Cyprinus carpio* fingerling in a confinement experiment at 500 fry in a plastic bag with 4 L water and 8 L pure oxygen

Attributes	Mortality rate (%) of common carp fry in different concentration of clove oil, µl/L				
	0.0	15.0	20.0	25.0	30.0
24 h confinement in treated plastic bag	$5.4±0.4^{a}$	$2.8±0.2^{b}$	$1.4±0.1^{c}$	$1.2±0.1^{c}$	$3.3±0.4^{ab}$
3 days after confinement in freshwater	$3.8±0.91^{a}$	$1.6±0.3^{b}$	$0.8±0.1^{c}$	$1.1±0.2^{bc}$	$2.5±0.2^{ab}$

Values with different superscripted letters are significant at $p<0.05$

Discussion

The qualities required of an anesthetic agent in sedating fish varies, depending on the nature, mode of application and species of fish. Most importantly, a quick induction and recovery time which allows for maximum manipulations of fish in culture medium is desirable. Despite this, anesthetics in aquaculture should be cheap, safe, easy to handle, readily available and accessible to the fish farmers in different parts of the country. In the present study, the maximum dose of clove oil was 500 µl/L and minimum of the same was 150 µl/L. It was found that induction time and clove oil had a direct relationship as the induction time significantly ($p<0.05$) reduced with increasing concentration of clove oil (Figure 1). On the other hand, the recovery time increased with increasing concentration of clove oil (Figure 2). While working on clove oil and other anesthetics, more or less similar trends were also reported (Durve 1975, Farid et al. 2008, Matin et al. 2009, Husen and Sharma 2015a).

In the present study, 250-300 µl/L concentration of clove oil was determined as the lowest concentration that induces anaesthesia in common carp in less than five minutes at temperature between 18.2-19.3°C. Husen and Sharma (2015a) reported the lower dose of clove oil required to produce anaesthetic in fry of rohu, *Labeo rohita* (50 µl/L) at temperature 24.7±0.11°C. The temperature preferred in this study is below optimal for common carp culture. Ross and Ross (2008) reported that increases in water temperature have a significant effect on the time induction and recovery time. When common carp fry are anaesthetized with clove oil, higher temperatures cause enhanced anaesthetic effects and shorter recovery times (Woolsey et al. 2004). In carp, a negative dependence of clove oil concentration on a water temperature and exposure time is reported by Hamáčková et al. (2006) and Hamáčková et al. (2004). The lowest calculated LC_{50} value (0.027 ml/L) was reached at combination of the highest applicated temperature (25°C) and longest tested exposure (40 min.) (Hamáčková et al. 2006). On the other hand, the highest LC_{50} value (0.752 ml/L) was gained at combination of lowest temperature (10°C) and shortest exposure (5 min.) (Chmel 2002). However, the results of the present study are consistent with those of other published studies involving juvenile and adult fish (Anderson et al. 1997).

Simulated concentration of clove oil (21.2±2.4 µl/L) for confinement (transportation) found in this study was higher than the reported for rohu (5.0 µl/L) (Husen and Sharma 2015a). Anaesthetic and sedation doses of clove oil vary with media temperature, fish species and size (Hamáčková et.al. 2000, Hamáčková et.al. 2004). The relatively high concentration of clove oil found in this study might have been related to the low water temperature, tolerance limit of species and size of fish.

Conclusion

The experiment confirmed the good possibilities of using clove oil as an anaesthetic when carrying out different manipulations on the common carp fingerling. Common carp lose their equilibrium but do not reach phase 3. The best results in regard to the time for reaching complete anaesthesia (277±80 seconds) and the time for complete recovery (315±66 seconds) are observed at a concentration between 250-300 µl/L of clove oil. The present finding demonstrated that the lowest effective dose of clove oil 21.2±2.4 µl/L (18.02 µl/L eugenol) at temperature between 23.1 to 23.7°C was found suitable for transportation purpose of common carp fingerling. These findings suggest that clove oil is promising, safe and effective to be used as anaesthetic and sedative for handling and transportation of common carp fingerling.

Acknowledgement
Authors are grateful to staff of the Fisheries Research Division, Lalitpur, Nepal for the management of experimental facilities. We also thank anonymous reviewers who gave helpful comments on the manuscript. This research was supported by Nepal Agricultural Research Council (NARC) to the first author.

References
Akar, M. A. A. 2011. Effects of clove oil on the response of blue tilapia (*Oreochromis aureus*) by transportation stress. Journal of the Arabian Aquaculture Society 6(1): 77-86.

Anderson, W.G., R.S. McKinlay and M. Colaveccia. 1997. The use of clove oil as an anaesthetic for rainbow trout and its effects on swimming performance. North American Journal of Fisheries Management 17(2): 301–307.

Barton, B.A. 2000. Stress. In: Encyclopedia of Aquaculture (ed. R.R. Stickney), pp. 892–898. John Wiley and Sons Inc., New York.

Brown, L.A. 1993. Anesthesia and restraint. In: Fish Medicine (ed. M.K. Stoskopf), pp. 79-90. WB Saunders Co., Philadelphia.

Chmel, J. 2002. Toxicity of two different anesthetics (2-phenoxyethanol and clove oil) for common carp. Diploma thesis, ZF JU České Budějovice 44 p. (Cz.).

Cho, G.K. and D.D. Heath. 2000. Comparison of tricane methane sulphonate (MS 222) and clove oil anaesthesia effects on the physiology of juvenile Chinook salmon *Oncorhynchus tshawytscha*. Aquaculture Research 31(6): 537-546.

Dobšíková, R., Z. Svobodová, J. Bláhov, H. Modrá and J. Velíšek. 2006. Stress response to long distance transportation of common carp (*Cyprinus carpio* L.), Acta Veterinaria Brno 75(3): 437-448.

Dobšíková, R., Z. Svobodová, J. Bláhov, H. Modrá and J. Velíšek. 2009. The effect of transport on biochemical and haematological indices of common carp (*Cyprinus carpio* L.). Czech Journal of Animal Science 54(11): 510–518.

Durve, V.S. 1975. Anesthetics in the transport of mullet seed. Aquaculture 5(1): 53-63.

Erdmann, M.V. 1999. Clove oil: an eco-friendly alternative to cyanide use in the live reef fish industry. SPC Live Reef fish Bulletin 5: 4–7.

Farid, S.M., M.M. Rahman, K.K. Shirin and N. N. Nur. 2008. Effect of clove oil as an anesthetic on *Labeo rohita* (Hamilton). Journal of Agro Forestry and Environment. 2:1-6.

Hamackova, J., J. Kouril, P. Kozak, Z. Stupka. 2006. Clove oil as an anaesthetic for different freshwater fish species. Bulgarian Journal of Agricultural Science 12(2): 185-194.

Hamáčková, J., J. Kouřil, Z. Stupka, E.V. Mikodina, M.A. Sedova, P. Lepič, P. Kozák, A. Lepičová and R. Vachta. 2004. Anesthesie of sterlet (*Acipenser ruthenus*) with clove oil in different water temperature. In: P. Spurný (Editor), *let rybářské specializace na MZLU v Brně,* Brno 30.11.-1.12.2004, pp. 105-113 (Czech).

Hamáčková, J., Z. Stupka, J. Kouřil and A. Lepičová. 2000. The effect of various anaesthetics on tench (*Tinca tinca*) related to its weight. Proceedings of abstract. III International workshop of Biology and Culture of the tench (*Tinca tinca* L.), Machern, Germany pp. 1.

Hernani, T.B. 1988. Analysis of patchouli oil and clove oil on chromatography. Media Research Sukamandi 6: 57-65.

Husen, M.A. and S. Sharma. 2014. Efficacy of anesthetics for reducing stress in fish during aquaculture practices- a review. Kathmandu University Journal of Science Engineering and Technology 10(I): 104-123.

Husen, M.A. and S. Sharma. 2015a. Immersion of rohu fingerlings in clove oil reduced handling and confinement stress and mortality. International Journal of Fisheries and Aquatic Studies 2(6): 299-305.

Husen, M.A. and S. Sharma. 2015b. Anaesthetics Efficacy of MS-222, Benzoak® vet, AQUI-S® and Clove oil on Common Carp (*Cyprinus carpio*) Fry. International Journal of Research in Fisheries and Aquaculture 5: 104-114.

Kamble, A.D., V.P. Saini and M.L. Ojha. 2014. The efficacy of clove oil as anesthetic in common carp (*Cyprinus carpio*) and its potential metabolism reducing capacity. International Journal of Fauna and Biological Studies 1(6): 1-6.

Matin, S.M.A., M.A. Hossain and M.A. Hashim. 2009. Clove oil anesthesia in singhi (*Heteropenustes fossilis*) and lata (*Channa punctatus*) fish. Bangladesh Veterinarian 26(2): 68-73.

Nagababu, E. and N. Lakshmaiah. 1992. Inhibitory effect of eugenol on no enzymatic lipid peroxidation in rat liver mitochondria. Journal of Biochemistry Pharmacology 43(11): 2393-2400.

Öğretmen, F., S. Golbasi and F. Kutluyer. 2016. Efficacy of clove oil, benzocaine, eugenol, 2-phenoxyethanol as anaesthetics on shabbout fish (*Barbus grypus* Heckel, 1843). Iranian Journal of Fisheries Sciences 15(1): 470-478.

Ross, L.G. and B. Ross. 2008. Anaesthetic and Sedative Techniques for Aquatic Animals. Blackwell Publishing, Oxford, UK. 222 pp.

Vartak, V. and R.K. Singh. 2006. Anesthetic effects of clove oil during handling and transportation of the freshwater prawn, *Macrobrachiun rosenbergii* (de Man). Bamidgeh 58(1): 46-54.

Woody, C.A., J. Nelson and K. Ramstad. 2001. Clove oil as an anaesthetic for adult sockeye salmon: field trails. Journal of Fish Biology 60(2): 340-347.

Woolsey, J., M. Holcomb, R.L. Ingermann. 2004. Effect of Temperature on Clove Oil Anesthesia in Steelhead Fry. North American Journal of Aquaculture 66(1): 35-41.

Nepalese Journal of Aquaculture and Fisheries
Vol. 3 & 4 (2016 & 2017) : 47-58

Biochemical assessment of dried fish products of Nepal

Neeta Pradhan[1]*, Madhav Kumar Shrestha[2], Sunila Rai[2], Dilip Kumar Jha[2] and Shyam Kishor Sah[2]
[1]Fisheries Research Division, Godawari, Kathmandu, Nepal
[2] Agriculture and Forestry University, Rampur, Chitwan, Nepal
*E-mail: pradhannita@yahoo.com

Abstract
Sun-dried and smoked fish are the commonly available dried fish products in Nepal. Quality of the dried-fish products available in the markets has been the major concern. A study was conducted to determine the proximate composition and microbial loads in ten species of sun-dried and six species of smoked fish samples collected from different markets of Nepal. The moisture, protein and fat content of the sun-dried fishes was in the range of 11.8±0.3 to 19.9±0.7%, 55.1±0.9 to 69.5±0.3% 3.7±0.0 to 15.4±0.3% respectively. The pH value ranged from 4.5 to 6.4. The total plate count (TPC) ranged from $5.3x10^2±2.3x10^2$ to $41.9x10^2±39.1x10^2$ cfu/g. The mold content ranged from $0.1x10^2±0.1x10^2$ to $8.6x10^2±6.9$ $x10^2$ cfu/g. In smoked fishes, moisture, protein and fat content ranged from 5.8±0.2 to 16.4±3.8%, 53.9±5.1to 79.7±0.5% and 0.9±0.0 to 16.0±3.0% while the pH value ranged from 5.4 to 6.2. The TPC ranged from $0.1x10^4±0.00$ to $3666.7x10^4±3179.7x10^4$ cfu/g and mold content ranged from 0.9 $x10^2±0.1$ $x10^2$ to $22.7x10^2±0.1x10^2$ cfu/g. The TPC and mold of the sun-dried fish sample did not exceed the permissible limit, while smoked fish samples were heavily contaminated with bacteria and mold, and that exceeded permissible limit. Openly displayed dried/smoked fish might have increased moisture content along with unhygienic processing, unhygienic drying, collection season and use of spoiled fish for processing might be the reasons for heavy microbial loads.

Key words: Sun-dried, smoked, proximate composition, microbial load

Introduction

Dried fish is a delicacy to many people. In Nepal dried fish products are mostly used by all communities and comprises as one of the essential food in festivals and wedding ceremony (Pradhan et al. 2006). Dried fish products are considered sacred and is offered to various deities during festivals (Pathak 2007). Dried fish is used as a substitute of fish at the scarcity of fresh fish in many countries (Bille and Shemkai 2006, Oyero et al. 2007, Chukwu and Shaba 2009, Oduor-Odote et al. 2010), including Nepal. Dried fish has a storage life of several years and is a great source of protein, essential fatty acids, vitamins and many minerals (Banu et al. 1985). Dried fish has become one of the major sources of protein supply in remote areas of Nepal, where transportation is poor (eg. high mountainous regions) and malnutrition is most severe.

Small fish such as *Puntius, Oxygaster, Anabas, Nandus* and *Channa* are dried in the sun more prominently in Terai region of Nepal (Shrestha 1999). These dried fish, locally known as Sidra, have a good taste and are used for pickles. Fish drying by smoking is common traditional activity widely used in different parts of the country. Locally processed indigenous fish are delivered to the consumers by fisher and other communities settled nearby wetland (rivers, lakes, etc). There is high demand of dried fish in the market for different uses such as in household, religious festivals, livestock, fisheries and poultry feed, instant noodle and flower nurseries (Pradhan et al. 2006). Very few have been supplied from some small market areas near riversides accessing highway. Different species of dried fish are also imported from India to fulfill the ever-increasing demand.

Fish is a nutritional rich food and a very good source of vitamins and mineral required by human (Ojutiku et al. 2009, Marimuthu et al. 2012) and the nutritional quality of dried fish remains intact, sometimes retains higher quality standard compared to fresh fish (as per unit weight). However, Fish products resulting from sun-drying and smoking are blamed for having variable nutrient composition, high load of microbes, incomplete drying, high moisture and thickness of fish muscle. Fish is a reservoir of large number of microorganisms; one of the major factors contributing to poor quality of the fish in retail trade is unhygienic handling and storage leading to off smell, physical damage and contamination with dirt and objectionable microorganisms (Sugumar et al. 2004). Presence of non-pathogenic microorganisms cause spoilage to fish while the presence of pathogenic microorganism causes food poisoning. Determination of microbiological quality of such processed fishes from the market is very important for guarding consumers' health and hygiene (Lilabati et al. 1999). The proximate composition is an important aspect of fish quality and it influences both the keeping quality and technological characteristics of the fish. The main chemical components such as moisture, crude protein, lipid and ash have the largest impact on the nutritive value, the functional properties, and the sensory quality and storage ability of flesh. Therefore, information on the nutritive value and its food quality are equally important regarding the proper utilization of products. The information on the nutritional and microbial status of sun-dried and smoked fish and their food safety values are meager in the country (Pradhan and Mishra 2012). The present study aimed to assess the nutritive value and examination of microbial load, and processing practices for sun-drying and smoking of those fish species.

Materials and methods

A total of ten sun-dried fish samples, namely: Kechaki (*Stolephorus* spp.), Bamala (*Harpodon nehereus*), Shrimp (*Penaeus* spp.), Nathali, Muwa (*Aspidoparia morar*), Pothia (*Puntius* spp.), Seto machha (Jalkapoor) (*Ompok bimaculatus*), Madali, Sidra (*Puntius sophero*) and Mali were observed in the markets. Fish processing, smoking and display in the market was also observed in Karnali, Chisapani, Kailali and Malekhu, Dhading and Lagankhel, Lalitpur and six smoked fish samples namely: Asala (*Schizothorax* spp.), Sahar (*Tor putitora*), Mahaseer (*Tor tor*), Rohu (*Labeo rohita*), Kalanch (*Labeo dyocheilus*) and Naini (*Cirrhinus mrigala*) were collected in three replicates from different markets (Table 1).

Table 1: Sample collection sites of sun-dried and smoked fish

S. No.	Sun-dried fish (species)	Collection site
1	Kechaki (*Stolephorus* spp.)	Balkhu, Kathmandu; Lagankhel, Lalitpur; Bharatpur, Chitwan;
2	Bamala (*Harpodon nehereus*)	Balkhu, Kathmandu; Lagankhel, Lalitpur; Bharatput, Chitwan
3	Shrimp (*Penaeus* spp.)	Balkhu, Kathmandu; Lagankhel, Lalitpur
4	Nathali	Balkhu, Kathmandu; Lagankhel, Lalitpur
5	Muwa (*Aspidoparia morar*)	Balkhu, Kathmandu; Lagankhel, Lalitpur
6	Pothia (*Puntius* spp.)	Balkhu, Kathmandu; Lagankhel, Lalitpur
7	Seto machha (*Ompok bimaculatus*)	Bharatpur, Chitwan; Balkhu, Kathmandu; Lagankhel, Lalitpur
8	Madali	Bharatpur, Chitwan; Balkhu, Kathmandu; Lagankhel, Lalitpur
9	Sidra (*Puntius sophore*)	Bharatpur, Chitwan; Balkhu, Kathmandu; Lagankhel, Lalitpur
10	Mali	Bharatpur, Chitwan; Balkhu, Kathmandu; Lagankhel, Lalitpur

	Smoked fish (species)	
11	Asala (*Schizothorax* spp.)	Karnali, Chisapani, Kailali
12	Sahar (*Tor putitora*)	Karnali, Chisapani, Kailali
13	Mahseer (*Tor tor*)	Karnali, Chisapani, Kailali
14	Rohu (*Labeo rohita*)	Karnali, Chisapani, Kailali
15	Kalanch (*Labeodyocheilus*)	Karnali, Chisapani, Kailali
16	Naini (*Cirrhinus mrigala*)	Malekhu, Dhading; Lagankhel, Lalitpur

The samples were packed in polyethylene bags during sample collection and brought to the laboratory for proximate, microbiological and chemical analysis. The proximate analysis was carried out in triplicates in all samples according to AOAC (2005). Analyses included for moisture, crude protein and crude fat content. The pH values were determined using a digital pH meter. The microbial load included enumeration of mold and total plate count (TPC).

Data were analyzed using Microsoft Excel and analyzed using SPSS for Windows version 16.0 software. Treatment means were compared using one-way Analysis of Variance (ANOVA) at 5% level of significance reported as mean standard error (±SE). Overall mean was compared using T-Test.

Results

Sun-dried fish

The results on moisture, protein and fat content of different sun-dried fish species are presented in Table 2. Overall mean moisture content was 15.2±0.7% with minimum of 11.8±0.3% in Sidra and maximum of 19.9±0.7% in Shrimp. Mean protein content was 60.6±0.9% with a minimum of 55.1±0.9% in Nathali and maximum of 69.5±0.3% in Madali. Similarly, mean fat content was 6.1±0.6% with a minimum of 3.7±0.0% in Muwa and maximum of 15.4±0.3% in Mali.

Table 2. Proximate value (moisture, protein and fat) in sun-dried fish collected from different markets (mean ± SE) (n = 3).

S. No.	Fish species	Moisture (%)	Protein (%)	Fat (%)
1	Shrimp (*Penaeus* spp.)	19.9±0.7^{c}	59.0±1.1^{abc}	4.6±1.2^{abc}
2	Kechaki (*Stolephorus* spp.)	13.1±0.5^{ab}	59.0±4.4^{abc}	5.5±0.4^{abc}
3	Nathali	16.0±0.3^{abc}	55.1±0.9^{a}	3.8±0.3^{ab}
4	Pothia (*Puntius* spp.)	18.6±2.0^{bc}	56.1±0.1^{a}	4.0±0.2^{abc}
5	Muwa (*A. morar*)	14.3±4.0^{abc}	62.9±1.8^{bc}	3.7±0.0^{a}
6	Bamala (*H. nehereus*)	16.8±3.2^{abc}	61.0±2.7^{abc}	5.8±0.3^{c}
7	Madali	15.8±0.3^{abc}	69.5±0.3^{d}	5.6±0.1^{bc}
8	Seto machha (*O. bimaculatus*)	12.8±0.0^{ab}	58.0±0.0^{ab}	5.2±0.1^{abc}
9	Mali	12.8±0.4^{ab}	60.6±0.6^{abc}	15.4±0.3^{e}
10	Sidra (*P. sophore*)	11.8±0.3^{a}	64.5±0.6^{cd}	7.7±1.2^{d}
	Over all mean	15.2±0.7	60.6±0.9	6.1±0.6

Values with same superscripts within column are not significantly different at α 0.05.

The results on microbial load and pH of different sun-dried fish species are presented in Table 3. Overall mean of TPC content was $13.6x10^2 \pm 7.2$ $x10^2$ with minimum of $5.3x10^2 \pm 2.3x10^2$ in Pothia and maximum of $41.9x10^2 \pm 39.1x10^2$ in Kechaki. Mean mold content was $3.0x10^2 \pm 2.0x10^2$ with a minimum of $0.1x10^2 \pm 0.1$ $x10^2$ in Shrimp and maximum of $8.6x10^2 \pm 6.9$ $x10^2$ in Nathali. Similarly, overall mean of pH was 5.1 with a range of 4.5 to 6.4.

Table 3: Microbial load (mean ± SE) and pH (mean and range) in sun-dried fish collected from different markets (n=3).

S. No.	Fish species	TPC (cfu/g)	Mold (cfu/g)	pH
1	Shrimp (*Penaeus* spp.)	$8.2x10^2 \pm 3.5x10^2$	$0.1x10^2 \pm 0.1x10^2$	6.2 (6.2-6.3)
2	Kechaki (*Stolephorus* spp.)	$41.9x10^2 \pm 39.1x1^2$	$7.6x10^2 \pm 7.2x10^2$	5.9 (5.5-6.4)
3	Nathali	$9.4x10^2 \pm 1.7x10^2$	$8.6x10^2 \pm 6.9x10^2$	6.2 (6.1-6.2)
4	Pothia (*Puntius* spp.)	$5.3x10^2 \pm 2.3x10^2$	$5.9x10^2 \pm 6.9x10^2$	5.4 (5.2-6.0)
5	Muwa (*A. morar*)	$17.8x10^2 \pm 13.7x1^2$	$1.5x10^2 \pm 0.9x10^2$	5.4 (5.0-6.2)
6	Bamala (*H. nehereus*)	5.8 $x10^2 \pm 2.7x10^2$	$0.3x10^2 \pm 0.2x10^2$	5.8 (5.6-6.1)
7	Madali	$11.5x10^2 \pm 2.3x10^2$	$0.9x10^2 \pm 0.2x10^2$	4.7 (4.5-5)
8	Seto machha (*O. bimaculatus*)	$10.9x10^2 \pm 0.5x10^2$	$0.2x10^2 \pm 0.0x10^2$	5.0 (4.9-5.0)
9	Mali	$12.0x10^2 \pm 0.6x10^2$	$0.8x10^2 \pm 0.1x10^2$	5.0 (5-5.2)
10	Sidra (*P. sophore*)	$16.3x10^2 \pm 6.3x10^2$	$1.5x10^2 \pm 0.7x10^2$	4.7 (4.5-5.0)
	Over all mean	13.6 $x10^2 \pm 7.2x10^2$	$3.0x10^2 \pm 2.0x10^2$	5.1

The bacterial load (TPC) in sun-dried fish showed negative correlation with moisture content (Figure 1). However, mold value did not show any correlation with moisture content (Figure 2).

