Proceedings of the 2nd NEFIS International Convention on "Sustainable Fisheries & Aquaculture Diversification"

A cross-sectoral conference for livelihoods, food security, aquatic ecosystems through sustainable inland aquaculture and fisheries

March 8-9, 2018
(Falgun 24-25, 2074)

Edited by

Narayan Prasad Pandit
Neeta Pradhan

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Organizer
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Nepal Agricultural Research Council
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Foreword

I am delighted to see this volume of proceedings focusing on Sustainable Fisheries & Aquaculture Diversification. This theme has great importance for fish production in landlocked countries. The fish production from natural rivers, lakes and other wetlands as well as from aquaculture gradually facing the impacts of climate change. Therefore, to face the changes of climate change such as heating up temperature or high precipitation resulting into the flooding has to be faced by fisheries and aquaculture sector. One of the way for sustainable fisheries and aquaculture production, the adoption and innovation of newer technologies and approaches could be one of the best solution. I hope the present volume of the proceedings has addressed those all the problems of climatic and non-climatic episodes hampering the fisheries, aquaculture and associated farmers and value-chain actors for sustainable growth of fisheries and aquaculture.

I would like to express my appreciations to all the authors of the papers and taking the pain to edit those all papers into the form of the proceedings to present editorial team. Hope this proceeding would be a valuable asset of information to all fisheries and aquaculture researchers, students and developmental professional and others.

Thanks

………………………..
Dr. Tek Bahadur Gurung
President NEFIS

Acknowledgements

Nepal Fisheries Society (NEFIS) express its gratitude to Hon. Prime Minister Mr. Puspa Kamal Dahal 'Prachanda' for inaugurating the 2nd NEFIS International Convention organized in Hotel Annapurna, Kathmandu, Nepal. We are also obliged for the gracious presence of Mr. Chitra Bahadur Shrestha, the President of Kisan Ayog in the meeting. The NEFIS is also thankful to Network of Aquaculture Centers in Asia (NACA) and Food and Agriculture Organization (FAO), Kathmandu office for offering the fund to organize the international convention. Similarly, NEFIS is also thankful to Nepal Agricultural Research Council, Agriculture and Forestry University and Fisheries Development Directorate for all support.

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International Conference on Sustainable Fisheries & Aquaculture Diversification

2nd NEFIS Convention

Opening Session PROGRAM

Day One: **8 March 2018, Thursday (24 Falgun 2074**), Venue: Hotel de'l Annapurna

Convenor: Ms. Paridhi Pathak

9:00-9:30	Registration	Remarks
9:30-9:45	Chairperson: President, Nepal Fisheries Society Chief Guest: Manniya Hon. Prachanda, the President of Nepal Communist Party (Center) and Former Prime Minister of Nepal Special Guest: Hon'ble President, Nepal Farmers Commission Special Guest: Secretary, Ministry of Land Management, Co-operatives & Agriculture (MoLMCA) Special Guest: Executive Director, Nepal Agriculture Research Council Special Guest: VC, Agriculture and Forestry University Guests: Director General, Department of Agriculture Guests: Director General, Department of Livestock Services Guests: President, Nepal Agriculture Federation	
9:45-9:50	Batch distribution to the Chief Guest and Special Guests	
9:50-9:55	Welcome and Objectives of the workshop	GS, NEFIS
9:55-10:00	Inauguration of the conference by the Chief Guest Hon. Prachanda by lighting the "Panas"	Chief Guest
10:00-10:10	Felicitation of eminent fisheries professional by offering shawl and appreciation certificate to following by the Honorable Chief Guest, • Mr. Sunder Bahadur Shrestha (for his dedication and leadership) • Mr. SurendraJha (for scaling up technology of carp breeding) • Dr. Bhola Ram Pradhan (for fisheries research leadership in Nepal) • Mr. Masao Wada (for his efforts to empower fisheries research and development as a Japanese Volunteer)	
10:10-10:15	Felicitation and offering Indira Bhusal Memorial Award by the Chief Guest	

10:15-10:25	**Few Words:** • Mr. Sunder Bahadur Shrestha • Mr. Masao Wada • Mr. Garvu Shah • NACA DG • Country Representative FAO • Secretary, MoLMCA	
10:25-10:40	Remarks	Chief Guest
10:40-10:45	Token of Memory to the guests, Vote of Thanks & Closing of the inauguration	Chairperson
10:45-10:50	Hi-tea	

Technical Session 1:
Chairperson: Prof. Dr. Madhav K. Shrestha
Rapporteurs: Agni P. Nepal & Narayan Giri

10:50-11:05	Inland aquaculture diversification for sustainable food and nutrition Security in Nepal: Tek Bahadur Gurung, Madhav Kumar Shreshtha, Ram Chandra Bhujel, Neeta Pradhan, Deep Bahadur Swar, Narayan Pandit, Sunila Rai, Baikuntha Adhikari, Madhav Bahadur Panta, Suresh Kumar Wagle
11:05-11:20	Status and development trend of aquaculture and fisheries in Nepal: Baikuntha Adhikari and Prabesh Singh Kunwar
11:20-11:35	Forewarned is forearmed: The case of information prognostics for restoration of *Schizothorax* fisheries in the Dal, Kashmir, India: Neha W. Qureshi and M. Krishan
11:35-11:50	Assessment of dried carp testes for success on hormonal sex reversal in Nile tilapia (*Oreochromis niloticus*): Ruchi Shrivastav, Madhav Kumar Shrestha, Nabin Babu Khanal and Narayan Prasad Pandit
11:50-12:05	Discussion & Chairperson's remarks

Technical Session 2:
Chairperson: Dr. Tek Bahadur Gurung
Rapporteurs: Dr. Arun Baidya & Sumitra Laudari

12:05-12:20	Effect of Tamoxifen, a non-steroidal aromatase inhibitor, on sex reversal of Nile tilapia (*Oreochromis niloticus*): Narayan Prasad Pandit, Rahul Ranjan, Ranjan Wagle, Ashok Kumar Yadav, Namraj Jaishi and Ishori Singh Mahato
12:20-12:35	Ichthyo-faunal diversity of Morang district, Nepal: Bharat Raj Subba, Nelson Pokharel and Manish Raj Pandey
12:35-12:50	Dynamics of Euglenophytes red bloom algae in relation to light intensity: Ram Bhajan Mandal, Sunila Rai, Madhav Kumar Shrestha, Dilip Kumar Jha and Narayan Prasad

	Pandit
12:50-13:00	Discussion & Chairperson's remarks
13:00-14:00	Lunch

Technical Session 3:
Chairperson: Dr. Ram Chandra Bhujel, AIT
Rapporteurs: Mahesh Chand Gupta & Neeta Pradhan

14:30-14:45	Potential impact of Tanahu hydropower project on fish faunal diversity in Seti River and measures for mitigation: Deep Bahadur Swar
14:45-15:00	Lake fisheries management practices in Pokhara valley for fish biodiversity conservation and sustainable yield: Md. Akbal Husen, Agni Prasad Nepal, Surendra Prasad, Tek Bahadur Gurung, Suresh Kumar Wagle
15:00-15:15	Gonadosomatic index, egg size and fecundity of the chocolate Mahseer, *Neolissochilus hexagonolepis* (McClelland, 1839) from Tamor River, Nepal: Suren Subba
15:15-15:30	Effect of different levels of probiotic additives on growth indices and body composition of juvenile *Labeo rohita* and *Cyprinus carpio*: Saadia Tabassum
15:30-15:45	Discussion & Chairperson's remarks
15:45-16:00	Hi-tea

Technical Session 4:
Chairperson: Dr. Bharat Subba
Rapporteurs: Arjun B. Thapa & Ruchi Shrivastav

16:00-16:15	Women in aquaculture (WiA) in Nepal after a decade: Ram Chandra Bhujel, Samantha Farquhar, Nisha Khanal, Madhav Kumar Shrestha and Matthew Farthing
16:15-16:30	Creating awareness on household nutrition through school pond education program in Nepal: Dilip KumarJha, Narayan Prasad Pandit, Nabin Babu Khanal, Ishori Singh Mahato, Madhav Kumar Shrestha, Rahul Ranjan, James S. Diana, Hillary Egna
16:30-16:45	Meeting the nutritional needs for the children: the role of fish from the emerging aquaculture of Nepal: Sudha Sapkota and Sumitra Laudari
16:45-17:00	Discussion & Chairperson's remarks
	End of Day One

Day Two: **9 March 2018, Friday**

Technical Session 5:

Chairperson: Dr. Cherdsak Virapat, NACA

Rapporteurs: Umita Sah & Sano Kaji Pachhain

9:30-9:45	Does Sahar *Tor putitora* quality to be a species for aquaculture? : Madhav Kumar Shrestha, Jay DevBista, Narayan Prasad Pandit, Rahul Ranjan, James S. Diana
9:45-10:00	Aquaponics integration: Vijaya Kumar Narayanan
10:00-10:15	Supply chain analysis of carp in Makwanpur, Chitwan and Nawalpur districts of Nepal: Kamala Adhikari, Sunila Rai, Ram Bhajan Mandal, Dilip Kuma Jha
10:15-10:30	Assessment of small-scale fish supply chain in Nepal's Terai: Value chain analysis and economic viability: Narayana Giri, Madhusudan Bhattarai
10:30-10:45	Discussion & Chairperson's remarks
10:45-11:00	Hi-tea

Technical Session 6:

Chairperson: Dr. Deep BahadurSwar

Rapporteurs: Gopal Prasad Lamsal & Jageswar Yadav

11:00-11:15	Diversity of edible aquatic mollusk and their nutritional contribution in selected Terai districts of Nepal: Suresh Kumar Wagle, Abhilasha Jha, Anita Gautam
11:15-11:30	The potentials and risks of Pangasius culture in Nepal: Bhagwat Prasad, Suresh Kumar Wagle, and Bainkunth Adhikari
11:30-11:45	Spawning response of Sahar (*Tor putitora*) in Terai region of Nepal: Subhash Kamal Jha
11:45-12:00	Breeding possibility of native species *Labeo bata* (Hamilton-Bochanan, 1822) in Nepal: Hare Ram Devkota, Mahendra Prasad Bhandari, Agni Prasad Nepal
12:00-12:15	Discussion & Chairperson's remarks

Technical Session 7: Recommendation

Chairperson: Dr. Tek Bahadur Gurung

Facilitation: Ramananda Mishra & Suresh Kumar Wagle

12:15-13:00	Plenary discussion and preparation of the workshop recommendation
13:00-13:10	Presentation
13:10-14:00	Lunch
	End of Convention

Declaration of 2nd NEFIS Convention

The 2nd NEFIS International Conference was held in Hotel Annapurna on the theme of 'Inland Aquaculture Diversification for Sustainable Food & Nutrition Security in Nepal' from 8 to 9 March 2018. The conference was organized with the support of MOALMC, Government of Nepal, Directorate of Fisheries Development (DoFD), Nepal Agricultural Research Council, Fisheries Research Division, Agriculture and Forestry University (AFU), Network of Aquaculture Centers in Asia-Pacific, and FAO Nepal office.

The conference hereby concluded with following declarations:

1. In present scenario of climate change, urbanization, gender and other allied issues for sustainability of fisheries and aquaculture sustainability in inland waters diversification on suitable policy, aquaculture species, practices, value chain should be incorporated in future development to keep the pace of fisheries and aquaculture development.
2. In present context of federal government system, the fisheries and aquaculture programs should be incorporated in all federal level programs for better opportunity of food and nutrition security in the country.
3. For diversification of the aquaculture research and development programs should also focus on shell fish and plant aquaculture in areas relevant for better food and nutritional security.
4. The diversification of fisheries and aquaculture should also be prioritized at policy level intervention. In education sector, there should be faculty level status, Fisheries Research Institute as envisaged by ADS under the NARC and Division level status in the related Ministry should be implemented.
5. The fisheries research and development programs should undertake rivers and other natural water bodies for productivity and production enhancement aspects.
6. Highly productive fish species Pangas and Tilapia along with Carps should be focused as primary fish for commercial aquaculture.
7. In order to catch niche market, sahar, trout, fresh water prawn, ornamental fishes, and aquaponics should be promoted.
8. Access to quality feed, seed and other production inputs and services should be strengthened.

9. Post-harvest /value addition parts of the aquaculture should also be focused for enhancing fisheries and aquaculture in the country with potential indigenous and exotic species.
10. The aquaculture farmers should also be equally subsidized as in other commodities for food and nutrition security in the country. For example power subsidy.
11. For sustainable fisheries and aquaculture development for economic enhancement of the country the academic areas should also be prioritized in Nepal. Specifically education for mid-level technician should be initiated.
12. Global co-ordination, collaboration and linkages for fisheries and aquaculture development in the country should be strengthened.
13. Fisheries policy should be finalized as soon as possible.
14. Provision of IEE and/or EIA should be in place for all kinds of developmental activities along the water basin. Potential impacts of planned hydroelectric and irrigation, and other development projects upon fishers and fish genetic resources should be assessed.
15. Programs related to resilient to aquaculture impacted by recent avalanche, historical earthquake and flood should be considered focusing on warm water pond culture, riverine and, wetland fishery and cold water aquaculture in impacted regions.
16. R&D related to fisheries and aquaculture should be prioritized in all seven provinces and 77 districts and local government for food and nutrition security, livelihood uplift and economic development of the country, wherever feasible.
17. Introductions of new species into aquatic systems would have serious consequences on existing resources. Fisheries institutions should take steps to establish mechanisms to ensure that an objective analysis of risks precedes the introduction of an aquatic organism into national waters. Genetic, behavioral and ecological data, as well as potential for introduction of disease, should be included in the risk analysis.

18. Environmental alteration (by pollution, siltation and erosion, etc.) is generally a more important threat to the preservation of fish genetic resources than their direct exploitation. Government Institutions should make every effort to ensure that environmental damage to natural waters is minimized to protect these resources.
19. The rules and directives of International Dams Commission must be followed.
20. Restructuring of fisheries organizations to have representation at the policy level and direct reach to the farmers and fishers communities needs immediate attention.
21. Implementation of Code of Conduct for Responsible Fisheries (CCRF) which Nepal is also a signatory should also be built in national programs.
22. Ecosystem based community management of natural water bodies should be included into the national policies.
23. Fisher's right must be guaranteed with fishing/boating/use/insurance right should be guaranteed
24. Mechanism should be established with under the leadership of fisheries organizations to ensure the supply of quality fish and fisheries products to the consumers.
25. Women groups/ and cooperative, mechanization in aquaculture and fish conservation should be prioritized in R&D.
26. Fish transport, auction system in fish marketing and marketing subsidy for internalize the value chain of domestic fish product should be prioritized in plan and policies.
27. Fish Sanctuary should be established wherever needed and recreational fisheries should be promoted.
28. Revisit the present aquatic animal protection act 1961 in context of federal system of the country and comprehensive aquaculture act should be formulated with suitable implementation mechanism.
29. To accelerate the growth of aquaculture sub sector, a comprehensive project has already been submitted in Ministry needs an immediate approval
30. For fine tuning this declaration 4 members committee representative from NEFIS, DoFD , NARC and AFU will be finalized.

Contents

Aquaculture diversification for sustainable livelihood in Nepal

Tek Bahadur Gurung[1]*, Madhav Kumar Shrestha[2], Ram Chandra Bhujel[3], Neeta Pradhan[4], Deep Bahadur Swar[5], Narayan Prasad Pandit[2], Sunila Rai[2], Suresh Kumar Wagle[4]

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Abstract

Nepal is known as 'the water tower' having seven tallest peaks of the world including Mt. Everest, as the point source of many rivers flowing down from high, mid hills and Terai agro-ecological zones endowed with finfish, shellfish and edible aquatic macrophytes. These resources of inland aquaculture contribute about 2% of GDP. However, climate change, seismic activities, urbanization, small holding and youth migration etc. are main challenges to sustainability of freshwater aquaculture. To overcome these challenges diversification of aquaculture might be one of the options. Therefore, we aim to elucidate common and potential aquaculture diversification applicable to all seven provinces of federal Nepal. For that we assessed present status, research activities; published papers; with performing simple mapping analysis of agro-ecological zones of Nepal with the mean temperature zones of the world. Evidences showed that carps, tilapia and rainbow trout are the major finfish used for aquaculture. For future aquaculture diversification, inclusion of Pangas, Arctic charr, Acipenser, prawn and several others commodities might be beneficial. Developing freshwater shellfish aquaculture might also be the options along with bio flock technology, recirculating aquaculture systems and use of army insect pupae as cheaper fish feed ingredients along with newer technologies for more opportunities. Micro-nutrient rich small fish are also recommended for aquaculture diversification, wherever adoptable in all provinces. The simple mapping analysis reflected that Nepal would be feasible for freshwater aquaculture technologies used elsewhere in the world because of her vertical gradients endowed with wide array of temperature variations and similarities in several ecological parameters.

Key words: Hydropower, Recreation, Finfish, Shellfish, Mapping

Introduction

Generally farming of freshwater organisms is inland aquaculture. Nepal has unique land linked landscape surrounded by India from south, east and west and China from north having subtropical flat land averaging 64 m elevation in south to world's highest mountain on earth of 8848 m in north within a width and length of 193 km and 885 km, respectively in the areas under active monsoon zone. These mountains along with the monsoon are the main sources of abundant water resources, offering ample opportunity for diverse aquaculture practices with finfish, amphibians, reptiles, crustaceans, mollusk, algae and plants in different agro-ecological locations. The finfish biodiversity is represented by about 232 fish species including numerous small indigenous species (SIS) Nepal. The SIS are generally argued to be contributing much higher nutritive values to global communities (Thilsted *et al.*, 1996; Hossain, 1999; HLPE, 2014; Belsan, 2016; Pradhan *et al.*, 2019). Aquaculture activities could be performed in all over Nepal, especially after the technological innovation of cold water technologies. However, its mountainous landscape, climate change, seismicity, rapid urbanization, land fragmentation, small holding, youth migration and theirs compounding interactions may poses threat and challenges to sustainability of inland aquaculture (Donnelly, 2011; ADB, 2011; Troell *et al.*, 2014; Tewabe, 2015). To overcome these challenges adopting diversification of aquaculture practices might be a surviving strategy (ICAR, 2010)

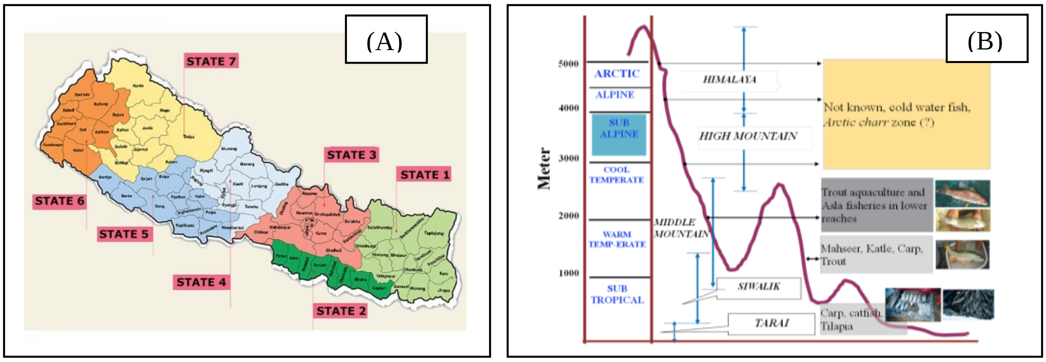

Figure 1. Map of Nepal (A) with vertical cross section of the country.

Methodology

Nepal in general is a mountainous country with the GPS coordinates of 28.3949° N, 84.1240° E having at least three layers of agro-ecological conditions along with many ecological niches and pockets with unique climatic features (Figure 1). Several research articles and reports on aquaculture in Nepal were consulted by desk work and internet search etc. Some of the preliminary information on cultivation, growth and breeding of Pangas, Tilapia, shellfish (Crab, prawn, snail and native shrimp) and summary reports were collected from published resources. To conceptualize the general idea for future potential of aquaculture diversification analysis, we obtained the air temperature distribution map of the world from internet and compared with general climatic conditions of at least three vertical layers of Nepal.

Findings

Present status of aquaculture diversification

Aquaculture is one of the fastest growing food sectors in Nepal (Gurung, 2016), having diversified fin fish farming practices limited to inland water such as cage and raceway aquaculture, fish culture in ponds in integration with, rice, poultry, horticulture, livestock (Gurung, 2012; Shrestha *et al.*, 2002). In Terai plains, fish species which are popularly used in aquaculture are Chinese and indigenous carp. On addition, recently Tilapia and cat fishes such as Pangasius and African catfish have intervened to be additional warm water aquaculture commodities.

In mid hill lakes and reservoir cage fish farming of planktivorous carp (Swar and Pradhan, 1992; Shrestha *et al.*, 2002; Gurung and Bista, 2003; Gurung *et al.*, 2005; 2009) is one of endeavors which required to be multiplied to enhance production for food and nutrition security (Funge-Smith, 2018). Recent studies have shown that SIS (Small Indigenous Species) of finfish could contribute to food and nutrition security and livelihood (Thilsted *et al.*, 1997; Rai *et al.*, 2012). More research towards the determination of nutrients and vitamins in small indigenous finfish for human nutrition would be desirable to highlight the importance of SIS. Similarly, the contribution of freshwater shellfish (snail, bivalve, crabs and shrimp) might add immense food and nutritional value in national total fisheries contribution (Gurung, 2016) as these are being consumed since ancient time among many ethnic communities in Nepal. Moreover, Nepalese finfish has potentiality to diversify towards ornamental, recreational and game fisheries to add benefits for society in multiple dimensions (Shrestha and Pant, 2009; Gurung and Thing, 2016).

It is mainly finfish (a true fish, as distinguished from a shellfish) which has been prioritized in policies and practices in union and provincial governmental agencies in Nepal. As a part of aquaculture, shellfish and aquatic plant farming have yet to be prioritized in national and provincial plans. However, shellfish and aquatic plant products are consumed since tradition in Nepal. The markets of aquatic plant products were well organized in ancient times especially across locations in Nepal bordering with Bihar of India (Jha, 2005).

The freshwater native shellfish such as shrimp, crab, mussel, and mollusk are collected from ditches, streams, canals, wetlands, lakes, ponds, rivers, rice fields as the food resources by rural women and men. The shellfish are one of primitive traditional foods of many ethnic communities' in southern Terai and mid hills of Nepal. Recently, trend of household consumption of shellfish are in increasing trend because of changing social dimension, awakening from food taboos to health and nutrition conscious population. These facts are evident that native shellfish are sold along with vegetables, meat and fish products in Kathmandu and numerous local, haat-bazar, the traditional market places of southern Terai. The prioritization of shell fish cultivation as a part of aquaculture diversification in government plans and policies might add additional opportunities for food and nutritional security in the country.

In mid hills and higher mountain, Rainbow trout farming has demonstrated to be successful enterprises (Gurung, 2008; 2010; Pokharel, 2014; Gurung *et al.*, 2017). While working with the rainbow trout, it was realized that this species do not well represent to all cold water agro ecological regions for commercial production. Instead only covering up to certain altitude and water temperature range, generally in an elevation upto 2800 meter having highest water temperature of about 12-14°C for rapid growth. The areas situated above 2800 m having mostly the water temperature range below 12-14°C demands newer species for commercial cultivation. Above 2800 m higher elevation cold water resources may be used for aquaculture, requiring cold tolerant species, such as Arctic charr for diversification. Since there are human settlements beyond 2800 m elevation, therefore opportunities prevail to utilize such agro-ecological areas to develop fish farming.

For finfish aquaculture diversification in higher altitude focus might be paid on developing cultivation technologies of three endemic species (*S. raraensis*, *S. macrophthalmus*, and *S. nepalensis*) inhabiting in Lake Rara (3600 m) as reported by Terashima (1983), Dimmicka and Edds (2002); and *Oxygymnocypris stewartii* of Lhasa River of Tibet (3,656 m) in China (Ng, 2010).