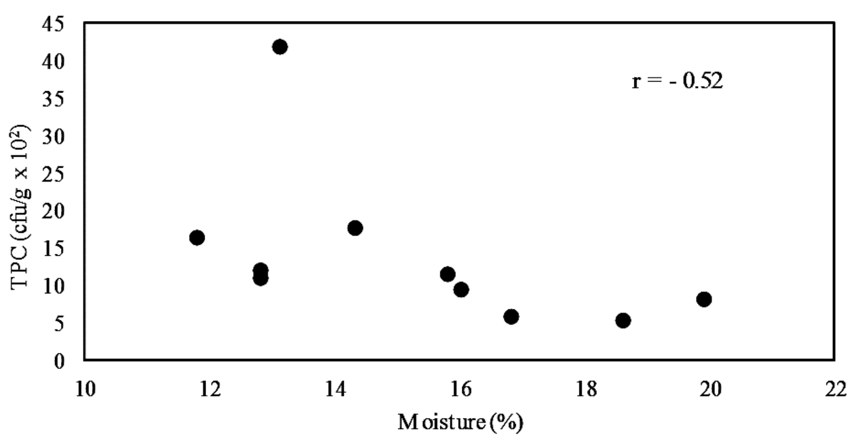


Figure 1: Correlation between TPC and moisture content in sun-dried fish samples.

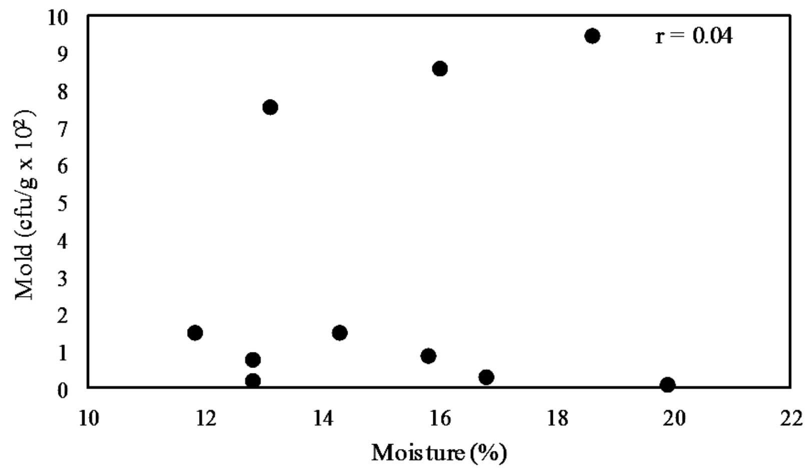


Figure 2: Correlation between mold and moisture content in sun-dried fish samples.

Smoked fish

The results on moisture, protein and fat content of different smoked fish species are presented in Table 4. Overall mean moisture content was 11.3±1.1% with minimum of 5.8±0.2% in Rohu and maximum of 16.4±3.8% in Kalanch. Mean protein content was 64.2±2.4% with a minimum of 53.9±5.1% in Sahar and maximum of 79.7±0.5% in Mahaseer. Similarly, mean fat content was 9.6±1.5% with a minimum of 0.9±0.0% in Mahaseer and maximum of 16.0±3.0% in Kalanch.

Table 4. Proximate value (moisture, protein and fat) in smoked fish collected from diferent markets (mean ± SE) (n=3).

S. No.	Fish species	Moisture (%)	Protein (%)	Fat (%)
1	Asala (*Schizothorax* spp.)	$8.5±0.0^{ab}$	$70.0±4.7^{c}$	$6.3±0.3^{b}$
2	Sahar (*T. putitora*)	$15.9±1.4^{c}$	$53.9±5.1^{a}$	$13.3±2.1^{c}$
3	Kalanch (*L. dyocheilus*)	$16.4±3.8^{c}$	$56.7±0.9^{a}$	$16.0±3.0^{c}$
4	Naini (*C. mrigala*)	$12.6±1.7^{bc}$	$58.3±1.5^{ab}$	$15.6±0.2^{c}$
5	Rohu (*L. rohita*)	$5.8±0.2^{a}$	$66.4±0.1^{bc}$	$5.8±0.1^{b}$
6	Mahseer (*T. tor*)	$8.8±0.1^{ab}$	$79.7±0.5^{d}$	$0.9±0.0^{a}$
	Over all mean	11.3±1.1	64.2±2.4	9.6±1.5

Values with same superscripts within column are not significantly different at α 0.05.

The results on microbial load and pH of different smoked fish species are presented in Table 5. Overall mean of TPC content was $1204.5x10^4±465.4x10^4$ with a minimum of $0.1x10^4±0.0x10^4$ in Rohu and maximum of $3666.7x10^4±3179.7x10^4$ in Sahar. Mean mold content was $6.2x10^2±3.6x10^2$ with a minimum of $0.9x10^2±0.1x10^2$ in Asala and maximum of $22.7x10^2±0.1x10^2$ in Naini. Similarly, overall mean pH was 5.7 with a minimum of 5.4 and maximum of 6.2.

Table 5: Microbial load (mean ± SE) and pH (mean and range) in smoked fish collected from different markets (n=3)

S. No.	Fish species	TPC (cfu/g)	Mold (cfu/g)	pH
1	Asala (*S.* spp.)	$1.3 \times 10^4 \pm 0.6 \times 10^4$	$0.9 \times 10^2 \pm 0.1 \times 10^2$	5.9 (5.8-6.2)
2	Sahar (*T. putitora*)	$3666.7 \times 10^4 \pm 3179.7 \times 10^4$	$3.9 \times 10^2 \pm 0.1 \times 10^2$	5.7 (5.6-5.8)
3	Kalanch (*L. dyocheilus*)	$2340.0 \times 10^4 \pm 37.9 \times 10^4$	$6.1 \times 10^2 \pm 0.1 \times 10^2$	5.5 (5.4-5.6)
4	Naini (*C. mrigala*)	$2423.3 \times 10^4 \pm 39.4 \times 10^4$	$22.7 \times 10^2 \pm 0.1 \times 10^2$	6.2 (5.9-6.7)
5	Rohu (*L. rohita*)	$0.1 \times 10^4 \pm 0.0 \times 10^4$	$2.3 \times 10^2 \pm 0.1 \times 10^2$	5.5 (5.5-5.6)
6	Mahseer (*T. tor*)	$0.1 \times 10^4 \pm 0.0 \times 10^4$	$1.4 \times 10^2 \pm 0.1 \times 10^2$	5.7 (5.6-5.8)
	Over all mean	$1204.5 \times 10^4 \pm 465.4 \times 10^4$	$6.2 \times 10^2 \pm 3.6 \times 10^2$	5.7

The microbial loads (TCP and molds) showd positive correlation with moiture content. (Figure 3 and 4).

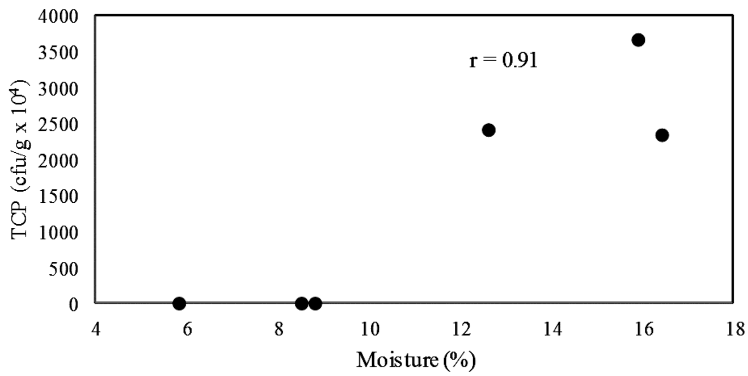


Figure 3: Correlation between TPC (cfu/g) and moisture content (%) in smoked fish.

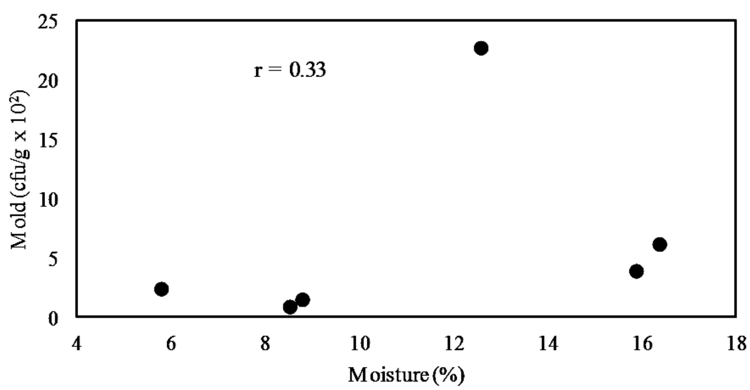


Figure 4: Correlation between mold (cfu/g) and moisture content (%) in smoked fish.

Sun-dried vs smoked fish

Mean comparison between sun-dried and smoked dried fish showed lower moisture content, higher crude protein and higher fat content in smoked fish compared to sun-dried fish (Figure 5). However, in terms of TPC and mold content it was higher in smoked fish than in sun-dried fish (Figure 6, and 7).

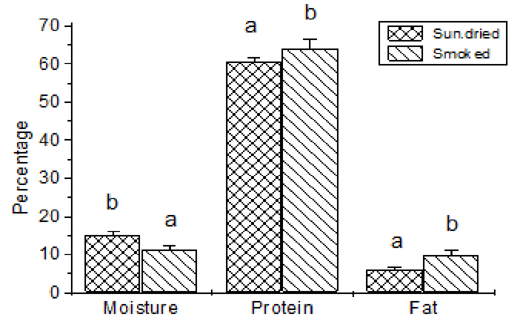


Figure 5: Comparison of moisture, protein and fat content between sun-dried and smoked fish. Different alphabet between sun-dried and smoked fish denotes significantly different ($p<0.05$).

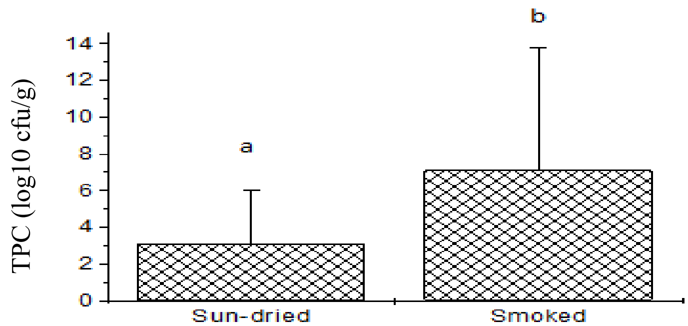


Figure 6: Comparison of mean TPC contents between sun-dried and smoked fish. Different alphabet denotes significantly different ($p<0.05$). Due to large difference in values, graph is presented in log value.

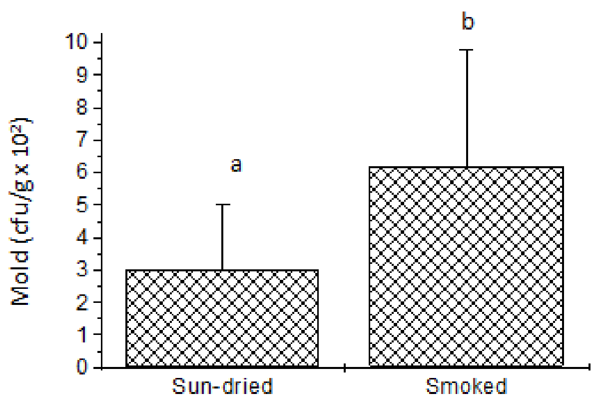


Figure 7. Comparison of mean mold contents between sun-dried and smoked fish. Different alphabet denotes significantly different ($p<0.05$).

Discussion

Imported sun-dried fish and smoked indigenous fish are the common dried fish products available in Nepal. Sun-dried fish normally contain 10-20% moisture, 60-80% crude protein, (Haque 2004), 0.20-25% fat (Love 1970). Our results of moisture percentage of the sun-dried and smoked fish were 12-20% and 5.8-16.4%, respectively, which is comparable with results of 19.2 to 23.1% of dried rohu (*Labeo rohita*), snakehead (*Channa striatus*), and catfish (*Wallago attu*) (Mansur et al. 2013), 9.6 to 18.6% in market samples of sun-dried *Gudusia chapra* (Bhattacharya et al. 1985), 18.2 to 23.6% in fourteen selected dried fishes (Azam et al. 2003),12.1% in *Puntius ticto* to 18.2% in *Palaemon* sp. (Flowra and Tumpa 2012), 7.8% in Phabaonga (*Puntius shalynius*) and the lowest was 2.8% in Goroi (*Channa punctatus)* (Ullah et al. 2016). However, lower moisture content of the dried fish, 2.8% in Seleng (*Barilius tileo*) to 8.9% in Mukanga (*Amblypharyngodon mola*) has been reported (Hazarika et al. 2016).

Higher percentage of protein is desirable as being an important factor for quality assessment. Our results in protein content ranged from 55.1 to 69.5% and 53.9 to 79.7% in sun-dried and smoked fishes, respectively. Protein contents of Shrimp, Kechaki, Nathali, Pothia and Seto machha were below the referred range and rest were within the referred range. However, a wide range of protein content values are reported, and it ranges from 27.5% in *Badis tuivaiei* to 56.8% in *Pethia shalynius* (Ullah et al. 2016); 28.6% in Ngamu (*Channa punctatus*) to 53.8% in Chanda (*Chanda ranga*) (Hazarika et al. 2016), 53.45 % in *Mystus vittatus* to 76.39% in *Trichiurus haumela* (Flowra et al. 2012), 28.20 % in *Wallago attu* to 51.19 % in *Palaemon* sp. (Flowra and Tumpa 2012), 49.23 to 62.85% in dried Rohu (*Labeo rohita*), snake headed fish (*Channa striatus*), catfish (*Wallago attu*) (Mansur et al. 2013), 42.4% in Puti (*Puntius sophore*) to 54.6% in Taki (*Channa punctatus*) (Majumdar 2017), 40.7% to 66.5% in fourteen selected dried fish species (Azam et al. 2003). Wide variations in protein content between species has been reported as we found variations between species in our results.

Our results of fat content ranged from 3.7 to 15.4% in sun-dried fish and 0.9 to 16.0% in smoked fish. Wide range of fat content among the dried fish species has been reported with a range of 3.7 to 17.8% (Kalaimani et al. 1988), 9.7 to 26.1% (Azam et al. 2003), 3.0% in Batashi fish (*Clupisoma atherinoides*) (Shahiduzzaman et al. 2004), 13.9% in dried *Rita* sp. (Mollah et al. 1998). Similarly, in marine fish fat content ranged from 1.9 to 8.6% in dried fish (*Harpodon nehereus, Johnius dussumieri* and *Lepturacanthus savala)* (Siddique et al. 2012), 6.8 to 9.2% in dried Bombay duck and Sin croaker samples (Bhuiyan 1992). Variation in fat content could be influenced by the variation of species, diet, temperature, salinity, selective mobilization and distribution (Lovern 1950). In our study the fat content of Mahseer (*Tor tor*) was very low (0.9±0) in comparison to other smoked fish species. The fat content varies greatly from species to species and also from individual to individual depending on age, sex, environment and season (Huss 1988 and 1995). Seasonal variation of crude fat in Golden Mahseer (*Tor putitora*) from hatchery fish pond, Bhimtal, Uttrakhund, India in different seasons were 0.62, 1.52 and 1.50% in October to January, February to May and June to September, respectively (Sarma et al. 2015). The fat content of shad (*Alosa fallax*) and horse mackerel (*Trachurus trachurus*) were 9.34% and 8.42%, respectively, in October and 25.24% and 12.15% respectively in March (Boran and Karaçam 2011). Variation in fat content in this study might be due to the variations in species, environment, season and age of sample collection.

pH is an indicator of the extent of microbial spoilage in fish and some proteolytic microbes produce acid after decomposition of carbohydrate, thereby increasing the acid level of the medium (Eyo 1993). pH value is a reliable indicator of the degree of freshness or spoilage. The pH in fresh freshwater fish flesh is almost neutral (Virta 2009). In the post-mortem period, decomposition of nitrogenous compounds leads to an increase in pH in the fish flesh (Shenderyuk and Bykowski 1989). The increase in pH indicates the loss of quality. The fish products are acceptable up to a pH of 6.8 but are considered to be spoiled above a pH of 7.0 (Huss 1988). In the present study pH value of sun-dried dried fish products were ranged from 4.5 (Sidra and Madali) to 6.4 (Kechaki).

Bacterial and molds infestation have been the prime concern for the quality of dried-fish along with its nutritional content. The International Commission on Microbiological Specifications for Foods (ICMSF 1982) indicated a limit of acceptability as less than 1.0×10^6 cfu/g for the total viable count and 1.0×10^4 cfu/g for mold in any food to be safe for consumption. In this study all sun-dried fish species were under permissible limit. Although Kechaki had highest TPC ($4.19 \times 10^3 \pm 3.91 \times 10^3$ cfu/g), however, did not exceed the permissible limit. A work carried out by Kumar (2008) and Sinduja et al. (2011) in dried fishes of Tuticorin fish market reported high bacteria count in *Sardinella fimbriata*. In Cochin market the bacterial count in dried fishes was less than 10^7/g (Sanjeev 1997). Saritha et al. (2012) reported high bacterial count in dried fishes of Cuddalore dry fish market. In Nigerian market the total bacterial count of dried beef and dried fish sample was $1.0 \times 10^5$and 1.0×10^9 respectively (Adesiyun and Kaminjolo 1992). In this study all dried fish species were under permissible limit of mold referred by ICMSF (1982). Although Nathali had highest mold content ($8.6 \times 10^2 \pm 6.9 \times 10^3$ cfu/g), but did not exceed the permissible limit. The relation between TPC and moisture content in sun-dried fish were negative (Figure 5), but with mold there was almost no relation (Figure 6). This result did not agree with Lilabathi et al. (1999) who reported the direct relationship between the microbial count (TPC and mold) and moisture content of the dried fish sample. This might be due to the reason that the samples of sun-dried fish available in the markets were transported at different season, some of them were might be quite old stock and some with new stock.

In smoked fish, highest TPC ($3666.7 \times 10^4 \pm 3179.7 \times 10^4$ cfu/g) was observed in Sahar followed by Naini ($2423.3 \times 10^4 \pm 39.4 \times 10^4$cfu/g) and Kalanch ($2340.0 \times 10^4 \pm 37.9 \times 10^4$ cfu/g), all of which are above the permissible limit according to ICMSF (1982). Lowest TPC was observed in Rohu ($0.1 \times 10^4 \pm 0.0 \times 10^4$cfu/g), which is within permissible limit. There is great variations in TCP values in different species of smoked fish. In Karnali Chisapani the fish used to smoke are only leftover fresh fish after preparing fish curry and deep-fried fish for consumers by the hoteliers. Usually the fish are smoked whole and displayed openly in the markets. The smoked fish are also stored without packing. Highest mold was observed in Naini ($22.7 \times 10^2 \pm 0.1 \times 10^2$ cfu/g) and lowest in Asala ($0.9 \times 10^2 \pm 0.1 \times 10^2$cfu/g) and all of which are within the permissible limit according to ICMSF (1982). The correlation between moisture content and TPC (Figure 3) and moisture content and mold (Figure 4) in smoked fish were positive. The study carried by Immaculate et al. (2013) in quality of dried fish in summer and monsoon sesean showed that dried fishes procured from fisher folk had bacterial count above the permissible limit and it was high in monsoon season due to high moisture content of the environment, and least bacterial load was observed in summer due to high temperature, low moisture and adequate drying. Lilabathi et al. (1999) reported the direct relationship between the microbial count and moisture content of the dried fish sample.

Conclusion

Dried fish products are popular where fresh fish are not reachable due to its perishable nature and lack of fresh fish storage facilities in markets of hills and mountains. The percentage of protein and fat content in the available sun-dried and smoked fishes is quite satisfactory though there were variations between species. Mean comparison between sun-dried and smoked fish showed significantly lower moisture, higher protein and higher fat in smoked fish than sun-dried fish sample ($p<0.05$). Similarly, TPC and mold content was significantly higher in smoked fish than sun-dried fish ($p<0.05$). The TPC and mold of the sun-dried fish sample did not exceed the permissible limit, while smoked fish samples were heavily contaminated with bacteria and mold, and that exceeded permissible limit. Openly displayed dried/smoked fish might have increased moisture content along with unhygienic processing, unhygienic drying, and use of spoiled fish for processing might be the reasons for heavy microbial loads. This study suggests for the further study on how quality of dried fish can be preserved along with increased shelf life in ambient temperature in Nepal.

Acknowledgements
This work is a part of PhD Dissertation funded by Nepal Agricultural Research Council. Authors express their sincere thanks to all staff members of Fisheries Research Division, Godawari for their assistance.

References

Adesiyun, A.A. and J.S. Kaminjolo. 1992. Susceptibility to antibiotics of *E. coli* strains isolated from diarrhea and non–diarrheic live stock in Trinidad. Revue d'elevage et de medecine veterinaire des pays tropicaux 45 (3-4): 260-262.

AOAC. 2005. Official Methods of Analysis of AOAC International. In 18th ed. Gathersburg, MD USA, 105 pp.

Kumar, P.A. 2008. Bacterial resistance to antimicrobial agents and microbiological quality among *Escherichia coli* isolated from the dry fishes in Southeast Coast of India. Roumanian Biotechnology Letter 13(6): 3984 - 3989.

Azam K., M.Z. Basher, M. Asaduzzaman, M.H. Hossain and M.Y. Ali. 2003. Biochemical quality assessment of fourteen selected dried fishes. University Journal of Zoology, Rajshahi University 22: 23-26.

Banu C. P., Nahar, B., and K. Ahmed. 1985. Studies on the protein, riboflavin and iron content of some fresh water fish and a prawn of Bangladesh. Bangladesh Journal of Zoology, 13, (1), 25-28. pp. 25-28.

Bhattacharyya S.K., J.K. Vandyopadhway and A.K. Chattopadhyay. 1985. Improved dried product on blanching *Gudusia chapra* prior to sun drying. In: Harvest and post-harvest technology of fish. Society of Fish Technologists (India). pp. 531.

Bille, G.P, and H.R. Shemkai. 2006. Process development, nutrition and sensory characteristics of spiced smoked and sun dried Dagaa (*Rastrineobola argentea*) from Lake Victoria, Tanzania. African Journal Food Agriculture. Nutrition Development 6 (2): 222-234.

Bhuiyan, M.R. 1992. Proximate biochemical analysis and spoilage patter of six marine fishes of Bay of bangal. Master's Thesis, Institute of Marine Science and Fisheries, University of Chittangong.

Boran, G. and H. Karaçam. 2011. Seasonal Changes in Proximate Composition of Some Fish Species from the Black Sea. Turkish Journal of Fisheries and Aquatic Sciences 11: 1-5.

Chukwu, O. and I.M. Shaba. 2009. Effects of drying methods on proximate compositions of catfish (*Clarias gariepinus*). World Journal of Agricultural Science 5(1): 114-116.

Eyo, A.A. 1993. Traditional and improved fish handling, preservation and processing techniques. Paper presented at national workshop on fish processing storage, marketing and utilization. Company, Philadelphia London.138:148 - 150, 164

Flowra, A.F. and S.A. Tumpa. 2012. Chemical composition of five selected dry fish species in Chalan Beel, Bangladesh. DAV International Journal of Science 1(2): 157-160.

Flowra, A.F., D.G. Nahar, S.A. Tumpa and M.T. Islam. 2012. Analysis of five dried fish species of Bangladesh. University Journal of Zoology, Rajshahi University 21:9-11.

Haque, E., 2004. Bangladesher Chhoto Machha (A book on small fishes of Bangladesh). Graphic Sign, Mymensingh, Bangladesh 2200: 81-84.

Hazarika, P., N. Ullah, and P. J. Handique. 2016. Assessment of Biochemical Quality of Ten Selected Dried Fish Products of North East India. International Advanced Research Journal in Science, Engineering and Technology 3 (3): 183-186.

Huss, HH. 1988 Fresh fish quality and quality changes: a training manual prepared for the FAO/DANIDA Training Programme on Fish Technology and Quality Control. FAO Fisheries Series no. 29, FAO, Rome, Italy, 132 pp.

Huss, H.H. 1995. Quality and Quality Changes in Fresh Fish. FAO Fiaheries Technical Paper Series no. 348. FAO. Rome. 195 pp.

ICMSF. 1982. Sampling for microbial analysis: principles and specific applications. Microorganisms in Foods 2. International Commission on Microbiological Specifications for Foods. University of Toronto Press, Toronto, Canada. 278 pp.

Immaculate, K., P. Sinduja, A. Velammal and J. Patterson. 2013. Quality and shelf life status of salted and sun-dried fishes of Tuticorin fishing villages in different seasons. International Food Research Journal 20(4): 1855-1859.

Kalaimani, N., K. Gopakumar and T.S.U. Nair. 1988. Quality characteristics of cured fish of commerce. Fishery Technology 25(1): 54-57.

Lilabati, H., W. Vishwanath and M. Singh. 1999. Changes in bacterial and fungal quality during storage, *Esomus danricus* of Manipur. Fishery Technology 36: 36-39.

Lovern, J.A., 1950. Some causes of variation in the composition of fish oils. Journal of the Society of Leather Trades and Chemists 34:7-23.

Love, R.M. 1970. The Chemical Biology of Fishes. Academic Press, Inc. London, UK. 547 pp.

Mansur, M.A., S. Rahman, M.N.A. Khan, M.S.R. Kamrunnahar, and S. Uga. 2013. Study on the quality and safety aspect of three sun-dried fish. African Journal of Agricultural Research 8(41): 5149-5155.

Marimuthu, K., M. Thilaga, S. Kathiresan, R. Xavier and R.H. Mas. 2012. Effect of different cooking methods on proximate and mineral composition of striped snakehead fish (*Channa striatus*, Bloch). Journal of Food Science Technology 49(3): 373-377.

Mollah, A.H., M.S. Rahman and M.T. Alam. 1998. Study of proximate chemical analysis of Bangladeshi freshwater fish *Rita rita* (Ham.) and seasonal variation of lipid, protein and related substances. University.Jouranl of. Zoology, Rajshahi University 17: 1- 6.

Majumdar BC .2017. Comparison of the changes in nutritional quality of three important small indigenous fish species in Bangladesh at Room temperature (27°-31°C): A review. Journal of Animal Research and Nutrition 2(2): 15.

Oduor-Odote, P.M., D. Shitanda, M. Obiero and G. Ituu. 2010. Drying characteristics and some quality attributes of *Rastrineobola argentea* (Omena) and *Stolephorus delicatulus* (Kimarawali). African Journal of Food, Agriculture, Nutrition and Development 10(8): 2998–3014

Ojutiku, R.O., R.J. Kolo, and M.L. Mohammed. 2009. Comparative study of sun-drying and solar tent drying of *Hyperopisus bebeoccidentalis*. Pakistan Journal of Nutrition, 8(7). 955-957.

Oyero, J.O., S.O.E. Sadiku, E.S.A. Ajisegiri and A.A. Eyo. 2007. Biochemical evaluation of enclosed solar dried and salted *Oreochromis niloticus*. Research Journal of Animal Sciences 1(3): 97- 101.

Pathak, J. 2007. Taste of Nepal, Hippocrene books. Inc., Newyork, USA. 470 pp.

Pradhan N., T.B. Gurung, A. Raymajhi and N.K. Roy. 2006. Dry fish market channel in Nepal. Paper presented in workshop on "7th National Workshop on Livestock and Fisheries Research Productivity Enhancement through Livestock and Fisheries Research" held on 25-27 June 2006.25-27 June 2017. NARC, Khumaltar.

Pradhan, N., and A. Mishra. 2012. Post Harvest Methods and Potential of Technological Intervention for Value Addition in Fish in Nepal. Proceedings of the Fourth SAS-N Convention*,* Society of Agricultural Scientists, Nepal (SAS-N) pp.333-344.

Sanjeev, S. 1997. Studies on coagulase positive *Staphylococcus* and *Vibrio parahaemolyticus* in selected items of fish. Crustaceans and fishery products, Cochin University of science and technology, Cochin, India 1: 37-40.

Saritha, K., J.K. Immaculate, A. Velammal and J. Patterson. 2012. Microbial and biochemical qualities of salted and sun-dried sea foods of Cuddalore, southeast coast of India. International Journal of Microbiological Research 3(2): 138-143.

Shahiduzzaman, M., N. Banu, M.M. Hossain, M.S. Islam, M.K. Alam and M.A. Hossain. 2004. Seasonal variation in proximate composition and mineral content of Clupisoma atherinoides. Bangladesh Journal of Life Science 16(1): 109-113.

Shenderyuk V.I. and Bykowski P.J., 1989. Salting and marinating of fish. In: Seafood Resources, Nutritional Composition and Preservation (Ed. Z.E. Sikorski). CRC Press Inc. Boca Raton, Florida.

Shrestha, J., 1999. Cold water fish and fisheries in Nepal. FAO Fisheries Technical Paper. No. 385. FAO, Rome: 13-40.

Siddique, M.A.M., P. Mojumder and H. Zamal. 2012. Proximate composition of three commercially available marine fishes (*Harpodon nehereus*, *Johnius dussumieri* and *Lepturacanthus savala*). American Journal of Food Technology 7(7): 429-436.

Sinduja P., I., Jeyasanta, R., Carol and J. Patterson. 2011. Microbial quality of salted and sun dried sea foods of Tuticorin dry fish market, southeast coast of India.International Journal of Microbiological Research 2: 188-195.

Sarma, D., P. Das, P. Das, S. Sanwar and M. S. Akhtar. 2015. Nutritional composition of golden mahseer (*Tor putitora*) in coldwater Himalayan region of India. SKUAST Journal of Research 17(1): 23-28.

Sugumar, G., B. Christolite, Balasaraswathy and Velayutham. 2004. Effect of handling and time lag on the bacterial flora of sardines landed at Thoothukudi coast. In: National Symposium on recent trends in Fisheries Education and Research. pp. 146-153.

Ullah N., P. Hazarika, PJ Handique. 2016. Biochemical quality assessment of ten selected dried fish species of north east India. International Advanced Research Journal in Science, Engineering and Technology 3 (1): 30-33.

Virta, S. 2009. Isolation and identification of rainbow trout spoiling microbiota. Bachelor's Thesis. Biotechnology and Food Technology, Turku University of Applied Science. 8pp. (Unpublished).

Nepalese Journal of Aquaculture and Fisheries
Vol. 3 & 4 (2016 & 2017) : 59-67

Estimation of fish catch and maximum sustained yield in a part of Kali Gandaki River for 10 years (2005-2015)

Arun Prasad Baidya* and Santa Kumar Shrestha
Kali Gandaki Fish Hatchery, Beltari, Syangja
*Email: apvaidya11@gmail.com

Abstract

In order to compare fish catch and maximum sustained yield (MSY) between upstream and downstream of Kali Gandaki hydro-dam, daily fish catch data was collected for 10 years from July 2005 to June 2015. The fish yield in upstream based on Henderson and Welcomme's, and Ryder's morphoedaphic model ranged from 36.2-42.8 kg/ha/y and 5.4-6.9 kg/ha/y respectively; but the actual fish yield estimated from daily fish catch data ranged from 4.8-20.0 kg/ha/y. Similarly, the fish yield in downstream based on Henderson and Welcomme's, and Ryder's morphoedaphic model ranged from 68.3-82.4 kg/ha/y and 10.2-12.3 kg/ha/y respectively, but the actual fish yield estimated from daily fish catch data ranged from 7.3-24.3 kg/ha/y. In upstream, Katle, *Neolissochilus hexagonolepis* was the dominant fish (26.2-58.1%) followed by Gardi, *Labeo dero* (16.6-54.0%) and Sahar, *Tor putitora* (3.2-25.3%). In downstream, Gardi was the dominant fish (43.8-85.1%) followed by Katle (0.2-9.9%) and Gounch, *Bagarius yarrellii* (1.1-8.8%), respectively. MSY value ranged from 41-209 kg/ha/y in upstream and 10-272 kg/ha/y in downstream.

Keywords: Fish catch, maximum sustained yield, morphoedaphic model, fishing effort

Introduction

Kali Gandaki River originates at the border with China and at the Nhubine Himal Glacier in the Mustang region of Nepal (29°17'0" N, 85°50'5" E, 6,268 masl.). The headwaters stream is named Chhuama Khola. The river flows southward through a world's deepest steep gorge known as the Kali Gandaki George or Andha Galchi, between the mountains Dhaulagiri to the west and Annapurna I to the east. South of the gorge, the river is joined by Raughat Khola at Galeshwor, Myagdi Khola at Beni, Modi Khola at Kushma and Seti Khola at Setibeni. The river joins with Andhi Khola at Mirmi of Syangja district. The dam was built across Kali Gandaki River at Mirmi forming a reservoir of 65 ha with depth ranging from 14 to 20 meters. The river is 47 km long from the dam site (27°58'41" N, 83°35'16" E, 514 masl.) to the powerhouse (27°55'28" N, 83°36'30" E, 382 masl.) of Kali Gandali "A" Hydroelectric Project. Between the dam and the powerhouse, Badigaad Khola at Rudrabeni and Ridi Khola at Ridi Bazaar join it. Turning south again and breaking through the Mahabharat range, the river is then joined by a major tributary the Trishuli River at Devghat (27°80'N, 84°41'E, 244 masl.) and becomes Narayani River into the terai plains of Nepal.

Multi species of indigenous fish exist in Kali Gandaki River and its tributaries (Edds 1986). Number of fish species inhabiting the various river systems increases with the increasing size of the river and river basin (Welcomme 1985). This is because the number of ecological niches remains greater in larger river systems than in small ones. It is necessary to have information on existing fish species, fish catch, catch per unit effort and fish yield per unit area for planning the fisheries management. Morphoedaphic index (MEI), which predicts potential fish harvest as a power function of total dissolved solids divided by mean depth, is regarded as a model of fish production systems. Henderson et al. (1973), and Henderson and Welcomme (1974) have demonstrated MEI as an estimation of fish yield and standing crop in reservoirs. The applicability of MEI to estimate fish yield in river is still unknown. The main objective of the study was to determine fish yield based on MEI models and compare its value with the actual fish catch data collected from up and downstream of Kali Gandaki hydro-dam and to estimate the maximum sustained yield.

The capture fish yield estimation in rivers is complex, thus not very known. Therefore, the estimation of fish yield in rivers is a pre-requisite to estimate the national capture fish yield. The present study on fish yield estimation from Kali Gandaki River is thus important and finding results could be applied to estimate the total fish production by capture fishery.

Materials and methods

Fish catch data were collected from the commercial fishermen all the year round for 10 years from July 2005 to June 2015. The study site lies within 27°58'41" N, 83°35'16" E, 514 masl. and 27°55'28" N, 83°36'30" E, 382 masl. Collected fish were sorted out according to species, and respective fish species were counted and weighed. Fishing effort was expressed as fishermen because fishing gears varied from cast net, gill net, lift nets to long lined loops and angling rods.

In upstream, the fishing area included Mirmi reservoir and Andhimuhan area and covered nearly 80 ha. The annual average depth was 17 m. In downstream, the fishing area covered 50.16 ha of Kali Gandaki River from Thulobagar to Tipua. The annual average depth was 5.2 m. The estimated fish yield was computed using MEI of Ryder (1965);

$$MEI = T/Z$$

$$\text{Yield} = 2\sqrt{MEI}$$

Where, T designated as the total dissolved solids, more conveniently calculated as the product of 0.65 and conductivity (µmhos/cm) and Z the average depth in meter. Henderson and Welcomme's morphoedaphic model was used to calculate fish catch (C) expressed as kg/ha/yr;

$$C = 14.3136 \times MEI^{0.4681}$$

Conductivity was measured every month at different depths to obtain the mean value. The average depth was determined measuring the mean cross sections of the river.

The maximum sustained yield (MSY) and optimum fishing effort (OFE) were calculated using the formulae;

$$MSY = a^2/4b \text{ and } OFE = a/2b$$

Where, a and b were intercept and slope of catch effort curve.

Results

The mean conductivity of upstream Mirmi reservoir area was ranged from 190-310µmhos/cm and the mean depth was 17 meter. Based on the MEI model (Henderson and Welcomme 1974) the fish catch in the upstream was ranged from 36.2-42.8kg/ha/y whereas the yield was ranged from 5.4-6.9 kg/ha/y based on Ryder's model, which was close to the actual fish catch which ranged from 4.8-20.0 kg/ha/y calculated based on daily fish catch data (Table 1).

The mean conductivity of downstream area was ranged from 208-303µmhos/cm and the mean depth was 5.2 m. Based on the MEI model (Henderson and Welcomme 1974) the fish catch in the downstream was ranged from 68.3-82.4 kg/ha/y whereas the yield was ranged from 10.2-12.3 kg/ha/y based on Ryder's model, which was close to the actual fish catch which ranged from 7.3-24.3 kg/ha/y calculated based on daily fish catch data (Table 2).

Table 1: Fish yield and maximum sustained yield in upstream of Kali Gandaki hydro-dam

Year	Conductivity	Fish Yield by MEI Model		Actual Yield	Maximum Sustained Yield (MSY)	Optimum Fishing Effort (OFE)
		Henderson and Wellcomme	Ryder			
	μmhos/cm	kg/ha/yr	kg/ha/yr	kg/ha/yr	kg/ha/yr	
2005/06	245	42.0	6.0	20.0	191	466
2006/07	197	36.7	5.5	12.9	98	122
2007/08	215	38.3	5.7	6.2	50	55
2008/09	260	42.2	6.3	13.3	209	234
2009/10	310	46.2	6.9	7.4	41	38
2010/11	266	42.7	6.4	5.7	60	20
2011/12	266	42.8	6.4	6.3	123	176
2012/13	238	40.5	6.0	4.8	59	60
2013/14	198	36.9	5.5	5.9	83	87
2014/15	191	36.2	5.4	6.9	138	112

Table 2: Fish yield and maximum sustained yield in downstream of Kali Gandaki hydro-dam

Year	Conductivity	Fish Yield by MEI Model		Actual Yield	Maximum Sustained Yield (MSY)	Optimum Fishing Effort (OFE)
		Henderson and Wellcomme	Ryder			
	μmhos/cm	kg/ha/yr	kg/ha/yr	kg/ha/yr	kg/ha/yr	
2005/06	276	78.0	11.0	12.0	272	419
2006/07	210	68.6	10.2	8.9	very low	very low
2007/08	233	72.2	10.7	14.2	very low	very low
2008/09	268	77.7	11.6	13.3	very low	very low
2009/10	303	82.4	12.3	7.8	very low	very low
2010/11	252	75.2	11.2	8.4	34	23
2011/12	278	79.0	11.8	9.7	10	10
2012/13	256	75.8	11.3	7.3	very low	very low
2013/14	209	68.6	10.2	14.3	very low	very low
2014/15	208	68.3	10.2	24.3	99	43

The concept of maximum sustained yield (MSY) is the optimum number of fish caught from the population without long–term changes in the fisheries management. MSY value was ranged from 41-209 kg/ha/y in upstream (Table 1). The actual fish yield was very low, just ranged from 4.8-20.0 kg/ha/y which was far below the value of MSY, an indication of under exploitation. In downstream, MSY value remained was ranged from 10-272 kg/ha/y but the actual fish yield was ranged from 7.3-24.3 kg/ha/y (Table 2).

In downstream, both catches and efforts remained highest from March to April corresponding to lower water velocity and upstream migration. In upstream, actual fishing efforts used was 216-3394 (Table 3). More use of gill nets with large mesh size than cast net with small mesh size and hooks might have built-up dense population of small fishes. Application of higher fishing efforts might overexploit fish resources but the exploitation rate was quite reasonable to maintain the desired fish population in the natural water. In downstream, actual fishing efforts used were 88-417 (Table 4). The average catch per unit effort was ranged from 0.33-2.07 kg/head/y which was very low compared to that of downstream (1.40-7.84 kg/head/y) (Table 3 and 4).

The estimated fish density based on collective catch data in upstream and downstream was ranged from 11-106/ha and 7-83/ha, respectively (Table 3 and 4). Fish stocking in the reservoir ranges from over 100 to 500 depending upon the natural food availability (Sreenivasan, 1977). Fish density seemed quite low for natural water body.

Table 3: Total number, weight and fishing effort of fish caught in upstream (80 ha)

Year	Fish catch data			Catch/effort	No./ha
	Total nos.	Total weight (kg)	Effort		
2005/06	8508	1544.8	3394	0.45	106
2006/07	3425	1028.6	1123	0.92	43
2007/08	873	497.7	520	0.96	11
2008/09	2983	1063.4	2040	0.52	37
2009/10	2323	589.1	284	2.07	29
2010/11	1660	459.4	216	2.13	21
2011/12	1762	501.0	1517	0.33	22
2012/13	1151	381.2	288	1.32	14
2013/14	2421	472.0	430	1.09	30
2014/15	1118	549.0	267	2.05	14

Regarding the species composition, Katle, *Neolissochilus hexagonolepis* was the dominant fish and contributing from 26.2% to58.1% followed by Gardi, *Labeo dero* (16.6-54.0%) and Sahar, *Tor putitora* (3.2-25.3%) to the total catch in upstream (Table 5). In downstream, Gardiwas the dominant fish (43.8-85.1%) followed by Katle (0.2-9.9%) and Gonch, *Bagarius yarrellii* (1.1-8.8%), respectively (Table 6). The reason might be regular stocking of Sahar, Katle and Gardi fingerlings to the river as a part of fisheries enhancement for mitigation program by Nepal Agricultural Research Council and Nepal Electricity Authority since 2002.

Table 4: Total number, weight and fishing effort of fish caught in downstream (50.16 ha)

Year	Fish catch data			Catch/effort	No./ha
	Total nos.	Total weight (kg)	Effort		
2005/06	1225	585.1	417	1.40	24
2006/07	769	445.1	349	1.28	15
2007/08	1817	714.3	242	2.95	36
2008/09	1150	666.7	191	3.49	23
2009/10	703	390.6	119	3.28	14
2010/11	556	421.3	116	3.63	11
2011/12	714	484.0	110	4.40	14
2012/13	351	364.0	88	4.14	7
2013/14	4144	713.3	91	7.84	83
2014/15	2663	1221.0	191	6.39	53

Table 5: Fish species composition (%) and dominant fish species in upstream of Kali Gandaki hydro-dam.

Year	No. of fish species	Fish species (%)										
		Neolissochilus hexagonolepis	*Tor putitora*	*Labeo dero*	*Schizothorax spp.*	*Eutropiichthys murius*	*Anguilla bengalensis*	*Bagarius yarrellii*	*Labeo pangusia*	*Labeo angra*	*Garra annandalei*	Others
2005/06	16	32.0	24.4	16.6	1.0	0.5	0.1	0	2.5	0	10.7	12.3
2006/07	11	43.0	25.3	18.9	0.2	1.0	0.4	0	0	0	6.5	4.7
2007/08	11	26.2	24.5	42.8	2.8	0.2	0.5	0	0	0	0	3.0
2008/09	9	49.7	7.4	37.8	0.5	2.5	0.1	0	1.2	0	0	0.8
2009/10	10	51.5	8.9	33.5	2.7	1.3	0.1	0.1	0	0	0.6	1.3
2010/11	6	58.1	3.2	37.3	0.9	0	0.4	0.1	0	0	0	0
2011/12	6	35.9	6.1	54.0	3.0	0.1	0	0	0.9	0	0	0
2012/13	9	53.6	8.6	35.1	0.5	0	0.2	0.3	0.3	0	0.4	1.1
2013/14	8	36.9	14.3	36.7	4.3	0.3	0	0	7.0	0.4	0	0.1
2014/15	7	41.4	5.9	40.6	0.4	0.5	0.1	0	11.2	0	0	0

Table 6: Fish species composition (%) and dominant fish species in downstream of Kali Gandaki hydro-dam.

Year	No. of fish species	Fish species (%)										
		Neolissochilus hexagonolepis	*Tor putitora*	*Labeo dero*	*Schizothorax spp.*	*Eutropiichthys murius*	*Anguilla bengalensis*	*Bagarius yarrellii*	*Labeo pangusia*	*Labeo angra*	*Garra annandalei*	Others
2005/06	16	9.9	1.6	43.8	2.6	22.0	2.5	2.1	0	0.1	3.1	12.2
2006/07	12	6.2	3.0	45.3	1.2	21.9	4.2	3.0	0	0.8	5.5	9.1
2007/08	11	0.9	1.0	85.1	0	5.2	2.3	1.1	0	0.4	0.9	3.1
2008/09	10	1.5	3.6	79.4	2.2	2.9	4.0	4.3	0	0	1.7	0.5
2009/10	6	3.1	3.3	49.5	0	39.3	1.3	3.6	0	0	0	0
2010/11	8	7.4	4.9	73.2	2.9	1.9	7.2	2.3	0	0.2	0	0
2011/12	8	0.6	1.4	74.2	0	12.6	3.6	2.4	0	0	5.0	0.1
2012/13	6	0.3	3.7	77.2	0	0	8.8	8.8	0	1.1	0	0
2013/14	8	0.2	0.6	61.1	0.2	10.8	0.6	1.2	0	25.3	0	0
2014/15	10	3.2	1.5	74.0	0.8	2.4	2.5	1.8	0.4	0.8	12.7	0

Discussion

The capture fish yield estimation in rivers is complex, thus not very known. Using morphoedaphic index (MEI) model suggested by Henderson and Welcomme (1974), it was shown that the present estimated capture yield of fish after restocking has increased upto 40 kg/ha/yr in upstream and 78 kg/ha/yr in downstream of Kali Gandaki Hydro-dam (Baidya et al. 2011a, Baidya and Devkota, 2014). Catches obtained from 6 flood plain rivers in Asia ranged from 24.17-78.17 kg/ha/y (Welcomme 1985), which was higher than the fish catch obtained both in up and downstream in the present study. Joshi and Nepal (2004) also reported higher fish yield from morphoedaphic model, actual fish yield and maximum sustained yield were 61 kg/ha/y, 53 kg/ha/y and 93 kg/ha/yr, respectively from Rijalghat, a part of Trishuli river, which is higher than calculated in the present study. Joshi and Nepal (2004) reported higher fish yield (61 kg/ha/y) from morphoedaphic model (Henderson and Welcomme, 1974), which was very close to the actual fish yield (53 kg/ha/y) from Rijalghat, a part of Trishuli river. They also reported that maximum sustained yield value was 93 kg/ha/y). Though fish yield of 4.8-24.3 kg/ha/y seemed low but the production in the reservoir increases over the pre-impoundment conditions. The fish catch increased with the increased efforts. Both catches and efforts remained highest from October until March. The highest catch and effort in those particular months was attributable to lower water velocity and upstream moving of fishes during spawning period. Fishing efforts declined in the monsoon due to heavy flood with silt and the catch dropped accordingly.