Principles of aquaculture diversification

The principle of aquaculture diversification is associated with food and nutrition security, livelihood, climate change, socio-economic development and sustainability of aquaculture. Therefore, aquaculture diversification is a tool for sustainable approach against climate change. In general, aquaculture diversification meant to reduce drudgery, support to poor and marginalized communities by promoting market opportunities of aquaculture products. Aquaculture diversification provides option associated with demand, knowledge gaps, market, business opportunities and climate change for using suitable fin- and shellfish species which might resist low or high water table, temperature, dissolved oxygen, turbidity, eutrophication and other water quality parameters in aquaculture systems.

It is indispensable that species selection for aquaculture diversification must be compatible with local agro ecological systems, national and international codes of conduct, conventions, laws and bylaws, reduction of pathogen and predator risk and promotion of the local aquatic biodiversity has been described by Pullin (1993). To be sustainable in aquaculture production, the diversification could be a useful tools and strategy, especially in countries like Nepal where climate change are likely to be major challenges for sustainable aquaculture.

Incorporation of institutional, managerial, value chain and policy diversification for aquaculture research and development would also certainly amplify the aquaculture diversification. Recently Agricultural Development Strategy (ADS, 2015) has emphasized strengthening of fisheries sector by establishing Fisheries Research Institute in the country. Such national institute is expected to be instrumental for aquaculture diversification for sustainable development supporting food and nutrition security in the country.

Policy and institutional interventions for aquaculture diversification

In Nepal, finfish has the priority in aquaculture sector; additionally there are opportunities to begin the research on shellfish farming, marketing and other value chain as 30% of total population consume varieties of shellfish in Nepal (Gurung, 2016). Earlier, Nepal has centralized unitary state; recently, the new constitution of Nepal has reformed the country into seven provincial states. This federal system might have advantages for aquaculture diversification at policy and institutional stages. For example the State No 2 might focus for investment on warm water fish cultivation and shellfish farming initiatives, while the provinces in covering mid hills and mountain areas may focus to produce cold water fish species as priority. Similarly other provinces may prioritize aquaculture diversification from their policies. It is strongly recommended that inclusion of shellfish should be initiated as one of the animal food contributors in human diets soon from provincial and union agriculture and livestock ministries.

Among the shellfish, crabs and shrimps are widely accepted as delicacies in all agro-ecological zones, while snail and mussel are consumed by majority of communities in Terai and inner foot hills. Snail as food is being popular in mid hills of Nepal. The shellfish has greater nutritional advantages as these possess high amount of Calcium (Ca) than in finfish species (Gurung, 2016). Freshwater shellfish are commercially cultivated in many countries (Cholik, 2001; Chopin *et al.*, 2011; Harvey *et al.*, 2017). Research on shellfish cultivation has been started; however, addition of these programs for promotion in national fisheries programs has yet to be initiated in Nepal for more promising aquaculture sustainability. Besides the shellfish, aquatic products such as water chest nut and Makhana are produced and harvested from wetlands of Terai and mid hills since time immortal (Gurung, 2016).

Main drives of aquaculture diversification

The primary drivers for aquaculture diversification are technologies, resilience, competitive advantages, sustainability, environment and market demand climate change, policy, market, economics and social factors (Table 1). Aquaculture diversification offers opportunities for fin and shellfish production at different agro-ecological zones for better economic, social and ecological assurance to aquaculture systems (Harvey *et al.*, 2017). At present, substantial amount of finfish are imported in Nepal (Gurung, 2014) indicating that there are ample market opportunities of aquaculture production, if suitable fish farming technologies were offered to Nepalese farmers. Recently, the Government of Nepal has declared that within few year fish production in Nepal would achieve self-sufficiency; to achieve such target the aquaculture diversification could also play much important roles.

Table 1. Main drivers of aquaculture diversifications.

Drivers	Opportunities
Climate change	Changing climate forcing to introduce and examine the potentiality of newer fish species for sustainability of inland aquaculture
Resilience	Being landlocked and mountainous country Nepal has limited opportunity to produce sustainable fish production for resilience, however, aquaculture diversity might increase the resilience opportunities
Competitive advantage	Nepal has diverse ecological niches where only limited volume of aquaculture production can be obtained, however, such niches in other hands might be advantageous offering competitive advantages for variety of fish production
Sustainability	Being mountainous scaling from 60 m elevation from sea level to highest peak on the earth aquaculture diversification is the only option for sustainable production.
Environment	Prevailing environment and agro ecology offers the immense opportunity of aquaculture diversification
Market demand	Day by the demand of fish has been immensely increased in the country almost importing fish equivalent to 3 billion each year.

Aquaculture diversification by using small indigenous species

Thilsted *et al.* (1997) and Rai *et al.* (2012) argued that small SIS are enriched with nutrients, vitamins, essential amino acids and fatty acids as an excellent sources for human health, thus such species should be prioritized for aquaculture species diversification. In general, focus on developing cultivation practices of small fish species has not been prioritized due to limited scientists and funding resources. Thus, there should be investment to develop farming practices of SIS from the government for aquaculture diversification. Generally, the advocacy for inclusion of new species in aquaculture practices should be performed by researchers and academicians. After developing technologies adaptable to local socio-economic conditions and ecology SIS commodities could be displayed into the farmer's field for adoption, uptake pathway, piloting and scaling up.

Aquaculture diversification potentialities in provinces

The Constitution of Nepal, Schedule 4 adopted on 20 September 2015 provisioned to have 7 federal states. In all these provinces there remain huge potentialities of aquaculture diversification, however, the existing aquaculture practices and potential diversification in provinces based on the agroecological zones has been shown in Table 2.

Table 2. Provinces, existing practices and future potential diversification in aquaculture.

Province No	Districts	Existing Aquaculture practices	Diversifications Potentialities
1.	Taplejung,Panchthar Ilam, SankhuwasabhaTerhathum, Dhankuta, Bhojpur, Khotang, Solukhumbu, Okhaldhunga, Udayapur, Jhapa, Morang, Sunsari	• Pond aquaculture with carp, Tilapia, catfish (Pangas and African cat fish) • Raceway aquaculture with rainbow trout • Integrated rice-fish farming an others	• Cold water aquaculture with *Arctic charr* • Shell fish farming • Pearl farming • Cage fish farming • Frog culture • Macrophyte aquaculture • Others
2.	Saptari, Siraha, Dhanusha, Mahottari, Sarlahi, Rautahat Bara, Parsa	• Pond aquaculture with carp, Tilapia, Catfish as above • Integrated rice-fish farming an others	• Shell fish farming • Pearl farming • Cage fish culture with tilapia • Frog culture • Macrophyte aquaculture • Others
3.	Dolakha, Ramechhap, Sindhuli, Kavre, Sindhupalchok, Rasuwa, Nuwakot, Dhading, ChitwanMakwanpur, Bhaktapur, Lalitpur Kathmandu	• Pond aquaculture with car Tilapia, catfish (Pangas an African cat fish) • Raceway aquaculture wit rainbow trout • Integrated rice-fish farming an others	• Cold water aquaculture with *Arctic charr* • Shell fish farming • Pearl farming • Cage fish farming • Frog culture • Macrophyte aquaculture • Others
4.	Gorkha, Lamjung, Tanahun Kaski, Manang, Mustang,	• Pond aquaculture with carp, Tilapia, catfish (Pangas and	• Cold water aquaculture with

Province No	**Districts**	**Existing Aquaculture practices**	**Diversifications Potentialities**
	Parbat, Syangja, Myagdi, Baglung Nawalparasi (east)	African cat fish). • Raceway aquaculture with rainbow trout • Integrated rice-fish farming an others	Arctic charr • Shell fish farming • Pearl farming • Cage fish farming • Frog culture • Macrophyte aquaculture • Others
5	Nawalparasi(west), Rupandehi, Kapilvastu, Palpa, Arghakhanchi, Gulm Rukum(east), Rolpa, Pyuthan, Dang Deukhuri, Banke, Bardiya	• Pond aquaculture with carp, Tilapia, catfish (Pangas and African cat fish). • Raceway aquaculture with rainbow trout. • Integrated rice-fish farming and others	• Cold water aquaculture with Arctic charr • Shell fish farming • Pearl farming • Cage fish farming • Frog culture • Macrophyte aquaculture • Others
6	Rukum (west), Salyan, Dolpa, Jumla, Mugu, Huml Kalikot, Jajarkot, Dailekh, Surkhet	• Pond aquaculture with carp, Tilapia, catfish (Pangas and African cat fish) • Raceway aquaculture with rainbow trout • Integrated rice-fish farming and others	• Cold water aquaculture with Arctic charr • Shell fish farming • Pearl farming • Cage fish farming • Frog culture • Macrophyte aquaculture • Others
7	Bajura, Bajhang, DotiAchham, Darchula, Baitadi, Dadeldhura, Kanchanpur, Kailali	• Pond aquaculture with carp, Tilapia, catfish (Pangas and African cat fish) • Raceway aquaculture with rainbow trout • Integrated rice-fish farming and others	• Cold water aquaculture with Arctic charr • Shell fish farming • Pearl farming • Cage fish farming • Frog culture • Macrophyte aquaculture • Others

To support the further aquaculture diversification, innovation on producing cheaper and nutrient rich pellet feed made up of rice, wheat & maize straw with other important additives would be a mile stone achievement. Nepal has developed the technological capacity to produce dry pellet feeds by the year 1993 (Gurung *et al.*, 2017) Some recent technological interventions for example use of black soldier fly (*Hermetia illucens*) maggots as protein sources in fish feed has been achieved elsewhere. It is learned that South African entrepreneur have earned millions US$ from maggot production for fish feed (Baker, 2015).

In general, it seems there are rarely identified finfish species which can perform in cool water (min 15 to max 25°C water temperature in pond conditions) regions of Nepal. For such water range conditions it is assumed that freshwater sturgeon (*Acipenser* sp.) might perform well, however, the target product of such aquaculture diversification should prioritized to be the high value caviar over the meat.

The other high important aquaculture diversification potentiality in near future would be prawn (*Macrobranchium* spp.) and pearl farming in most provinces of Nepal. Experimentation on pearl cultivation using the indigenous bivalve species has already been started. The experiment at Pokhara Fisheries Research Station showing positive signs of pearl deposition in the shells, such diversification could be applied in most provinces bordering with southern warmer and mid hill mountain areas.

Constraints of aquaculture diversification

- Cost involvement in new species research and development activities
- Long time involvement to develop aquaculture diversification
- Regulation and biodiversity laws
- Area for farming could be limited with increasing aquaculture diversification
- Limited trained human resources
- Issues of diversification on environment and ecosystem

Way forward

Many studies (Paudel, 2016; Gurung, 2017) revealed that Nepal is endowed with diverse agro-ecological conditions. Such agro-ecology is comparative to diverse global agro ecological conditions available in the world (Figure 2). Ensuring that Nepal could adopt the aquaculture technologies practicing elsewhere as has been projected using simple mapping analysis based on the temperature suitability. In general, because of higher altitudinal gradient, Nepal is known to be representing most of average annual temperature range found in worldwide. Therefore, we assumed that the aquaculture technologies developed elsewhere could be adopted with further research and verifications in specific local conditions.

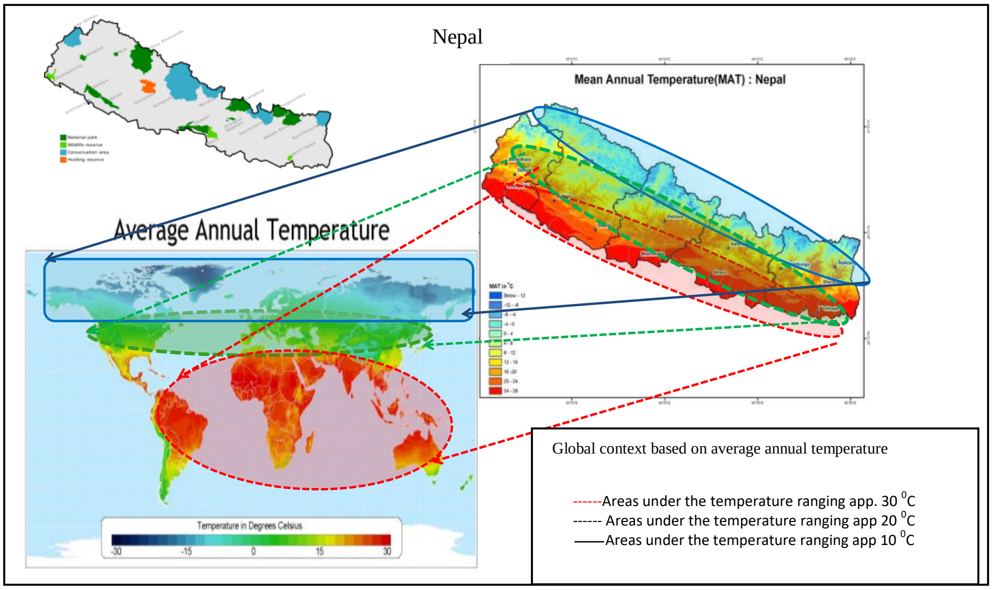

Figure 2. A comparative agro-ecological similarity based on average annual temperature worldwide and Nepal.

Recently, Song *et al.* (2018) suggested that inland fisheries are closely impacted by other essential human activities such as hydroelectricity generation, irrigation activities, agricultural run-off etc. Therefore, an understanding of aquaculture interactions with many other sectors would be pre requisite for intersectoral governance for achieving sustainability.

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Women in Aquaculture (WiA) in Nepal after a Decade: Lessons Learned

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Abstract

Fish farming practices have been expanding in neighbouring villages in and around Kathar, Chitwan where a small-scale aquaculture project called "Women in Aquaculture (WiA)" was initiated with 26 farmers in 2000. Many households started their farms independently, without external support indicating that it has real impacts in the community. An updated assessment of the socioeconomic impacts of household aquaculture on women's livelihoods was needed. A household survey was therefore conducted from January to April 2017 in which 71 women from Kathar village of Chitwan were interviewed in order to investigate the impacts of the Women in Aquaculture (WiA) project after a decade and half of the its completion. Results show that fish farming families consumed significantly more protein per year—an average of 50 kg more - compared to non-fish farmers. Additionally, fish farmers generated an additional mean of $265 in net profit, almost 40% of the per capita GDP, annually from the selling of fish. Women in the fish farming cooperative reported feeling increased levels of happiness, self-confidence, and an increase in their education and skill set. Even after the essential WiA project ceased, aquaculture practice continued to spread throughout the village. Non-project supported fish farmers started their farms after seeing their neighbours and their farms became just as successful. Overall, the WiA project is deemed a success due to its long-lasting socio-economic impacts and further expansion; it should undoubtably serve as a role model for further development efforts in Nepal.

Keywords: Women in aquaculture, Fish farming, Small-scale aquaculture, Nutrition security, Socio-economic impacts

Introduction

Nepal is a land-locked country with the population of nearly 30 million. It is sandwiched between two giant neighbours; China and India. Because Nepal is well-known as a Himalayan and high terrain country, there is a common perception among the international communities that aquaculture is not suitable for the country and it can't play a major role in terms of contributing to the UN's sustainable development goals (SDGs), especially related to poverty (SDG1), hunger (SDG2), health and wellbeing (SDG3), gender equality (SDG5), employment (SDG8), life below water (SDG14) among others. According to ADB data (ADB, 2019), only about 20% population resides in the urban areas and the remaining population living in peri-urban and rural areas are dependent on agriculture-based livelihoods. About 20% of the population is still below national poverty line. There are rare opportunities of employment the rural areas as a result many young and adults have to migrate to cities or abroad to work. About half of the population, struggle with malnourishment. Nearly one-third households have limited food consumption (Gurung, 2016). About 36% children under-5 suffer from malnutrition in Nepal (ADB, 2019). These facts and figures indicate that the country is in urgent need of concrete actions if the government is committed to achieving the SDGs by 2030 set by the United Nations.

Most families in rural Nepal face food shortages and unbalanced diets. Food security of the people in Nepal depends mostly on subsistence crop and livestock farming methods. Productivity of which is substantially low. More alarmingly, effects of global climate change have further decreased the productivity of traditional agriculture (Bhujel *et al.*, 2008). As a result, malnutrition is rampant in Nepal. The most common forms are short supply of protein, iodine, iron and vitamin A (Rai *et al.*, 2014). The national average protein consumption is only about 10%, which is considerably lower than the 33% recommended for proper nutrition (AIT, 1994; Bhujel *et al.*, 2008). The effects of malnutrition and food insecurity are especially evident amongst Nepali women and children. Over 36% (approx. 1 million) children under the age of 5 years are stunted or severely stunted, whilst 36% of women aged 15-49 are anemic (World Bank, 2016). Therefore, Nepal needs effective and sustainable local solutions to these problems. Fish is the best source of animal protein and can be caught in rivers, rice fields, lakes, and swamps but concerns grow as wild stocks are declining. The per capita consumption of fish is about 3 kg/year, which is about seven times lower than the world average (Bhujel, 2014; Gurung, 2016).

The government of Nepal has been promoting aquaculture as it has been recognized as a popular tool to combat household malnutrition and low economic opportunity (Shrestha *et al.*, 2012). Aquaculture is growing at 8.4% annually, producing over 80,000 mt. However, over 90% of the aquaculture activities are in the Terai region. About two-third country is covered by mid-hills where aquaculture has not been expanded. There is potential for the expansion of small-scale aquaculture in mid-hills especially with the involvement and empowerment of women to combat malnutrition in Nepal. Some efforts e.g. trout farming and other efforts e.g. Aquaculture without Frontiers (AwF) are being expanded, but more is needed (Bhujel *et al.*, 2014; Rai *et al.*, 2005). Aquaculture is limited mostly to traditional culture of carps. Over 95% production comes from Chinese carps, Common carp and Indian carps (Bhujel *et al.*, 2008). Recently, Nile tilapia and Pangasius have become popular because they grow fast and their meat has no small bones. As only finfish are cultured in Nepal, they are limited to pond culture, except few lakes and reservoirs. Rainbow trout was introduced to utilize the cold water of mountainous areas (Rai *et al.*, 2005) but its volume is still small i.e. less than 1,000 mt/year. Whereas in other Asia countries especially China, India, Indonesia, Bangladesh, Thailand, Vietnam and others have considerable development of aquaculture where its contributions to SDGs are well recognized and high priority is given to it in their national plans. They are the leading countries in the world. Whereas in Nepal there its potential is yet to realize. Hence, more efforts similar to WiA and others are needed to justify its role and highlight its need. Present paper and the information provided should be useful to policy makers, researchers and educators.

Brief history of the project

Globally, aquaculture was expanding since 1980s, and many countries were giving high priorities. Commercial aquaculture was booming. However, small-scale aquaculture projects were considered failures as they rarely succeeded in addressing the problems of poverty and food insecurity. In Nepal, ADB/N had launched project in 1980/81 which required minimum size of 2,000 m^2 pond to be eligible for loans and technical support. The average size of land holding in Nepal is about 5,000 m^2, thus people could not consider digging a pond that would occupy 2,000 m^2 just to join the project. Therefore, small-scale farmers, who are the majority, were left-out and aquaculture was only considered suitable for more prominent landowners who could afford to give up their land for fish farming. With the goal of developing a replicable model of small-scale aquaculture and promoting it, the project team recommended 200 m^2 ponds as an ideal size for the engagement of Tharu women in Kathar Chitwan. The Tharu community has traditionally caught fish from swamps, streams, rivers, and rice fields. They consider fish as a good sign of luck and it is commonly offered to family guests. However, the Tharu community began facing the problem of decline in catch. Thus, aquaculture was thought to be a culturally appropriate and relevant livelihood diversification tool. The 2-year pilot project entitled, “Women in Aquaculture (WiA)” was launched in 2000 jointly by the Asian Institute of Technology (AIT), Thailand and the Agriculture and Forestry University (AFU), formerly, Institute of Agriculture and Animal Sciences (IAAS), Chitwan, Nepal (Fig 1, Bhujel *et al.*, 2008). It was also encouraged at various levels by the Directorate of Fisheries Development (DoFD) and Nepal Agricultural Research Council (NARC). Together professionals from AIT, AFU, DoFD, and NARC acted as the implementing Project Team. The project was funded by the German NGO, “Women's World Day of Prayer” (WDP) with the objectives of improving rural food security, generating supplemental income, and empowering ethnic women by forming groups for small-scale fish farming. After providing training and covering 50% of the digging cost, 26 women constructed new ponds. The project achieved its targets of producing an average of 78 kg fish per family per year, out of which 21 kg (60%) was consumed by each family and the remaining 40% was sold to generate an average income of US$33 per family. The average size of the pond was 175 m^2 and the productivity was 4.5 ton/ha/year which largely contributed to local food security and income generation. More importantly, the project taught new skills and was well-received by the community. Therefore, WDP extended the second phase (another two years) during which additional 53 ponds were constructed which included the expansion to Kawasoti village of an adjacent district, Nawalparasi. Despite the success, WDP had to stop their funding after second phase as their rule.

The Project Team managed to carry on the project further with support from the Canadian Cooperation Office (CCO) in Kathmandu. With this new support, efforts were made to introduce and develop prawn culture, and to establish women's cooperatives which can create revolving funds for their groups’ long-term sustainability. By 2007, three cooperatives were formed, 30 additional ponds were constructed, and prawns were successfully farmed with the introduction of post-larvae from Thailand. At the end of 2007, the CCO's support also ended, and the women of Kathar were left to manage their ponds on their own through their cooperatives. Despite the lack of outside assistance, small-scale aquaculture activities spread to other nearby communities. Over 120 fishponds belonging to 106 households in Kathar village of Chitwan alone were reported in 2011. Since then, fish farming has also been observed in neighboring villages without any external support. More groups, relatives of project farmers and other individuals, adopted the small-scale aquaculture practices as they saw the benefits. However, no comprehensive study was done so far to assess this increasing trend of aquaculture. Therefore, there was a need to assess the impacts, benefits, and sustainability of the WiA project idea in the communities beyond the project period. More importantly, an assessment of the socioeconomic benefits to the society and the impacts women's livelihoods was required as well as the need to identify problems and needs so that the expansion of small-scale aquaculture could continue.

Figure 1. A farmer expanding his pond (left) and women's group harvesting fish (right)

Objectives of the study

The main purpose was to assess the production, consumption, and income generated, and of the overall changes in socioeconomic status from the WIA project. More specifically, it was to see whether there were any differences between project-supported and non-project-supported fish farmers in terms of pond size, fish production, yield efficiency, family fish consumption, fish sales, and net income after about a decade.

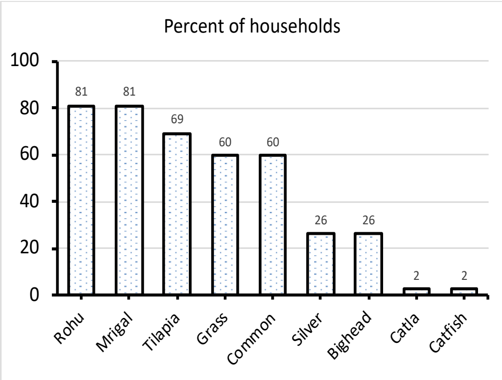
Methodology

A field survey was conducted over the period of four months i.e. January - April 2017 using semi-structured questionnaires. Researchers traveled to Kathar village and went house-to-house asking to speak to individual women household heads who had fish farms on their property. A total of total 71 respondents residing in Kathar village, Chitwan, Nepal were interviewed, out of them, 41 farmers were project supported and 30 were non-project supported. In order to compare the descriptive statistics of pond area, fish production, consumption, sales, costs, and incomes were initially compiled in the MS Excel. Family income from fish sales was initially collected in local currency (Nepali rupees, NPR) and then was converted to US dollars using an average exchange rate (USD1=NPRs104) of the data collection period.

Key Findings

Results of the survey revealed that at least 54% farmers interviewed started their fishponds in 2000 i.e. the first year of the project launching, while the remaining ponds were constructed during 2001-2015. Average size of ponds was found to be 437 m^2 (range 120-2,664 m^2). Fish farmers who did not receive project support had larger ponds (477±143 m^2, Mean±SD,) than those supported by the project (423±110 m^2) as shown in Table 1. Rohu (*Labeo rohita*), Mrigal (*Cirrhina mrigala*), Nile tilapia (*Oreochromis niloticus*), Grass carp (*Ctenopharyngodon idella*), Common carp (*Cyprinus carpio*), Silver carp (*Hypophthalmichthys molitrix*), Bighead carp (*Aristichthys nobilis*), Katla (*Catla catla*) and catfish (*Clarias* sp.), were the species cultured by the farmers. They grew 1-7 species, 93% of them were having more than three species together in single pond. Most farmers with no project support cultured five species, while most project-supported farmers cultured four. The most commonly cultured species amongst all fish farmers were Rohu and Mrigal (81% each, Fig 2, left) followed by Nile tilapia (69%), Grass carp (60%) and Common carp (60%). Tilapia and catfish were culture mostly using monoculture.

The most common feed ingredients used by all farmers were mustard oil cake (95%) and rice bran (90%), followed by rice (29%) and banana leaves (26%) as shown in Figure 2 (right). The number of feeds per day ranged from one to three, with an average of 1.6 (±0.5 SD). There was no considerable difference in the feeding practices between project-supported and non- project-supported farmers. All farmers acquired seed stock from the Bhandara Government Farm, Chitwan apart from one tilapia farmer who was producing his own seed stock assisted by the Institute of Agriculture and Animal Science (IAAS). At least 24% of all fish farmers interviewed said that the transportation and accessibility of seed stock was the most difficult problem in maintaining their fish farm. Fish production per family ranged from 15 to 1,200 kg/yr for all fish farmers.



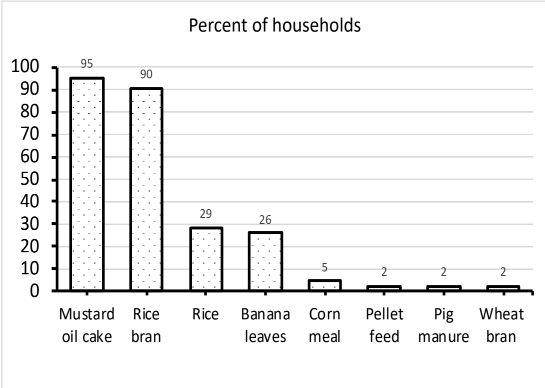


Figure 2. Percent of households having different fish species (left) and feed ingredients used (right)

Table 1. Summary of results of the survey (Mean and Standard Error, SE) (Source Farquhar et. al., 2019)

Parameters / Indicators	No./Mean ± SE	Project Supported Fish Farmers	Non-Project Supported Fish Farmers	All Fish Farmers	Non-Fish Farmers
Women farmers interviewed	(n)	30	11	41	30
Cooperative members	(n)	30	9	39	-
Total no. of ponds	(n)	32	15	47	-
Ponds per family	Mean	1.1	1.0	1.1	-
	SE	0.0	0.0	0.0	-
Pond size (m^2)	Mean	423	477	437	-
	SE	110	143	14	-
Fish production (kg/yr)	Mean	129	109	123	-
	SE	38	81	28	-
Yield efficiency (kg/yr/m^2)	Mean	0.35	0.26	0.33	-
	SE	0.01	0.01	0.00	-
Fish consumption (kg/yr)	Mean	78	70	76	30
	SE	6	8	5	6
Fish sales (kg/yr)	Mean	82	74	80	-
	SE	37	25	28	-
Net profits (NRs/yr)	Mean	29,954	21,113	27,581	-
	SE	8,366	4,652	6,165	-
Net profits (US$/yr)	Mean	289	203	266	-
	SE	81	45	59	-

Note: Net profits were calculated deducting labour and feed costs from revenue generated.

The average fish production per family was 123 kg/yr for all fish farms (Table 1). The farmers supported by the project had 18% higher average fish production per family (129 kg/yr) than farmers with no project support (109 kg/yr). Productivity of the ponds of the farmers (3.5 ton/ha/yr) who received project support was 35% higher than pond of the farmers (2.6 ton/ha/yr) who did not receive the support. Average fish consumption of fish farming families (80 kg/yr) was 50 kg higher i.e. approximately 2.5 times more than that of non-fish farmers (30 kg/yr). Project-supported households consumed 12% more fish per year than non-project-supported households (Table 1). Nearly one-third (29%) of the households who had fish farming consumed more fish than they produced. All the fish farmers reported seeing health benefits from consuming fish. The price of fish was species dependent, ranging from NPRs 200-300 (US$1.9-2.9)/kg. Only 7% of all fish farmers sold more fish than their household consumed per year. At least 77% of fish farmers reported selling their fish to neighbors or to the market. About 22% of fish farmers reported that they did not sell any fish as their families consumed. The families which consumed all, actually saved NPRs 15,000 (US$ 144)/year, if they had to buy from the market.

The survey also revealed that the average cost to start a household fish farm was NPRs 9,524±5,666 (mean±SD) or US$92. The average amount of financial startup support provided by the project to supported households was NPRs 2,747±658 (approx. USD 26) which was approximately one-third (29%) of the star-up cost. All the surveyed farmers except one were members of cooperatives. Less than half (42%) of fish farmers had taken a loan from the cooperative, with the average amount being NPRs 28,667±19,452, approx. USD 276). The average cost for monthly maintenance was NPR 3,017 (approx. USD 29). On average, fish farmers spent less than 1 hour working on the fish farm per day (52±26 minute). Nearly, 90% of participants spent 1 hr or less working on their fishpond per day. Non-fish farmers typically spent 4-12 hrs working on agricultural activities per day. In addition to working on their fish farms, 80% of participants reported working on household activities and 73% also engaged in other forms of agricultural work. Agriculture was the primary source of income amongst all fish farmers, apart from three individuals who had other businesses or employment. Over 90% of fish farmers explicitly reported that fish farming had helped them economically. On average, each fish farming household earned NPRs 27,581 (approx. USD 265) in net profit per year. Farmers spent this supplemental income mainly on children's education (33%) and to buy agricultural inputs (33%).

Almost two-third (65%) of the participants interviewed were from the Tharu ethnic minority followed by 23% from the Newari and 12% others. All women indicated receiving help from family members and felt happy owning a fish farm. Over 92% of fish farmers were part of the local cooperative which encouraged a sense of community coherence, built individual’s self-confidence, and gave the women opportunity to access the training workshops to develop skills. At least 40% of fish farmers interviewed indicated that they would like to expand fish farming either by making their existing pond(s) bigger or adding more ponds. However, half of the respondents did not have land available. More interestingly, two-thirds (66%) of non-fish farmers indicated that due to lack of land, they had not tried fish farming, while 10% said it was due to a lack of knowledge. At least 37% of non- fish farmers indicated that they would like to learn more about fish farming and the support given to fish farmers. Among those who have ponds, water scarcity was the most common problem (86%), followed by fingerling transportation (26%), predation (21%), and market competition (21%). Access to quality fish feed, theft, and fish diseases were also minor problems pointed out some farmers.

Discussion

In Nepal, the common sources of protein are vegetable sources i.e. lentils, milk and curd on a regular basis. Mutton is highly preferred choice among the animal protein sources. Its demand and the prices are very high; about four times of the price of fish. Other sources are chicken, chicken eggs, buffalo meat and pork. Even in the project area, 80% of the non-fish farming households did not know about fish farming and only 20% knew and consumed fish on regular basis. This indicated that dissemination of proven aquaculture technology and exchange of knowledge was still weak even within the same district. Among fish-consuming families, 83% confirmed that they had noticed the health benefits from eating fish, which underpinned the WiA project team's mission of expanding small-scale aquaculture throughout the country.

One of the objectives of the project was to empower the women and their communities and to help produce fish as a high-quality diet to fight against malnutrition. The project has been successful in its intervention as the project farmers have been capable of producing about 129 kg of fish per family annually, the majority of which (60% or 78 kg) was used for the consumption by the family itself. The amount is approx. 16 kg per capita per annum assuming an average family size of five members and is close to the world average of 20 kg, which is more than two-folds of the apparent seafood consumption in low-income, food-deficit countries (LIFDC), and nearly five times the national average of Nepal (FAO, 2016; Gurung, 2016). It serves as an important source of protein for the community that otherwise relies mainly on traditional sources. Small amounts of other animal meats are consumed, but still the average consumption of protein from animal sources in Nepal is far below the recommended one-third level for good health (AIT, 1994). Fish farming provides a ready supply of fish for purchase in the vicinity not only for fish farmers' families, but also for non-fish farming community members. Most fish farming households typically sell their fish from their pond sites or homes to neighbors in the community at a cheaper than market prices. Therefore, small-scale aquaculture has of course improved the food and nutrition security of the communities. The WiA project has been highly successful to make impacts on economic benefits as was indicated by overwhelming percent (95%) of female fish farmers surveyed. The project farmers earned a good average net profit of NPR 27,581 (USD 265) per family per year from the average pond size of 423 m^2 which are 8 and 2.4 times higher than those of the farmers during the initial phase of the project (Bhujel *et al.*, 2008). If the farmers could double the size of pond to approximately 850 m^2, a female farmer may earn a net profit sufficient to come out of the poverty benchmark set by the World Bank of USD 1.25/day or USD 456.25/year (World Bank, 2016). If there are four members in a family, then about 3,600 m^2 of pond would be required, so that a fishpond of one third of a hectare could easily eliminate rural poverty in those areas where fish farming is possible. Farmers who sold fish, earned NPRs. 15,430-40,478 (US$203-266) per year which was generally spent on their children's education. Even households which consumed all the fish they produced, on average directly saved approx. NPRs. 15,000 (USD 144) in fish purchases for family consumption. More importantly, these earnings were possible simply working in ponds for an hour per day only as fish farming requires relatively low inputs and management. This can be done still while undertaking other agricultural and household chores.

It is apparent that the small-scale aquaculture implemented in Kathar, Chitwan was a great success and was regarded as model to show other farmers of other districts brought by the government and NGOs. It also showed it was expanding in its area also helped grow in other areas. The average pond size of the project-supported fish farmers (423 m^2) in this study was found to be 2.4 times bigger than the size of their fishponds during the initial stage (175 m^2) reported by Bhujel *et al.*, (2008). More importantly, success was also evident and realized when 11 households subsequently initiated their own fishponds without any supports from the WiA project.

Non-project supported farmers emulated the farming practices of the project-supported farmers, culturing the same species and using similar feed items. These non-project farmers had relatively larger (11%) ponds than the project-supported farmers. This indicates that the financially better off members of the community adopted the aquaculture to do more commercially so that they could take advantage of economy of scale. However, the average annual fish production or yield per unit area of ponds of non-project farmers was lower than those of the WiA project-supported farmers. This suggests that planned training and workshops provided to the project farmers during the project implementation period had good impacts to make them more effective fish farmers, and they might have opportunity to improve and further enhance their fish farming skill over the long period than the unsupported farmers. On the other hand, non-project fish farmers mentioned that they also benefited from their fish farms although there was more room for improvement in the culture practices and to maximize their income. Provision of technical inputs, organizing well-planned training, and workshops are the recommendations. Furthermore, the productivity of both project-supported and unsupported farmers was found to be quite low (2.6 and 3.5 ton/ha/year) as they depended on farm byproducts and kitchen wastes as feed. Provision of feeds, and improved management of feeding and water quality in addition to preventing predation during early stages could result in a higher productivity. These valuable lessons should be considered while planning for the aquaculture development.

The WiA project empowered the women by equipping them with technical as well as social skills. Firstly, the project gave the women of an opportunity of owning the fishponds and its benefits to them for which they were proud of and showed strong feelings of happiness and social image. The training and workshops provided the woman the technical skill of managing the ponds to produce fish at home which was a new development. In addition to these, becoming a part of a cooperative increased their self-confidence and enhanced their sense of community. Almost all of them (97%) interviewed were members of a cooperative and few of them developed their leadership skill in handling the revolving funds of the cooperatives, and even became political leaders. Their cooperative membership also gave women access to additional training, workshops, and more opportunities for acquiring other skills.

Project clearly proved that aquaculture has been a good alternative to rural communities. However, due to shortage of land and human resource, most non-fish farmers are not doing to. However, 37% of non-fish farming households would like to know more about it and the support given to fish farmers so that they could pursue aquaculture as an alternative occupation and business as at least 40% of the non-fish farming households said they felt financially and nutritionally insecure about their future.

This survey also identified the problems of the small-scale, household fish farmers. The main problem was water scarcity. Most farmers rely on rainfall to fill their ponds. Water is lost due to evaporation and seepage. Evaporation is common for all, but seepage is worse in if ponds are poorly constructed in sandy areas. There is a growing concern about the uneven distribution of monsoonal rain due to climate change and global warming. Fish farmers might face bigger problems in the future. Water shortage is particularly problematic for the farmers who can't afford pumps to lift the large volume of water from boreholes and nearby streams. If a cheap, sensible solution to this problem could be found, more farmers could readily adopt the aquaculture and also expand the size and number of fishponds. Other minor problems pointed out by fish farmers were the limited availability and accessibility to fish fry or fingerlings, high cost of transportation, and fish losses due to predation by birds and other animals in the pond as well as some diseases. In summary, major obstacles are the lack of access to land, water scarcity, predation, and shortage of fingerling, their transportation and know-how. Planners need to address these if aquaculture expansion is to be done for food and nutrition security, employment creation, poverty reduction in Nepal or to achieve UN's SDG goals.

Conclusions and recommendations

1. Well-designed, small-scale aquaculture projects, such as the one detailed here, can be scaled over time and have lasting impacts without continued outside support
2. Producing over 100 kg fish per family per year is possible from a pond of about 400 m^2.

3. Farmers supported by a project with proper training can remain impactful even after a decade as evidenced by the project farmers having 18% higher average fish production per family as compared to the farmers with no project support.
4. The impact of project was even higher in terms of productivity as seen in this study that the farmers who received project support had 35% higher fish productivity (3.5 ton/ha/yr/) than farmers who did not receive support (2.6 ton/ha/yr)
5. The average fish consumption of a fish farming household (80 kg/yr) was approx. 2.5 times that of non-fish farmers (30 kg/yr).
6. Fish farmers generated an additional net profit of approximately $265 annually by selling fish.
7. Fish farming is less rigorous and management intensive than traditional agriculture, and the average fish farmer spent less than 1 hr per day mainly for fish feeding and related works. Whereas non-fish farmers invested much larger amounts of time in traditional agriculture, but still a large proportion (40%) felt both nutritionally and economically insecure about their future.
8. Almost all women fish farmers that were part of the local cooperative report increased feelings of self-confidence, independence, and actively learning
9. Small-scale aquaculture should continue to be scaled-up throughout the Terai region as well as the mid-hills.
10. This project has the potential to expand if the access to land, water, feed and technologies are made available in conjunction with support to reduce losses due to predation, diseases and others.
11. Availability and access to land is a big problem. Attempts should be made to utilize underused and unproductive rice fields or swamps. If only 1% of those underused rice fields could be converted into fish farms, fish production would double.
12. In order to solve the water scarcity issues, storage dams across the seasonal streams are suggested to be constructed. Additionally, ponds could be made deeper to avoid the impact of water losses through leakages.
13. Proper pond construction by compacting the soil bottom layer and incorporation of plastic sheets or liner, should be the focus of future pond constructions.

14. The provision of large water tanks or reservoir ponds to store rainwater collection during summer could also serve as water supply for small tank culture using water recycling aquaculture systems (RAS or Biofloc system).
15. More support for extension and training of existing farmers could help in maximizing their productivity and economic benefits.
16. Shortage of seed and costly transportation are major problems. The Government of Nepal should encourage and provide supports to the farmers to establish more hatcheries in certain pocket areas so that fingerlings do not need to be transported long distances and are produced adequately whenever farmers needed.
17. Farmers should be provided with bigger fingerlings (>5g) so that their survival would be higher in the pond. To do so, hatchling should be nursed in hapas with net cover before stocking directly into the pond. It would increase survival so the higher would be the supply of seed.
18. Overall, the project was successful and sustainable in the long term; therefore, it can be used as a tool to improve food/nutrition and economic security which are associated with the UN's SDGs especially SDG1, 2, 3, 4, 5, 8, 14 among others.
19. The project should undoubtedly serve as a model for further development efforts in Nepal and other countries especially for food and nutrition security of the rural people.
20. Recognizing the role of small-scale aquaculture, prioritizing it in the planning for launching the similar projects nationwide could have tremendous impacts on the enhancement of food and financial security in rural communities.

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

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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, Nepal. There were a total of 8 mollusks (bivalves and gastropods) species found and identified in the area. The mollusks served as food and as source of livelihood by residents in the region. 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/100 g) and iron (95.6±4.3 mg/100 g) content. Survey revealed that the mean consumption of edible aquatic mollusks was 42.6 kg per household in the region. The mollusks were preferred by most of the native residents but not limited to them. 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.

Key words: Bivalve, Consumption, 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 (Terai) 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 Terai, 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 Terai 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 Terai. 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 also aims 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).

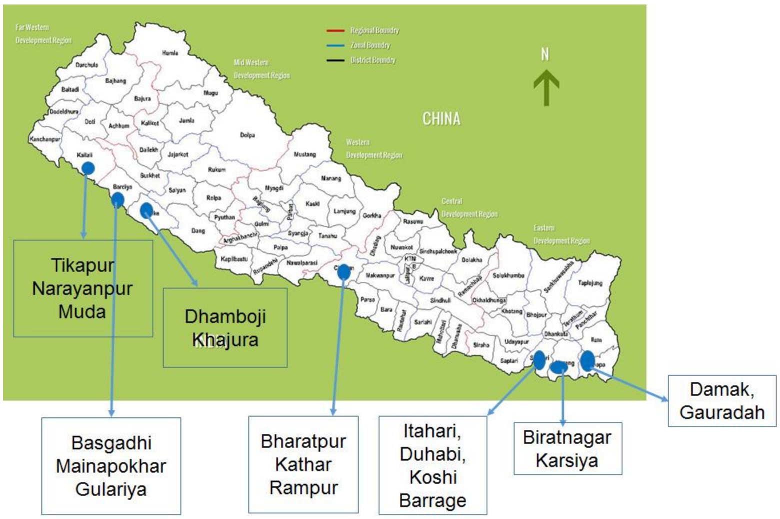

Figure 1. Map of Nepal showing the survey locations of edible aquatic mollusks.

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 f.ovulis*	-	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 from the flesh of different molluscan species 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 (*Indoplanorbis exustus*) 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%).

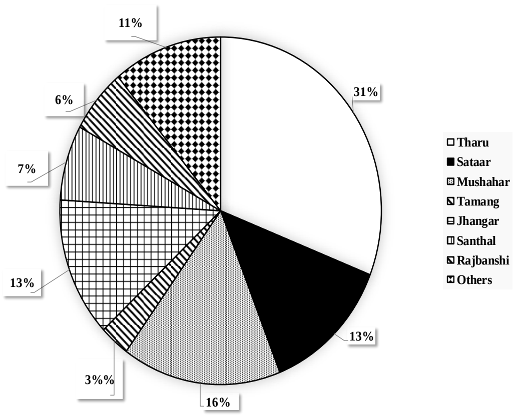

Figure 2. Major consumers of aquatic mollusk (percent of respondents).

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 exist between 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%).

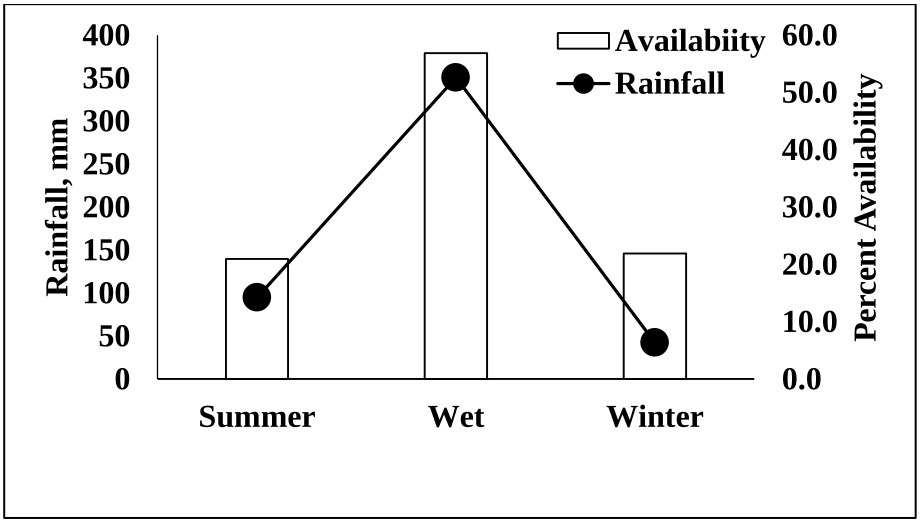

Figure 3. Relationship between intensity of rainfall and availability of mollusk.

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 large being 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.

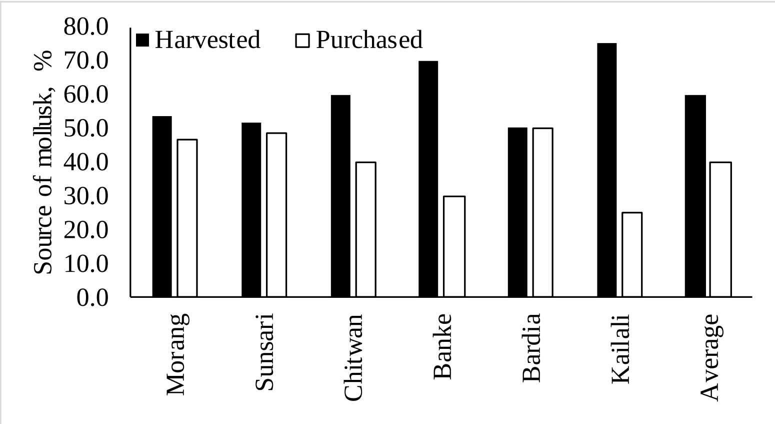

Figure 4. Proportion of harvested and purchased mollusk by consumers in different districts.

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.

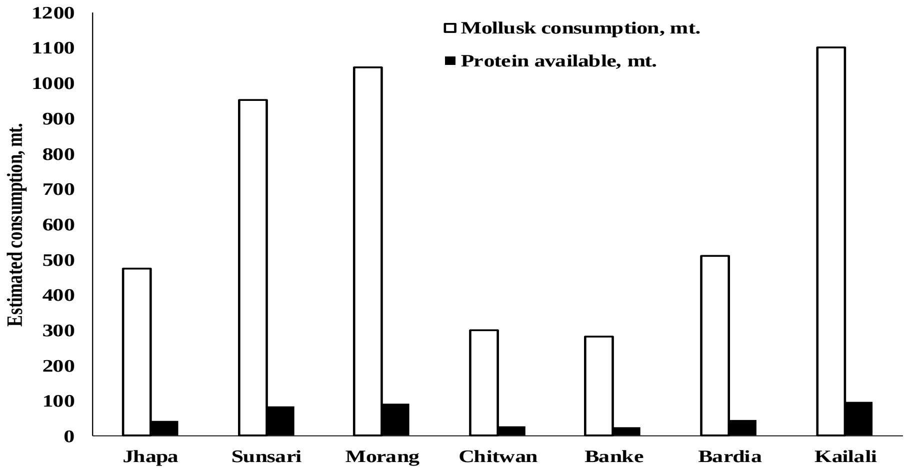

Figure 5. Estimated annual consumption of mollusk and corresponding intake of protein in surveyed districts.