The updated list of indigenous fish of Nepal has listed a number of 230 fish species (Rajbanshi 2012). Edds (1986) reported 39 fish species from temperate middle hills during the study on Kali Gandaki/Narayani River System. Shrestha (1999) reported 35 cold water fish species from Kali Gandaki River. Wagle et al. (2000) collected 24 fish species from Kali Gandaki and Aandhi Khola near Kali Gandaki hydro-dam. Out of 157 fish species reported from the Kali Gandaki River System (Shrestha 1992), 57 species of fish were recorded in Kali Gandaki River during different level of studies conducted by the Kali Gandaki 'A' Hydroelectric Project (NEAEIA 1996, NEA 2003, Shrestha and Chaudhary 2004). Rayamajhi et al. (2010) have characterized and identified altogether 22 fish species from Kali Gandaki reservoir and its tributaries. Nineteen species of fish were collected in up and down stream of Kali Gandaki hydro-dam in May 2005 to April 2006 (Baidya et al. 2011a). Ten species of fish were collected in up and downstream of Kali Gandaki hydro-dam in July 2011 to June 2012 (Baidya and Devkota. 2014). In the present study, ten species of fish were collected in up and downstream of Kali Gandaki hydro-dam in July 2014 to June 2015. The present result is more or less similar with the findings of different level of studies conducted by Environmental Assessment (NEA 2013). According to Environmental Assessment (EA) Final Draft of NEA, twelve to sixteen fish species are reported from different stations of Kali Gandaki River during the different survey on pre and post construction of Kali Gandaki hydro-dam (NEA 2013).

On the basis of migratory behavior, Shrestha and Chaudhary (2004) divided fishes into three major groups such as longdistance migrant: *Tor tor*, *Tor putitora*, *Anguilla bengalensis bengalensis*, *Bagarius yarrellii*, *Clupisoma garua*; midrange migrant: *Neolissochilus hexagonolepis*, *Labeo dero*, *Labeo pangusia*, *Labeo angra, Schizothorax spp*., *Schizothoraichthys spp*. and resident species: *Garra gotyla gotyla*, *Botia almorhae*, *Schistrura rupicola*, *Barilius barila* etc. After the construction of hydro-dam at Kali Gandaki River, population of long distance migrant species such as *Anguilla bengalensis bengalensis* and *Bagarius yarrellii* were declining form upstream of hydro-dam during the study period.

After the operation of Kali Gandaki Fish Hatchery (KGFH) by Nepal Agricultural Research Council (NARC), four species of previous vulnerable status (Shrestha 1995) such as Sahar, *Tor putitora*; Katle, *Neolissochilus hexagonolepis*; Buchhe Asala, *Schizothorax richardsonii* and Rewa, *Changunius changunio* have been successfully bred (Baidya et al. 2011b, Baidya and Devkota 2012) in this KGFH as project mitigation activities. Recently, only one endangered species in Nepal (Shrestha 2012) and near threatened (NT) in IUCN Red List Status (IUCN 2014), Deep-bodied Mahseer, *Tor tor* was successfully spawned for the first time in Nepal on 29th September 2014 (Baidya and Shrestha 2015). In the last 12 years, 12 fish species have been successfully bred in the KGFH. Every year about 1 million fish fry and fingerlings are released in up and downstream of the hydro-dam after successful breeding activities performed in the KGFH. Other species of same status are also under domestication for further propagation. Routine stocking of declining fish seeds will definitely help to revive fish population and fish biodiversity in up and downstream of the hydro-dam. After the hatchery operation and mass seed production of indigenous fishes, Katle and Gardi were the dominant fish species in upstream and downstream of the hydro-dam, respectively.

Conclusion

This study concluded that actual fish yield was higher (ranged from 7.3-24.3 kg/ha/y) in downstream than in upstream (ranged from 4.8-20.0 kg/ha/y) based on daily fish catch data for 10 years. Regarding the species composition, Katle, *Neolissochilus hexagonolepis* and Gardi, *Labeo dero* were the dominant fish species in upstream and downstream of Kali Gandaki hydro-dam, respectively. Other fish species commonly caught in Kali Gandaki river during study period were Sahar, *Tor putitora*; Asala, *Schizothorax and Schizothoraichthys spp*.; Jalkapur, *Eutropiichthys murius*; Rajbam, *Anguilla bengalensis bengalensis*; Gonch, *Bagarius spp.*; Hade, *Labeo pangusia*; Thend, *Labeo angra* and Lahare, *Garra annandalei*.

Acknowledgements

This study was supported by the funding obtained by Nepal Electricity Authority (NEA) and Nepal Agricultural Research Council (NARC) under Project No.: 62365002. The authors gratefully acknowledge the contributions, guidance and encouragement of Mr. Purushottam Lal Joshi. The authors would like to thank Mr. Agni Nepal (2005-2006), Mr. Chudamani Bhusal (2006-2008), Mr. Kamalesh Srivastav (2008-2010), Mr. Hareram Devkota (2010-2012) who spent time helping gather fish catch data during different period for the study. We wish to thank Mr. Rajkapur Napit for coordinates measurement of the dam site and the powerhouse of the Kali Gandaki "A" Hydroelectric Project. We wish to thank all the staffs of Kali Gandaki Fish Hatchery, Beltari, Syangja, for their cooperation for this study.

References

Baidya, A.P. and H.R. Devkota. 2012. Breeding performance of indigenous fishes for conservation of wild population. In: Proceedings of the Consultative Workshop on Fish Conservation in Nepal. (eds. S. K. Wagle and N. Pradhan). Fisheries Research Division, Godawari, NARC. 132-142.

Baidya, A.P. and H.R. Devkota. 2014. Estimation of fish catch and maximum sustained yield in a part of Kali Gandaki River. Nepalese Journal of Aquaculture and Fisheries 1: 124-131.

Baidya, A.P. and S.K. Shrestha. 2015. Successful semi-artificial breeding of pond reared Mahseer, *Tor tor* and its initial growth performance. Nepalese Journal of Aquaculture and Fisheries 2: 48-55.

Baidya, A.P., P.L. Joshi and A.P. Nepal. 2011a. Actual fish yield and estimation of maximum sustainable (MSY) in up and downstream of Kali Gandaki hydro-dam. In: Proceedings of the 8th National Workshop on Livestock and Fisheries Research (eds. T.B. Gurung, P.K. Pokharel, C.R. Upreti, B.R. Joshi, S.M. Pradhan and S.K. Wagle). National Animal Science Research Institute, Nepal Agricultural Research Council, Lalitpur, Nepal. ISBN: 978-9937-2-3960-8: 44-48.

Baidya, A. P., J. D. Bista and T. B. Gurung. 2011b. Domestication and breeding status of some native fishes in Nepal. In: Proceedings of the Workshop on Indigenous Fish Stock and Livelihood in Nepal (eds. S.K. Wagle, T.B. Gurung and N. Pradhan), Fisheries Research Division, Godawari, NARC. 55-71.

Edds, D. R. 1986. Fishes of Kali Gandaki/ Narayani River, Nepal. Journal of Natural History Museum 10(1-4): 13-22.

EIA. 1996. Environmental Impact Assessment of Kali Gandaki 'A' Hydroelectric Project. Vol.1.

NEA, 1996. Environmental Impact Assessment (EIA) Report. Kaligandaki 'A' Hydroelectric Project. Environmental and Social Studies Department (ESSD)/ Nepal Electriciy Authority (NEA). Associates: Morisson Knudsen Corporation. USA. Norconsult International. Norway and IVO International Ltd. Finland.

Henderson, H.E., R.A. Ryder and A.W. Kudhongania. 1973. Assessing fisher potentials of lakes and reservoirs. Journals of the Fisheries Research Board of Canada 30(12): 2000-2009.

Henderson, H.E. and R.L. Welcomme. 1974. The Relationship of Yield to Morphoedaphic Index and Numbers of Fishermen in Africa Inland Fisheries. CIFA (Committee for Inland Fisheries of Africa). Occasional Paper (1). FAO Rome. 19 pp.

IUCN. 2014. IUCN Red List of Threatened Species. Version 2014.

Joshi, P.L. and A.P. Nepal. 2004. Fish catch data and estimation of maximum sustained yield in a part of Trishuli River. In: Proceedings Nepal Animal Science Association: 81-84.

NEA. 2003. Post Construction Environmental Impact Assessment Audit Study of Kali Gandaki 'A' Hydroelectric Project. Kathmandu: Environmental and Social Studies Department (ESSD)/ Nepal Electricity Authority (NEA).

NEA. 2013. Environmental Assessment (EA) Final Draft. Kali Gandaki 'A' Hydropower Plant Rehabilitation Project. Environmental and Social Studies Department (ESSD)/ Nepal Electricity Authority (NEA). 61 pp.

Rajbanshi, K.G. 2012. Bio-diversity and distribution of fresh water fishes of Central/Nepal Himalayan Region. Nepal Fisheries Society (NEFIS). 136 pp.

Rayamajhi, A., A.P. Nepal, S.K. Wagle and A.P. Baidya. 2010. Fishes in Kali Gandaki Hydro-dam and surrounding tributaries. In: Sustainable use of biological resources in Nepal, pp. 152-160. Ecological Society (ECOS).

Ryder, R.A. 1965. A manual for estimating the potential fish production of north-temperate. Transactions of American Fisheries Society 94(3): 214-218.

Shrestha, J. 1992. The role, scope and importance of natural water resources for increased fish production in Nepal. In: Proceedings of Workshop on Human Resources Development in Fisheries Research, Nepal. HMG/FDD, IDRC: 158-182.

Shrestha, J. 1995. Enumeration of the fishes of Nepal. Technical Publication, No. 10. Biodiversity Profile Project, HMG, Department of National Parks and Wildlife Conservation. 64 pp.

Shrestha, J. 1999. Coldwater fish and fisheries in Nepal. Fish and fisheries at higher altitudes: Asia. FAO Fisheries Technical Paper No. 385. FAO, Rome. pp. 13-40.

Shrestha, J. 2012. Threat status of indigenous fish species of Nepal. In: Proceedings of the Consultative Workshop on Fish Conservation in Nepal (eds. S.K. Wagle and N. Pradhan). Fisheries Research Division, Godawari, NARC. pp. 13-34.

Shrestha, J. and R. Chaudhary. 2004. Fish diversity in Kali Gandaki River before and after the project construction. In: Proceedings of Nepal Agricultural Research Council/Nepal Electricity Authority (NARC/NEA), Kathmandu, Nepal: 20-31.

Sreenivasan, R.A. 1977. Fishery of Cauvery River System, Tamilnadu, India. Proc. IPFC, 17(3): 167-172.

Wagle, S.K., J.D. Bista, A.P. Baidya and R.M. Mulmi, 2000. Survey of fish species and environmental variables in Kali Gandaki River near dam of Kali Gandaki 'A' Hydroelectricity project. Proceedings Livestock and Fisheries Research in Nepal. 24-29.

Welcomme, R.L. 1985. River fisheries. FAO Fisheries Technical Paper 262. 330 pp.

Nepalese Journal of Aquaculture and Fisheries
Vol. 3 & 4 (2016 & 2017) : 68-76

Rediscovery of sisorid catfish *Glyptothorax kashmirensis* (Hora) (Teleostei: Siluriformes: Sisoridae) from Narayani River, Central Nepal

Asha Rayamajhi
Fisheries Research Division, Godawari, Kathmandu, Nepal
*Email: rayamajhiasha@hotmail.com

Abstract
Glyptothorax kashmirensis (Hora 1923) Kashmir catfish a critically endangered fresh water fish species in Asiawas first described from the type locality Kashmir Valley. Wild populations of *G. kashmirensis* are severely declined due to habitat loss, dam construction and over-exploitation leading to an alarming situation, which deserves high conservation consideration. In Nepal this species was earlier reported in Mahakali, Chamelia (far western region) and Babai River (mid-western region) of Ghaghra basin. Further *Glyptothorax kashmirensis* has reported from Narayani River but not supported by voucher specimens and exact distribution range. But, present study rediscovered the occurrence of *Glyptothorax kashmirensis* (Hora) (voucher specimen) from the freshwater habitat of Narayani River at buffer zone of Chitwan National Park, border of Nawalparasi and Chitwan districts and exposed the verification of its distribution in Ganges River drainage.

Key words: Rediscovery, Narayani River, Ganges River basin, critically endangered, conservation.

Introduction

Glyptothorax kashmirensis is a benthopelagic freshwater fish belonging to family Sisoridae) and known for its good taste and has local trade value (Molur and Walker 1998). *Glyptothorax kashmirensis* (Hora 1923) was originally described from Kashmir valley reaches about 10 cm SL. Kullander et al. (1999) has described this species as a little-known rheophilic and benthic species of small dimensions. Afterwards *Glypthorax kashmirensis was* described by Mukerji (1936) (species locality: Jhelum River and Shadiput Kashmir) and Menon (1954) (from Kashmir and Punjab, Western Himalayas). *Glyptothorax kashmirensis* was reported by Mirza and Hameed (1974) from the Kishen Ganga and by Misra (1976) from India and Pakistan. Talwar and Jhingran (1991) have listed this species from Kashmir valley, Pakistan: Indus River and Rashida and Saleem (1996) recorded from Kurram River in North West Pakistan.

In Nepal, distribution of *Glyptothorax kashmirensis* was earlier known from Mahakali River and Chamelia River in far western region and Babai River in mid-western region (Shrestha 2008, Shrestha 2011) as tributaries of Karnali River of Ghaghra basin. Jha (2012) reported *Glyptothorax kashmirensis* as in uncommon state from Karnali River, Kailai district in check list of cat fishes. Further Shrestha (2012) and Rajbanshi (2012a) has also listed this species in checklist. IUCN red list mentioned the distribution of *Glyptothorax kashmirensis* in the Ganges River drainage (Narayani River) in Nepal but the IUCN red list has remarked its status in Nepal as requires further confirmation (IUCN, 2014). The first record of *Glyptothorax kashmirensis* outside its type locality (Kashmir valley) was made by Rajbanshi (2012b) from the Narayani River, but this report was not supported by voucher specimens and exact distribution range. As in Asia *Glyptothorax kashmirensis* is now under critically endangered category of IUCN (IUCN 2015, Ng 2010, Singh et al. 2015) predicting a decline of greater than 80% over the next five to ten years if present threats (damming, introduction of exotic species, overexploitation etc.) continuous in future (Ng 2010). So, urgent efforts are needed to sustain the wild population of *G. kashmirensis*. This species is on the verge of extinction due to habitat loss (breeding and nursery grounds). Therefore, present paper enlightens the description of *G. kashmirensis*, reviewed the previous work done and recommends conservation actions that should be taken into account towards the preservation of the wild population of *G. kashmirensis* (Singh et al. 2015)**.** In the present paper description of *Glyptothorax kashmirensis* (Locally called as ‘Ketingi or Tite) was based on one specimen, like many other species within this genus, *Glyptothorax kashmirensis* has been poorly represented in collections from Narayani River near Ghadiyal project, Village Development Committee Amaltari, boundary of Nawalparasi and Chitwan, buffer zone of Chitwan National Park at 133 msl, 27°33’15”N and 84°07’12”E, Central Nepal and herein *Glyptothorax kashmirensis*is described as rediscovery and confirmation of its distribution range in central region (Ganges River basin).

Materials and Methods

Fish specimen was collected using cast net in February, 2014 from Narayani River which lies in buffer zone area of Chitwan National Park, at village development committee Amaltari, boundary of Nawalparasi and Chitwan districts. Specimen was photographed in the field and then preserved in 10% formalin for morphometric and meristic studies. Preserved specimen is kept in Fish Museum of Fisheries Research Division (FRDFM) under Nepal Agricultural Research Council, Kathmandu, Nepal. Measurements were made point to point using digital caliper (0.01 mm accuracy). Methods used for counts and measurements follow those of Ng and Kottelat (1998). Sub units of the head are presented as proportions of head length (HL). Head length and measurements of body parts are given as proportion of standard length (SL). A total of 53 morphometric characters and 10 meristic counts were taken (Table 1 and 2). Physico-chemical parameters; temperature (°C), pH, dissolved oxygen (mg/l), conductivity (ms) and TDS (ppt) were taken. GPS co-ordinates and altitudes were taken using GPS recorder (Model Oregon). Abbreviation used herein include SL=standard length, HL=head length, FRDFM= Fisheries Research Division Fish Museum in Godawari, Lalitpur, Kathmandu, Nepal.

Results

Glyptohorax kashmirensis Hora

Figures 1-3, Tables 1-2

Figure 1: *Glyptothorax kashmirensis*: dorsal and ventral views, locality from Narayani River, buffer zone of CNP, boundary of District; Nawalparasi and Chitwan, central Nepal.

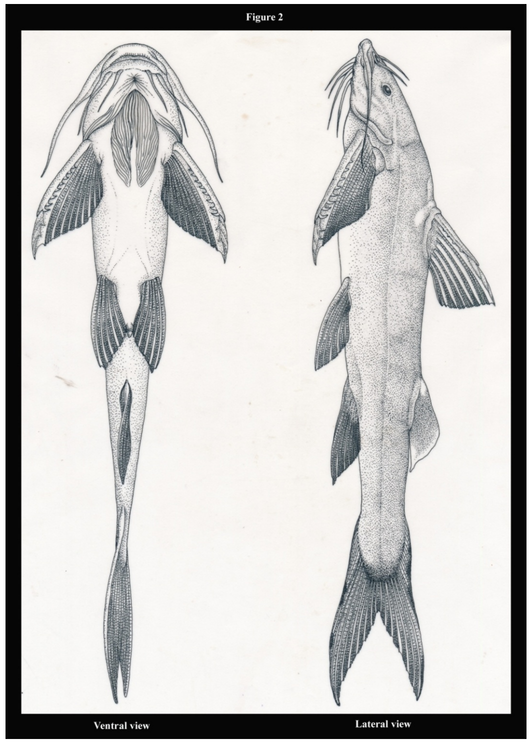


Figure 2: *Glyptothorax kashmirensis*: Ventral and Lateral views, collected locality from Narayani River buffer zone of CNP, boundary of District; Nawalparasi and Chitwan, central Nepal.

Figure 3: Locality of *Glyptothorax kashmirensis*: Narayani River at buffer zone of Chitwan National Park, boundary of District; Nawalparasi and Chitwan, central Nepal.

Table 1: Morphometric data of *Glyptothorax kashmirensis* from Narayani River, border of District; Nawalparasi and Chitwan, buffer zone of CNP, central Nepal (n=1).

No.	Morphometric measurements	(mm)
A.	Standard length (SL)	62.32
B.	**In percent of standard length**	
1	Snout to urocentrum	95.73
2	Pre-anal length	63.05
3	Pre-dorsal fin length	32.40
4	Pre-pelvic fin length	43.76
5	Pre-pectoral fin length	18.18
6	Lower jaw to isthmus	21.24
7	Peduncle length	19.78
8	Dorsal-fin origin to pelvic-fin insertion	22.42
9	Dorsal spinous height	14.35
10	Anal-fin height	21.13
11	Peduncle depth	7.91
12	Caudal-fin length	33.14
13	Dorsal-fin height	22.34
14	Pectoral-fin length	23.67
15	Pelvic-fin length	17.78
16	Occiput to dorsal-fin origin	10.46
17	Occiput to pectoral-fin insertion	13.61
18	Occiput to pelvic-fin insertion	28.92
19	Dorsal-fin insertion to pelvic-fin insertion	15.04
20	Dorsal-fin origin to pectoral-fin insertion	13.77
21	Dorsal-fin origin to anal-fin origin	36.73
22	Dorsal-fin insertion to caudal-fin base	49.36
23	Dorsal-fin insertion to anal-fin origin	23.75
24	Dorsal-fin insertion to anal-fin insertion	32.64
25	Dorsal-fin base length	10.57
26	Anal-fin base length	11.99
27	Adipose fin base length	11.95
28	Pectoral-fin insertion to pelvic insertion	22.82
29	Pectoral-fin insertion to anal-fin origin	39.33
30	Pelvic-fin insertion to anal-fin origin	16.00
31	Head length	25.47
32	Total length	121.89
33	Post dorsal length	63.56
34	Body depth	16.25
35	Distance b/w pectoral-fin to vent	39.30
36	Distance b/w pelvic-fin to vent	12.00
C.	**In percent of head length**	
37	Pre occipital length	86.01
38	Snout to opercle	84.56
39	Upper jaw length	17.33
40	Snout length	43.48

41	Pre-nasal length	6.99
42	Orbit width	9.77
43	Inter orbital width	26.59
44	Inter nasal width	10.96
45	Head width	71.58
46	Gap width	36.67
47	Head depth at nostril	14.62
48	Head depth at pupil	39.38
49	Head depth at occiput	51.42
50	Maxillary barbel length	81.85
51	Mandibular outer barbel	49.46
52	Mandibular inner barbel	26.40
53	Nasal barbel length	34.66

Table 2: Meristic characters of *Glyptothorax kashmirensis* (n=1).

No	**Meristic counts**	
1	Unbranched dorsal fin rays	I
2	Branched dorsal fin rays	6
3	Unbranched anal fin rays	ii
4	Branched anal fin rays	9
5	Unbranched pelvic fin rays	i
6	Branched pelvic fin rays	5
7	Unbranched pectoral fin rays	I
8	Branched pectoral fin rays	9
9	Antrose teeth on Pectoral fin spine	10
10	Caudal fin rays	14

Materials Examined

FRDFM-6, (1 ex.) 62.32 mm SL. Narayani River, Argoili-5, Near Ghadiyal project, VDC Amaltari, Border of District; Nawalparasi and Chitwan, buffer zone of CNP. (27^{o} 33'15"N, 084^{o} 07'12"E and 133 msl), deposited in FRDFM, Kathmandu. Collected by Asha Rayamajhi, 24 February, 2014.

Diagnosis

This species is characterized by a thoracic adhesive apparatus about oval in outline, made up of elongated folds interiorly arranged longitudinally, posteriorly more latero medially extended with distinct central depression.

Description

Morphometric and meristic measurements are given in Table 1 and Table 2 respectively. Count and measurement is from 1 specimen 62.32 mm SL. Body elongate, slightly tapering from mid body to caudal fin; its depth at dorsal origin is 16.25% SL. Head short, wide and depressed**;** its length is 25.47% SL. Head depth at nostril is 14.62% HL, at orbit 39.38% HL and at occiput 51.42% HL. Eyes are dorsal, minute, not visible from below the ventral surface of head. Orbit width is 9.77% HL and interorbital width 26.59% HL. Median longitudinal groove on head is not reaching the base of occipital process. Four pairs of barbells are not annulated; maxillary barbell having broad and thick base and is reaching beyond pectoral fin base. Length of maxillary barbel is 81.85% HL. Nasal barbel reaches to middle of eye; its length is 34.66% HL; outer mandibular reaches to pectoral fin base; its length is 49.46% HL. Inner pair is shorter than width of mouth, its length is 26.40% HL. Thoracic adhesive apparatus longer than broad about oval outlined, made up of elongate folds arranged longitudinally and having central depression which is incomplete posteriorly (Figure 1 and 2). Gill membranes are united with isthmus.