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 of mollusks from a 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 Terai 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; Gosh *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 (Gosh *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; Gosh *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 (Gosh *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, which 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 were 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.

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

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Abstract
Tamoxifen is a non-steroidal aromatase inhibitor showing ability to reverse sex in fish. The present study assessed the efficacy of tamoxifen for inducing masculinization in Nile tilapia. Nine days post hatch tilapia fry were reared in fine meshed nylon hapas suspended in a green cemented pond and fed with tamoxifen (Cytotam 20) @ 150, 200, 250, 300, 350, 500 and 1000 mg/kg diet for 30 and 40 days. In 30 days treatment group, the percentage of male fed with tamoxifen @ 150, 200, 250, 300, 350, 500 and 1000 mg/kg diet were 67.6±0.5, 72.4±2.7, 84.5±1.8, 87.5±1.4, 89.7±1.0, 90.0±1.3 and 96.7±1.2, respectively, while control group showed 50.8% males. Although the treatment with tamoxifen in lower concentrations (150-500 mg/kg diet) significantly increased the male proportion compared to control diet, the percentage of males was significantly highest in treatment with 1000 mg/kg diet. There was no significant differences in percentage of males between 30 and 40 days of treatment durations. There was no significant difference in the survival of Nile tilapia fries among control and treatment groups. The present study showed that tamoxifen has potential for production of all-male Nile tilapia. The best dose of tamoxifen based on present study is 1000 mg/kg feed. However, increased dose of tamoxifen should be tested to induce 100% masculinization.

Keywords: Nile tilapia, Cytotam 20, Aromatase inhibitor, Masculinization

Introduction
Nile tilapia (*Oreochromis niloticus*) is an important aquaculture species which is ranked in the second most produced farmedfish with an annual world production of 5.6 million tons per annum (Fitzsimmons, 2016). Monosex culture of male Nile tilapia is preferred due to sexual size dimorphism, males being substantially larger than females (Beardmore *et al.*, 2001). In addition, precocious

reproduction of this species also leads to low growth performance of stocked fish as a consequence of over-crowding and feed competition in production ponds. Currently male monosex populations are produced mainly by androgen (17-α methyltestosterone) treatment. However, the use of steroids in aquacultures is not desirable and it is avoided in many countries, on account of its adverse environmental effects (Baroiller *et al.*, 2009). Furthermore, the 17-α methyltestosterone is quite expensive and not easily available in many developing countries like Nepal. As an alternative, the non-steroidal aromatase inhibitors (AIs) might be useful tools for sex reversal in fish.

Previous studies reported the possibility of using non-steroidal AIs in sex reversal of fishes(Kitano *et al.*, 2000;Nakamura *et al.*, 2003; Kwon *et al.*, 2000; Afonso *et al.*, 2001; Uchida *et al.*, 2004;Alam *et al.*, 2006; Ruksana *et al.*, 2010; Pandit and Nakamura, 2015). For example, fadrozole and exemestane have 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; Ruksana *et al.*, 2010; Pandit and Nakamura, 2015) and sex-changing protogynous species (Nakamura *et al.*, 2003; Bhandari *et al.*, 2004; Alam *et al.*, 2006). 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 aromatase enzyme (P450arom) is the key enzyme for biosynthesis of estradiol-17β (E_2) from testosterone (Nakamura *et al.*, 2003). The principle behind masculinization in fishes using AIs is that they block P450 aromatase enzyme activity, leading to reductions in the production of estrogen (Steele *et al.*, 1987).

Tamoxifen has been used in the treatment of breast and ovarian cancer in postmenopausal women (Nazarali and Narod, 2014). It is cheaper than 17-α methyltestosterone and easily available in Nepal. Although few studies reported the possibility of using tamoxifen in successful masculinization of fishes, there are no reports about the use of tamoxifen for mass production of monosex male tilapia fry in commercial scale. If this technology can be successfully applied in commercial scale, it will be a good alternative of 17-α methyltestosterone to produce all-male tilapia fry in easier and cheaper way. Thus, the purpose of this study was to determine the efficacy, dose and duration of tamoxifen treatment for mass production of monosex male population in Nile tilapia.

Materials and methods

This experiment was conducted at the research facilities of the Fisheries Program, Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal. The experiment included two phases, the first phase with three low doses (150, 200 and 250 mg/kg feed) of tamoxifen and the second phase with four high doses (300, 350, 500 and 1000 mg/kg feed) of tamoxifen based on the results of the first phase experiment. For both experiments, rearing of Nile tilapia broods was done in nylon hapa placed in a cemented pond and were allowed to breed naturally in hapa. Fertilized eggs were collected from the mouth of several females, randomly mixed, counted and placed in jar incubation system (Ranjan *et al.*, 2015).

In the first phase experiment, 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 pond (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. In the second phase experiment, a total of 3750 individuals at 8 days after hatching (dah) were used, with 250 fries in each of 15 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 pond (4.9 m x 4.5m x 1.25 m). The second phase experiment was also conducted in a completely randomized design (CRD). There were five treatments with three replications of each treatment. The treatments were: (1) Feed without tamoxifen (control; only fishmeal); (2) Tamoxifen at the rate of 300 mg/kg feed; (3) Tamoxifen at the rate of 350 mg/kg feed; (4) Tamoxifen at the rate of 500 mg/kg feed; and (5) Tamoxifen at the rate of 1000 mg/kg feed.

To prepare the treatment diet, Tamoxifen Citrate Tablets IP 20 mg (Cytotam 20; Cipla Ltd., India) was dissolved in 95-100% 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 for 30 and 40 days, whereas control groups were fed with normal dry fishmeal. 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.

In the first phase experiment, after 30 days of treatment, half of the fishes from all treatments were taken and cultured in another hapa for 45 days feeding with normal feed, 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 and experimental period. Gonadal status of fish was observed by scarifying all fishes from each group anaesthetizing with clove oil. The gonads were excised and squashed with aceto-carmine to determine the sex of fish (Guerrero and Shelton, 1974). The procedure of second phase experiment was similar to the first phase experiment. In the second phase experiment, after 30 days of treatment, fish were reared for additional 30 days feeding with normal diet and then growth survival and gonadal status was observed as described above for the first phase experiment. Data were analyzed statistically by analysis of variance (ANOVA) using SPSS (version 21.0) statistical software package. All means were given with ± standard error (S.E.).

Results

Phase-1

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 (Table 1). Similarly, 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, respectively, while control group showed 51.5±0.3% males (Table 1). In both treatment durations, the percentages 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 and 40 days on sex ratio of Nile tilapia during gonadal sex differentiation.

	Male (%)	
Treatments	**30 days treatment**	**40 days treatment**
Control feed	50.8 ± 0.9^{a}	51.5 ± 0.3^{a}
Tamoxifen @ 150 mg/kg diet	67.6 ± 0.5^{b}	68.6 ± 3.1^{b}
Tamoxifen @ 200 mg/kg diet	72.4 ± 2.7^{c}	74.7 ± 1.6^{c}
Tamoxifen @ 250 mg/kg diet	84.5 ± 1.8^{d}	84.8 ± 0.8^{d}

Mean values with different superscript in the same column are significantly different ($p<0.05$)

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 2). There was no significant difference in the survival of Nile tilapia fries among control and treatment groups ($p>0.05$). 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 2). There was no significant difference in the survival of Nile tilapia fries among control and various treatment categories ($p>0.05$).

Table 2. Effects of tamoxifen treatment on growth and survival of Nile tilapia fry at the end of treatment period of 30 and 40 days.

	30 days		40 days	
Treatments	**Weight (g/fish)**	**Survival (%)**	**Weight (g/fish)**	**Survival (%)**
Control feed	0.6±0.0	92.4±4.6	0.7±0.0	91.7±4.5
Tamoxifen @ 150 mg/kg diet	0.5±0.0	92.8±4.4	0.7±0.1	91.0±4.2
Tamoxifen @ 200 mg/kg diet	0.6±0.1	88.0±3.2	0.7±0.1	87.2±3.2
Tamoxifen @ 250 mg/kg diet	0.5±0.0	84.8±4.2	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 cm, respectively (Table 3).

Table 3. Weekly mean and range of water quality parameters of experimental pond. 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)

Phase-2

The first phase experiment showed that there was no significant difference in percentage of male between 30 and 40 days of treatment durations. Thus, in the second phase fish were treated only for 30 days. The percentage of male fed with tamoxifen @ 300, 350, 500 and 1000 mg/kg diet were 87.5±1.4, 89.7±1.0, 90.0±1.3 and 96.7±1.2, respectively, while control group showed 50.9±1.4% males (Table 4). The percentages for males in tamoxifen @ 1000 mg/kg diet were significantly higher than control and other treatments ($p<0.05$). There was no significant difference in percentage of male among 300, 350 and 500 mg/kg diet treatments ($p>0.05$; Table 4).

Table 4. Effects of Tamoxifen treatment at different doses for 30 days on sex ratio of Nile tilapia during gonadal sex differentiation.

Treatments	Male (%)
Control feed	50.9±1.4^{a}
Tamoxifen @ 300 mg/kg diet	87.5±1.4^{b}
Tamoxifen @ 350 mg/kg diet	89.7±1.0^{b}
Tamoxifen @ 500 mg/kg diet	90.0±1.3^{b}
Tamoxifen @ 1000 mg/kg diet	96.7±1.2^{c}

Mean values with different superscript in the same column are significantly different ($p<0.05$)

At the end of experiment, the survival percentage of treatment group fed with tamoxifen @ 300, 350, 500 and 1000 mg/kg diet were 90.8±2.7, 86.0±1.0, 83.5±3.1 and 83.9±4.7, respectively, while control group showed 90.5±3.6% survival (Table 5). There was no significant difference in the survival of Nile tilapia fries among control and various treatment groups ($p>0.05$; Table 5).

Table 5. Effects of Tamoxifen treatment on body weight and survival of Nile tilapia at the end of treatment period of 30 days.

Treatments	Weight (g/fish)	Survival (%)
Control feed	0.6±0.1	90.5±3.6
Tamoxifen @ 300 mg/kg diet	0.5±0.0	90.8±2.7
Tamoxifen @ 350 mg/kg diet	0.5±0.1	86.0±1.0
Tamoxifen @ 500 mg/kg diet	0.6±0.0	83.5±3.1
Tamoxifen @ 1000 mg/kg diet	0.6±0.1	83.9±4.7

The weekly average water temperature, dissolved oxygen, pH and Secchi disk depth of the cemented tank where the experimental hapa were set were 26.5±0.4 °C, 5.9±0.4mg/L, 7.7 and 33.3±2.1 cm, respectively (Table 6).

Table 6. Weekly mean and range of water quality parameters of experimental pond. Values in the parenthesis are range values.

Parameters	Mean±SE
Water temperature (°C)	26.5±0.4(24.7-28.0)
Dissolved oxygen (mg/L)	5.9±0.4(4.0-7.0)
pH	7.7(7.4-8.0)
Secchi disk depth (cm)	33.3±2.1(27.4-38.2)

Discussion

The present study assessed the efficacy of tamoxifen, a non-steroidal aromatase inhibitor, for inducing masculinization in Nile tilapia. Results showed that dietary administration of tamoxifen dose-dependently induce masculinization of the sexually undifferentiated Nile tilapia. Although the treatment with tamoxifen in lower concentrations (150-500 mg/kg diet) significantly increased the male proportion compared to control, the percentage of males was significantly higher in highest dose treatment (1000 mg/kg diet) than lower dose treatments. After 30 days of treatment, the percentage of male fed with tamoxifen @ 150, 200, 250, 300, 350,500 and 1000 mg/kg diet were 67.6±0.5, 72.4±2.7, 84.5±1.8, 87.5±1.4, 89.7±1.0, 90.0±1.3 and 96.7±1.2, respectively. The finding of the present study is similar to 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 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). In contrast to the present finding, Guiguen *et al.* (1999) reported that in rainbow trout and Nile tilapia, tamoxifen does not induce the masculinization in the gonadal sex differentiation. These results show that there is a difference of sensitivity for tamoxifen in gonadal sex differentiation among several fish species.

Some previous studies applied longer treatment duration (more than 60 days treatment) of tamoxifen and obtained slightly higher percentage of male than the present experiment (Singh *et al.*, 2012). In contrast to these results, the present study showed there were 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.

The present experiment showed that tamoxifen feeding does not significantly affect the growth and survival of fry. However, tamoxifen fed group showed slightly 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).

Conclusion

The present study demonstrated the possibility of using tamoxifen as an alternative of 17-α methyltestosterone for monosex male production of Nile tilapia. In Nepalese context, the tamoxifen is about eight times cheaper than 17-α methyltestosterone as well as it is easily available in the country. The best dose of tamoxifen among treated treatments was 1000 mg/kg feed. However, increased dose of tamoxifen should be tested to induce 100% masculinization.

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

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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, fry were reared in outside hapa for a period of three months. Although not significant ($p>0.05$) among treatments, 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. There was insignificant effect of feeding duration on survival while daily weight gain (DWG) and specific growth rates (SGR) were higher in sex reversed Nile tilapia. Dried carp testes can effectively masculanize 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 methyl testosterone (MT) (Little, 1989). However, the cost of methyl testosterone 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. One hundred-fifty 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. Continues aeration and 90% water change after every 2 days was maintained. Feed was supplied four times a day in dried powder form. First feeding state of Nile Tilapia fry were fed to ad-libitum at each feeding with absolute dose of dried carp testes. After completion of treatment days, 100 fry 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 markets in Narayangadh and Tandi, Chitwan, 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 was 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 analyzed through one-way ANOVA for differences in mean of the treatments. 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 quality parameters were analyzed in MS-Excel. Means were expressed as mean±SE.

Results

Feeding response on 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 Common carp testes (CCT) powder for different durations (Mean±SE). Means with different superscripts within a column differ significantly at $p<0.05$.

Parameters	Aquarium			Hapa		
	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}

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 treatments both in aquarium and in hapa.

Feeding response on 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 of 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. Rate of sex reversal of Nile Tilapia fry fed with Common carp testes (CCT) in different feeding duration.

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.

Table 3. Average DO, pH and representative Max-Min temperature in aquarium.

Parameters	Treatment				
	T1	T2	T3	T4	T5
DO (mg/L)	7.4±0.13[a]	6.7±0.12[b]	6.8±0.12[b]	6.9±0.11[b]	7.0±0.09[b]
	6.5-8.4	6.1-7.5	6.2-7.9	6.0-8.3	6.1-8.4
pH	7.7	7.8	7.7	7.7	7.7
	7.2-8	7.6-8	7.2-7.9	7.4-7.9	7.2-8
Temperature	29.1±0.13 (Maximum)			23.0±0.19 (Minimum)	

Table 4. Average total ammonium nitrogen during treatment phase.

Parameter	Day	Average
NH_4^+	1	1.1±0.28
	2	1.9±0.62
	3	2.9±0.78
	4	3.5±0.72
NH_3	1	0.9±0.31
	2	1.7±0.63
	3	2.4±0.98
	4	3.2±0.97

Table 5. Average DO, pH and temperature in hapa.

Parameter	Treatment		
	P1	P2	P3
Temperature (°C)	30.8±1.3[b]	30.8±1.3[b]	30.6±1.3[b]
DO (mg/L)	5.3±1.0[b]	5.3±1.2[b]	5.3±1.0[b]
pH	7.4	7.5	7.4

Discussion

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. In the present study, 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 in the present study, 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 was also obtained with synthetic androgens methyl testosterone (MT) reported by other studies (Phelps *et al.,* 1996; Macinthos *et al.,* 1993). 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, Macinthos *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 by the fry under longer durations of feeding. After certain duration of feeding, decrease in palatability was observed in the present study. 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). In this study 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/catfish testes produced only 83.3% 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 on survivability of fish fry showing a range of 72 to 77%. Benli and Köksal (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 greater than 2 mg/l; however, mortality will be reduced to half or less when they are gradually acclimatized to a level as high as 3 mg/l for 3 or 4 days. In the present study, after water change total ammonium nitrogen level gradually increased from Day 1 to Day 4 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.

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Gonadosomatic index, egg size and fecundity of Chocolate Mahseer, *Neolissochilus hexagonolepis* (McClelland, 1839) in Tamor River, Nepal

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Abstract

The present study was carried out to assess gonadosomatic index (GSI), egg size and fecundity of chocolate mahseer, *Neolissochilus hexagonolepis* from Tamor river, Nepal. The fish samples were collected from the river every month from December 2014 till November 2016. The mean GSI of female fish varied from minimum (0.19±0.05) in January to maximum (7.75±4.40) in July and the mean GSI of male fish varied from minimum (0.25±0.17) in December to maximum (2.33±1.38) in July. Absolute fecundity ranged from 2038.56 for fish of total length (TL-cm) 33.2 cm, total weight (TW-g) 430 g and gonad weight (GW-g) 3.58 g to 11423.9 for fish of total length (TL-cm) 37 cm, total weight (TW-g) 710 g and gonad weight (GW-g) 102 g. The mean value of fecundity was (6262.25 ± 3004.75) with a mean total length of (36.45cm ± 4.79) and mean body weight (586.5 g ± 261.14 g). The smallest size of eggs measuring 1.22±0.07 mm in diameter was recorded from a fish sample that measured 33.2 cm total length, 430 g total weight, 3.58 g gonad weight and with 2038.56 numbers of eggs. The largest eggs measuring 2.34±0.05 mm in diameter were recorded in a fish that measured 33.6 cm total length, 442 g total weight, 38.42 g gonad weight and with 4092 numbers of eggs.Fecundity showed positive correlations with total weight ($r= 0.30$), total length ($r= 0.44$) and gonad weight ($r= 0.78$). Egg size (ES) was found to have significant moderate positive correlation with GW ($r=0.65$, $P<0.05$) but very weak positive correlation with fecundity ($r= 0.25$). Gonadosomatic index (GSI) and gonad weight (GW) were positively correlated for both the sexes with the value of correlation coefficient (r) for female equal to 0.92 and that for male equal to 0.55.

Keywords: *Neolissochilus hexagonolepis*, Gonadosomatic index, Egg size, Fecundity, Tamor River.

Introduction

To write the Introduction part, give introductory notes may one or half para about the subject matter you are dealing. Then give a para about the statement of the problem in short with giving some review on past studies, if possible give the findings of most recent studies, suggestion and gaps which is require to enhance the knowledge on such approaches. Then state about the objective in short why you perform this study. *Neolissochilus hexagonolepis* is considered as a near threatened species and hence due care and attention should be given for its conservation (Arunachalam, 2017). The present investigation is also an attempt to do little, if not much, for the conservation of this species.

Neolissochilus hexagonolepis, locally known as katle, is one among the dominating species in snow fed torrential rivers of Nepal. Unfortunately, its population is in sharp decline due to the loss of its habitat and over-exploitation.The total number of eggs present in the ovary of a gravid fish just prior to spawning is termed as fecundity. The evaluation of fecundity finds an important place in monitoring the reproductive potential of a species and study of its biology. Several ichthyologists have defined fecundity and emphasized its significance from time to time.Simpson (1951) came up with the idea that the data pertaining to fecundity are useful in determining the density dependent factor affecting population size and for separating different fish stocks from the same population. Lagler (1956) opined that the knowledge about fecundity of a fish is essential for evaluating the commercial practical culture and actual management of the fishery potentialities of its stock, life history.

Gonadosomatic Index (GSI) gives indication of the gonadal development of a species and is thus, useful in the study of reproductive biology of the species. Shabana *et al.* (2013) opined that the Gonadosomatic Index (GSI) is widely used by biologists to indicate the maturity and periodicity of spawning and predicting the breeding season of a fish. The fishes which produce a large number of eggs and deposit them over a short period of time are referred to as total spawners. On the other hand, multiple spawners have a longer breeding period and deposit only a portion of eggs during one spawning. Total spawners are said to have a higher GSI than multiple spawners (Wooton, 1990).

Various imminent ichthyologists have studied fecundity and gonadosomatic index of cold water fishes from time to time. Swar (1994) assessed the fecundity of

Neolissochilus hexagonolepis in a Nepalese reservoir and river and reported that the species is a low fecund fish. Mahapatra and Vinod (2011) studied the reproductive biology and artificial propagation of *N.hexagonolepis* in Meghalaya, India and reported on the GSI and fecundity. Among other hill stream fishes, Shabana *et al.* (2013), Kharat and Khillare (2013), Ulfat Jan *et al.* (2014), Wagle (2014) and Jan and Imtiaz (2016) studied the fecundity of *Tor Putitora* (Ham.), *Nemacheilus moreh, and Schizothorax esocinus and S. niger, Schizothorax richardsonii and S. plagiostomus* respectively.

Although studies on reproductive biology of cold water fishes, encompassing gonadal cycle, GSI, fecundity and egg size have been reported frequently it appears that there is poor reporting on the biology of this species. The present study is, therefore, an attempt to fill up this void situation.

Materials and Methods

Fish sampling and sex identification

The study area for this research is the Tamor River, which lies approximately between latitude and longitude coordinates of 26.916667 and 87.166667, respectively and is a major river in eastern Nepal. Originating around the mountain Kanchenjunga, this river meets the confluence of Arun and Sunkoshi at Tribenighat to drain into the giant Saptakoshi which flows through Mahabharat Range on to the Gangetic plain. The total length of this river is about 190 km with 5817 km^2 catchment area. This river also serves as the suitable home and breeding ground for *Neolissochilus hexagonolepis*. Fish sampling was carried out for two years commencing from the second week of December 2014 till the end of November 2016. Samples were collected from the catch landings of fishermen who used hooks, cast net, gill net, traps and other conventional local techniques for catching the fishes.

The samples were sexed and total weight and total length measured for each of the collected specimen. Total weight (including gut and gonads) was measured using a digital balance with the precision of 0.01 g. Total length was measured from the tip of snout to the distal tip of the longest caudal fin ray. The measurement was taken in full stretched condition to the nearest 1mm using a measuring tape and graduated ruler. The samples were sexed by external observation and later confirmed through observation of gonads after dissection.

Fecundity assessment

For fecundity assessment, ovaries from matured fishes were preserved in Gilson's fluid for over two weeks to loosen the tissue surrounding the eggs during which the eggs were agitated several times. The eggs were then washed with distilled water and gently teased with needle and forceps until they became disentangled from ovarian tissues. The eggs were then spread over blotting paper to remove excess moisture and the clamped eggs gently separated. The eggs were then air dried.

Gravimetric method was followed to estimate the fecundity. The eggs liberated from the ovarian tissue were thoroughly washed and spread on blotting paper to dry in air. Three sub-samples of eggs, each weighing 1 gram and each obtained from the anterior, middle and posterior part of the ovary were considered. After that the number of eggs in each sub-sample was counted and mean value of the three sub-samples calculated. The total number of eggs in the ovary was then weighed. Absolute fecundity was then calculated by using the formula:

Fecundity (F) = Total weight of eggs in the ovary * Mean count in 1 gm of egg mass

In case of the ovaries weighing less than 10 gm, count in 1 gm egg mass was simply multiplied with the total weight of eggs in the ovary to work out the absolute fecundity.

During the present investigation, the advanced yolked oocytes or vitellogenic oocytes were only included in the count. The vitellogenic oocytes were easily identified through visual observation as they appeared larger and yellowish opaque due to the accumulation of yolk and cortical alveoli.Fifteen eggs were randomly selected from ovaries staged at III (Ripening), IV (Mature) and V (Spawning) and diameters measured to the nearest 0.01 mm. After that, the eggs were grouped in the intervals of 0.2 mm and their frequencies calculated. The least – squares linear regressions between fecundity (F) and total body weight (TW), total length (TL) and gonad weight (GW) were calculated according to the method described by Bagenal (1968).

Egg size

To determine the size of eggs, fifteen eggs from each of ovary were picked at random. The eggs were spread on blotting paper and dried and the diameter of the eggs measured using a calibrated micrometer mounted on the eyepiece of a monocular microscope (1 division = 0.05mm). From this, the mean size of egg for each ovary was then estimated.

Gonado-somatic index (GSI)

The gonado-somatic index (GSI) was calculated for each fish every month using the formula:

GSI=Weight of the gonad / Total weight of the fish * 100

Results

A total of 198 samples were examined from December 2014 till the end of November 2016, among which 89 were males and 109 were females. The overall male to female ratio was 1: 1.2.

Among many dominating speciesin the torrential rivers of the hilly region of the country *Neolissochilus hexagonolepis* is one. However, its population is in sharp decline during recent years as is confirmed by several studies. The present investigation was carried out to unravel the prevailing situation of the fish in Tamor River so as to work out the ways for its conservation in the days to come.

Gonado-somatic index

The Gonado-somatic index ranged from 0.084 % (in a fish of total length 19.5 cm and total weight 95 g) to 16.47 % (in a fish of total length 30.2 cm and total weight 340 g) for female fish and 0.086% (in a fish of total length 16.5 cm and 70g) to 4.78 % (in a fish of total length 19.5 cm and 85 g) for male.The mean GSI of female fish varied from minimum (0.19±0.05) in January to maximum (7.75±4.40) in July and the mean GSI of male fish varied from minimum (0.25±0.17) in December to maximum (2.33±1.38) in July (Figure 1).

Gonadosomatic index (GSI) and gonad weight (GW) were positively correlated for both the sexes with the value of correlation coefficient (r) for female equal to 0.92 and that for male equal to 0.55.

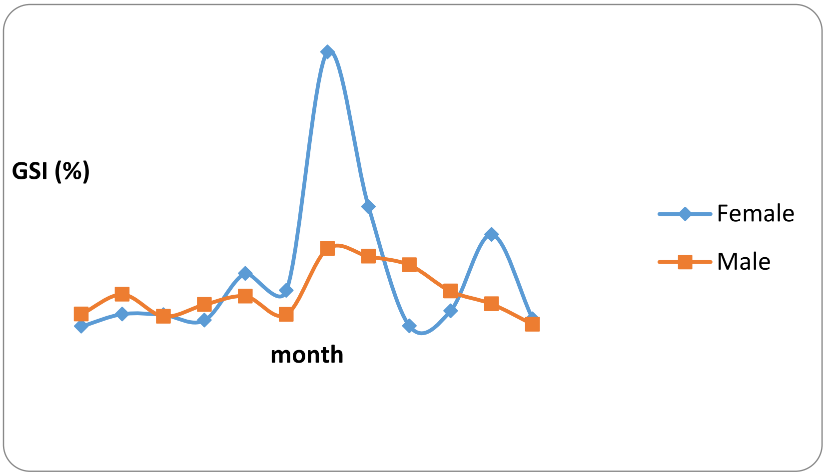

Figure 1. Graph showing monthly Mean GSI of male and female *Neolissochilus hexagonolepis* from Tamor River, Nepal.

Fecundity and egg size

Absolute fecundity of 18 gravid stage female individuals of *Neolissochilus hexagonolepis* ranged from 2038.56 for fish of total length 33.2 cm, total weight 430 g and gonad weight 3.58 g to 11423.9 for fish of total length 37 cm, total weight 710 g and gonad weight 102 g.

The mean value of fecundity was (6262.25 ± 2868.43) with a mean total length of (35.6cm ± 4.22cm) and mean body weight (586.5g ± 261.14 g). The relative fecundity to weight ranged from 4.74 for a fish with total length 33.2 cm and weight 430 g to 18.60 for a fish with total length 30.2 cm and weight 340 g. The relative fecundity to length ranged from 61.40 for a fish with total length 33.2 cm and weight 430 g to 308.76 for a fish with total length 30.2 cm and weight 340 g. Fecundity showed positive correlations with total weight (r= 0.30), total length (r= 0.44) and gonad weight (r= 0.78).

When log transformation of fecundity was regressed against log transformations of total length, a significant linear relationship emerged (Figure 2). The slope of the relationship was estimated to be 3.1059 with a standard error of 0.7957. Similarly, when log transformations of fecundity were regressed against log transformation of total weight, a significant relationship emerged (Figure 3) with slope of the relationship 0.9250 with a standard error of 0.2291.

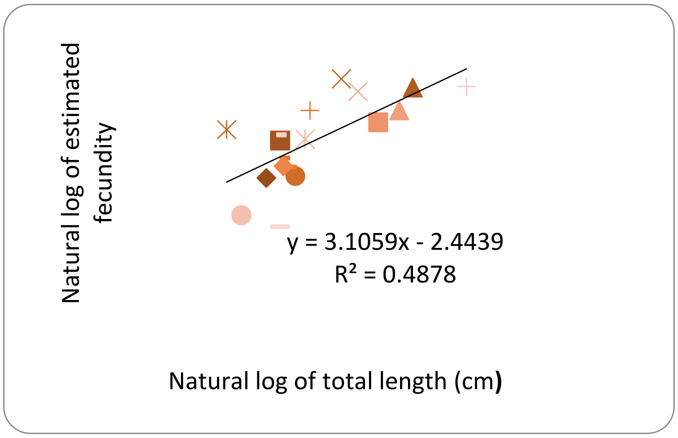

Figure 2. Graph with regression equation and coefficient of determination (R^2) showing estimated linear relationship (indicated by solid line) of log transformations of estimated fecundity with total length of 18 mature Katle from Tamor River, Nepal.

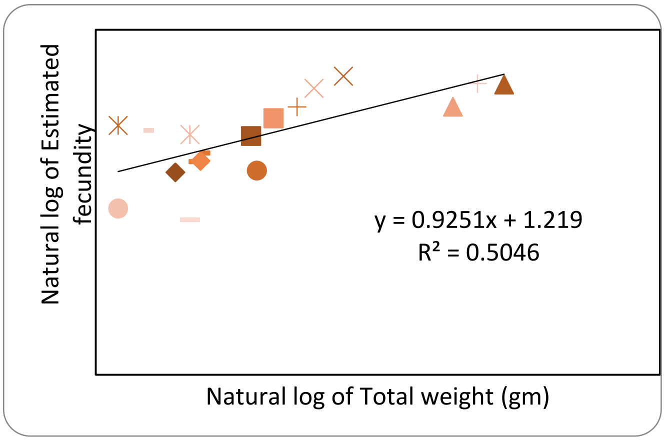

Figure 3. Graph with regression equation and coefficient of determination (R^2) showing estimated linear relationship (indicated by solid line) of log transformations of estimated fecundity with total weight of 18 mature Katle from Tamor River, Nepal.

The smallest size of eggs measuring 1.22 ± 0.07 mm in diameter was recorded from a fish sample that measured 33.2 cm total length, 430 g total weight, 3.58 g gonad weight and with 2038.56 numbers of eggs. The largest eggs measuring 2.34 ± 0.05 mm in diameter were recorded in a fish that measured 33.6 cm total length, 442 g total weight, 38.42 g gonad weight and with 4092 numbers of eggs. Most of the eggs were in the size range of 1.21 to 1.4 mm in the ovaries at III or ripening stage. No eggs larger than 2.2 mm in size were encountered at stage III.

At stage IV (Mature) majority of the eggs were in the range of 2.21 to 2.4 mm. No eggs in the range of 1.01 to 1.80 mm in size were encountered at this stage.

Similarly, at stage V (Spawning) majority of the eggs were in the range of 1.81 to 2.0 mm. No eggs in the range of 1.01 to 1.2, 1.21 to 1.4 and 2.41 to 2.6 mm in size were encountered at this stage.Frequencies of egg size ranges in the ovaries of katle at III (Ripening), IV (Mature) and V (Spawning) stages are presented in figure 4.Egg size (ES) was found to have significantly moderate positive correlation with GW ($r=0.65$, $P<0.05$) but very weak positive correlation with fecundity ($r= 0.25$). It showed negative correlations with TL ($r = -0.26$) and TW ($r=- 0.22$). Egg size showed strong positive correlation with GSI ($r= 0.73$).

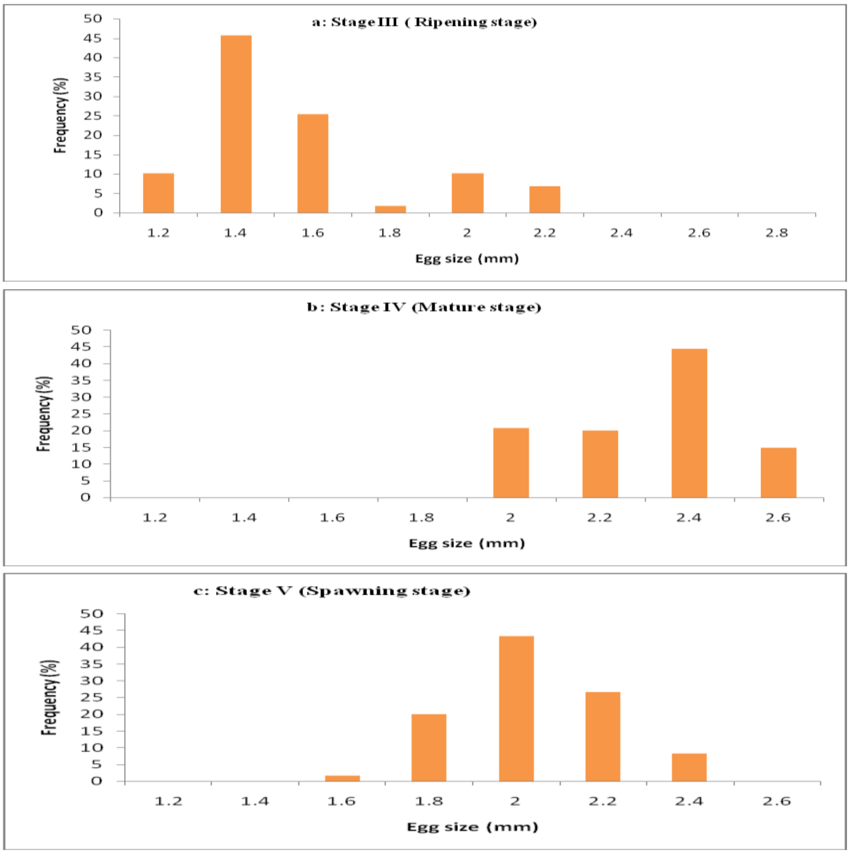

Figure 4. Frequency of egg size ranges in the ovaries of katle at III (Ripening), IV (Mature) and V (Spawning) stages (Egg size ranges: 1.2 = 1.01-1.2; 1.4 = 1.21-1.4; 1.6 = 1.41-1.6; 1.8 = 1.61- 1.8; 2 = 1.81-2; 2.2 = 2.01-2.2; 2.4 = 2.21-2.4 and 2.6 = 2.41-2.6 mm).

Discussion

The mean value of fecundity of *Neolissochilus hexagonolepis* (6262.25 ± 2868.43) in the present study was rather low as compared to that for the same species in Meghalaya, India (Mahapatra and Vinod, 2011).This variation may be due to the difference in size of the samples investigated. Swar (1994) reported that the fecundity of *N. hexagonolepis* varied considerably from individual to individual ranging from 1,387 to 33, 270 in reservoir populations while from 760 to 8,951 in riverine population. He found that the fishes from river were lessfecund than those from reservoir. He attributed this variation to the difference in feeding conditions. Nikolsky (1963) reported that the food consumed by the fish plays an important role not only in the fecundity but also the quality of the eggs and their fertilization.The average relative fecundity was estimated to be 10.94 ± 3.77 eggs per gram in the present study. In comparison to this, the finding of Swar (1994) was higher where he reported 19.13 ± 1.56 eggs per gram for reservoir population and 22.57 ± 1.41 for riverine population. The difference in average relative fecundity from that of the present study may be attributed to the difference in the size range of the fishes investigated and also upon the variations in environmental factors. Kharrat and Khillare (2013) reported that a wide variation in fecundity exists in fishes even of same length and weight and opined that a considerable variation in the fecundity between fish of equal length is a common and it may be due to environmental factors including temperature, availability of food and differences in the genetics.

As with other species, Khaironizam and Ismail (2013) reported that the absolute fecundity of *N. soroides* (Duncker, 1904) ranged from 803 to 6218 which is quite low as compared to that for Katle in the present study.Jan and Imtiaz (2016) reported the mean fecundity of *Schizothorax plagiostomus* from a river in Kashmir to be equal to 12964.62 ± 7385.54 with relative fecundity ranging from 9.696 to 56.704 per gram and 64.52 to 834 per cm. These values were way too higher compared to that of the species investigated. They suggested that the contributing factors for the variation in the fecundity among the fishes of the same as well as different species include size, age and condition of fish and also the food intake and the space.

Still comparing with other species, the fecundity of a fresh water hill stream teleost *Nemacheilus moreh* (Sykes) ranged from 142 to 1197 with the mean value of 546 (Kharat and Khillare, 2013) is rather low. Similar situation was also seen when compared with the fecundity range of of *Barilius bendelisis* (Ham.1809) from Manas River, Assam, India (Jabeen *et al.*, 2016).But, the value of absolute fecundity of the fish under study was little lower as compared to that of *Ompak pabo* (Ham.1822), with the mean value of 9857.315, from Gomati river of Tripura (Bhattacharya and Banik, 2015).

Hence, the present study revealed that *N. hexagonolepis* is a low fecund fish as compared to other high fecund fish with tens of thousands of eggs. This finding agreed in terms with the finding of Dasgupta (1988) who described Katle as a low fecund fish, although a prolific breeder.

Fecundity showed positive significant correlations with total body weight and total length. However, more positive correlation observed between the fecundity and total length compared to that between the former and total body weight showed that the total length is the better predictor of fecundity of the fish. This may be due to the fact that the increment in length of the body allows the gonad (ovary) to accommodate larger number of eggs before increasing the overall weight of the fish.

As with other species, positive relation was found between the fecundity and length and weight of *Schizothorax niger* and *S. esocinus* from Kashmir (Ulfat *et al.*, 2014), similar to the finding of the present study. Similar results were also reported for *S. plagiostomus* from Kasmir, India (Jan and Imtiaz, 2016). Shabana *et al.* (2013) also reported increase of fecundity with the increase in size of *Tor putitora* (Ham.). Simpson (1951) opined that fecundity is directly proportional to body weight of a fish and suggested that the environmental factors and food supply might affect the fecundity of fish. The higher values of gonadosomatic index (GSI) recorded for both male and female during the months of July and August suggest that these months indicate the spawning season of *N. heaxagonolepis* in Tamor River. Kharat and Khillare (2013) suggested that the increase in GSI during the period of gonad maturation is mainly due to the deposition of large amounts of proteins and lipids directly from ingested food during the active feeding season.

Age of fish may be considered as an important factor for the determination of size of its eggs as also opined by Nikolskii (1965). Oocytes of different size groups were found in the mature ovaries of the fish which clearly suggests that this species spawns more than one time in a single breeding season. Similar result was reported for the species from Meghalaya, India (Mahapatra and Vinod, 2011).Swar and Craig (2014) suggested that the multiple spawning behaviour of *Neolissochilus hexagonolepis* may have developed as an adaptation to unsuitable spawning conditions.The rapid accumulation of yolk protein or vitellogenin in the developing oocytes adds to the diameter of the oocytes, thereby increasing the weight of the ovaries.When the egg size was regressed against different body parameters, it was found that the egg size is more dependent on the gonad weight as compared to other parameters. Negative correlation between the egg size and total body weight (TW) suggested that larger fishes do not necessarily carry larger eggs in their ovaries. Bagenal and Brian (1978) suggested that fecundity and egg size are negatively correlated. In contrast to this, in the present study the two parameters were seen to show a weak positive correlation (r = 0.25) suggesting that the rise in the number of eggs does not necessarily correspond to the decrease in their number.Elevated GSI values accompanied with the maximum egg size in the month of July and August suggested that these months corresponded to the breeding season of the fish. These months actually fall in the peak monsoon time of the region, during which the river experiences flood with increased water current. Flood water with abundant food nutrients and high water current seemed to trigger the spawning activity for the fish.

Conclusion

The prime objective of the present investigation was to assess gonadosomatic index (GSI) and to enumerate the fecundity and size of eggs of *Neolissochilus hexagonolepis* with the aim to generate additional knowledge on the reproductive biology of the species.

The finding of the present investigation revealed the fish to be a low fecund compared to other high fecund fishes with tens of thousands of eggs. Since fecundity showed more positive correlation with gonad weight as compared to others morphometric parameters, it was concluded that among all, gonad weight (ovary weight) does the better prediction of the fecundity of the fish.

The higher values of Gonadosomatic index (GSI) recorded for both male and female katle during July and August suggested their spawning season during these months

Oocytes of different size groups were found in the ovaries of mature fish. This suggested that katle spawns more than once in a single breeding season. The fractional spawning nature of this fish can be seen as an adaptive behaviour of the fish to compensate higher mortality of its juveniles in the hill streams due to high monsoon flooding.

Maximum egg size recorded in the months of July and August accompanied by elevated GSI values during these months also confirmed the coincidence of breeding season of the fish during this time of the year. Egg size is more dependent on weight of the gonad (ovary weight) compared to others. Since, the egg size bore negative correlations with length of the body, it was confirmed that larger the fish in length smaller the size of eggs within its ovary. Similarly, as egg size was found to be negatively correlated with total body weight it was concluded that the larger fishes do not necessarily carry larger eggs in their ovaries. Moreover, it was concluded that the rise in the number of eggs (Fecundity) does not necessarily correspond to the decrease in the size of the eggs, as indicated by the negative correlation established between the two parameters.

It was observed that the breeding season of the fish during the peak monsoon season, it could be concluded that the flood water with abundant food nutrients and high current trigger the spawning activity for katle in the river.

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Meeting the nutritional needs for the children: the role of fish from the emerging aquaculture of Nepal

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Abstracts

The contributions of the fishery in the daily lifestyles for the nutritional security of the children were examined. Primary and secondary data were collected and analyzed. Literature review was done for the trend analysis and individual questionnaire was provided to children's group of 5 to 13 years, about the issues of fish dishes, preferences and nutritional awareness. The per capita fish consumption per person in Nepal is about 3.36 kg as compared to world (19 kg) and least developing countries (11 kg). The trend analysis shows that the population growth rate was 1.10% whereas the total fish growth rate was 11.52% with contribution of 4.29% in the AGDP and 1.3% in the GDP in the year of 2017. Scenario of fish pond culture shows that the 95.5% of the area was shown in the Terai, 2.48% in hills whereas, only 0.39% of the pond fish culture have been found in the mountain. Fried fish by 91.2%, fish pakauda by 50%, fish gravy by 41.4% were the three most preferred dishes for the children of age group of 5 to 13. Among different dishes fish ball was the most preferred dishes by the children of age group of 5 to 8 is due to less chance of spine in the food. Multiple regressions were run to find out the significance level of difference among the dishes prepared in Nepali kitchen. Tilapia, trout and pangasius are the emerging fish species; therefore protein and calcium level of cooked fish after proximate analysis were analyzed. Result with the p value 0.001 showed that the protein content of tilapia was lower in comparison to the cooked trout and pangasius. Whereas, result with the p value of 0.27 showed the higher level of calcium in cooked tilapia in comparison to cooked item on trout and pangasius. The highest share in area for pond culture as well as the opportunity of more calcium intake depicted the importance of tilapia to promote for the increased calcium level of children.

Keywords: Fishery, Nutrition, Children, Pangasius

Introduction

Nepal being a landlocked country to fulfill the aquaculture needs has to depend on the fresh water products. Approximately 5% of the total area of the country is known to be occupied by different freshwater aquatic habitats in the form of river, lake, swamps, ponds and rice fields which occupy 398,000ha (DoFD, 2017). Although Nepal's aquatic resources are limited to inland fishery production but the presence of 232 indigenous fish species depicted the richness in aquatic biodiversity and shows the abundance opportunities (Shrestha, 2008). Out of such area Nepal's total production of fish is 83897mt in the year of 2017 (DoFD, 2017). These figures point out the increased trend, awareness and interest of peoples about the importance of fishery. Increase in product definitely is a triggering factor for the outlet of the product. Increased fish production needs fish to be consumed, as a consequence of population increase and increasing per capita consumption among certain sectors (driven by the health benefits of consuming fish) will have to be provided primarily through aquaculture. Beside this several health benefits ω-3 or N-3 fatty acids, protein, amino acids, folic acids and vitamins found in fish food to all age group (Gurung, 2016; Limbu *et al.*, 2012) have been reported. Study done in Malawi also support the view that individuals fed intervention diets containing significantly more soft-boned fishes had lower incidences of anemia and common infections than those in the control group (Gibson *et al.*, 2003). But history shows that the per capita fish consumption of Nepal has been ranked 144th within the group of 160 countries in the year of (FAO, 2013). For the increased rate of nutrition balanced food intake; still there is a gap of awareness and food intake habits. The study therefore designed to show the information regarding the preference of fish item by the children so thatan intervene can be directed for increased nutritional availability for the children through aquaculture products.

Objectives

- To depict the trend of fishery development and identify the fish intake level of the children and recommend the ways for the increased nutritional level.
- To promote the intake of fish food in children's diet by creating enabling the fish product fits to children's diet

Methodology

This study comprises with the two parts; review of literature for the secondary data to depict ecology wise scenario of fish production and the nutritional availability in the cooked food after proximate analysis. Second part of the study was based on the personal interview with the children for their interest in fish product. With the age range of 5 to 13 years of 100 children's has been selected randomly and asked about the preference of fish dishes. Rainbow trout, Pangasius and Tilapia were the three species of fishes selected and purposively reviewed the data about the proximate analysis.

Fat, protein fiber, calcium, magnesium and phosphorus were the nutrients found after the proximate analysis in the cooked fish.Based on the basic requirement of protein and calcium intake for children and fish could be a means for supplement. The study was purposively taken for the nutritional awareness of the children therefore; only protein and the calcium content of the three fishes were done and found the R square value to compare the protein and calcium content. SPSS and Excel were the statistical package to analyze the data.

Results and discussion

Ecology wise scenario of fish production, scenario of fish production, importance and preference of fish food in children's diet has been summarized in the result and discussion part.

Ecology wise Scenario of fish production

Fish species may differ in different water temperature and mineral content of water; also the nutritional availability vary with the ecological segregation. Given the table below shows Terai ecological belt of the country with 9,488ha of area have (95.5%) the highest area coverage. Scenario of fish pond culture shows that the 95.5% of the area was shown in the Terai, 2.48% in hills whereas, only 0.39% of the pond fish culture have been found in the mountain.

Table 1. Scenario of pond fish culture.

Particulars	Pond (Nos.)	Total Area (ha)	Fish Production (mt.)	Yield/Kg/ha	Coverage area (%)
A. Fish Production from Aquaculture Practices			55,500		
A1 Pond Fish culture	39,308	9,934	48,543	4,887	
Mountain	154	11	28	2,545	0.39
Hill	4,203	435	1,205	2,770	2.48
Terai	34,951	9,488	47,310	4,986	95.5

Source: Annual book, DoFD (2017).

Fishe production in Nepal

Human population growth rate as well as fish population growth rate is shown in the Table 2. Fish population growth with 10.8% increment is quite high shows the volume of production will be encouraging to search for new niche for marketing through established a fish delivery network over the country.

Table 2. Scenario of fishery production of Nepal.

Year	Total population	Population growth rate	Total fish production	Total fish growth	Contribution of fish on AGDP
2014			57520	14.3	2.71
2015	28,656,282	1	65770	5.7	4.9
2016	28,982,771	1.10	69500	10.8	6.31
2017	29,304,998	1.08	77000		11.8

Source: Annual book, DoFD (2017).