Fins counts are: dorsal fin rays i-6(1); pectoral fin rays i-9(1); pelvic fin rays i-5(1); anal fin rays ii-9(1); caudal fin rays 14(1). Pre-dorsal distance 32.40% SL and pre-pelvic fin distance 43.76% SL. Rayed dorsal fin inserted above half of the pectoral fin, its position nearly midway between end of snout and adipose fin origin. The length of dorsal fin is 22.34% SL. Dorsal spine serrated along its inner margin and between each spines having wide space. Length of dorsal spine is 14.35% SL.
Pectoral fin broad, flattened and long, with strong spine and it is serrated posteriorly having antrose 9 teeth along inner margin. Length of pectoral fin is 23.67% SL. Pectoral fin not reaching pelvic fin origin. Pelvic fin almost near to anal fin base; its length 17.78% SL. Length of anal fin is 21.13% SL. Distance between pectoral fin to vent 39.30% SL. Distance between pelvic fin to vent 12.00% SL. Distance between pectoral fin insertion to pelvic fin insertion 22.82% SL and distance between pelvic fin insertion to anal fin origin 16.00% SL. Post dorsal fin length is 63.56% SL. Adipose fin position is opposite of anal fin, both adipose fin and anal have similarities in shape and their base length; 11.95% SL and 11.99% SL respectively. The adipose dorsal fin is separated from the rayed dorsal fin by an interspace. Caudal fin deeply forked; its length is 33.14% SL (not prolonged). Skin profusely granulated, including body and fin bases are covered with small elongate tubercles. The cubito humeral process is inconspicuous, hidden by skin. Lateral line is complete.

Coloration
Above portion of body is uniformly dark brown and becoming lighter in colour at below portion. All fin bases with faint black spots.

Remark
The specimen collected in the present survey from Narayani River shows one dissimilarity that was in anal fin ray counts having 11 rays though it has described as having 9 rays (Jayram 2006).

Site description at Chitwan National Park (CNP) Buffer Zone
The Narayani River (Gandak River) which receives numerous tributaries of high mountainous areas with glaciers and snow melt and finally drains into Ganges River. Small boulders and sand are the dominant substrates in Narayani River at near Ghadiyal project, VDC Amaltari, boundary of district; Nawalparasi and Chitwan, buffer zone of Chitwan National Park (CNP) (Figure 3). Pools and runs are the major habitat types. River depth ranges from 0.5-1 m and elevation was 133 msl (27°33.258'N 084°07.205'E) at fish collection site. Air temperature and water temperature in Narayani River at Argoili -5, were 21°C and 28°C respectively and dissolved oxygen (DO), pH, conductivity and total dissolved solids were 8.4 mg/l, 7.7, 0.01 mhos and 0.2 ppt respectively in February 24, 2014 at 12:05 PM noon. Other fish species collected from this area are; *Tor mosal, Garra mullya, Botiya dayi, Glypthorax cavia, Glypthorax pectinopterus, Macrognathus pancalus* and *Chanda nama.*

Discussion
Globally, *Glyptothorax* is represented by more than 70 species (Anganthoibi and Vishwanath 2010). Earlier in Nepal, Edds (2007) reported *Glyptothorax cavia*, *Glyptothorax gracilis* and *Glyptothorax indicus* from Bardia National Park, mid-western region and *Glyptothorax gracilis* from Shukla Phanta Wildlife Reserve, far western region. Recent review by Shrestha (2012) and Rajbanshi (2012a and

2012b) listed twelve valid species in Nepal: *Glyptothorax alaknandi* (Tilak 1969), *Glyptothorax annandalei* (Hora 1923), *Glyptothorax botius* (Hamilton-Buchanan 1822), *Glyptothorax cavia* (Hamilton-Buchanan 1822), *Glyptothorax conirostre* (Steindachner 1867), *Glyptothorax garhwali* (Tilak 1969), *Glyptothorax gracilis* (Gunther 1864), *Glyptothorax indicus* (Talwar and Jhingran 1991), *Glyptothorax kashmirensis* (Hora 1923), *Glyptothorax pectinopterus* (McClelland 1839), *Glyptothorax telchilta* (Hamilton-Buchanan 1822) and *Glyptothorax trilineatus* (Blyth 1860).

Among congeners of *Glyptothorax*; three species namely *Glyptothorax cavia* (Hamilton), *Glyptothorax indicus* (Talwar) and *Glyptothorax pectinopterus* (McCllend) has been listed from different sites of Narayani River at Chitwan and Nawalparasi district (Jha and Bhujel 2014). Dhital and Jha (2002) have listed *Glyptothorax horai* (Shaw & Shebbeare) and *Glyptothorax trilineatus* (Blyth) from Narayani River at localities Devghat and Narayanghat, Chitwan, central Nepal. Additionally, Shrestha (2011) has listed *Glyptothorax telchitta* (Hamilton) from Narayani River in fish check list.

In previous ichthyological surveys in Nepal, the workers such as Edds (2007), Shrestha (2008), Shrestha (2011), Dhital and Jha (2002), Jha (2012) and Jha and Bhujel (2014) did not report *Glyptothorax kashmirensis* from Narayani River. Recently, *Glyptothorax kashmirensis* was discovered in the locality of Narayani River in Nepal and revealed the authentication of this species in Ganges River basin. In Nepal, the status of *Glyptothorax kashmirensis* is listed as of insufficient known (Shrestha 2012) as well as this species is listed as critically endangered in the IUCN Red List of Threatened Species (Ng 2010, IUCN 2014, IUCN 2015). Based on the updated information on distribution and threats (Ng 2010, IUCN 2014, IUCN 2015), more research about the population size and trends, distribution and the biology of this species is needed, as there is insufficient information available.

As *Glyptothorax kashmirensis* is naturally adapted to inhabit fast flowing waters, any obstruction like construction dam will alter the regime of flowing water dramatically which eventually affect its population (Molur and Walker 1998, Ng 2010). Other anthropogenic activities, pesticides run off, and overexploitation and local trade are also reasons for declining the population of this species (Molur and Walker 1998). Therefore, a comprehensive strategy, needs to be evolved to conserve the species under high risk of extinction.

Acknowledgement
Sincere gratitude goes to the Professor Arunachalam, M. of Sri Paramakalyani Centre for Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi - 627 412, Tamil Nadu, India for editing the manuscript. I am also grateful to Mr. S. Mariappan, drawing master of Sri Paramakalyani Centre for Environmental Sciences, Manonmaniam Sundaranar University for line drawings of specimen *Glyptothorax kashmirensis* collected by author. Thankful to the fishermen of Tharu community of Home Stay area, Nawalparasi for their cooperation in fish collection.

References

Anganthoibi, N. and W. Vishwanath 2010. Two new species of *Glyptothorax* from the Koladyne basin, Mizoram, India (Teleostei: Sisoridae). Ichthyological Exploration of the Freshwaters 21(4): 323-330.

Dhital, R.R. and D.K. Jha 2002. Fish fauna of the Narayani River System and their impact on the Fishermen communities in Chitwan, Nepal. In: Cold water fishes of Trans-Himalayan countries (eds. T. Peter and D.B. Swar), FAO Fisheries Technical Paper No. 431, FAO, Rome. pp 119-128.

Edds, D.R. 2007. Fishes in Nepal: Ichthyofaunal surveys in seven nature reserves. Ichthyological Exploration of Freshwater 18(3): 277-287.

Hora, S.L. 1923. Notes on the fishes in the Indian Museum. On the composite genus Glyptosternun McClelland. Records of Indian Museum25 (1): 29. pp. 1-44.

IUCN, 2014: IUCN Red List of Threatened Species. Version 2014.2. Updated in August 2014.

IUCN, 2015. The IUCN Red List of Threatened Species.

Jayaram, K.C. 2006. Catfishes of India. Narendra Publishing House, New Delhi, xxii+383+11pls pp.

Jha, D.K. 2012. Indigenous catfishes and their diversification for aquaculture. In: Proceeding of the Consultative Workshop on Fish Conservation in Nepal (July 4, 2011), Fisheries Research Division (FRD), Godawari, Lalitpur, Nepal. pp 111-122.

Jha, D.K. and R.C. Bhujel 2014. Fish diversity of Narayani River System in Nepal. Nepalese Journal of Aquaculture and Fisheries 1: 94-108.

Kullander, S.O., F. Fang, B. Dellingand and E. Ahlander 1999. The fishes of the Kashmir valley. In: River Jhelum, Kashmir Valley, Impacts on the Aquatic Environment (ed. L. Nyman), pp.99-167. Swedmar, Goteberg, Sweden.

Menon, M.A.S. 1954. Notes on fishes of the genus *Glyptothorax* Blyth. Records of the Indian Museum 52: 27-54.

Mirza, M.R. and Hameed, K. 1974. Sisorid Fishes (Osteichthyes, Sisoridae) of Pakistan and Azad Kashmir. Biologia (Lahore) 20: 83–89.

Misra, K.S. 1976. The fauna of India and the adjacent countries. Pisces (Second edition) Vol. III Teleostomi: Cypriniformes; Siluri. Zoological Survey of India, Calcutta. pp. 1-367.

Molur, S. and S. Walker. 1998. Report of the Workshop "Conservation Assessment and Management Plan for Freshwater Fishes of India", Zoo Outreach Organisation, Conservation Breeding Specialist Group, India, Coimbatore, India. 156 pp.

Mukerji, D.D. 1936. Report on fishes. Part II: Sisoridae and Cypriniidae. Memoirs of the Connecticut Academy of Arts and Sciences 10: 323-359.

Ng, H.H. 2010. *Glyptothorax kashmirensis*. The IUCN Red List of Threatened species.

Ng, H.H. and M. Kottelat. 1998. The catfish genus *Akysis* Bleeker (Teleostei: Akysidae) in Indochina, with descriptions of six new species. Journal Natural History 32(7): 1057-1097.

Rajbanshi, K.G. 2012a. Indigenous fishes of Nepal and the need of their conservation for their sustainable. In: Proceeding of the Consultative Workshop on Fish Conservation in Nepal, (July 4, 2011), Fisheries Research Division (FRD), Godawari, Lalitpur, Nepal. pp. 35-63.

Rajbanshi, K.G. 2012b. Bio-diversity and distribution of fresh water fishes of central/Nepal Himalayan region. pp. 1-136.

Mirza, M.R. and M. Saleem 1996. A contribution to the systematics and biology of *Glyptothorax kashmirensis* Hora (Pisces: Sisoridae) from Pakistan and Azad Kashmir. Biologia 42(1-2): 59-60.

Shrestha, J. 2012. Threat status of indigenous fish species of Nepal. In: Proceeding of the Consultative Workshop on Fish Conservation in Nepal, (July 4, 2011), Fisheries Research Division (FRD), Godawari, Lalitpur, Nepal. pp. 13-34.

Shrestha, T.K. 2008. Ichthyology of Nepal. A study of fishes of the Himalayan waters. Himalayan Ecosphere. Kathmandu, Nepal. 388 pp.

Shrestha, T.K. 2011. Fish diversity of Nepal and potentiality of indigenous fishes for future development of aquaculture. In: Proceeding of the workshop on indigenous fish stock and livelihood in Nepal (eds. S.K. Wagle, T.B. Gurung and N. Pradhan) (June 5, 2008), Fisheries Research Division (FRD), Godawari, Lalitpur, Nepal. pp 4-31.

Singh, R., S. Akhtar, N. Pandey, J.I. Mir and N.K. Sharma 2015. Threatened Fishes of the World: *Glyptothorax kashmirensis* (Hora, 1923) (Siluriformes: Sisoridae) a mini review. Journal of Fisheries and Livestock Production 3: 147. doi:10.4172/2332-2608.1000147.

Talwar, P.K. and A.G. Jhingran. 1991. Inland fishes of India and adjacent countries. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India, p. 1158.

Nepalese Journal of Aquaculture and Fisheries
Vol. 3 & 4 (2016 & 2017) : 77-90

Diversity of edible aquatic mollusk and their nutritional contribution in selected Terai districts of Nepal

Suresh Kumar Wagle[1*], Abhilasha Jha[2] and Anita Gautam[1]
[1]Fisheries Research Division, P. O. Box 13342, Godawari, Lalitpur, Nepal
[2]Regional Agriculture Research Station, Tarahara, Sunsari, Nepal
*Email: waglesk@yahoo.com

Abstract
Freshwater mollusks play an important role in lotic and lentic ecosystems. Every year significant quantities of edible aquatic mollusks are harvested by ethnic communities of Terai for consumption with relish. The gastropods and bivalves have not been the subject of intense studies despite the presence of rich diversity of edible and commercial species in Nepal. This work assessed, described and identified some economically important mollusks in eastern Terai region of Nepal. There were a total of 8 mollusks (bivalves and gastropods) species identified which is served as food. Proximate analysis showed that gastropod muscle contains 16.2% crude protein (CP) and low in fat (1.0%), while bivalve accounts for 11.0% CP and 0.61% fat. They were also rich in calcium (189.2±21.2 mg/100g) and iron (95.6±4.3 mg/100g) content. Survey revealed that the mean consumption of edible aquatic mollusks was 42.6 kg per household in the region. Presence of micro-organisms (e.g., *Salmonella*) in mollusks found during microscopic observation warned for improvement in processing and recipe preparation. However, due to inevitable increase in commercial demand and overexploitation it may result to a possible depletion of mollusk resources. Hence, native community should be equipped with the proper scientific knowledge on the preservation and conservation of these resources.

Keywords: Bivalve, ethnic community, diversity, gastropod, mollusk

Introduction

The accepted definition of food security is: food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life (FAO 2003). Consumption of fish and most of the livestock products is directly account for nutritional food security primarily for many ethnic community living around the natural water bodies of the country. For good human health at least 33% of the total protein should be from animal sources (Bhujel 2007). Animal protein intake in Nepal is about 14% of the total protein requirement. To meet the nutritional requirement of the country it makes sense to strengthen the fisheries and livestock sector, because nutritionally animal protein has a higher value than that of plants on account of its larger quantity of essential amino acids as well as micronutrients like minerals and vitamins. Thus animal-based food stuffs could be used to alleviate the mild to moderate protein malnutrition prevalent throughout the developing world (Neumann et al. 2002). However, due to rising costs of conventional animal protein sources, a general shift from animal protein to plant sources could affect the nutritional state of the country. This scenario justifies the need for alternative cheap and nutritional sources of animal origin.

Molluscs are one of the larger invertebrate groups in the freshwater habitat of Nepal. Nesemann and Sharma (2003) have reported existence of 45 species of aquatic molluscs (25 gastropods and 20 bivalves) from lowland (Tarai) regions of Nepal of which 25 species are edible (Subba 2012). They are ecologically important because of their widespread distribution and biological filtration activity (Kasprzak 1986) and also economically, used as food and production of freshwater pearls (Subba Rao and Dey 1989). The use of freshwater molluscs as protein-rich food is very much in practice in a number of countries viz. India (Subba Rao 1989), Mexico (Flores-Garza et al. 2012), Taiwan, Formosa (Baby et al. 2010), The Philippines (Talavera and Faustino 1933) and Thailand (Kaewajam 1986). Freshwater mollusks play a vital role in the economy and tradition of West Bengal in India serving as a food of 80.81% families belonging to more than 30 castes of general schedule and tribal peoples (Baby et al. 2010). Several species of freshwater mollusk serve as food to the ethnic people of Tarai, Nepal (Subba 2012). Other communities are also beginning to appreciate the taste of molluskan meat. Edible mollusks are cheap non-conventional source of protein for huge population of poor ethnic peoples (53 castes living in 21 districts of Tarai in Nepal), from time immemorial (Subba 2012). Importance of gastropods and bivalves in mitigating the protein deficiency in poor countries like Nepal cannot be overlooked. Every year significant quantities of edible mollusks are harvested by ethnic communities of Tarai. Production of edible mollusks in Nepal is negligible as mollusk culture has not yet been started in Nepal. Rampant harvest of gastropods and bivalve from various water resources has led several species of mollusks closer to extinct (Subba 2012). The gastropods and bivalves have not been the subject of intense studies despite the presence of rich diversity of edible and commercial species in Nepal. The aim of this study was to document the diversity, nutritional contribution and conservation threats of freshwater edible mollusk to help in planning judicious exploitation and conservation measures. This paper alsoaims to justify the need to introduce aquatic edible mollusks into mainstream farming systems and attempts to rationalize the concept of snail farming in the country.

Materials and Methods

The survey on diversity and nutritional contribution of edible aquatic mollusks was carried out in 19 locations of 7 Terai districts of Nepal during July 2015 to December 2016 (Figure 1).

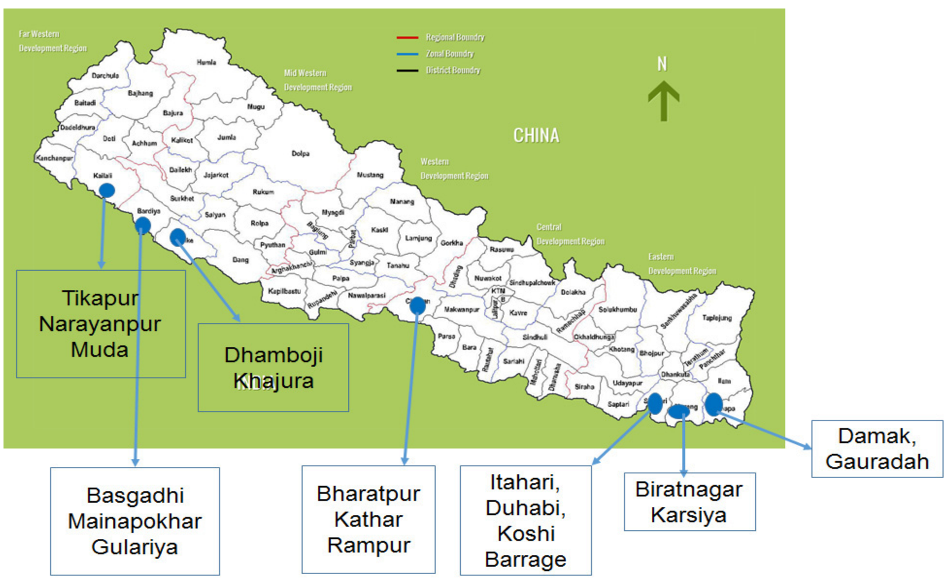


Figure 1: Map of Nepal showing the survey locations of edible aquatic mollusks

For the species diversity study, major habitats such as ponds, ghols and river banks will be surveyed. Samples of mollusk (gastropod and bivalve) was collected using a kick net (D-shaped, 30 cm width, 400 µm mesh) for identification. Sampling was limited to the littoral zone of Taltalaiya wetlands (water depths of less than 50 cm), perennial stream and ponds in Sunsari and Morang districts by kicking up the substrate and then sweeping above the disturbed area. In the laboratory, each sample was washed through a 400 mm sieve and the resulting material will be preserved with 80% ethanol. Individuals was identified through visual and microscopic observations at genus and species level, by using the extensive malacological literature available (Preston 1915, Hyman 1967, Subba Rao 1989).

The proximate composition of the experimental diets was determined following standard methods of AOAC (1995) in Food Research Division and Animal Nutrition Division, Khumaltar, Nepal. Nitrogen was determined by the Micro-Kjeldahl method as described by Pearson (1976) and the percentage nitrogen was converted to crude protein by multiplying by 6.25. Fat content was determined by the method of Bligh and Dyer (1959). The ash (obtained from both of molluscs flesh and shells) was dissolved in standard flasks with distilled, de-ionized water containing a few drops of concentrated hydrochloric acid. After warming and evaporation to dryness on boiling water bath the ash became free of acid. After filtrating through the Whatman No. 40 filter paper, the suitable aliquots were then taken for the estimation of calcium and phosphorus.

Samples of edible gastropods and bivalves was collected for parasitic zoonosis study. Each collection was kept on ice and transported to the Animal Health Research Division, Khumaltar for examination. The anterior and posterior muscles of each mollusc was cut and the shell opened. The haemolymph, approximately 0.5 ml, was collected with a pipette from the adductor muscle into a clean well labeled test tube. The muscle was placed in 2 ml of phosphate buffered saline (PBS) pH 7.4 in an appropriately labeled test tube, washed and left for 30 min. The organs was then vortexed using a vorter mixer and removed. A drop of the sediment from mollusk was be carefully smeared on a properly labeled clean glass slide. The slides were air-dried overnight and stained using modified acid fast staining technique. The prepared slides were examined with a binocular microscope for the presence of parasites and microorganisms.

Data was collected through participatory rural appraisal (PRA) and questionnaire interview with mollusk harvester and traders (Table 1). PRA tools including focus group discussion (FGD), transect walk, daily activity and seasonally were conducted with target people. Several FGD sessions were conducted where each group size was between 4 and 6 farmers. Cross check interviews were conducted with key informants. The interview was sought to collect information and data on types of aquatic edible mollusk, seasonality, volume of trade, household consumption, price variation, ethnic value and conservation threats. Several case studies were also made with different stakeholders.

Table 1: Number of respondents by selected districts

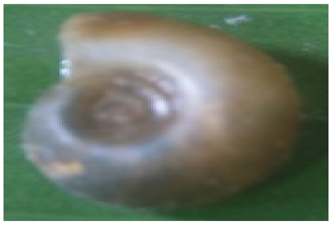
District	Number of respondent		
	Collector	Trader	Consumer
Jhapa	5	2	-
Morang	9	3	16
Sunsari	16	13	31
Chitwan	13	4	22
Banke	6	2	10
Bardiya	11	2	13
Kailali	18	5	26
Total	78	31	118

Tabulation and illustrations of both laboratory and survey data and estimation of mean of consumption related parameters and indicators was prepared using Microsoft Excel.

Results

Eight species of edible aquatic mollusks were identified with their taxonomic details. Six species of gastropods belonging to five family and the two species of bivalve represented by a single family were found to inhabit in various types of wetland across Terai region of Nepal (Table 2). The most common types of gastropod for consumption as flesh/meat were *Indoplanorbis exustus, Bellamya bengalensis* and *Thiara tuberculate.* Although the two species of bivalve were found in collection sites and market, the species *Lamellidens marginalis* (Lamarck) was most common for consumption.

Table 2: Types of aquatic mollusk with their generic name collected during field survey

Class	Family	Scientific name	Local name	Size (H x W)	Photo
Gastropod	Planorhidae	*Indoplanorbis exustus*	Goghi	H= 15mm, W= 11mm	
Gastropod	Pilidae	*Bellamya bengalensis*	Goghi	H= 28mm, W= 21mm	
Gastropod	Thiaridae	*Thiara tuberculata*	Aithawa	H= 33mm, W= 9mm	
Gastropod	Lymnaeidae	*Lymnaea luteola f.typica*	-	H= 28mm, W= 16mm	
Gastropod	Lymnaeidae	*Lymnaea luteola fovulis*	-	H= 19mm, W= 12mm	
Gastropod	Thiaridae	*Thiara scabra (Muller)*	-	H= 33mm, W= 9mm	

Bivalvia	Unionidae	*Lamellidens marginalis (Lamarck)*	Sipi, Situwa	L= 64mm, W= 35mm	
Bivalvia	Unionidae	*Parreysia favidens (Benson)*	Sipi, Situwa	L= 67mm, W= 47mm	

Proximate analysis revealed that about three-fourth of the flesh of mollusks is water by weight, ranging grossly from 72.4% in bivalve to 74.7% in gastropod (Table 3). The analysis of the nutrients results that mollusks are moderate sources of protein. Among the molluskan groups the highest amount of protein (16.2±1.3) was recorded from gastropod and lowest (11.0±0.9) for bivalve (*Lamellidens marginalis*). The present study showed that these mollusks are a negligible source of fat. Crude fat and fibre content was low in both gastropod and bivalve ranged from 0.6 to 1.0% and 0.3 to 0.4%, respectively.