Importance of fish food in child's food

Nutrition level and the mode of consumption is always a buzz in most of the part of Nepal where live fish is not easily accessible. How we could include fish food in children's food is a common question raised inside the parents those who hadn't included fish food during their childhood. It has been reported that nearly all fish contain trace amounts of methyl mercury, an environmental contaminant. In large amounts, all forms of mercury are toxic to nerve cells and can cause vision problems (Weisenberger, 2017). It creates controversy for the amount and the species of fish intake of inland fishery for low level of mercury level. The US Food and Drug Administration and Environmental Protection Agency advice serving 1 to 2-ounce servings of fish to children per week, beginning at age two. Fish lowest in mercury includes Salmon and Tilapia (Weisenberger, 2017). Fish is food for the brain as well as good protein therefore; Small amount of fish could be beneficial for the significant change on the dietary requirement of amino acids and are present very low amount in vegetarian diet (FAO, 2018). Based on all the review and availability context study on preference of cooked fish food of Trout, Pangasius and Tilapia was taken.

Children's preference regarding the food item

Dry fish, fish ball, fish burger, fish gravy, fish pakauda, fish pickel, fried fish were the most preferred fish dishes reported by the respondents. Among them the most preferred dish by the age group of 5 to 7 was the fried fish 39.4% and the fish ball 30%. Preference of age group of 8 to 10 was fish gravy and fried fish whereas, the age group of 11 to 13 preferred fish pakauda 50% and fish pakauda 45.5%. Among the respondents 80% reported that the preference of children towards these kinds of fish dishes are due to less chance of spines in the dishes.

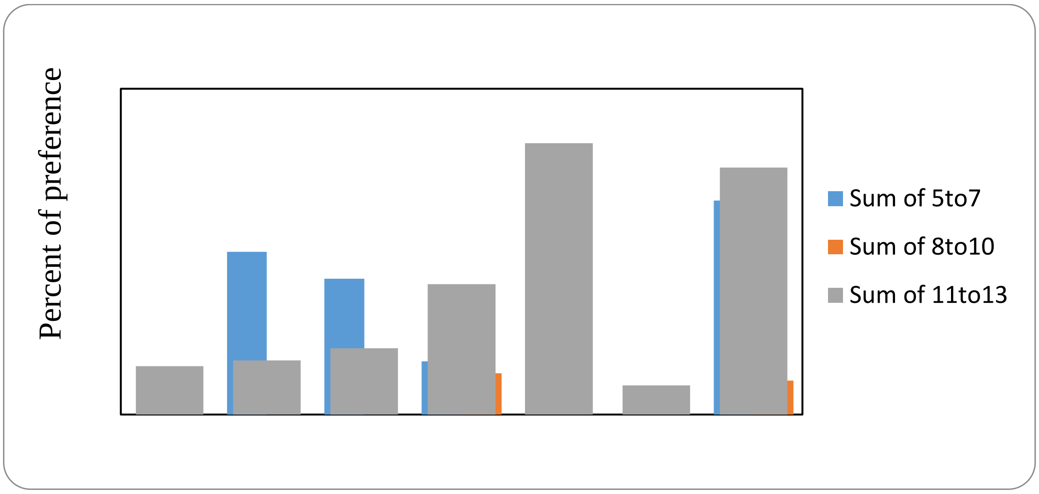

Figure 1. Fish dish preference by the children of age group of 5 to 13.
Source: School student of age group of 5 to 13

Nutrition availability on three different fish dishes

Trout, Pangasius and the Tilapia were the three fish species used during fish dishes preparation. Tilapia tacos, blackened tilapia with butter carrots, garlicky grilled tilapia with cousous, ham wrapped tilapia, oriental rainbow trout, trout curry, deep fried rainbow trout, smoked rainbow trout, steamed rainbow trout, easy lemon cheese baked pangasius, pangasius fillets in light crust, florentine pangasius fillets were the cooked dishes taken as a sample and done proximate analysis (Pradhan *et. al.*, 2072). Among various nutrient value comes from proximate value only protein and calcium content of the dishes were calculated and Table 3 shows calcium content in tilapia was highly significant than trout and Pangasiusspecies. Whereas, protein content of trout was highly significant to other two species of fish.

It is reported that lower level of protein on the diet can retard growth and development of babies and toddlers need to play and learn. Every cell in the body contains protein, it makes up enzymes needed for chemical reactions and 10 percent of a child's energy comes from protein. Dietary proteins are digested into amino acids that are used to make body proteins to grow and maintain the bones, muscles, blood, skin, hair and organs. Of the 22 amino acids, babies and toddlers can make 13 and must get the others from protein-rich foods, such as breast milk, formula, meat, eggs and fish. Tilapia fish is considered as a fatty fish and is different research has proved that fatty fish contain high level of Omega-3 fats and less mercury content (Weisenberger, 2017). Omega-3 fatty acids are healthy fats that lower inflammation and blood triglycerides. They have also been associated with a reduced risk of heart disease.Nepal, one of the poorest nations in both way due to slow economic growth and lack of marine product people suffers severe malnutrition problems especially among young children. Tilapia, fish suitable for Terai region which have large pie in production have possibility of profitable in small scale farmers as well as could provide cheap source of protein, calcium and omega -3. Beside all those benefits precautionary measures should be followed during fish consumption for reduction of stuck of spines on children.

Table 3. Status of protein and calcium content of fish.

Variable	Coefficients	Standard Error	t Stat	P-value
Constant	11.531	5.174	2.228	0.049
Protein	0.019	0.004	4.322	0.001
Calcium	-7.198	6.27	-1.148	0.277

Observation=13
R^2=0.653
F statistics=9.4

Recommendation

Develop a plan to incorporate fish food in school children's diet.Make aware to fish processor to develop fish product without the fish bones by introducing crushing fish to a paste whole fish meat paste milling machine.

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Botanicals based dietary effects of feed additives on growth performance and body composition of goldfish (*Carassius auratus*) and red cap oranda (*Carassius auratus auratus)*fingerlings

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Abstract

This experiment was carried out in the aquarium (12” × 24” × 12”) of Aquaculture lab at IAAS Paklihawa, Rupendehi District from 28 February 2017 to 28 April 2017. The growth performance of carp fish species i.e. Goldfish (*Carassius auratus*) and Red cap oranda (*Carassius auratus auratus*) were carried out on the aquarium based culture system. Four types of feed were fed to the fishes i.e. Rice bran and mustard oil cake in T1 (Control), Rice bran and mustard oil cake with Cassava leaves powder 5% in T2, Rice bran and mustard oil cake with Sweet Potato leaves powder 5% in T3 and Rice bran and mustard oil cake with *Colocassia* leaves powder 5% in T4 in aquarium during the experimental period. The average harvest weight of single goldfish was found to be 0.015 kg and that of red cap was found to be 0.006 kg. The initial weight of goldfish (kg) in T1, T2, T3, T4 was found to be 0.018±0.0015, 0.021±0.00057, 0.018±0.00100, 0.016 ±0.00450, respectively and final weight of same fish (kg) was found to be 0.034±0.0568, 0.037±0.00435, 0.028±0.00057, 0.027±0.00624, respectively. Similarly initial weight of red cap fish (kg) was found to be 0.0137±0.00096, 0.0133±0.00050, 0.0125±0.00058, 0.0132±0.00083 and final weight of same fish (kg) was found to be 0.0235±0.00493, 0.0175±0.00790, 0.0233±0.00328, 0.0214±0.00590 in T1, T2, T3, T4, respectively. There was no significantly difference in FCR, SGR %, Survivability % of Redcap, Goldfish and both fishes. There were no significantly different in water quality parameters in terms of pH, DO and Temperature in different four treatments in the period of two months of experimental period.

Key words: Goldfish, Red cap oranda fish, Feed additives, Fish growth

Introduction

Ornamental fish keeping is one of the most popular hobbies in the world today. With the expansion of global ornamental fish trade, increased attention is being paid to the nutritional requirements of ornamental fish. Nutrition is one of the key factors in improving production efficiency of ornamental fish. The other factors include growth, health, body colour and breeding of these fishes. Nutritional requirements and feed management needs in ornamental fish are determined mainly based on the information of these and the experiences of successful aquarist in the line.Fish feed is the most expensive input during aquaculture operations. The high cost of feed arises from extensive reliance on animal protein sources, such as fishmeal and shrimp meal (Omoregie, 2001). Shortage and high cost of pelleted feed severely constrains the development of low cost aquaculture systems suitable especially for small-scale farmers. Therefore, there is a need to assess the potential of non-conventional raw ingredients before use in fish diets. Good nutrition in animal production systems is essential to economically produce a healthy and high quality product. Fish nutrition has advanced dramatically in recent years Omoregie and Ogbemudia (1993) with the development of new, balanced commercial diets that promote optimal fish growth and health. However, as the cost of fish production continue to escalate due to soaring feed prices owing to extensive use of expensive animal protein like fish meal, aquaculture production becomes a less or non-profitable enterprise (El-Sayed, 2006). It is of primary importance for fish farmers to find affordable and high quality fish feeds through the use of locally available plant ingredients. Therefore, it is necessary to explore utilization of plant proteins in fish feeds as substitutes for expensive animal protein materials (Omoregie and Ogbemudia, 1993).Fish meal has become the most essential protein for commercial aquaculture feeds. It provides the fish with high quality protein, an essential amino acid profile and has high palatability (Li *et al.*, 2006). However, fish meal is an expensive source of protein and is inaccessible to small scale fish farmers because of other valuable competing uses including human consumption. Therefore, replacement of fish meal with cheaper ingredients of plant origin in fish feed is necessary because of rising costs and uncertain availability of fish meal (Higgs *et al.,* 1995). Plant proteins are likely candidates because of local availability and low cost (Lim and Webster, 2006). However, substituting fishmeal with plant protein ingredients mostly results in reduction in fish growth (Francis *et al.,* 2001). The current study, therefore, aims at evaluating the nutritional potential of plantingredients despite its associated challenges. The future development of small-scale aquaculture system depends on the use of available local ingredients which will reduce feed cost (Edwards and Allan, 2004). However, for plant ingredients to be incorporated into least cost-diets, an assessment of nutritional value is vital. It is imperative to systematically characterize the biological value of plant raw materials (Olele, 2011).

Cassava (*Manihot esculenta*, Crantz) as an all – seasoncrop as food in several parts of Africa (Nigeria inclusive), Asia and Latin America is well documented (Longe, 1980; Rosling, 1987; Bradbury *et al.*, 1991). Cassava leaves, a byproduct of cassava root harvest is (depending on the varieties) rich in protein (14 - 40% Dry Matter), minerals, Vitamin B1, B2, C and carotenes (Eggum, 1970; Adewusi and Bradbury, 1993).

Colocasia is widely produced throughout theworld for its underground corms (Njintang *et al.*, 2007).The nutritional value is the main concern when a crop is being considered as a food source. Due to the emphasis placed on the nutritional value of food by consumers, a great need exists for information on the nutritional contents of root crops (Huang, *et al.*, 2007). Starch is the most important component (73-80%) of taro (Njintang *et al.*, 2007). It contains about 11%protein on a dry weight basis. This is more than yam, cassava or sweet potato. The protein fraction is rich in essential amino acids of trionine, leucine, arganine, valine and phnylalanine. Among the essential amino acids methionine, lycine, cystine, phnylalanine and leucine are relatively abundant in the leaf than the corm (FAO, 1999).

Sweet potato,among other root and tuber crops, contains higher contents of carbohydrates, various vitamins,minerals, and protein than other vegetables (Shih *et al.*, 2007).It also contains much higher levels of provitamin A, vitamin C and minerals than rice or wheat (Wang *et al.*, 1997).

Objectives

- To assess the feeding trial of goldfish and red cap oranda fish species in aquarium rearing.
- Specific objectives
- To compare the growth performance of goldfish and red cap oranda fish in different botanical feeding treatments
- To assess the water quality parameter in aquarium system of fish rearing

- To assess the overall feed performance in different botanical treatment and replications

Materials and methods

Experimental setup: The experiment was carried out in the aquarium of Aquaculture lab at IAAS Paklihawa, Rupendehi District on 28 February 2017 to 28 April 2017. The growth performance of carp fish species i.e. Goldfish (*Carassius auratus*) and red cap oranda (*Carassius auratus auratus*) were carried out on the aquarium based culture system.

Aquarium preparation: Twelve glass aquarium tanks (12” × 24” × 12”), each ¾ filled with water were aerated continuously using an air compressor. 24 farm-raised *Carassius auratus* fingerling (with an average initial weight of 0.009 kg) and 48 farm raised *Carassius auratus auratus* (with an average initial weight of 0.003 kg) were acclimated to laboratory conditions for 14 days before being distributed randomly into the twelve tanks representing three dietary treatments (5% per kg of feed additives) respectively and a control (0 % per kg of feed additive). Fish were fed at 2-3% of their body weight per day between 8:00-9:00. All fish were weighed and counted fortnightly and feeding rates were adjusted accordingly.

Procurement of research materials: The aquarium, aerator as well as fingerlings of Goldfish and Red cap oranda were brought from Prashan Aquarium and Plantation, Bhairahawa.

Experimental design: The experiment was setup in a Completely Randomized Design (CRD) with 4 treatments and 3 replications.

Feed preparation: Four types of feed were provided to test the growth rate of the carp fishes.

Local Feed (Rice Bran + Mustard Oil Cake): Mustard oil cake (MOC) was soaked for 12 hours in water and then 250 gm of water soaked MOC and 250 gm Rice Bran in 1:1 ratio were mixed thoroughly in the tray. The thoroughly mixed feed was grind manually to appropriate size for fingerling feeding and then the feed was oven dried at 121 °C for 10 minutes.

Cassava (5 %): MOC and Rice bran (1:1) mixed with Cassava leaves (5%) thoroughly in the tray. The thoroughly mixed feed was grinded manually to appropriate size for fingerling feeding and then oven dried at 121 °C for 10 minutes.

Sweet potato (5 %): MOC and Rice bran (1:1) mixed with Sweet Potato leaves (5%) thoroughly in the tray. The thoroughly mixed feed was grinded manually to appropriate size for fingerling feeding and then oven dried at 121 °C for 10 minutes.

Colocasia (5 %): MOC and Rice bran (1:1) mixed with Colocasia leaves (5%) thoroughly in the tray. The thoroughly mixed feed was grinded manually to appropriate size for fingerling feeding and then oven dried at 121 °C for 10 minutes.

Fish seed stocking: The fingerlings were brought in 2 plastic bags containing gold fish and red cap oranda fish in each from Prashan aquarium and plantation to the aquaculture lab. It was difficult for the fingerlings to survive outside the environment immediately. In order to acclimatize them with favourable environmental condition they were placed on the pond surface along with the plastic bag for certain time period (approximately 15 mins). When the environmental condition favoured them (i.e. after 15 mins) they were slowly released from the plastic bag, counted and kept inside the aquarium as per the stocking density. The number of stocking density of fishes in each aquarium consists of 2 Goldfish and 4 Red Cap Oranda fish.

The stocking of fingerlings were done according to the 4 treatments along with 3 replications as:

(1) T1 - Fish fed with local feed (Control)
(2) T2 - Fish fed with local feed (Cassava leaves 5 %+Local feed)
(3) T3 - Fish fed with local feed (Sweet potato leaves 5%+Local feed)
(4) T4 - Fish fed with local feed (Colocasia leaves 5%+Local feed)

Table 1. Treatment and Replications in each Aquarium.

Treatment	**T1**	**T2**	**T3**	**T4**
R1	T1R1 (1)	T2R1(2)	T3R1 (3)	T4R1 (4)
R2	T1R2 (5)	T2R2 (6)	T3R2 (7)	T4R2 (8)
R3	T1R3 (9)	T2R3 (10)	T3R3 (11)	T4R3 (12)

Feeding: The fingerlings were given feed on the basis of average weight of the two speciesSo, before stocking the average weight was taken and the feed was calculated as 3 % of the body weight which was calculated as follows:

Table 2. Calculation of average weight of fingerlings in each aquarium.

Number of Goldfish	Weight of Goldfish	Number of Red cap	Weight of Red cap
2	X (say)	4	Y (say)
1	X/2	1	Y/4
24	X/2*24	48	Y/4*48

Average weight of the fingerlings in each aquarium = Average weight of Goldfish+ Average weight of Red cap oranda fish i.e Average weight of the fingerlings in each aquarium = X/2*24+Y/4*48

Therefore, Amount of feed in each aquarium = 3% of Body weight of the fingerlings in each aquarium

Water quality analysis

Daily measured water quality parameters were:

pH : The P^H of the water was measured daily at 8:00-9:00am with the help of digital pH meter. It was dipped in the aquarium, slowly shaken and the reading so obtained was recorded on the note.

Dissolved Oxygen (DO): Dissolved oxygen was recorded daily at 8:00-9:00 am. The oxygen meter was calibrated first and then dipped into the water and DO was measured and noted.

Temperature: The temperature was measured with the calibrated DO meter at the same time of measuring of DO. It was noted in °C.

Water quality maintenance: The water quality and transparency was maintained by changing water of the aquarium weekly.

Fish sampling and growth measurement:

The fish sampling was done fortnightly by using electronic balance, scoop net and plastic bucket. By the help of scoop net the fish was trapped and placed in the plastic bucket filled with water. Initially small amount of water was taken in the bucket placed on the electronic balance and weights of two types of fishes were noted separately.

The total weight of fish in each aquarium was calculated as:

Total wt. of fish in each aquarium= Av. wt. of Goldfish + Av. wt. of Red cap Oranda fish

Statistical analysis:

The statistical analysis of data was performed by using SPSS (version 16.0).Microsoft excel computer program was used for data tabulation and figure preparation. Microsoft word was used for the preparation of report.

Table 3. Water quality parameters measured during experimental period.

Parameters	Measured unit	Method/Instruments
DO	mg/L	Lutron Oxygen meter Model DO 5510.
Temperature	°C	Lutron Oxygen meter Model DO 5510.
pH		Lutron pocket type Model pH-201.

Results

Fish yield

In the Table 4, there was no significantly different in initial weight of Goldfish in T1 than T2, T3 and T4. But it was significantly different in T2 than T4. Same way, there was no significantly different among all treatments. But we can see, there was significant different T1 than T2. Furthermore, there was no significant difference in final weight of Goldfish in T1 among all the treatments. But it is seemed that there was significant different in T2 than T3. Same way T3 is significantly different than T2 and T4 is also significantly different than T2 in case of final weight of goldfish. In case of initial weight of redcap fish, there was significantly different in T1 than T3 and T3 than T1, respectively. But there was no significant different in T2 and T4 with other treatments. Similarly, it is found that there was no significant difference in all the treatments in case of final weight in red cap fish and Goldfish.

Table 4. Stocking and harvest weight of Redcap and Goldfish (Mean± SE).

Parameters	T1	T2	T3	T4
Total No. of Redcap	12	12	12	12
Ini. Wt. of Redcap (kg)	0.013±0.0009^{a}	0.013±0.0005^{ab}	0.0125±0.0005^{b}	0.0132±0.0008^{ab}
Final Wt. of Redcap (kg)	0.023±0.0049	0.017±0.0079	0.0233±0.0032	0.0214±0.0059
Total No. of Goldfish	6	6	6	6
Ini. Wt. Goldfish (kg)	0.018±0.0015^{ab}	0.021±0.0005^{b}	0.018±0.0010^{ab}	0.016 ±0.0(
Final Wt. Goldfish (kg)	0.034±0.0568^{abc}	0.037±0.0043^{a}	0.028±0.0005^{bc}	0.027±0.0062^{c}
Ini. Wt. of both fishes (kg)	0.032±0.0034	0.033±0.0009	0.0298±0.0045	0.0318±0.0033
Final Wt. of both fishes (kg)	0.055±0.0093	0.045±0.0066	0.0520±0.0088	0.0510±0.0088

Water quality analysis

Weekly and fortnightly means of water quality parameters are presented. Daily mean pH was recorded from 2017/2/28 to 2017/3/15 based on the different treatments. During the period, the mean pH was found highest with 9.202 in T3R2 and lowest with 9.123 in T1R2 (Figure 1). At the same time, daily dissolved oxygen was recorded during the same time period within the different experimental period was found 6.50 mg/L in T2R1 and 5.647 mg/L in T2R3 respectively (Figure 2). Based on the Figure 3, the daily mean temperature was recorded 23.42 ^{O}C in T1R1 and 23.24 ^{O}C in T3R3, respectively. Based on the Table 3, there were no significantly different in water quality parameters in terms of pH, DO and Temperature in different four treatments in the period of two months of experimental period.

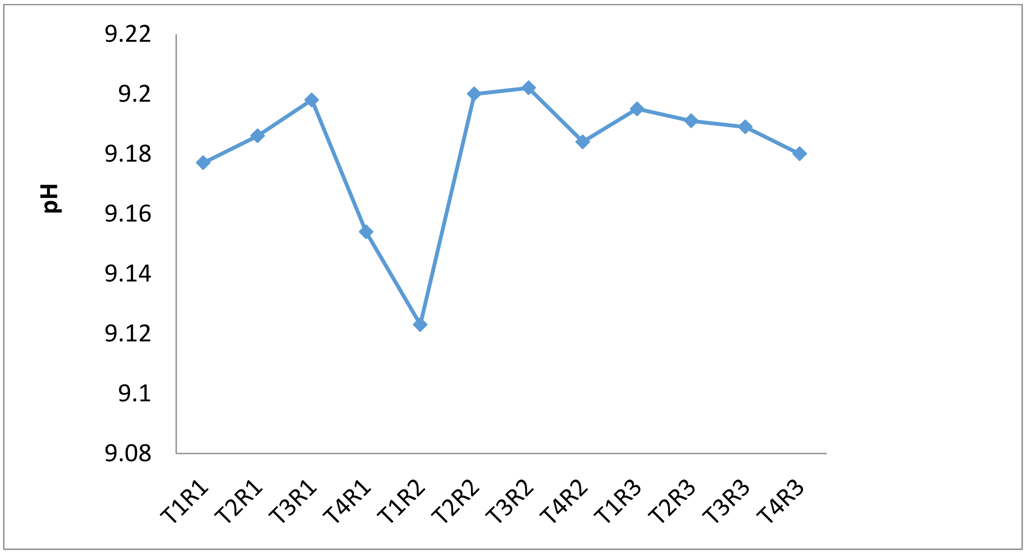

Figure 1. Daily mean pH of aquarium water in each treatment during the experimental period.

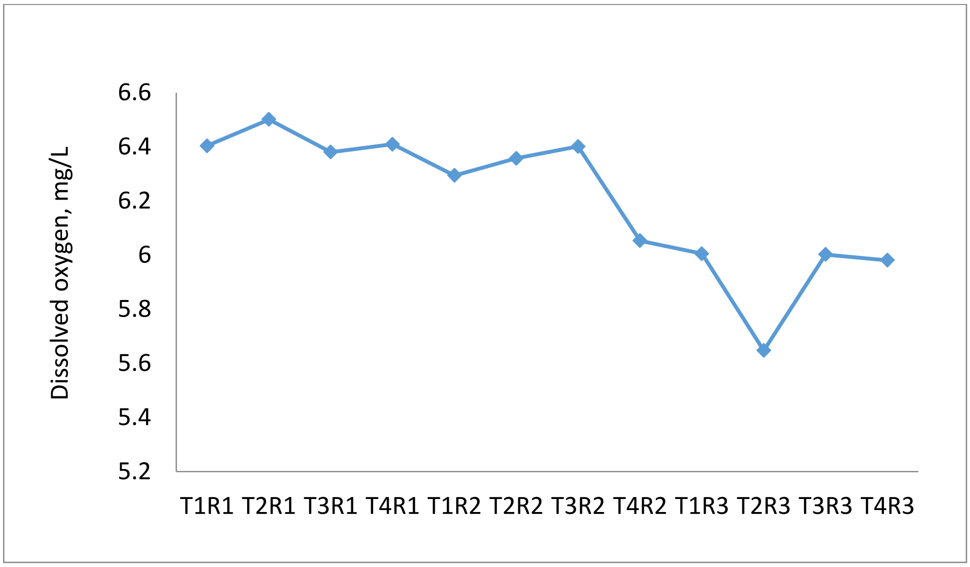

Figure 2. Daily mean dissolved oxygen of aquarium water in each treatment during the experimental period.

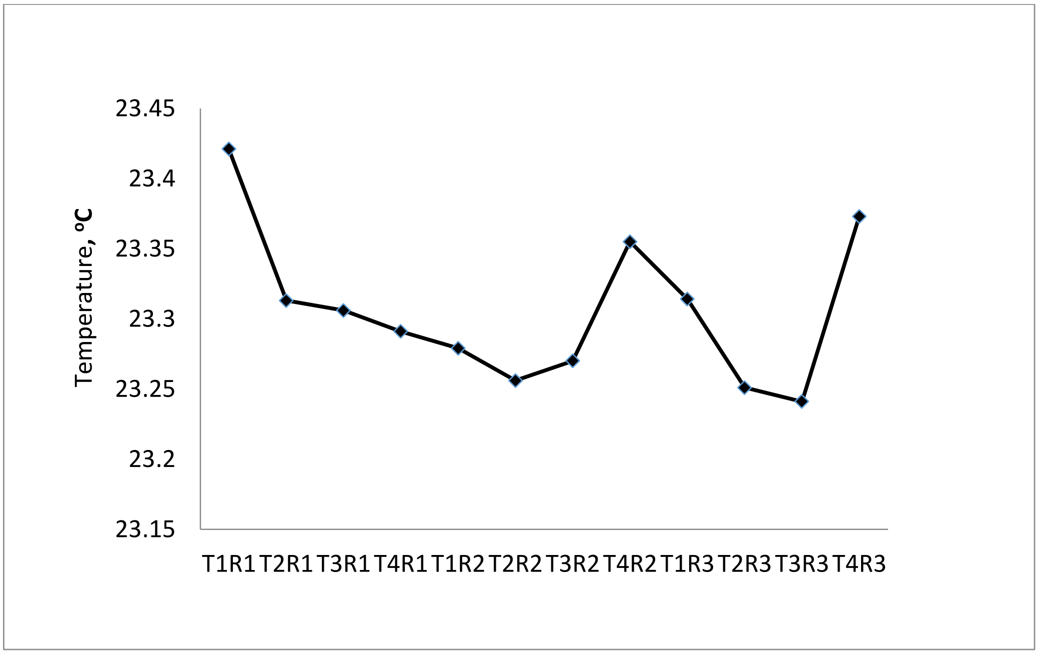

Figure 3. Daily mean temperature (^{O}C) of aquarium water in each treatment during the experimental period.

Discussion

Growth performance of fishes

In case of final average weight of Goldfish, T3 is significantly different than T2 and T4 is also significantly different than T2 in case of final weight of goldfish. The performance of Sweet potato leaves has remarkable outcome than the feed mixed with cassava and colocasia. In case of final weight of Redcap, it is found that there was no significant difference in all the treatments, it is due to the average size of individual Red cap fish is comparatively smaller with 0.003 kg than Goldfish having 0.009 kg in stocking time period. Similarly it is also addressed with the justification that the handling stress during the time of changing water through the direct deep bored artisanal water system might have affected the accidental fluctuation in water quality parameters. As the fish size is estimated directly influence by sudden changes in water quality deterioration. Furthermore, it is justified with the result that In case of initial weight of redcap fish, there was significantly different in T1 than T3 and T3 than T1, respectively. There is no substantial increment in final average weight of both fishes with the same feedstuffs. The stocking average weight of single goldfish was found to be 0.009 kg and that of red cap was found to be 0.003 kg. The average harvest weight of single goldfish was found to be 0.015 kg and that of red cap was found to be 0.006 kg. The initial weight of goldfish (kg) in T1, T2, T3, T4 was found to be 0.018±0.0015, 0.021±0.00057, 0.018±0.00100, 0.016 ±0.00450, respectively and final weight of same fish (kg) was found to be 0.034±0.0568, 0.037±0.00435, 0.028±0.00057, 0.027±0.00624. Similarly initial weight of red cap fish (kg) was found to be 0.0137±0.00096, 0.0133±0.00050, 0.0125±0.00058, 0.0132±0.00083 and final weight of same fish (kg) was found to be 0.0235±0.00493, 0.0175±0.00790, 0.0233±0.00328 and 0.0214±0.00590. There was no significantly difference in FCR, SGR% and Survivability% of Redcap, Goldfish and both fishes. There were no significantly different in water quality parameters in terms of pH, DO and Temperature in different four treatments in the period of two months of experimental period.

Water quality

The mortality of red cap oranda goldfish might be due to the presence of comparatively bigger sized goldfish which intake more feed than the small fishes and they had also shown aggressive nature towards the small fishes in feed consumption. Additionally, we had seen the effect of low DO on the red cap oranda goldfish which were more prone to low DO than the big goldfish.

When the DO was lower than 4 mg/L, the small red cap started moving irregularly due to suffocation and started to float on the surface of the aquarium water. The pHwas about normal i.e. 8-9 during the experimental period which was normal and was generally accepted by the fishes. The DO of water was seen decreasing with increase in temperature due to seasonal variation of temperature.The average pH varied in each aquarium from 9.123-9.202 in two months while the DO varied from 5.646-6.501 mg/L and the temperature varied from 23.241-23.421 ºC.

Conclusion

Use of botanicals in fish feed was found to be an innovative and low cost feeding technology for proper growth and development of goldfish and red cap oranda fish due to the feed availability anywhere in the country with reasonable price of the low cost botanicals. We used four types feed prepared from botanicals including, cassava leaves, sweet potato leaves, colocasia leaves in mixture with MOC, Rice bran in definite proportion and fed as 3% of body weight for two months. The water was changed in weekly basis and weight was measured fortnightly.

By the above result, we can conclude that if average size of Goldfish can directly influenced by the outcome of sweet potato leaves contained feed additives than other botanicals supplement used in feedstuffs for the proper growth of Goldfish. We can also conclude that in the same way with the stocking of maintaining average size of Redcap for proper growth.

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Spawning response of Sahar (*Tor putitora*) in Terai region of Nepal

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Abstract

Sahar, *Tor putitora* is a highly valued and important indigenous riverine species initiated to culture in ponds. Limited seed production of this species has restricted for expansion in culture to increase the availability of sahar seed. An experiment was conducted at the Department of Aquaculture and Fisheries, Agriculture and Forestry University, Rampur, Chitwan from 1st August 2014 to 30th April 2015 to explore and assess the breeding performance of sahar in terai region of Nepal. Twenty eight male broods (0.5-1.5 kg) and 35 (0.8-2.5 kg) female broods were reared in ponds at the rate of 1000 kg/ha with 35 % supplementary feed. Maturity observation was conducted biweekly during off-season and the frequency was increased to every third day as breeding season approaches. Males of about 1-2 year were found ready to spawn almost in all months during breeding season. A female sahar of 3-5 years old was ready for breeding in March without using any artificial hormonal (natural breeding) when the water temperature was 23.3-25.2°C. In the same month, another female brood responded by inducing hormone (ovaprim) at the rate of 0.5 ml/kg (induced breeding) when the temperature was 25.3-28.7°C. Ova from mature female were obtained by simple hand stripping method and fertilized with milt collected from male manually. In both natural breeding and induced breeding, the number of eggs was 94 and 103 per gram of brood, fertilization rate was 98% and 99%, hatching rate was 95% and 97%, hatchling survival was 81% and 90% and time to yolk sac absorption period was 6 and 5 days respectively. The size of eggs and larvae produced by females were also investigated in detail in both natural and artificial breeding. The mean fertilized egg diameter, mean fertilized egg weight, mean newly hatched larvae length, mean newly hatched larvae weight, mean yolk sac absorbed larvae length, mean yolk sac absorbed larvae weight, mean length of jump up fry and mean weight of jump up fry of natural and induced breeding were 2.9±0.2 mm and 3.1±0.3 mm, 12.37±0.80 mg and 12.69±0.78 mg, 9.4±1.2 mm and 8.9±0.7 mm, 13.01±0.53 mg and 13.19±0.49 mg, 11.5±0.5 mm and

11.5±0.5 mm, 10.09±1.12 mg and 9.87±1.41 mg, 14.6±0.52 mm and 13.3±0.5 mm and 20.96±1.08 mg and 14.04±0.40, respectively. Out of 35 brood, only two brood have responded for breeding and rest of the brood have gone over ripped in different months (November-March), which was due to less frequently maturity observation. This study indicates that most of the sahar brood responded well during February-March than September-October. This study concludes that higher spawning success rate can be achieved by determining the optimum timing for egg stripping by monitoring and frequent checking of brood fish for ovulation under cultured condition. Thus, it concludes that natural and induced breeding and fry rearing is possible in Terai region of Nepal. However, synchronization of breeding time and mass production of fries are recommended for further studies.

Keywords: Sahar, Indigenous fish, Stripping, Natural breeding, Induced breeding

Introduction

Sahar (*Tor putitora*), a golden sahar also known as "Mahseer", is one of the massive fish species of the torrential water of the Himalaya. It is very popular, economically important, high-value indigenous fish species of Nepal. Sahar is game as well as food fish and widely distributed in rivers, streams and lakes of Nepal (Rai *et al.*, 1997). The price of sahar in the Nepalese market is almost double, compared to the commonly cultivated carps and tilapia species. Sahar is still taken in capture fisheries in lakes and rivers, and no commercial cultivation has begun in Nepal. This species is declining from its natural habitat mainly due to urbanization, illegal encroachment, over-fishing, and ecological alterations of physical, chemical, and biological conditions in the natural environment (Bista *et al.*, 2007). Hence, need for conservation of this species has been realized. In recent years, the success in its artificial breeding has provided the additional enthusiasms towards the development of this species for commercial cultivation as well as the rehabilitation of its population in natural waters (Rai *et al.*, 2006). Attempts to culture and conserve this species have been initiated in Nepal with major efforts to develop culture technology and propagate the species (Gurung *et al.*, 2002; Joshi *et al.*, 2002). This has led to a better knowledge of spawning biology, ecology, and behavior of this species, as well as preliminary growth performance in captive conditions. Enhanced growth in tropical and subtropical ponds, as well as the recent breeding success in hatcheries, has raised new hopes on the prospects of sahar aquaculture in Nepal (Shrestha *et al.*, 2005, 2007; Bista *et al.*, 2001; 2007; Rai, 2008). In addition to culture of fish to adult size for consumption, these new developments can contribute to rearing individuals that can be stocked into natural waters to replenish populations there. Due to its omnivorous and predatory feeding, sahar has also proved to be a good candidate to co-culture with mixed-sex tilapia to control tilapia recruits in a pond and provide better size at harvest and yield of tilapia (Shrestha *et al.*, 2011). Inclusion of sahar in polyculture of mixed-sex tilapia with carps has enhanced production in these ponds (Jaiswal, 2012).Sahar is known to be an intermittent in spawning behavior. It can spawn in most months, except January, under cultured conditions, but in natural waters, it spawns during the monsoon when rivers and streams are at peak flows. Sahar typically migrate a long distance from large rivers to streams for spawning. The Fisheries Research Center in Pokhara is the only center that produces sahar fry in limited quantity. Demand for sahar fry has increased for restocking in rivers and lakes, as well as for aquaculture production. Lack of availability of fish seed is a major bottleneck for commercial production and conservation.

Materials and methods

The experiment for sahar breeding activity was conducted at the Aquaculture farm of Department of Aquaculture and Fisheries, Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal for 9 months from 1st August 2014 to 30th April 2015. For response studies, female sahar brood fish carried from fisheries research center, Pokhara were reared in controlled condition. Male brood fish more than one year age were collected from the aquaculture farm of department of aquaculture and fisheries. Females of approximately 1.0-2.5 kg body weight were stocked at the rate of 1000 kg/ha. Brood fish were fed with 30-40 % protein diets. The feeding rate was 2-5 % of the total body weight. Pond water quality parameters such as temperature, pH, and dissolve oxygen were measured every morning using Lutron Oxygen Meter DO-5510 and Lutron YK-21 PH model. Maturity of brood was monitored in regular interval. The brood fish were checked biweekly before the breeding season (May–August). The male broods were always found matured with oozing milt after pressing their belly but females were not in those months (May-August).As the breeding season approaches maturity test frequency was increased during August- November and February-May to every third day.For the remaining two months (December and January), maturity observation was performed once in the month. The broods collected from ponds were hold in hammock and readiness of brood for spawning was examined by applying gentle hand pressure near the genital opening. Male released milt and a female brood released ova on slight pressure on 9th March during maturity test. The female brood and two males were transported to the hatchery. The clean and dried female brood was stripped gently to receive eggs in clean and dried bowl. Milt from both males was also collected in another clean and dried bowl which was mixed with eggs for dry fertilization. The fertilized eggs were washed several times and spread in Atkin's incubators by allowing one layer of eggs to settle on single mesh screen in flow through system, where water flow was maintained 7-9 L/minute. The incubation trays were covered with towel to make dark over the tray. The fertilized eggs during incubation were observed after 24 hrs and unfertilized eggs were counted. Dead eggs were counted and removed each day with help of feather to protect the healthy eggs from fungal infection. After 4 days (96 hrs) hatching occurred and was completed within 24 hrs with distinct eyes seen in hatchling. Early hatched larvae has large amount of yolk sac and settled around the stone kept or near corners of the incubation tray. After attaining free-swimming stage the larvae were transferred into a tank of 2.5m x 0.40m x 0.30m dimension.On 26th March, 2 female brood fish were injected with ovaprim hormone at the rate of 0.5 mg/kg body weight. Along with those female broods 4 matured male broods were also kept in the spawning tank with continuous showering of water. After 26 hrs, when the brood was checked by pressing gently on the stomach, one female brood released eggs. Milt from two males were used to fertilize the eggs. Fertilized eggs were incubated as before and similar process repeated. Hatching occurred after 60-72 hrs. Following reproductive parameters were measured to analyze the breeding performance.

Fecundity:

$$\text{Relative fecundity (eggs/kg)} = \frac{\text{number of eggs (estimated)}}{\text{weight of female , kg}} \text{ x } 1000$$

Fertilization rate:

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs}} \text{x } 100$$

Hatching rate:

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatchlings}}{\text{Total number of fertilized eggs}} \text{ x } 100$$

Survival rate:

$$\text{Survival rate (\%)} = \frac{\text{Number of fry harvested}}{\text{Number of larvae stocked}} \text{ x}100$$

Besides the above parameters other reproductive performance were measured by different equipment and at different time. After fertilization total number of eggs and egg number per kg body weight were calculated. Egg size, mean weight of egg and mean weight of swim up fry were measured by scale and electronic balance. Fertilization rate, hatching rates, incubation period, hatchling survival rate, yolk absorption time and time of hatchling to fry were recorded at their respective time.

Results and Discussion

Out of 35 female brood were stocked, one brood was observed over matured on 21st November 2014 another on 24th November 2014. On 8th December 2014 two broods were found over matured and also on 9th December 2014 again two broods showed over maturation. Another brood showed over maturation on 4th January 2015. In February, altogether four broods were over matured. Similarly, in March four broods were over matured. In total, 15 female broods were found over matured. Regular maturity observation of sahar during present study showed that getting correct time for maturity is critical in breeding of sahar and thus examination of brood for maturity must be done more frequently. As reported by Bista *et al.* (2010), pond reared sahar shows intermittent spawning characteristics and thus determining the optimum timing for egg stripping by monitoring and frequent checking of brood fish may result into spawning success rate of more than 50%. Although frequent examination of brood for maturity was carried out and 15 broods were found over matured from 21st November, 2014 to 27th March, 2015 while success on breeding was attained only in two broods. Two consecutive spawning occurred on 9th March, 2015 and 27th March, 2015. Overripe females were recorded even when temperature ranged between 15.5-28.7°C from last week of November to last week of February.

Table 1. Result of maturity test in different month during research period.

Date	No. of over matured brood	No. of spawned brood	Water temperature of brood fish pond (°C)
21st November 2014	1		21.2-23.0
24th November 2014	1		19.7-22.8
8th December 2014	2		18.2-21.2
9th December 2014	2		17.5-20.5
4th January 2015	1		15.5-17.2
21st February 2015	1		19.1-21.3
24th February 2015	2		19.4-22.0
27th February 2015	1		20.6-22.8
4th March 2015	2		22.5-26.0
7th March 2015	1		22.2-25.4
9th March 2015	1	1	23.3-25.2
27th March 2015		1	25.3-28.7

One sahar was ready to spawn without injecting hormone (Natural breeding) under captive condition on 9th March 2015 when minimum temperature of water was 23.3°C and maximum temperature was 25.2°C. Similarly, breeding of hormone induced (Induced breeding) fish occurred when minimum temperature was 25.3°C and maximum temperature was 28.7°C (Table 2).Body weight of female that spawned without injecting hormone (Natural breeding) (B1) was 1.2 kg and two males were of 0.65 kg and 0.80 kg. Similarly for hormone induced breeding, female (B2) weight was 1.3 kg and 0.72 kg and 87 kg of male were used (Table 2).Total egg number of eggs obtained from natural breeding female was 2585 while 4738 was in case of hormone induced breeding. Similarly, the egg number per kg body weight was 2119 and 3746 for natural and hormone induced breeding, respectively. In case of natural breeding 1g of ovulated egg contained 94 eggs while in hormone induced breeding 103 ovulated eggs were in 1 g of egg (Table 2).Fertilization rate of natural breeding was 98% and in hormone induced breeding that was 99%. It took 96-104 hrs for incubation period in natural breeding. Similarly in case of induced breeding incubation period was completed in 80-88 hrs (Table 2).In case of natural breeding hatching rate was 95% while in hormone induced breeding that was 97%. Hatchling survival of natural breeding was 81% whereas in hormone induced breed hatchling survival was upto 90% (Table 2). Newly hatched larvae length (mm) and weight (mg) of natural breeding were 9.4±1.2and 13.01±0.53, respectively. Similarly, newly hatched larvae length (mm) and weight (mg) of hormone induced breeding were 8.9±0.7 and 13.19±0.49, respectively (Table 3).It took 6 days to complete yolk sac absorption in natural breeding at 19.4-26.2 °C. Similarly in hormone induced breeding it took 5 days to absorb yolk sac at 24.8-27.2 °C completely. Average yolk sac absorbed larvae length (mm) and weight (mg) of natural breeding were 11.5±0.5 and 10.09±1.12, respectively. Similarly, average yolk sac absorbed larvae length (mm) and weight (mg) of hormone induced breeding were 11.5±0.5 and 9.87±1.41, respectively (Table 3).

Table 2. Breeding performance of sahar with and without using inducing hormone.

Description	**Natural breeding**	**Induced breeding**
Date	9th March 2015	27th March 2015
Temperature (°C)	23.3 -25.2	25.3-28.7
Female body wt (kg)	1.2	1.3
Male body wt (kg)	0.65, 0.80	0.72, 0.87
Total egg spawned	2585	4738
Egg number per kg body wt	2119	3746
Ovulated eggs per g	94	103
Fertilization rate (%)	98	99
Incubation period (hour)	96-104	80-88
Hatching rate (%)	95	97
Hatchling survival (%)	81	90
Yolk sac absorption period (Days)	6	5
Time to swim up fry (Days)	17	17

First successful spawning occurred on 9th March, 2015 without injecting hormone (Natural breeding) when water temperature was 23.3-25.2°C. While second spawning occurred on 27th March, 2015 after injection of Ovaprim (0.5 ml/kg body weight) when water temperature was 25.3-28.7°C. Bista *et al.* (2010) reported that spawning occurred when temperature ranged between 26-27.4°C on one occasion and 20-21°C on second occasion in Pokhara with higher number of spawners in February- March compared to September- October. Pandey *et al.* (1998) reported successful spawning by hormonal injection when water temperature in pond was 18-24°C. However, dose administered was comparatively lower (0.2 ml/kg body weight) than present study. Bista *et al.* (2010) have also reported that diameter and weight of fertilized eggs were 2.87±0.13 to 2.98±0.08 mm and 13.90±0.91 to 15.38±1.26 mg respectively. Also, Bista *et al.* (2010) reported incubation period of 45-125 hours at water temperature 19-28°C. Incubation period of common eggs decreases with increase in temperature (Shrestha & Pandit, 2012). Thus lower incubation period for second lot of eggs compared to first lot of eggs can be attributed to increase in temperature. The result of length and weight of newly hatched larvae was accordance with Bista *et al.* (2010) which were 10.1-10.5 mm and 9.76-10.22 mg respectively. Similarly the length and weight of yolk sac absorbed larvae to be 11.5-12.0 mm and 10.5-11.3 mg, respectively was reported by Bista *et al.* (2010).

Brood rearing during present study was carried out in temperature ranging between 14.3 to38.2°C, DO ranging from 1.4-13.5 mg/L and average pH ranging from 5.9 to 10.4. Natural spawning occurred when temperature of pond was 23.3°C minimum and 25.2°C maximum and induced spawning occurred when temperature of pond was 25.3°C minimum and 28.7°C maximum. Bista *et al.* (2011) reported that Sahar female responded to natural breeding in autumn when temperature was 22-27°C and in spring when temperature was 19-25°C. Similarly, Pandey *et al.* (1998) reported that induced breeding of Sahar was successful when temperature was 18-24°C. Bista *et al.* (2010) also reported that pH was 7-9 while DO was 3-9 when natural breeding occurred.

Table 3. Mean and range of egg diameter, length and weight (mean±SE) of larvae & jump fry of two breeding parents.

Parameters	Brood	
	Natural breeding	Induced breeding
Mean Fertilized eggs diameter (mm)	2.9±0.2 (2.8-3.5)	3.1±0.3 (2.8-3.3)
Mean Fertilized eggs weight (mg)	12.37±0.80 (11.57-13.17)	12.69±0.78 (11.97-13.47)
Mean Newly hatched larvae length (mm)	9.4±1.2 (8.2-10.6)	8.9±0.7 (8.2-9.6)
Mean Newly hatched larvae weight (mg)	13.01±0.53 (12.48-13.54)	13.19±0.49 (12.70-13.68)
Mean yolk sac absorbed larvae length (mm)	11.5±0.5 (11-12)	11.5±0.5 (11-12)
Mean Yolk sac absorbed larvae weight (mg)	10.09±1.12 (8.97-11.21)	9.87±1.41 (8.46-11.28)
Mean swim up fry (17 days) length (mm)	14.6±0.5 (14.08-15.12)	13.3±0.5 (12.8-13.8)
Mean swim up fry (17 days) weight (mg)	20.96±1.08 (19.1-22.5)	14.04±0.40 (13.5-14.6)

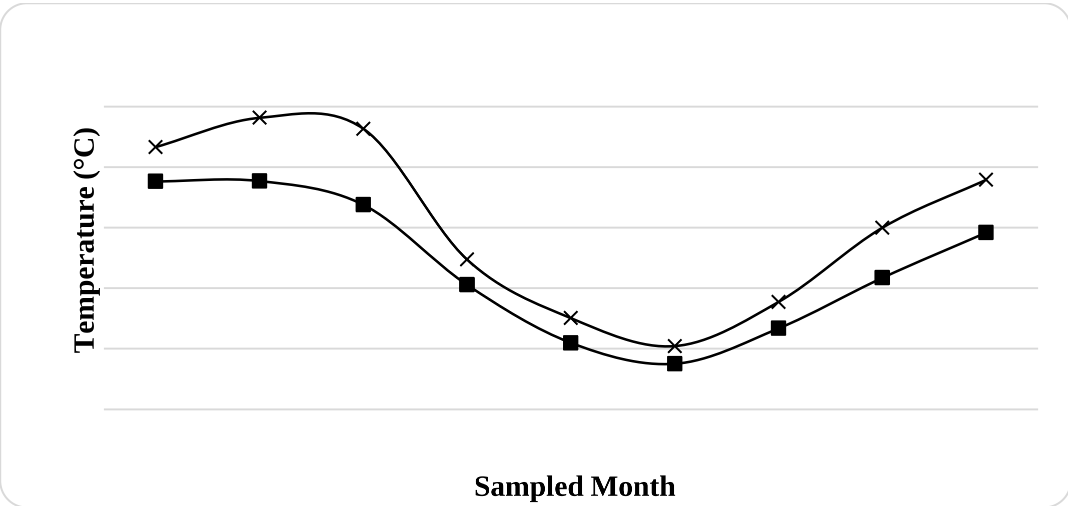

Figure 1. Average water temperature of brood pond at morning and evening in different month during brood rearing.