The analysis of the minerals in flesh of different molluskan species results that mollusks are good sources of minerals (Table 3). The mean calcium content in gastropod and bivalve flesh was 174.2 and 204.2 mg, respectively, per 100 g dried sample. There was a large quantity of calcium in molluskan shells and the mean value is 909±50 mg per 100 g sample. The mean value of iron content per 100 g of dried sample (95.6±4.3 mg) in muscle was significantly ($P<0.05$) higher than the mean value of iron (7.3±1.5 mg) in shell.

Table 3: Proximate composition flesh and shell of gastropod and bivalve

Nutrients	Flesh/muscle		Shell	
	Gastropod	Bivalve	Gastropod	Bivalve
Moisture, %	74.7±2.7	72.4±1.8		
Dry matter, %	25.3±0.8	27.6±1.1		
Crude Protein, %	16.2±1.3	11.0±0.9		
Crude fat, %	1.0±0.2	0.6±0.1		
Crude Fibre, %	0.3±0.07	0.4±0.06		
Total ash, %	7.6±0.4	7.2±0.2	71.2±3.6	77.7±4.1
Iron (mg/100 g)	98.7±6.1	92.6±4.4	6.3±0.3	8.4±0.2
Calcium (mg/100 g)	174.2±11.3	204.2±8.7	873.3±18.4	944.8±10.7

Microscopic observations unrevealed the presence of protozoan parasites and helminths in the seasonal samples of both gastropod and bivalve. However, the presence of several species of pathogenic bacteria was evidenced in the gastropod samples (Table 4). According to the mollusk harvesters and meat consumers, they did not find any diseases in mollusks. Their ability to identify diseased animal was very poor.

Table 4: Presence of pathogenic organisms in mollusks sampled during the survey

Organisms	Mollusk	
	Gastropod	Bivalve
Protozoan parasites	—	—
Helminths	—	—
Microorganisms		
Websiella spp	+	—
Salmonella spp	+	+
Becillus spp	+	—
Kiebsiella spp	+	—
Proteus spp	+	—
Pseudomonas spp	+	+
Staphylococcus spp	+	—

Mollusk harvesters were mainly the tribal community of Terai region and they harvest by hand and nets from ponds, irrigation canals, shallow steams, inundated paddy fields and riparian areas of rivers. The major community involved in mollusk value chain market was Tharu, Statar, Mushahar, Jhangar, Santhal, Rajbanshi and others (Figure 2). Field survey showed that mollusk consumption was dominated by Tharu (31%), Satar and Santhal (13% each), Tamang (11%) and others (16%).

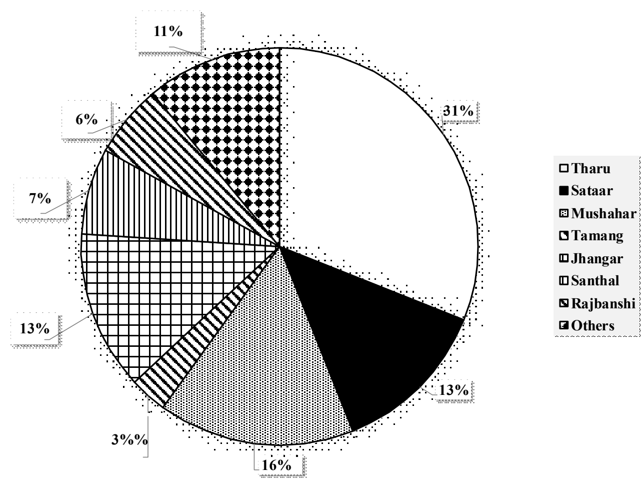


Figure 2: Major consumers of aquatic mollusk (percent of respondents)

Mean consumption of mollusk by consumer respondent was 42.6 kg/house hold and 6.8 kg/caput in surveyed districts. According to respondent the mollusk consumption per consumer's household was high in Chitwan (61.7 kg/year) followed by Sunsari (53.3 kg/year) and the lowest was in Jhapa (28.7 kg/year). Per caput consumption of mollusk by consumers' family was high in Sunsari (8.5 kg/year) followed by Chitwan (7.5 kg/year) and the lowest in Jhapa (5.9 kg/year). Consumption of mollusk was affected by the availability, propagation period of different species of mollusk and the climatic condition. Mean consumption of mollusk was high (56.9%) during wet followed by winter (21.9%) and summer (21.2%) season (Table 5).

Table 5: Consumption of aquatic mollusk by respondent in different districts season

District	Consumption by respondent (kg)		Seasonal consumption (%)		
	Household	Per person	Summer	Wet	Winter
Jhapa	28.7	5.9	-	-	-
Morang	32.5	7.2	25.0	55.0	20.0
Sunsari	53.3	8.5	14.1	57.5	30.1
Chitwan	61.7	7.5	22.0	56.0	22.0
Banke	35.4	5.7	25.0	55.0	20.0
Bardia	41.8	6.0	15.0	75.0	10.0
Kailali	45.0	7.1	24.7	42.7	29.3
Average	42.6	6.8	21.0	56.9	21.9

Harvesters reported that mollusks are abundant when the fields are inundated with water. Correlation existbetween rainfall intensity and collection of mollusk (Figure 3). Analysis of availability of mollusk in natural field and rainfall pattern showed that the collection of mollusk was high (56.9%) during wet months (May to August) and low during winter (21.9%) and summer months (21.0%).

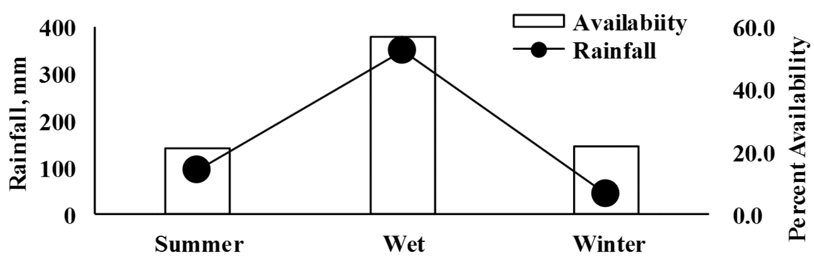


Figure 3: Relationship between intensity of rainfall and availability of mollusk

The proportion of mollusk comes from harvest and purchased was 1.5:1 across surveyed districts (Figure 4). The major sources of mollusk collection were rice field, pond, ghol and littoral zone of stream and river.The proportion of harvested mollusk in total consumption by respondent was usually largebeing highest in Kailali (75%), Banke (70%) and lowest in Sunsari (51.6%). Presence of large number of perennial wetlands in Kailali could have provided opportunity for the harvest of significant volume of mollusk.

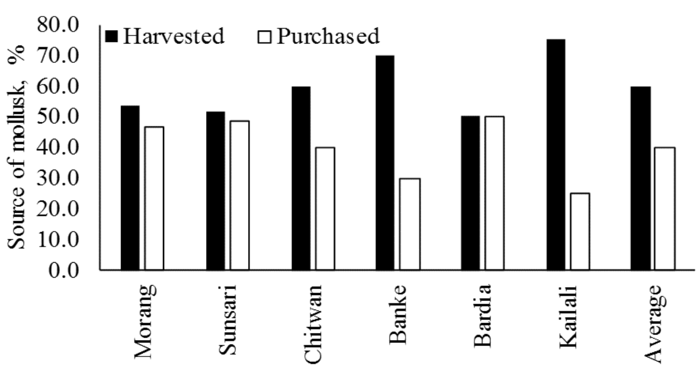


Figure 4: Proportion of harvested and purchased mollusk by consumers in different districts

Market price of aquatic edible mollusk was found to vary greatly among districts (Table 6). Observation of weekly market (haat bazar) indicated that the price usually hikes in winter and summer when mollusk harvesting is poor and the demand remains consistent throughout the year. Annual average price of mollusk was NRs 51.3±17.8 being low in Banke (NRs 30.0/kg) and high in Chitwan (NRs 83.3/kg). Most of the mollusk in Chitwan was found marketed in weekly market organized in Bharatpur sub metropolitan city where large number of consumers from diverse community over poured for limited volume of mollusk. Hence, according to the sellers' the price remains usually high in Chitwan. Unlike Chitwan, in other districts it was observed that the mollusk collected nearby wetlands are marketed in rural haat bazar and the customer mostly comprised of selected tribal community. Estimated household expenditure on mollusk including own harvest and purchased was found vary among districts which was governed by market price and consumption rate. The customers of Chitwan invest more (NRs 5139.6/HH and NRs 626.8/caput) whereas the customers of Banke (NRs 1062.0/HH) and Bardia (NRs 238.8/caput) invest lees on the purchase of mollusk.

Table 6: Market price of mollusk and annual expenditure on mollusk purchase by respondent

District	Market price, NRs	Annual expenditure, NRs	
		Household	Per person
Jhapa	45.3	1300.1	265.3
Morang	47.5	1543.7	343.1
Sunsari	46.3	2467.8	391.7
Chitwan	83.3	5139.6	626.8
Banke	30.0	1062.0	171.3
Bardia	40.0	1672.0	238.8
Kailali	66.7	3001.5	476.4
Mean±standard deviation	51.3±17.8	2186.8±210.6	350.9±18.4

A sum of 31 traders from seven selected districts of Terai were interviewed for the for the volume of mollusk trade and number of customer visit in a year. Market observations were also made to verify the information provided by the traders. According to them mollusk market comprised of 81.5% by gastropod and 18.5% by bivalve. Analysis of three representative weekly market showed that wide variation exists in the trade of gastropod and number of customer visit in a year (Table 7). Weekly market of Narayanpur, Kailali had the highest estimated volume of mollusk trade (16.1 mt/year) and corresponding number of customer visit (10000/year) while weekly market of Bharatpur, Chitwan had low volume of mollusk trade (4.0 mt/year) and number of customer (1450/year) during the year 2016. Sellers informed that the volume of mollusk traded in each weekly market was highly influenced by their availability. Disappearance of mollusk in several weekly markets during late winter and the beginning of summer was observed. In general, the estimated average sales of gastropod and bivalve (9.0 mt/market/year) and the number of customer (4600/market/year)received services from these representative weekly markets indicates the significant contribution in nutritional food security among rural community of Nepal.

Table 7: Annual sales of mollusk and number of customer in selected weekly market (haat bazar)

Weekly market	Annual sale of mollusk, tons			Number of customer/year
	Gastropod	Bivalve	Total	
Duhabi, Sunsari	5.7	1.2	6.9	2250
Narayanpur, Kailali	13.8	2.3	16.1	10000
Bharatpur, Chitwan	2.5	1.5	4.0	1450
Average	7.33	1.67	9.0	4600

Annual consumption of mollusk and corresponding intake of protein in surveyed districts was estimated based on per capita consumption of respondent (Table 5) and percent of population (tribes & ethnic community) in the district that prefer mollusk meat. Estimated mollusk consumption was ranged between 281.5 to 1100 metric tons per year being highest in Kailali and lowest in Banke. Supply of mollusk protein estimated by averaging the protein content (14.5%) of gastropod and bivalve followed similar trends of mollusk consumption (Figure 5). Total protein intake in the form of mollusks meat was estimated to be 405.7 metric tons in these seven districts.

Average availability of mollusk protein and fish protein to the whole population of each district was compared (Table 8). The availability of mollusks protein (0.34g/day/person) exceeded the protein availability from fish (0.27g/day/person) in Kailali district. Mollusk contributed the lowest (10.4%) and the highest (55.7%) percentage of protein in Chitwan and Kailali districts, respectively, to the total protein available from aquatic products. The mean protein availability from mollusk estimated was 0.23g/day/person across surveyed districts which was 23.5% of the total protein available from fish and mollusks.

Percentage contribution of protein from mollusk and fish to the daily requirement of protein of animal origin was also compared among surveyed districts. Total protein requirement for a person is 51g/day of which 15.3 g/day need to be fulfilled by animal origin (animal and vegetable protein ratio 30:70). Based on this parameter, mollusk meat contributed 2.26% in Kailali and 0.83% protein in Chitwan to the animal protein requirement (Table 8). Fish and mollusk cumulatively contributed 6.49% of daily protein requirement of animal origin and mollusk alone shared 1.5% in surveyed 7 districts.

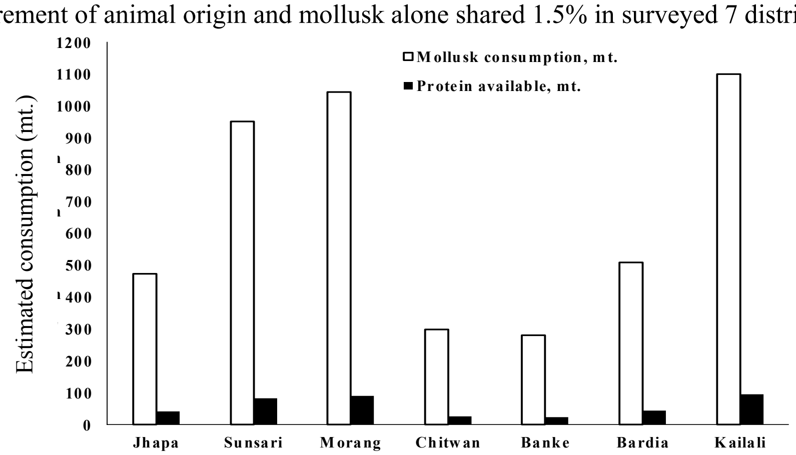


Figure 5: Estimated annual consumption of mollusk and corresponding intake of protein in surveyed districts

Table 8: Nutritional contribution of mollusk and fish to the selected Terai districts of Nepal

Attributes	Jhapa	Sunsari	Morang	Chitwan	Banke	Bardia	Kailali	Average
Population as of 2013	810636	751125	964709	566661	493017	426946	770279	683339
Fish production & nutrient contribution								
Fish Production, mt. (2015/16)	1149.1	2683.2	1280.2	1937.8	1140.8	1345.0	633.3	1452.8
Fish protein, mt	137.9	322.0	153.6	232.5	136.9	161.4	76.0	174.3
Fish protein, g/day/person	0.47	1.17	0.44	1.12	0.76	1.04	0.27	0.75
Mollusk harvest & nutrient contribution								
Mollusk harvest, mt. (present survey)	474.8	953.2	1045.1	298.5	281.5	509.9	1100.4	666.2
Mollusk Protein, mt.	41.3	82.9	90.9	26.0	24.5	44.4	95.7	58.0
Mollusk protein, g/day/person	0.14	0.30	0.26	0.13	0.14	0.28	0.34	0.23
Contribution to animal protein requirement (15.3 g/day/person), %								
Fish protein	3.09	7.78	2.89	7.45	5.04	6.86	1.79	4.98
Mollusk protein	0.92	2.00	1.71	0.83	0.90	1.89	2.26	1.50
Fish+mollusk protein	4.01	9.78	4.60	8.28	5.94	8.74	4.05	6.49

The meats with or without shell of mollusk were found sold fresh. Sellers and consumer informed that the quality of meat remain acceptable to the consumers for 2 days without any freezing and preservation, then started to deteriorate and could be detected by bad smell. According to consumer, gastropods are cooked in traditional way to prepare chatani, gravy, curry, light fry and chop dishes. The fresh gastropod with shell are left overnight in a vessel with water to get rid of the soil and waste inside the mollusk shells.Next morning, tail of the gastropod is cut which makes it easier to suck the meat out of the shell when it is cooked.Mollusk are boiled and cooked in the way other curries are cooked.Most essential part is to add the ground linseed which gives consistency to the gravy as well as enhances the taste.Mollusk is served with rice and this combination, for several communities in Terai,has been a staple food.

Figure 6: Dishes from gastropod and bivalve

The information on traditional knowledge and belief associated with eating mollusks collected during the study revealed that mollusk provide various health benefits:

- provides the immunity power to the indigenous people to fight against malaria,

- build stronger bones as mollusk contains high calcium,
- act as facial cleanser and prevent hair fall when washed with water containing soil and mucus released by mollusk,
- protein supplement to pregnant women, and
- clear the bowel movement.
-

Several threats for the conservation of mollusks was raised by the respondents and these were similar in all surveyed districts. The threats include:

- decreasing trend of natural production of mollusks,
- irregular monsoon and long drought killed hibernating mollusks,
- seasonal and permanent loss of habitat (ghols and swamps),
- climate change impact (rise in temperature),
- pesticide leachate from agricultural land,
- overexploitation of natural population of mollusks,
- neglected commodity in plan and policy,
- lack of technology for ex-situ conservation and farming exploitation, and
- highly vulnerable dependent community to adopt the conservation plan, if any.

Discussion

In the present survey only eight species of mollusk were identified from seven Terai districts (Table 2). However, Subba and Ghosh (2000) have reported that 20 edible species out of 50 aquatic species ofmollusks from an extensive survey made across Terai region.

During the field study period aquatic edible gastropod and bivalve were selected as commercially important and their nutrient values were analyzed. The moisture content recorded (74.6 to 85.9%) by Fagbuaro et al. (2006) and Baby et al. (2010)supported the findings of the present study. In the present study the recorded amounts of moisture in the samples were ranging from 72.4% to 74.7% (Table 3). The amount of proteins in the flesh of mollusks in the present study was ranged between 11.0% to 16.2% which was lower than reported protein percent (18.66% to 20.56%) by Fagbuaro et al. (2006) and higher than recorded protein percent of 6.46 % to 12.93% by Baby et al. (2010). This is the most significant difference among these two studies and the most possible explanation for the difference in protein content may be due to differences in species, region, diet and environment. A little ash was detected in the study of Fagbuaro et al. (2006) and Baby et al. (2010) whereas in the present study the ash content was found to be from 7.2% to 7.6%. However, the ash content measured in this study was corroborated with the findings of Shafakatullah et al. (2013). The mineral composition of the flesh and shell of gastropod and bivalve studied by Fagbuaro et al. (2006), Subba et al. (2009) and Baby et al. (2010) supports the findings of the present study, specifically for the proportion of calcium and iron.

Information collected from interviews and questionnaires in this study showed that eight major castes living in Tarai consume mollusks (Figure 2). Subba (2012) reported that more than 56 sub-caste totaling 25 to 30% of the Terai population involve in harvesting and consumption of mollusks. According to the census of 2011, we estimated 14% of the population in surveyed districts of Terai do consume mollusks meat. The decrease in mollusk consumers over the time could be due to the migration of non-consumers from hilly region to Terai.

The food situation of the future is dominated by discussions on possible protein deficiencies rather than the availability of carbohydrates and fats. It is therefore protein-rich food items that receive the most attention in attempts to identify alternative food resources. The poorer sections of a population can often not afford conventional protein-rich products, e.g., common meats, and have to make do with nutritionally inferior food stuffs. Mollusks can serve as an alternative and economic source of protein (Solaiman et al. 2006, Ghosh et al. 2016). Present study showed that gastropod contains 16.2±1.3% and freshwater bivalve contains 11.0±0.9% protein by wet basis which are comparable to fish. The quality of the protein depends on its amino acid composition and mollusks protein contain all the essential amino acids required by humans (Ghosh et al. 2016). The diet of Neal and South Asian countries is dominated by rice, wheat or maize, grains in which lysine is a limiting amino acid. Gastropods meat, however, can be regarded as a good source of lysine (Cagiltay et al. 2011, Ogungbenle et al. 2012). Cagiltay et al. (2011) suggested about 100g of gastropod meat can satisfy 30% of the daily essential amino acid requirements of a 75 kg person. As indicated by this and several other studies (Fagbuaro et al. 2006; Subba et al. 2009; Baby et al. 2010; Subba 2012; Ghosh et al. 2016), mollusks are also an excellent source of minerals. Iron needs to be mentioned, because it is often deficient in infants, children, adolescents and women of child bearing age, especially when pregnant. The situation with regard to iron supplies is more critical in developing (including Nepal) than developed countries.

This study also tried to understand the status of health and disease of mollusk meat consumers. In general, the consumers do not feel any problem due to eating meat rather they think that they are healthy due to consume mollusk meat. However, this study revealed the presence various pathological micro-organisms (Table 4). One of the major considerable issues related to mollusk eating is safety. Some cases of angiostrongylus infection have been reported to have possibly occurred in connection with the consumption of raw or undercooked mollusks (Shan et al. 2012, Tsai et al. 2001).Processing methods of food mollusks is important and it is recommended that the gut content of the mollusks be removed by starvation and by feeding the species with wheat bran and water for some days prior to processing (Ghosh et al. 2016).

In Nepal, mollusks are almost always directly harvested from the wild and this practice can threaten at least some species' continued existence because of overexploitation. Thus, research should focus on methods to farm the most sought-after species. Local community remained unaware of the possible ecological and economical consequences if these resources are not sustainably managed. It has been demonstrated in many instances, that often management of resources only began after resources are depleted. Proper education and convergence of indigenous knowledge of the community with the professional scientific knowledge should be harmonized for the harmonious conservation of natural resources. With this, future studies about the extent of harvesting of these commercially important mollusks should be evaluated and that policy and regulation on this issue be formulated and implemented to prevent ecological imbalance and possible depletion of these rich natural resources.

Conclusion

The present study shows that there was a total of 8 aquatic edible mollusks (2 bivalves and 6 gastropods) species found and identified in Terai of Nepal. These were valued as food, had significant contribution in daily dietary protein supply for several ethnic community and served as a source of livelihood in the area. Due to habitat degradation, inevitable increase in commercial demand and overexploitation, it may result to a possible depletion of these resources. Hence, it is recommended for future studies to develop the conservation policy of these economically important freshwater bivalves and gastropods. To face the challenge of future food shortages, mollusks farming could be of assistance. For this proposition would be awareness of the nutritional as well as economic benefits of mollusk farming, involving in particular small land holders or enterprises. Research institutions of the country need to develop farming techniques for molluskan species and bring the scientific know-how to the attention of the common people.

Acknowledgements
The authors would like to extend their heartfelt gratitude to the Animal Health Research Division, Food Research Division and Animal Nutrition Division of NARC for the assistance. We also thank Dr. Bharat Subba, Biratnagar Multiple Campus for the identification of mollusk specimen. This study was supported by Nepal Agricultural Research Council (NARC) through a research project.

References

AOAC. 1995. Official Methods of Analysis*,* 16th edition. Association of Official Analytical Chemists, Washington DC, USA. 1234 pp.

Baby, R.L., I. Hasan, K.A. Kabir and M.N. Naser. 2010. Nutrient analysis of some commercially important mollusks of Bangladesh. Journal of Scientific Research 2(2): 380-396.

Bhujel, R.C. 2007. Problems and prospects of Aquaculture in Nepal. Aquaculture and Aquatic Resources Management (AARM), School of Environment, Resources and Development (SERD), Asian Institute of Technology (AIT), Thailand.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of lipid extraction and purification. Canadian Journal of Biochemistry and Physiology. 37: 911-917.

Cagiltay, F., N. Erkan, D. Tosu and A. Selcuk. 2011. Amino acid, fatty acid, vitamin and mineral contents of the edible garden snail (*Helix* Ogungbenle *aspersa*). Journal of FisheriesSciences.com. 5(4): 354-363.

Fagbuaro, O., J.A. Oso, J.B. Edward and R.F. Ogunleye. 2006. Nutritional status of four species of giant land snails in Nigeria. Journal of Zhejiang University-Science B Zhejiang Univ. 7(9): 686-689.

FAO. 2003. World agriculture: towards 2015/2030: A FAO perspective. Earthscan. Rome, FAO. 432 pp.

Flores-Garza, R., S. Garcia-Ibanez, P. Flores-Rodriguz, C. Torreblanca-Ramirez, L. Galeana-Rebolledo and A. Valdés-González. 2012. Commercially important marine molluscs for human consumption in Acapulco, Mexico. Natural Resources 3(1): 11-17.

Ghosh, S., C. Jung and V.B. Meyer-Rochow. 2016. Snail farming: An Indian perspective of a potential tool for food security. Annals of Aquaculture and Research 3(3): 1024-1029.

Hyman, L.H. 1967. The Invertebrates: Mollusca I-Aplacophora, Polyplacophora, Monoplacophora, Gastropoda-The Coelomate Bilateria. v. 6. New York, McGraw-Hill. 792pp.

Kasprzak, K. 1986. Role of Unionidae and Sphaeriidae (Mollusca, Bivalvia) in the Eutrophic lake Zbechy and its outflow. International Revue Gesamten Hydrobiologie71: 315-334.

Keawjam, R.S. 1986. The apple snails in Thailand: distribution, habitat and shell morphology. Malacological Review. 19(1-2): 61-81.

Neumann, C., D.M. Harris and L.M. Rogers. 2002. Contribution of animal source foods in improving diet quality and function in children in the developing world. Nutrition Research. 22(1): 193-220.

Nesemann, H. and S. Sharma. 2003. Population dynamics and distribution of the aquatic molluscs (Gastropods, Bivalvia) from Nepal. Paper presented at the International Conference on Himalayan Biodiversity, Kathmandu, Nepal; 2003 February 26-28.

Ogungbenle, H.N. and B.M. Omowole. 2012. Chemical, functional and amino acid composition of Periwinkle (*Tympanotonus fuscatus var radula*) meat. International Journal of Pharmaceutical Sciences Review and Research 13(2): 128-132.

Pearson, D. 1976. Chemical Analysis of Foods. 7th edition. Churchill Livingstones, London. xii+575 pp.

Preston, H.B. 1915. The Fauna of British India including Ceylon and Burma. Mollusca(Freshwater Gastropoda and Pelecypoda). Taylor and Francis, Red Lion Court Fleet, Bombay. xix +244pp.

Shafakatullah, N., S. Shetty, R.O. Lobo and M. Krishnamoorthy. 2013. Nutritional analysis of freshwater bivalves, *Lamellidens* spp. from River Tunga, Karnataka, India. Research Journal of Recent Sciences 2: 120-123.

Lv, S., Y. Zhang, H.X. Liu, K. Yang, P. Steinmann, Z. Chen, L.Y. Wang, J. Utzinger and X. N. Zhou. 2009. Invasive snails and an emerging infectious disease: results from the first national survey on Angiostrongylus cantonensis in China. PLoS Neglected Tropical Diseases 3(2): 368.

Solaiman, M., K.A. Huq, A.R. Faruk and M.A.R. Hossain. 2006. Mollusc marketing system through value chain analysis with special emphasis on livelihood of the poor fishermen. Bangladesh Fisheries Research Forum (BFRF): 55-85.

Subba, B., K.P. Limbu and B.K. Rai. 2009. Food value of edible Molluscs of eastern Nepal. Project Report submitted to National Academy of Science and Technology (NAST), Kathmandu, Nepal. 45 pp.

Subba, B. R. 2012. Impact of climate change on food value of molluscs in Nepal. Nepalese Journalof Biosciences 2: 98-108.

Subba, B.R. and T.K. Ghosh. 2000. Some freshwater molluscs from eastern and central Nepal. Journal-Bombay Natural History Society 97(3): 452-455.

Subba Rao, N.V. 1989. Handbook of freshwater mollusks of India. Zoological Survey of India, Calcutta, India. 289 pp.

Subba Rao, N.V. and A. Dey. 1989. Freshwater molluscs in aquaculture. Zoological Survey of India, Calcutta, 225-232.

Talavera, F. and A.L. Faustino. 1933. Edible mollusks of Manila. Philippine Journal of Science 50 (1): 1-48.

Tsai, T.H., Y.C. Liu, S.R. Wann, W.R. Lin, S.J. Lee, H.H. Lin, Y.S. Chen, M.Y. Yen and C.M. Yen. 2001. An outbreak of meningitis caused by *Angiostrongylus cantonensis* in Kaohsiung. Journal of Microbiology, Immunology and Infection 34(1): 50-56.

Nepalese Journal of Aquaculture and Fisheries
Vol. 3 & 4 (2016 & 2017) : 91-96

Efficacy of veterinary drugs on coccidiosis control in rainbow trout, *Onchorhynchus mykiss*

Abhilasha Jha[1], Suresh Kumar Wagle[2*] and Swoyam Prakash Shrestha[3]
[1]Regional Agricultural Research Station, Tarahara, Sunsari, Nepal
[2]Fisheries Research Division, Godawari, Lalitpur, Nepal
[3]Animal Health Research Division, Khumaltar, Lalitpur, Nepal
*Email: waglesk@yahoo.com

Abstract
The efficacy of three anticoccidial drugs commonly used in poultry and livestock were tested for the prevention and treatment of *Emeria aurati* infection in rainbow trout (*Oncorhynchus mykiss*). Trout with an average weight of 157±29.3 g infected with the parasite were stocked at a density 24 fish/raceway of four private trout farms in Nuwakot and Dhading. The fish were fed with the respective drug treated pellet feed at 3% of biomass for seven days. The dose of drugs Prolium-K, Anvicoc and Metronidazole was 600 mg, 500 mg and 1000 mg per kg of fish biomass, respectively. Mortality of trout was significantly reduced ($p<0.05$) in fish fed with drugs treated feed after 7 days of exposure compared to control fish. The mortality of fish was 0.0%, 0.1%, 0.6% and 9.0% on day 21 after exposure with Prolium-K, Anvicoc, Metronidazole and untreated, respectively. No harboured oocysts of *Emeria aurati* were found in the mucus and epithelium of the gut and fecal samples of fish on day 7 and 21 after exposure with the Prolium-K. Result of the present study suggested that the Prolium-K and Anvicoc could be used for controlling the prevalence of coccidiosis caused by *Emeria aurati* in rainbow trout.

Keywords: Anticoccidial, coccidial enteritis, *Emeria aurati*, prevalence, rainbow trout

Introduction

The recent growth of the rainbow trout (*Oncorhynchus mykiss*) aquaculture in Nepal in intensive rearing systems, has led to increased occurrence of pathogens that cause serious problems and limit development. Parasitic diseases are of particular importance because of their high incidence in trout occasioned by the sub-tropical environmental conditions under which the farmer operates. Studies have established the economic importance of coccidiosis as a major parasitic disease of trout in Nepal (Rayamajhi and Dhital 2008). *Eimeria* is a genus of apicomplexan parasites that includes 130 species capable of causing the coccidiosis disease in fish, among other animals (Davies and Ball 1993). The most prevalent species of *Eimeria* that cause coccidiosis in trout has been identified as *E. aurati* (Rayamajhi and Shrestha 2011). *Eimeria* is a protozoan parasite affecting both marine and cultured freshwater fish that are subjected to crowding and intensive management and other factors contributing to diseases in fish, i.e. age, immune status, nutrition, and stress (Awadalla 1998). Among fresh water fish, Eimeriosis caused by *Eimeria* is a long known and rather common disease of rainbow trout (*Oncorhynchus mykiss*) and transmission of this coccidial infection to cultured fish may be direct by oral ingestion of oocyst that are shed with contaminated faces in environment (Brown et al. 1996). Symptoms of *Eimeria* infection include bloody diarrhea due to intestinal epithelium dying off when a large number of oocysts and merozoites burst out of the cells. The severity of the disease is directly dependent on the number of infective *Eimeria* oocysts that are ingested by the bovine. In light infections the damage to the gut might only be minimal and be rapidly repaired as cells are rapidly replaced by the body. However, in heavy infections it may only take two weeks for many intestinal epithelial cells to be infected with either *Eimeria* meronts or gametocytes. These cause the epithelial cells to burst, which causes significant damage to the intestine epithelial layer, and releases blood, fluid, and electrolytes into the intestine (Mass 2014). The parasite multiply in the intestine and causes tissue damage, lowered feed intake, poor absorption of nutrients from the feed, dehydration and blood loss (Seifert 2006).

High mortality rates are associated with the absence of adequate treatments for coldwater fish. In contrast to mammalian therapeutics, the use of pharmaceutical substances, particularly antiparasitic drugs, is limited in fish (Golomazou et al. 2006). Control of animal coccidians is based on the use of different coccidiostatics or coccidiocides, but information regarding fish coccidia is very scarce (Rayamajhi et al. 2010). Supercox, Amprol and Coctreat-EP, commonly used to treat poultry coccidian, have been tested to treat fish cocidia (Rayamajhi and Shrestha 2011). Emergence of drug-resistant strains of coccidian has made the anticoccidials less effective and this has threatened the economic stability of the aquaculture, especially in developing countries where the problem has become a major concern to resource-poor farmers (Hanan et al. 2009). Therefore, some of other anticoccidial sensitivity testing needs to be studied to minimize the risk of resistance build-up that has been seen in recent past with Supercox, Amprol and Coctreat-EP. Thus, the aim of this study was to recognize the drugs for the control of coccidiosis disease caused by the parasite *E. aurati* in trout.

Materials and Methods

The study on efficacy of anticoccidial drugs was carried out in four private trout farms, with series type raceways, located in Nuwakot and Dhading districts for the two consecutive years during April-May 2013 and 2014. Three vet drugs namely, Prolium-K, Anvicoc and Metronidazole were tested as they are already used as preventive anticoccidian drugs for livestock. Each selected trout farm involved for a specific drug test in Nuwakot by allocating four adjoining raceways; two as control and the other for treatment. Complete set of treatments was applied in a trout farm in Dhading.

The dose of drugs Prolium-K, Anvicoc and Metronidazole applied was 600 mg, 500 mg and 1000 mg/kg fish biomass, respectively (Table 1). The drug compounds were of commercial grade, diluted in soybean oil and coated onto commercial feed pellets (30% crude protein) by manual mixing. The inclusion level of soybean oil was 7% of the feed quantity. Approximately 6 to 8 months old rainbow trout naturally infected with coccidiosis were selected as test animal. Trout with an average weight of 157.0±29.3 g were stocked at a density 24 fish/raceway (Table 2). The stocked fish were fed with the respective drug treated pellet feed at 3% of the biomass of fish for seven days. Fish in control raceway were fed a regular non-medicated diet. Control fish were fed the same diet mixed with the same quantity of soybean oil. The diets were freshly prepared before feeding and fed to the fish by hand.

Table 1: Active ingredients, trade name and manufacturers of anticoccidial agents tested for efficacy in controlling eimeriosis caused by *Eimeria* species

Generic or Chemical name	Trade name	Composition	Route of application	Use level (mg)
1-(4-amino-2-n-propyl-5-pyrimidinylmethyl)-2-picolinium chloride hydrochloride	Prolium-K	Amproliun+ Vitamin K	Feed	600
1-(4-amino-2-n-propyl-5-pyrimidinylmethyl)-2-picolinium chloride hydrochloride	Anvicoc	Amprolium	Feed	500
$C_6H_9N_3O_3$	Metronidazole	Metronidazole	Feed	1000

Table 2: Raceway and fish size, drug dose and feeding strategy

Attributes	ProliumK	Anvicoc	Metronidazole
Raceway area, m^2	16.4	13.5	12.4
Fish size, g	182.0	125.0	165.0
Stocking Density, fish/m^2	26.5	22.2	22.0
Feeding rate, % of biomass	3	3	3
Drug dose, mg/kg of fish	600	500	1000
Feeding days	7	7	7

Three replicate intestine and fecal samples were collected before exposure (day 1), at the end of exposure of drugs (day 7) and 15 days after exposure (day 21) from each experimental unit. Fecal sample was collected by hand squeezing near to the anal opening of the fish. The fish was sacrificed and incised to collect anterior and posterior part of the intestine. Both fecal and intestine samples were separately kept at 2.5% Potassium dichromate solution for sporulation at room temperature. Presence of parasite *E. aurati* in fecal and intestine samples was examined under a compound microscope in Animal Health Research Division (AHRD), Khumaltar. Mortality and the presence of clinical signs were recorded daily throughout the experiment.

Analysis of variance (ANOVA) was used to test for statistically significant changes in mortality rate over time and among drug groups. None Parametric test (NPT, χ^2) was used to compare differences in presence of parasite in intestine and fecal samples over the time. Analyses were performed with the SPSS V20.0) and differences were considered statistically significant when $p<0.05$.

Results

Although, variation of fish size (125.0 to 182.0 g) for drug treatment among farms was large, fish irrespective of size and management were found infected with coccidiosis at various degree of infestation at the beginning of drug treatment. Mean prevalence rate of coccidiosis in trout at the beginning of drug exposure was 26.9±4.5%. The prevalence rate decreased sharply in all the drug treated fish being lowest in fish fed with Prolium-K treated feed (0.3%) followed by Anvicoc (3.2%) and Metronidazole (9.1%) after 21 days of drug exposure (Figure 1). Difference in prevalence rate between treated and control fish was significant ($p<0.05$).

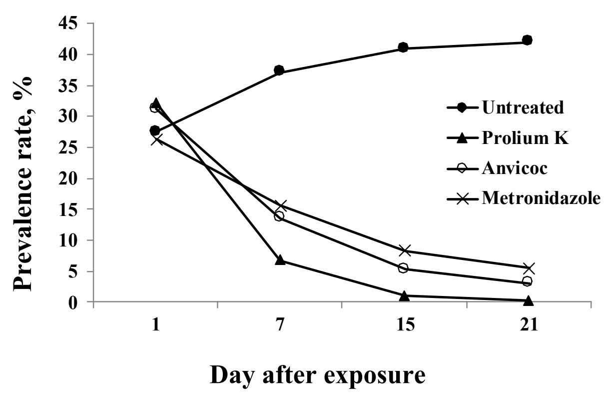


Figure 1: Coccidiosis prevalence rate in trout, *Onchorhynchus mykiss* after the exposure of anticoccidial drugs

Recovery from coccidiosis was significantly high ($p<0.05$) in fish fed with drugs treated feed over the time compared to control fish. Mean mortality rate of trout at day 1, just before the initiation of drug treatment, was 3.3±0.5% across selected trout farms (Table 2). Mortality of trout was significantly reduced ($p<0.05$) in fish fed with drugs treated feed after 7, 15 and 21 days of exposure compared to the control fish. The mortality of fish was 0.0%, 0.1%, 0.6% and 9.0% on day 21 after exposure with Prolium-K, Anvicoc, Metronidazole and control, respectively (Table 3). Although the mortality rate of fish treated with Prolium-K was 0% after day 15 and onward, but not significantly different ($p>0.05$) from the mortality rate recorded for Anvicoc at day 21.

Table 3: Mortality rate of trout in different drug treatment against coccidiosis

Attributes	Untreated (control)	ProliumK	Anvicoc	Metronidazole
Mortality % (at day 1 before drug exposure)	3.4 ± 0.3^{Aa}	3.6 ± 0.8^{Aa}	2.6 ± 0.3^{Aa}	3.7 ± 0.9^{Aa}
Mortality % (days after drug exposure)				
Day 7	5.7 ± 0.4^{Ab}	0.3 ± 0.02^{Cb}	2.78 ± 0.2^{Ba}	2.2 ± 0.2^{Bb}
Day 15	6.5 ± 0.5^{Ab}	0.0 ± 0.0^{Cb}	0.7 ± 0.05^{Bb}	0.9 ± 0.1^{Bc}
Day 21	9.0 ± 1.0^{Ac}	0.0 ± 0.0^{Bb}	0.1 ± 0.06^{Bc}	0.6 ± 0.1^{Cc}

Different superscripted letters in upper case within row are significantly different at $p<0.05$.
Different superscripted letters in lower case within column are significantly different at $p<0.05$.

Microscopic examination revealed that no harboured oocysts of *Emeria aurati* were found in the mucus and epithelium of the gut and fecal samples of fish on day 7 and 21 after exposure with the Prolium-K. None Parametric χ^2 test revealed that the results with Prolium-K were significantly different ($p<0.01$) to that of the prevalence rate found in untreated fish and treated with other drugs. *Emeria aurati* was not present in intestine samples at day 7 and onward in fish treated with ProliumK and Anvicoc while absence of the parasite was evident only at 21st day of treatment with metronidazole. In fecal sample, the parasite was detected in one sample out of three samples for Metronidazole while none of the fecal sample indicated presence of *Emeria aurati* for Prolium-K and Anvicoc treated fish at 21 day of drug treatment (Table 4).

Table 4: *Emeria aurati* prevalence in intestine and fecal samples of rainbow trout, *Oncorhynchus mykiss*

Days after treatment	Untreated (control)	Prolium K	Anvicoc	Metronidazole
Intestine				
Day 1	+ve	+ve	+ve	+ve
Day 7	+ve	-ve*	-ve*	+ve
Day 21	+ve	-ve*	-ve*	-ve*
Fecal				
Day 1	+ve	+ve	+ve	+ve
Day 7	+ve	-ve*	±ve¶	±ve¶
Day 21	+ve	-ve*	-ve*	±ve¶

¶Positive in one fish and negative in two fish out of three samples
* denotes significant difference ($p<0.05$) within row.
+ve = present, -ve = absent

On the basis of this experiment, it can be stated that the preventive feeding of two anticoccidials (Prolium-K and Anvicoc) at the doses applied consistently prevented the development of *E. aurati* infection, while the feeding of the other drug (Metronidazole) proved to be ineffective or of low efficacy. The partial efficacy of the latter drug was suggested by the observation that the infection developing in the group medicated with this agent was consistently of lower degree than that found in the former groups.

Discussion

Although coccidiosis is a long known and rather common disease of fish, little attempt has been made to treat this disease and to find a drug effective against it. Coccidiostatic drugs Supercox and Coctreat has been recommended in the past to control *E. aurati* infection in trout (Rayamajhi and Shrestha 2011). Emergence of drug resistance in coccidiosis has become a great problem (Bafundo and Jeffers 1990, Chapman and Shirley 1989) and the previously recommended drugs were of no exception. In search of new drugs, the results of present study suggest that some of the anticoccidials widely used for the therapy and prevention of coccidiosis in poultry can be used successfully for the control and prevention of coccidial infections of fish as well. In our field experiments, the feeding of two anticoccidial drugs (Prolium-K and Anvicoc) completely controlled the development of enteritis caused by *E. aurati* and provided complete protection against it. Among anticoccidials, coccidiostat or coccidiocidal drugs can be utilized. Coccidiostat serves to retard the life cycle or reduce the population of pathogenic coccidian to the point that disease is minimized and host develops immunity. Drugs with a coccidiostatic mode of action arrest the development of certain parasite stages in a reversible way and their withdrawal will still lead to the completion of the coccidiosis life cycle. In contrast, coccidiocidal drugs kill or irreversibly damage most parasite stages with no signs of disease relapse after drug withdrawal (Rayamajhi and Shrestha 2011). Some products can have coccidiostatic and coccidiocidal properties at the same time (Peek and Landman 2011). Microscopic examination of intestinal and fecal samples in the present study indicated that the coccidiostatic drugs Prolium-K and Anvicoc arrested the life cycle of oocyst and kill the parasite. Both drugs contain amprolium [1-(4-amino-2-n-propyl-5-pyrimidinylmethyl)-2-picolinium chloride hydrochloride] which is most active against several species of *Eimeria* including *E. aurati* and to lesser extent *E. maxima* (Kant et al. 2013). Absense of *Emeria* oocyst in both intestine and fecal samples of infected trout fed with Prolium-K (contains vitamin K) suggests that amprolium drug mixed with other drugs and vitamin would have synergism for better effectiveness (Bhatia 2000). Continuous use of drugs containing amprolium is resulting into the development of drug resistance which is a major problem and limiting their use (Kant et al. 2013). Studies of these two drugs in combination with sulphaquinoxaline and other drugs would be needed to prolong the effectiveness against *E. aurati* in trout.

Metronidazole (MNZ) is an antibiotic and antiprotozoal medication (Ursing et al. 1982). It is widely used to treat infections of *Giardia* in pet and other animals, although it does not reliably clear infection with this organism and is being supplanted by fenbendazole for this purpose in animals (Kraft et al. 1989). In the present study, metronidazole as an antibiotic does not stop the infection with *E. aurati* as observed in intestinal and fecal samples of trout after the exposure of this drug.

Conclusion

Results presented in this paper suggest that some of the coccidiostat/coccidiocidal drugs widely used for the therapy and prevention of coccidiosis in poultry can be used successfully for the control and prevention of coccidial infections of fish as well. From the results of the present study, it can be concluded that veterinary drugs Prolium K and Anvicoc are effective and gave promising results for controlling *Eimeria* infections in trout. Metronidazole was not effective in controlling and prevention of the Eimeriosis in trout.

Acknowledgement
Authors are grateful to trout farmers of Nuwakot and Dhading districts who participated in this study. We also thank anonymous reviewers who gave helpful comments on the manuscript. This research was supported by Nepal Agricultural Research Council (NARC).

References

Awadalla, S.F. 1998. Effect of some stressors on pathogenicity of *Eimeria tenella* in broiler chickens. Journal of Egyptian Society of Parasitology 28(3): 683-690.

Bafundo, K. W. and T.K. Jeffers. 1990. Selection for resistance to monensin, nicarbazin and monensin plus nicarbazin combinations. Poultry Science 69(9): 1458-1490.

Bhatia, B.B. 2000. Textbook of Veterinary Protozoology. Indian Council of Agricultural Research, New Delhi, India. pp. 197-207.

Brown, W.D., W.J. Sherman and D. Wise. 1996. Intestinal coccidiosis of channel catfish, *Ictalurus punctatus*. Journal of Veterinary Diagnostic Investigation 8(4): 510-512.

Chapman, H.D. and M.W. Shirley. 1989. Sensitivity of field isolates of *Eimeria* species to monensin and lasalocid in the chicken. Research in Veterinary Science 46(1): 114-117.

Davies, A.J. and S.J. Ball. 1993. The biology of fish coccidia. Advances in Parasitology 32: 293-366.

El-Sadawy, H.A., R.M. El-Khateb and M.A. Kutkat. 2009. A preliminary in vitro on the efficacy of products of *Xenorhabdus* and *Photorhabdus* spp. on *Eimeria* oocyst. Global Veterinaria 3(6): 489-494.

Golomazou, E., F. Athanassopoulou, E. Karagouni, S. Vagianou, H. Tsantilas and D. Karamanis. 2006. Efficacy and toxicity of orally administrated anti-coccidial drug treatment on *Enteromyxum leei* infections in sharpsnout seabream (*Diplodus puntazzo* C.). Bamidgeh 58(3): 157-169.

Kant, V., P. Singh, P. K. Verma, I. Bais, M. S. Parmar, A. Gopal and V. Gupta. 2013. Anticoccidial drugs used in the poultry: An overview. Science International, 1 (7): 261-265.

Kraft, M.Y., P.M. Kochergin, A.M. Tsyganova and V.S. Shlikhunova. 1989. Synthesis of metronidazole from ethylenediamine. Pharmaceutical Chemistry Journal 23(10): 861–863.

Maas, J. 2014. Coccidiosis in Cattle. California Cattlemen's Magazine. Retrieved 24 April 2014. http://www.calcattlemen.org/cca_news/california_cattleman_magazine.aspx

Peek, H.W., W.J.M. Landman. 2011. Coccidiosis in poultry: anticoccidial products, vaccines and other prevention strategies. Journal Veterinary Quarterly, 31(3): 143– 161.

Rayamajhi, A., R.R. Dhital. 2008. An evaluation on rainbow trout (*Oncorhynchus mykiss*) diseases and their control measures in Nepal. In: Proceedings of 1st National Workshop on Scaling-up of rainbow trout (*Oncorhynchus mykiss*) Farming Strategies in Nepal, NARC/JICA/DOA/NEFIS: 52-56.

Rayamajhi, A., N. Pradhan and N.K. Roy. 2010. Diagnosis and innovative treatments of coccidian (Apicomplexa) infection in Rainbow trout (*Onchorhynchus mykiss*) in Nepal. In: Proceedings of the 8th National Workshop on Livestock and Fisheries Research (June 7-8, 2010), pp. 24-25, National Animal Science Research Institute (NASRI), Khumaltar, Lalitpur.

Rayamajhi, A. and S.P. Shrestha. 2011. Isolation of *Eimeria aurati* from rainbow trout in Nepal and efficacy of different anticoccidial drugs on Eimeriosis. Nepalese Veterinary Journal 30: 59-67.

Seifert, H. 2006. Tropical Animal Health. Kluwee Academic Publishers, Boston. 57 pp.

Ursing B., T. Alm, F. Barany, I. Bergelin, K. Ganrot-Norlin et al. 1982. A comparative study of metronidazole and sulfasalazine for active Crohn's disease: The Cooperative Crohn's Disease Study in Sweden. Gastroenterology 83(3): 550-562.

Nepalese Journal of Aquaculture and Fisheries
Vol. 3 & 4 (2016 & 2017) : 97-103

Use of tamoxifen, a non-steroidal aromatase inhibitor, on sex reversal of Nile tilapia (O*reochromis niloticus*)

Narayan Prasad Pandit*, Rahul Ranjan, Ranjan Wagle, Ashok Kumar Yadav, Namraj Jaishi and Ishori Singh Mahato
Department of Aquaculture, Agriculture and Forestry University, Rampur, Chitwan, Nepal
*Email: panditnp@gmail.com

Abstract
The potential of tamoxifen, a non-steroidal aromatase inhibitor, for all-male production in Nile tilapia was assessed. Nine days post hatch fry were reared in nylon hapa placed in green cemented pond and fed with tamoxifen @ 150, 200 and 250 mg/kg diet for 30 and 40 days. In 30 days treatment group, the percentage of male fed with tamoxifen @ 150, 200 and 250 mg/kg diet were 67.6±0.5, 72.4±2.7 and 84.5±1.8, respectively, while control group showed 50.8±0.9% males. In 40 days treatment group, the percentage of male fed with tamoxifen @ 150, 200 and 250 mg/kg diet were 68.6±3.1, 74.7±1.6 and 84.8±0.8, with 51.5±0.3% males in control. In both treatment durations, the percentage for males in tamoxifen @ 250 mg/kg diet were significantly higher than control and lower dose treatments ($p<0.05$). There was no significant differences percentage of male between 30 and 40 days treatment durations ($p>0.05$). There was no significant difference in the survival of Nile tilapia fries among control and treatment groups ($p>0.05$). The tamoxifen evaluated in this study showed potential for commercial use for production of all-male Nile tilapia. Further studies are needed to determine an optimum treatment regime with this agent for induction of 100% sex reversal.

Key words: Tamoxifen; non-steroidal aromatase inhibitor; Nile tilapia; sex reversal

Introduction

Tilapias are currently the second most farmed group of fish with an annual world production of 5.57 million tons production per annum in 2016 (Fitzsimmons, 2016). This production relies on all-male populations in order to avoid pond over-crowding due to their precocious sexual maturity and continuous reproduction, associated to an elaborated parental care as well as to benefit from male's higher growth-rate (Beardmore et al., 2001). Currently male monosex populations are produced mainly by androgen (17-α methyltestosterone) treatments. The use of steroids in aquacultures is not desirable and it is avoided in many countries including US, on account of its adverse environmental effects (Baroiller, 2009). Moreover, the 17-α methyltestosterone is quite expensive and not easily available in many developing countries. As an alternative, the non-steroidal aromatase inhibitors (AIs) might be useful tools for sex reversal in fish.

Non-steroidal AIs have been widely used in studies of sex reversal in fishes since two decades. For example, fadrozole has been used for the masculinization of genetic females in many species of gonochoristic fish (Kitano et al., 2000; Kwon et al., 2000; Afonso et al., 2001; Uchida et al., 2004; Komatsu et al., 2006; Pandit and Nakamura, 2015) and sex-changing protogynous species (Higa et al., 2003; Nakamura et al., 2003; Bhandari et al., 2004; Alam et al., 2006). Unfortunately, fadrozole is no longer available commercially. Another non-steroidal AI tamoxifen has also been used for successful masculinization of Japanese medaka (Chikae et al., 2004), Japanese flounder (Kitano et al., 2007), bagrid catfish (Park et al., 2003), guppy (Chakraborty et al., 2012) and Nile tilapia (Singh et al., 2012).

The hormone estrogen plays a key role in ovarian differentiation in fish (Nakamura et al., 1998). Estrogen is synthesized from testosterone. The aromatase enzyme (P450arom) is the key enzyme for biosynthesis of estradiol-17β (E_2) from testosterone. The P450arom also involved in sex differentiation and gonadal development in teleost fish (Deng et al., 2009). Analysis of the role of estrogen in ovarian differentiation therefore is central to understanding the process and mechanism of sex differentiation in fish. Aromatase inhibitors (AIs) are chemicals that block P450arom activity, leading to reductions in the production of estrogen (Steele et al., 1987). The ability of AI to induce sex reversal in fish makes this class of chemical a valuable tool for analyzing the role of estrogen in the processes of sex differentiation and sex change.

Tamoxifen has been used in the treatment of breast and ovarian cancer in postmenopausal women. It is highly cheaper than 17-α methyltestosterone and easily available in Nepalese medical shops. There are no reports about the usefulness of tamoxifen for mass production of monosex tilapia fry in commercial scale in the Nepalese context. If this technology works, all-male tilapia fry production will be easy and cheaper. Thus, the main objective of this study was to determine the potential, dose and duration of tamoxifen treatment for mass production of monosex male population in tilapia.

Materials and Methods

This experiment was conducted at the Aquaculture and Fisheries Program, Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal. Rearing of Nile tilapia broods was done in hapa placed in a cemented pond and were allowed to breed naturally in hapa. Fertilized eggs were collected from the mouth of four females, randomly mixed, counted and placed in jar incubation system (Ranjan et al., 2015).

A total of 3000 individuals at 8 days after hatching (dah) were used, with 250 fries in each of 12 nylon hapa of 0.5 m^3 (0.5m x 0.5m x 1.0 m) size. The hapa were placed in a green water cemented tank (4.9 m x 4.5m x 1.25 m). The experiment was conducted in a completely randomized design (CRD). There were four treatments with three replications of each treatment. The treatments were:(1) Feed without Tamoxifen (control; only fishmeal); (2) Tamoxifen at the rate of 150 mg/kg feed; (3) Tamoxifen at the rate of 200 mg/kg feed; and (4) Tamoxifen at the rate of 250 mg/kg feed.

To prepare the treatment diet, Tamoxifen Citrate Tablets IP 20 mg (Cytotam 20; Cipla Ltd., India) was dissolved in 100% ethanol and mixed to the dry fish meal powder, which was then dried overnight at room temperature to completely evaporate the ethanol (Ruksana et al., 2010). For experimental treatments, fish were fed with Tamoxifen containing feed from 9 days after hatch (dah) for 30 and 40 days, whereas control groups were fed with normal dry fishmeal feed. Fish were fed four times daily (7.00 am, 11.00 am, 2.00 pm and 5.00 pm) @ 20%, 15% and 10% of the total body weight per day in first week, second week and rest of the time, respectively. Water temperature, dissolved oxygen, pH and transparency were measured weekly at 7.00-8.00 am. After 30 days of treatment, half of the fishes from all groups were removed and cultured in another hapa for 45 days feeding with normal diet, while the remaining half of fishes were continued to feeding Tamoxifen until 40 days. After 40 days of treatment, fish were reared for additional 35 days feeding with normal diet. Fish growth and survival were calculated at the end of treatment period and experimental period. Gonadal status of fish was observed by sacrifying all fishes from each group anaesthetizing with clove oil. The gonads were excised and squashed with acetocarmine to determine the sex of fish (Guerrero and Shelton, 1974).

Data were analyzed statistically by analysis of variance (ANOVA) using SPSS (version 16.0) statistical software package (SPSS Inc., Chicago). All means were given with ± standard error (S.E.).

Results

A dose dependent effect of Tamoxifen was observed on sex reversal of Nile tilapia. In 30 days treatment group, the percentage of male fed with Tamoxifen @ 150, 200and 250 mg/kg diet were 67.6±0.5, 72.4±2.7 and 84.5±1.8, respectively, while control group showed 50.8±0.9% males (Table 1). Similarly, in 40 days treatment group, the percentage of male fed with Tamoxifen @ 150, 200and 250 mg/kg diet were 68.6±3.1, 74.7±1.6 and 84.8±0.8, respectively, while control group showed 51.5±0.3% males (Table 2). In both treatment durations, the percentage for males in Tamoxifen @ 250 mg/kg diet were significantly higher than control and lower dose treatments ($p<0.05$). There was no significant difference in percentage of male between 30 and 40 days of treatment durations ($p>0.05$).

Table 1: Effects of Tamoxifen treatment at different doses for 30 days on sex ratio of Nile tilapia during gonadal sex differentiation.

Treatments	Male	Female	Male (%)
Control feed	150	145	50.8±0.9[a]
Tamoxifen @ 150 mg/kg diet	145	67	67.6±0.5[b]
Tamoxifen @ 200 mg/kg diet	144	56	72.4±2.7[c]
Tamoxifen @ 250 mg/kg diet	162	30	84.5±1.8[d]

Table 2: Effects of Tamoxifen treatment at different doses for 40 days on sex ratio of Nile tilapia during gonadal sex differentiation.

Treatments	Male	Female	Male (%)
Control feed	150	141	51.5±0.3[a]
Tamoxifen @ 150 mg/kg diet	190	91	68.6±3.1[b]
Tamoxifen @ 200 mg/kg diet	229	77	74.7±1.6[c]
Tamoxifen @ 250 mg/kg diet	242	43	84.8±0.8[d]

At the end of experiment, the survival percentage of 30 days treatment group fed with Tamoxifen @ 150, 200 and 250 mg/kg diet were 92.8±4.4, 88.0±3.2 and 84.8±4.2, respectively, while control group showed 92.4±4.6% survival (Table 3). There was no significant difference in the survival of Nile tilapia fries among control and various treatment categories ($p>0.05$). Similarly, the survival percentage of 40 days treatment group fed with tamoxifen @ 150, 200 and 250 mg/kg diet were 91.0±4.2, 87.2±3.2 and 84.6±4.1, respectively, while control group showed 91.7±4.5% survival (Table 4). There was no significant difference in the survival of Nile tilapia fries among control and various treatment categories ($p>0.05$). There was no significant difference in the weight and length of Nile tilapia fries among control and treatment groups ($p>0.05$) (Table 3 and 4).

Table 3: Effects of Tamoxifen treatment on length, weight and survival of Nile tilapia at the end of treatment period of 30 days.

Treatments	Total length	Total weight	Survival (%)
Control feed	3.3±0.0	0.6±0.0	92.4±4.6
Tamoxifen @ 150 mg/kg diet	3.2±0.1	0.5±0.0	92.8±4.4
Tamoxifen @ 200 mg/kg diet	3.4±0.2	0.6±0.1	88.0±3.2
Tamoxifen @ 250 mg/kg diet	3.3±0.1	0.5±0.0	84.8±4.2

Table 4: Effects of Tamoxifen treatment on length, weight and survival of Nile tilapia at the end of treatment period of 40 days.

Treatments	Total length	Total weight	Survival (%)
Control feed	3.5±0.0	0.7±0.0	91.7±4.5
Tamoxifen @ 150 mg/kg diet	3.4±0.1	0.7±0.1	91.0±4.2
Tamoxifen @ 200 mg/kg diet	3.6±0.2	0.7±0.1	87.2±3.2
Tamoxifen @ 250 mg/kg diet	3.5±0.1	0.6±0.0	84.6±4.1

The weekly average water temperature, dissolved oxygen, pH and Secchi disk depth of the cemented tank where the experimental hapa were set were 29.8±0.5°C, 5.7±0.2mg/L, 7.6 and 29.3±1.1, respectively (Table 5).

Table 5: Mean (±SE) and range of daily water quality parameters in cemented tank. Values in the parenthesis are range values.

Parameters	Mean±SE
Water temperature (°C)	29.8±0.5 (27.5-31.2)
Dissolved oxygen (mg/L)	5.7±0.2 (4.2-6.9)
pH	7.6 (7.3-7.9)
Secchi disk depth (cm)	29.3±1.1 (25.5-34.2)

Discussion

The present study examined the possibility of using Tamoxifen for monosex male production of Nile tilapia in commercial scale as an alternative of 17-α methyltestosterone. For the same concentration, Tamoxifen is about 8 times cheaper than 17-α methyltestosterone and is easily available in Nepal. Tamoxifen is a nonsteroidal anti-estrogen, which blocks up competitively the binding of estrogen-receptor with estrogens (Sun et al., 2007). It has been reported to be effective in inducing female-to-male sex reversal in many fish species such as Japanese flounder (*Paralichthys olivaceus*) (Kitano et al., 2000) and Southern catfish *Silurus meridionalis* (Liu et al., 2007).

In the present study, we confirmed that tamoxifen, incorporated into the food, dose-dependently induced masculinization of the sexually undifferentiated larvae in Nile tilapia. Although the treatment with tamoxifen in lower concentrations (150 and 200 mg/kg diet) significantly increased the male proportion compared to control, the percentage of males was significantly higher in high dose treatment (250 mg/kg diet) than lower dose treatments. After 30 days of treatment, the percentage of male fed with tamoxifen @ 150, 200 and 250 mg/kg diet were 67.6±0.5, 72.4±2.7 and 84.5±1.8, respectively. The finding of the present study is similar to many previous studies in different fish species. A dose-dependent increase in percentage of males was observed in bagrid catfish fed diets treated with tamoxifen where the highest dose of 200 ppm produced 90% males (Park et al., 2003). On the other hand, dietary administration of tamoxifen at a dose of 2 mg/g diet to 8 days post hatch Nile tilapia fry for 150 days have resulted in gonads with both testicular and ovarian tissue (Nakamura et al., 2004). In an immersion experiment, tamoxifen treatment of Nile tilapia juveniles with 200 µg/L for 60 days produced 90% male (Singh et al., 2012). Moreover, at high concentration tamoxifen has been found to inhibit the normal vitellogenin induction in female medaka (*Oryzias latipes*) during oral administration (Chikae et al., 2004) and immersion experiments (Sun et al., 2007). Such masculinizing effect of tamoxifen was associated with blockage of estrogen function as it competes with endogenous estradiol for binding with estrogen receptor (Liu et al., 2010), and suppression of cyp19a expression (Kitano et al., 2007).

Most of the previous studies used more than 60 days of treatment with tamoxifen with slightly higher percentage of male than the present experiment (Singh et al., 2012). However, the present study showed there was no significant differences in percentage of male between 30 and 40 days of treatment durations. This indicates that 30 days of treatment duration is sufficient for tamoxifen treatment.

Though there was no significant difference ($p>0.05$) in survival among the different treatment groups, fish fed diets containing tamoxifen showed a comparatively higher mortality than the control diet. Similar high mortality was observed in Nile tilapia after dietary treatment with tamoxifen (Nakamura et al., 2003; Chikae et al., 2004). A dose-dependent cumulative mortality was observed in *Pseudobagrus fulvidraco* fed diets treated with tamoxifen (Park et al., 2003). In *Oryzias latipes* as well, the hatchability of fertilized eggs and time of hatching were significantly delayed after exposure to high concentration of tamoxifen (Sun et al., 2007). No significant difference in the weight and length of Nile tilapia fries among control and treatment groups in the present study indicates tamoxifen feeding has no adverse effect on the growth of fish.

Conclusion

The present study demonstrated that tamoxifen can be used as an alternative of 17-α methyltestosterone for monosex male production of Nile tilapia in commercial scale. However, the dose of tamoxifen up to 250 mg/kg diet is not sufficient to induce 100% masculinization. Further experiments are needed to test the efficacy of higher doses of tamoxifen to induce high rate of masculinization.

Acknowledgements

The authors wish to acknowledge the help of the Department of Aquaculture and Fisheries, Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal for providing research and laboratory facilities. This work was financially supported by the Fish Farm (AFU) and Aqua-Fish Innovation Lab Project.

References

Afonso, L.O.B., G.J. Wassermann and R.T.D. Oliveira. 2001. Sex reversal in Nile tilapia *Oreochromis niloticus* using a non-steroidal aromatase inhibitor. Journal of Experimental Zoology 290: 177–181.

Alam, M.A., R.K. Bhandari, Y. Kobayashi, K. Soyano and M. Nakamura. 2006. Induction of sex change within two full moons during breeding season and spawning in grouper. Aquaculture 255: 532–535.

Baroiller, J.F., H. D'Cotta, E. Bezault, S. Wessels and G. Hoerstgen-Schwark. 2009. Tilapia sex determination: Where temperature and genetics meet. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 153: 30-38.

Beardmore, J.A., G.C. Mair, and R.I. Lewis. 2001. Monosex male production in finfish as exemplified by tilapia: applications, problems, and prospects. Aquaculture 197: 283–301.

Bhandari, R.K., M. Higa, S. Nakamura and M. Nakamura. 2004. Aromatase inhibitor induces complete sex in protogynous honeycomb grouper, *Epinephelus merra*. Molecular Reproduction and Development 67: 303–307.

Chakraborty, S.B., T. Molnár and C. Hancz. 2012. Effects of methyltestosterone, tamoxifen, genistein and *Basella alba* extract on masculinization of guppy (*Poecilia reticulata*). Journal of Applied Pharmaceutical Science 2(12): 48-52.

Chikae M., R. Ikeda, Q. Hasan, Y. Morita and E. Tamiya. 2004. Effects of tamoxifen, 17α-ethynylestradiol, flutamide, and methyltestosterone on plasma vitellogenin levels of male and female Japanese medaka (*Oryzias latipes*). Environmental Toxicology and Pharmacology 17: 29-33.

Deng, S.-P., S.-L Chen, J.-Y. Xu and B.-W. Liu. 2009. Molecular cloning, characterization and expression analysis of gonadal P450 aromatase in the half-smooth tongue-sole. *Cynoglossuss emilaevis* Aquaculture 287: 211–218.

Fitzsimmons, K. 2016. Supply and demand in global tilapia markets 2015. World Aquaculture Conference, February, 2016, Book of Abstracts, World Aquaculture 2016, Las Vegas, USA.

Guerrero, R.D. and W.L. Shelton. 1974. An aceto-carmine squash method for sexing juvenile fishes. Progressive Fish-Culturist 36: 56.

Higa, M., K. Ogasawara, A. Sakaguchi, Y. Nagahama and M. Nakamura. 2003. Role of steroid hormone in sex change of protogynous wrasse. Fish Physiology and Biochemistry 28: 149–150.

Kitano, T., K. Takamune, Y. Nagahama and S.-I. Abe. 2000. Aromatase inhibitor and 17α-methyltestosterone cause sex-reversal from genetical females to phenotypic males and suppression of P450 aromatase in Japanese flounder *Paralichthys olivaceus*. Molecular Reproduction and Development 56: 1–5.

Kitano T., N. Yoshinaga, E. Shiraishi, T. Koyanagi and S. Abe. 2007. Tamoxifen induces masculinization of genetic females and regulates P450 aromatase and Müllerian inhibiting substance mRNA expression in Japanese flounder (*Paralichthys olivaceus*). Molecular Reproduction and Development 74: 1171-1177.

Kwon, J.Y., V. Haghpanah, L.M. Kogson-Hurtado, B.J. McAndrew and D.J. Penman. 2000. Masculinization of genetic female Nile tilapia *Oreochromis niloticus* by dietary administration of an aromatase inhibitor during sexual differentiation. Journal of Experimental Zoology 287: 46–53.

Komatsu, T., S. Nakamura and M. Nakamura. 2006. Masculinization of female golden rabbitfish *Siganus guttatus* using an aromatase inhibitor treatment during sex differentiation. Comparative Biochemistry and Physiology Part C 143: 402–409.

Liu, Z.H., F.R. Wu, B.W. Jiao, X.Y. Zhang, C.J. Hu, B.F. Huang, L.Y. Zhou, X.G. Huang, Z.J. Wang, Y.G. Zhang, Y. Nagahama, C.H.K. Cheng and D.S. Wang. 2007. Molecular cloning of Dmrt1, Foxl2 and Cyp19 in Southern catfish and their possible roles in sex differentiation. Journal of Endocrinology 194: 223–241.

Liu, Z.H., Y.G. Zhang and D.S. Wang. 2010. Studies on feminization, sex determination, and differentiation of the Southern catfish, *Silurus meridionalis*: a review. Fish Physiology and Biochemistry 36: 223-235.

Nakamura, M., R.K. Bhandari and M. Higa. 2003. The role estrogens play in sex differentiation and sex changes of fish. Fish Physiology and Biochemistry 28: 113–117.

Nakamura, M., T. Kobayashi, X.-T. Chang and Y. Nagahama. 1998. Gonadal sex differentiation in fish. Journal of Experimental Zoology 281: 362–372.

Pandit, N.P. and M. Nakamura. 2015. Effect of Aromatase inhibitor on gonadal development and growth of female Nile tilapia (*Oreochromis niloticus*). Nepalese Journal of Aquaculture and Fisheries 2: 40-47.

Park, I.S., H.S. Oh and J.G. Koo. 2003. Effect of oral tamoxifen on growth and survival in the bagrid catfish *Pseudobagrus fulvidraco*. Aquaculture Research 34: 1471-1474.

Ranjan, R., M.K. Shrestha, N.P. Pandit and N.B. Khanal. 2015. Efficacy of common carp (*Cyprinus carpio*) testis on sex reversal of Nile tilapia (*Oreochromis niloticus*) fry. Nepalese Journal of Aquaculture and Fisheries 2: 92-100.

Ruksana, S., N. P. Pandit and M. Nakamura. 2010. Efficacy of Exemestane, a new generation of aromatase inhibitor, on sex differentiation in a gonochoristic fish. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 152: 69-74.*

Singh R., A.K. Singh and M. Tripathi. 2012. Effect of a non-steroidal tamoxifen on the gonad and sex differentiation in Nile tilapia, *Oreochromis niloticus*. Journal of Environmental Biology 33: 799-803.

Steele, R.E., L.B. Mellor, W.K. Sawyer, J.M. Wasvary and L.J. Browne. 1987. In vitro and in vivo studies demonstrating potent and selective estrogen inhibition by the nonsteroidal aromatase inhibitor CGS16949A. Steroids 50: 147–161.

Sun L., J. Zha, P.A. Spear and Z. Wang. 2007. Tamoxifen effects on the early life stages and reproduction of Japanese medaka (*Oryzias latipes*). Environmental Toxicology and Pharmacology 24: 23–29.

Uchida, D., M. Yamashita, T. Kitano, and T. Iguchi. 2004. An aromatase inhibitor or high water temperature induce oocyte apoptosis and depletion of P450 aromatase activity in the gonads of genetic female zebrafish during sex-reversal. Comparative Biochemistry and Physiology 137A, 11–20.

Print & Design : Graphic Design & Printing Support, graphic_dps@yahoo.com