Conclusion

The male and female shows sexual dimorphism during spawning season. During spawning season (September-November and February-April) fine tubercles were seen on the head and snout of the male and in female genital papillae becomes pinkish. Though distinct sexual characters are seen in spawning season but in December and January respond for the maturity test. During maturity test it was seen that maturity time holds for very short period (12 hours to 24 hours) and test should be done very frequently to catch the spawning time. It is seen that sahar can breed naturally in most of the months of the year when water temperature lies between 17.5°C and 28°C in Chitwan. The experiment also gives the result for induced breeding of sahar. It is seen that 0.5 ml/kg ovaprim hormone is effective for induced breeding of sahar in Chitwan. In the context of Chitwan, March month is seen favorable for the natural or induced breeding when the temperature lies between 23°C-28°C.

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Carp feed management and feeding practices in eastern Terai region of Nepal

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Abstract
A questionnaire-based survey was carried out to examine feeding practices in carp polyculture systems of Eastern Terai of Nepal. For the purpose eighteen farmers involving in carp farming in ponds from four eastern Terai districts, viz Jhapa, Morang, Sunsari and Sarlahi were selected for the assessment of farmers based feeding practices. The study revealed that mash feed was the most popular and widely used feed type.Raw rice bran was used as the primary feed ingredient followed by oil cake. Over Ninety-four percent farmers reported using rice bran and mustard/linseed oil cake, and 41% used wheat flour as basic ingredients to prepare mash feed. Commercial pellet feeds used by 16.7% farmers as sole source feed or to complement mash feed. Majority farmers (over 90%) practicing hand feeding to all sizes of fish, 14% used feed tray for rearing fish larvae. The FCR recorded across all the culture systems ranged between 1.8 to 3.1:1 with a combination of mash-pellet feed, and 2.6 to 4.2:1 with mash feed. Feed related major issues and constraints to the production of carp from polyculture systems and research areas were identified, and some recommendations were made in optimizing feed use and management.The poor quality of the mash feed ingredients, specifically adulteration in rice bran and fungal development in oil cake was an important issue of concern to the farmers.

Keywords: Carps, Polyculture, Mash feed, Food conversion ratio

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Introduction
Supply of quality fish seed and feeding play central and essential role in the sustained development of carp aquaculture. As feed alone represents one of the highest operating costs in aquaculture systems (Hasan, 2007), feed choice and feed management practices have a significant impact on the economic performance of a production system. In Nepal, most aquaculture fish production is based on low-input systems relaying on low protein agricultural by-products. It

has been observed that farmers relying on single ingredient, and mash feed prepared from several ingredients to feed the farmed fish. Feeding practices are not well developed to satisfy the need of fish, and both underfeeding and overfeeding is common. As a consequence, low productivity of fish (1.03 to 5.79 tons/ha) from carp polyculture has been reported (Shrestha, 2014). There is large information and data gap on feed management and feeding practices adopted by the farmers and their impact on fish production. In Eastern Terai of Nepal, most aquaculture fish production is based on low-input systems relaying on low protein agricultural by-products. There is large information and data gap on feed management and feeding practices adopted by the farmers and their impact on fish production. A reliable database in these aspects is an essential prerequisite for planning sustainable aquaculture development. The present survey report, therefore, attempted to examine and document feeding practices in carp polyculture of Eastern Terai Region (ETR), and to identify the major issues that need to be addressed to build the capacity of aquafarmers to optimize the use of feed and feed additives.

Material and Methods

A semi-structured questionnaire survey of 18 randomly selected farmers was carried out in four districts viz. Jhapa, Sunsari, Morang and Sarlahi between July 2016 and June 2017 to understand the on-farm carp feed management and feeding practices that are applied to the various production systems adopted by the farmers. Primary data collection included information related to mash feed ingredients, commercial pellet feeds, feeding practices, feeding methods, feeding rates, FCR, on-farm storage, and issues related to feed quality. Participatory Rural Appraisal (PRA) tools including Focus Group Discussion (FGD) was conducted with 18 fish farmers to get an overview of particular issues of fish feed and feeding practices to identify areas that require future intervention and research.

Eighteen feed samples including individual ingredients, mash feed comprised of different ingredients and pelleted feed were collected directly from the farm sites for proximate analysis. Nutrient composition of all collected feeds were analyzed in Animal Nutrition Division of NARC, Khumaltar. The data pertaining to the different stages of production, i.e. grow-out, fingerling, fry and larvae, were analyzed independently. Data were processed using Microsoft Excel and analyzed by using tabular and descriptive statistical methods. The technique of analysis included the classification of tables into consequential results by arithmetic mean, percentage and ratios.

Results

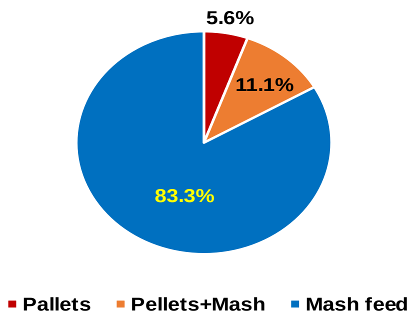

Figure 1. Types of feed used by the carp polyculture farmers.

The survey revealed that 83.3% of farmers used mash feed as their sole feed source for the production of fish (Figure 1). A further 11.1% of farmers used a combination of mash and pelleted feeds, and 5.6% of farmers reported using pellets as their sole feed source.

Rice bran and mustard/linseed oil cake was used by most of the surveyed farmers (94.4%), followed by wheat flour (41%) and soybean flour (22.2%) (Figure 2). The data of present survey indicate that rice bran, oil cake and wheat flour were the primary three ingredients used in mash feeds. The survey also revealed that the use of mass feed was gradually declining in areas where commercial pellet feeds are available, which was demonstrated by the facts that 11.1% and 5.6% of the farmers reported using combinations of pelleted and mash feeds, and pelleted feeds, respectively as sole source of feed.

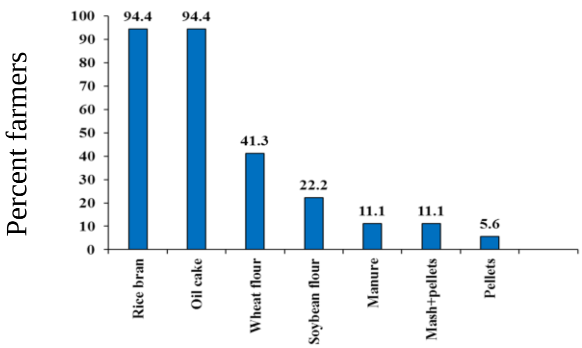

Figure 2. Feed ingredients used in carp polyculture by the farmers.

All the farmers, irrespective of stages of fish, reported feeding carps at least once a day. The survey revealed that 65% farmer feed the larvae two times/day, while a significant number of farmers (35%) were still prefer one time feeding to the larvae (Figure 3). Fingerling and grow-out fish were fed once in a day by more than 70% of the farmers. Generally, feeding was undertaken in the morning for all development stages of fish under one time feeding by 68.8% of the farmers. While there was no technical or scientific rationale for feeding in the morning, it became a standard practice as it was a convenient time for the farmers. Of those farmers that used more than one feeding frequency, 23.1% and 8.1% reported feeding a second and third ration between 13:0 and 15:0 hours and 17:0 to 19:0 hours, respectively. It was reported during the survey, in most of the cases the farmers themselves involved in feed preparation, feeding and monitoring of the feed response. Two time feeding for rearing fry was practiced by 65% of farmers, and fingerling and grow-out fish were fed once in a day by more than 70% of the farmers.

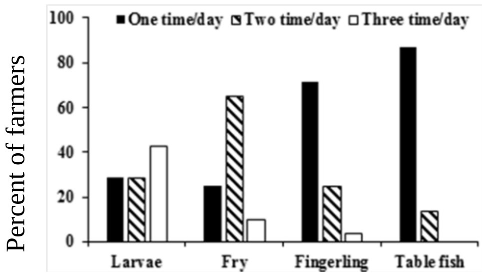

Figure 3. Feeding frequency used by the farmer for different development stages of carp fish.

Survey revealed that hand feeding to all sizes of fish was practiced by over 85% of the farmer (Figure 4). A few farmers use feed tray (14%) and feed bags (4%) for feeding larvae and table size fish. Farmers reported that the feed was distributed in the form of moist feed ball of mash feed. In general, the farmers use between 3 to 4 feeding points per hectare at the start of the production cycle when the feed requirements are low. As the feed requirements increase, they gradually increase the number of feed points in the pond; usually a maximum of six feed points per hectare are used.

Survey revealed that the farmers base their feed rations on their personal experience. Large variation exists in daily feeding rates for similar size classes of fish. Survey data indicated that one summer old fish (200 g) were fed between 2.0 and 4.0% of body weight per day. Similar feeding regime was also applied for larger fish (500 g and above). As a result, there is unsubstantiated claims that the production cost of fish are high accompanied with high feed conversion ratio (FCR) probably because of the wasting of significant quantities of feed. Farmers reported that disintegration of the major part of the

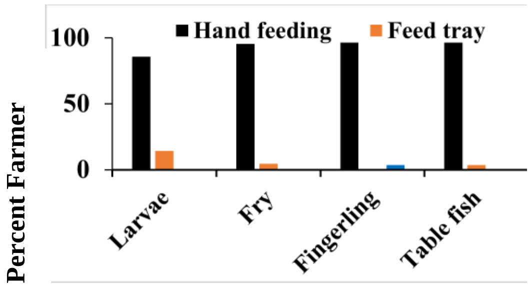

Figure 4. Feeding methods practiced by farmers

provided moist feed is not properly utilized by the fish. The daily quantity of feed required for a production system is judged by the farmers based on a number of factors which includes fish growth trends, concurrent fish biomass, average fish weight and weather condition. Most of the farmers have a poor understating to estimate the feed requirement for different size classes of each carp species.

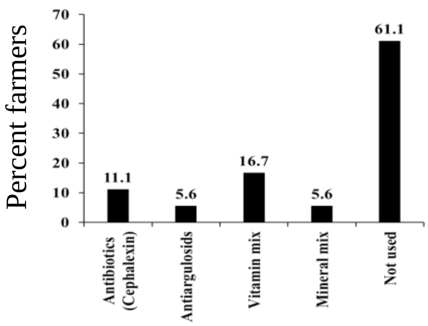

Figure 5. Usage of feed additives by carp farmers

Feed additives that were used by the farmers using mash feed are illustrated in Figure 5. The most common feed additive was vitamin mix used by 16.7% of the farmers. The second frequently used feed additive was antibiotics that were used by 11.1% of the farmer. Antibiotics were used to treat bacterial diseases (red spot and epizootic ulcerative syndrome, EUS). The requirement of vitamin and mineral in fishes varies with species, age, environment and culture system as well. Many farmed fish species, including carps, are filter-feeders and derive much of vitamin and mineral requirements by consuming fine particulate matter such as phytoplankton, zooplankton, bacteria and detritus. Taking into account of availability of particulate matter and biosynthesis of some vitamins by fish itself, further study is warned on whether the addition of mineral and vitamin mixtures to the mash diets has any measurable effect in terms of increasing feed efficiency and optimizing growth.

Proximate composition of ingredients used for mash feed preparation, major groups of mash feed and pelleted feeds are presented in Table 1. Proximate analysis in laboratory revealed that mean moisture content of mash feed was 11.1%. The mash feeds used by majority of farmers comprised of oil cake+ rice bran at proportion 40:60 contained 20.0% crude protein and 7.6% crude fat. Survey revealed that the commercial pellet feeds used by the farmers were originated from Nepal (2 brand) and India (6 brand). These pellet feeds contained over 90% dry matter, 16.1% to 21.5% crude protein and relatively high levels (9.8 to 12.2%) of crude fat. Survey data indicated that the large variation exist in dry matter and nutrient composition of feed ingredients and prepared mash feed. These differences are supposed to be largely governed by ingredients type and quality, composition of mash feed, storage condition and weather condition at times when feed samples were collected.

Table 1. Proximate composition analysis (% as fed basis) of mash feed and pelleted feed used in Eastern Terai of Nepal

Nutrients	Carp			Pangas (India, 3)
	Mash feed (10)	Pelleted feed		
		Nepal (2)	India (3)	
Moisture, %	11.1	8.6	9.6	7.5
Crude protein, %	20.0	16.1	19.7	21.5
Ash, %	11.5	6.7	9.3	14.5
Crude fat, %	7.6	12.2	11.1	9.8

The survey revealed that 81.4% of the farmers had fish health and water quality related problems. These includes water pollution (33.9%), oxygen deficiency (30.5%) and occurrence of fish disease and parasites reported by 16.9% of the farmers. Among fish diseases, epizootic ulcerative syndrome (EUS), argulosis and red spot disease were the major problems reported by farmers using mash feed. Other disease reported by the farmers were exophthalmia (protruded eye), trichodina parasites and dropsy.

The combined production characteristics of the different aquaculture production systems that were surveyed are presented in Tables 2. Basically two types of culture systems adopted by the surveyed farmers, viz. low density carp polycultures with yearlings, and high density carp polycultures using fingerlings. In high and low density semi-intensive polyculture systems, seven (Indigenous major carp, Chinese carp and common carp) carp species are cultured. Irrespective of production systems, the ponds received 2.5 to 6.0 tons of cow dung/ha/annum and 55 to 122 kg of inorganic fertilizer/ha/annum. The recorded productivity was varied greatly among production systems and the types of feed applied. The survey data indicated that the production volume range between 5.25 tons/ha with mash feed to 6.35 tons/ha with combination of mash and pellet feed in low density while the production volume was relatively low in high density polyculture system ranging from 4.48 tons/ha with mash to 4.75 tons/ha with mash+pellet feed.

Table 2. Production characteristics of semi-intensive carp aquaculture production systems in Eastern Terai of Nepal.

Type of culture	**Feed type**	**Feeding frequency, times/day**	**FCR**	**Organic manure, t/ha**	**Inorganic fertilizer, kg/ha**	**Average production (t/ha)**
Low density carp polyculture	Mash	1-2	2.6-4.2	2.5-5.6	60-90	5.25
	Mash +Pellet	1-2	1.9-2.7	2.8-4.3	55-85	6.35
High density carp polyculture	Mash	1	2.2-3.8	3.0-6.0	60-122	4.48
	Mash +Pellet	1-2	1.8-3.1	2.5-4.0	55-90	4.75

The FCR recorded across all the culture systems ranged between 1.8 to 4.2:1. The most efficient food conversion was recorded in the low density polyculture using mass+pellet feed. Irrespective of culture systems, the combination of maas and pellet feed gave the lowest FCR (1.8:1) followed by mash feed (2.2:1) comprised of oil cake (40%) and rice bran (60%). In all the production systems, the FCR cannot be recognized to the single use of the external feeds. In every production system, phytoplankton and zooplankton and other natural food organisms significantly contribute to the nutrition of the fish. Considering the FCR and lower feed wastage that is attained by feeding commercial pellets, priority could be given to developing high quality pellet feeds to be used in combination with the feed ingredients used in the traditional mash feeds.

Discussion

Present survey indicated that the most of farmers practicing one time feeding for adult fish. De Silva and Davy (1992) reported that feed utilization can be optimized by increasing feeding frequencies, and that the growth response to differential feeding frequencies is both species-specific and specific to the life history stage of the fish. High feeding frequency has significant positive impact on body weight gain of common carp (Stankovic *et al.*, 2010). In an experiment conducted in Bulgaria, they found that the body weight gain is 41.47% in single feeding, 50.42% in fish fed two times, and 87.79% in fish fed three times, respectively, in relation to the stocking biomass. Effects of such feeding practices on fish growth and yield has not yet been evaluated in carp polycultures of the country to best utilize the given feed.

Survey revealed that feeding rates applied by the farmers were varied greatly among similar size class of fish. Wagle and Giri (2016) reported that the daily feeding rate of 15-20% for larvae, 8-10% for advanced fry, 5-7% for fingerling and 2-4% of the standing biomass of grow out carps would be sufficient. Although it is often postulated that feeding carp fish at 2-3 times with 25-30% protein content feed, feed forms and feeding regime relative to size will result in more efficient feed utilization (Nekoubin and Sudagar, 2012), research need to be conducted to validate this hypothesis for carp polyculture with feed types and quality available in the country.

Farmers had shown concerns of increasing problems of water quality and fish disease during the survey. Over and underfeeding and poor quality feed can be detrimental to the health of the fish and may cause a marked deterioration in water quality, reduced weight, poor food utilization, and increased susceptibility to infection (Nekoubin and Sudagar, 2012). Single feeding practice with mass feed and sinking pellet may lead to the release of ammonia from unconsumed feed, thereby polluting the water since the primary nitrogenous waste produced by fish from protein digestion is ammonia (Durborow *et al.*, 1997). Farmers in the ETR frequently complained about that their fish (usually bottom feeder-common carp) disappeared without recognized mortality. It is assumed that high level of ammonia resulting from single fed disintegrated mass feed could have been the cause of unseen loss of fish. Correlation between feeding practice (quality, forms and feeding frequency) and the changes in pond environment has yet to be established with respect to carp polyculture for environment friendly efficient and effective use of feed. Consequently, specific growth rates and the efficiency of feed conversion can be directly related to feed ration and frequency.

In the case of increasing occurrence and severity of disease the use of drugs is inevitable. Aquaculture drugs are not readily available in the market of ETR. Farmers are compelled to use drugs that are registered for the control of livestock diseases. Livestock drugs and other chemicals such as Toximar, Sokrena, Cifax and Wasorich and Mittha used by the farmers for the treatment of a specific disease problem have not yet been tested, verified and recommended to the local environment (Jha and Wagle, 2016). This indicates that continual research efforts are necessary to verify the effectiveness of these chemicals and other commercial products claimed for fish disease treatment.

Proximate composition analysis revealed that the mean crude protein content of mash feed and pelleted feed was 20% and 19.1%, respectively (Table 1).The dietary protein requirement of the carp is varied greatly depending on stages of development. The level of dietary protein requirement of carp is ranged between 35 to 47%, 30 to 43% and 23 to 36%, respectively for fry, fingerling and adult stages (ICAR, 2013; Mahapatra *et al.*, 2012). In the absence of natural feeds, stunted carp yearlings have been shown to require a dietary formulation comprising 25% protein and 37% carbohydrate; in the presence of natural feeds, the protein component of the dietary formulation can be reduced to 20% (Nandeesha *et al.*, 1994). The optimum dietary requirement of carbohydrate ranged between 22 to 56% for different species of carp fish (Mahapatra *et al.*, 2012). There is no information to describe the optimum levels of dietary carbohydrate required for carps production in semi-intensive, fed and fertilized systems, such as those practiced in Nepal. The carbohydrate level of typical supplementary feeds (mash) used in carp culture is about 45% (Ramkrishna *et al.*, 2013). This represents a higher dietary carbohydrate level considering the natural food derived from pond production. Growth retardation and reduced feed efficiency can result when carbohydrate levels for specified carp species exceed in diet. Ramkrishna *et al.* (2013) observed that excess dietary carbohydrate results in excessive fat deposition in the viscera of the larger pond-cultured adult catla and rohu.

The FCR of the present survey are comparable with the reported FCR of 1.8–3.4:1 and 2.3–4.1:1 using commercially manufactured pellets and farm-made feeds in India (Ramkrishna *et al.*, 2013). In all the production systems, the FCR cannot be recognized to the single use of the external feeds. In every production system, phytoplankton and zooplankton and other natural food organisms significantly contribute to the nutrition of the fish (Ramkrishna, 2013). It is evident from the survey that the use of pelleted feeds generally improves the FCR recorded in these systems. The proximate analysis revealed that the pellets contain similar crude protein content to the mash feed used by the farmers of ETR (Table 2). Pellet feeds are based on different formulations to those used in the mash feeds, as a result this may account for the improved FCR recorded when pelleted feeds are used. Edwards (2008) reported that the high average FCR (2.5–3.5:1) obtained in carp culture system in Andra Pradesh, India represents low levels of nutritional efficiency, and reported that an FCR of 1.0:1 is attainable in experimental fertilized ponds in which the fish are fed commercial pelleted feeds. Those farmers using mash-pellets and pellet feed reported that they had fewer problems with water quality and outbreak of fish diseases.

During the survey with regard to use of feed types, most of the farmers were unaware about the commercial pellet feeds and many farmers raised concern of high cost of pelleted feed. In most of the cases the farmers reported that, in terms of growth and economic returns, they were satisfied with the mash feeds that they used. Considering the FCR and lower feed wastage that is attained by feeding commercial pellets, priority could be given to developing high quality pellet feeds to be used in combination with the feed ingredients used in the traditional mash feeds. However, the production and economic efficiencies of these types of combined feeding regimes needs to be evaluated and verified in farmers' field.

In the present study, farmers were mostly found lack of knowledge on feed management and have inadequate opportunities to improve management skills. Their ability to respond effectively to fish feed and feeding problem is also very limited. As a result, they suffered from the loss of feed quality and quantity and associated financial losses due to improper feed management. Training and the dissemination of information to farmers that have limited exposure to the latest technological and management developments is an issue that needs to be addressed (FAO, 2010). Most farmers using mash feed have relatively limited education, thus the transfer of technical messages is problematic and requires continuous Attention. Strong extension and information dissemination networks result in high adoption rates of feed formulation technologies and better management practices. In addition, farmers require access to information pertaining to species-specific feed formulations and ingredient inclusion rates.

Farmers complained about the quality of the rice bran and oil cake that they contained high moisture and adulteration with rice husk in rice bran and contamination with fungal growth in oil cake. Farmers also responded on the quality of pelleted feed, that they had doubt on protein content labeled in feed bag in the background of poor growth of fish and high FCR from pellet feeding experienced by them. The monitoring mechanism of feed quality and adulteration of feed ingredients remains an important issue that needs to be resolved. The government needs to establish diagnostic laboratories at least at regional level to detect cases of feed adulteration and regulate quality. In the light of these issues, the role of feed regulation and governance in ensuring the quality of feed and feed ingredients and optimizing production becomes pertinent (FAO, 2010).

Farmer's response to disease problems was generally application of chemicals in feed with little understanding of their effectiveness. They largely depend on advice received from neighbor farmers and local veterinary drug and chemical stores for the treatment (Jha and Wagle, 2016). Most of the drugs and feed additives used by farmers at present for the treatment of a specific disease problem and to promote growth of fish have not yet been tested, verified and recommended to the local environment. This indicates that continual research efforts are necessary to verify the effectiveness of these chemicals and other commercial products claimed for fish disease treatment and growth promoter.

Recommendations

The following recommendations can be derived based on the issues and constraints highlighted in this study:

- Undertake research on improving the nutritional quality of farm-made mash feeds.
- Conduct research on nutrient requirements of seven species carp polyculture system under intensive pond culture conditions, including the role of natural productivity.
- Establish the dose, efficacy and cost-effectiveness of the chemicals and materials used as feed additives.
- Improve feed and feed management practices. Optimize feeding schedules, and develop protocols to reduce feed losses.
- Train the farmers about the importance of feed management practices in optimizing production parameters.

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Effect of water depth on retention and quality of water and fish productivity in pond of Terai region, Nepal

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Abstract

Water depth in a fishpond is one of the key environmental factors for improving water quality and fish yield. An experiment was carried out to investigate the trend of water loss and associated change in water quality and fish productivity in earthen ponds having different water depths in Ratna Nagar Municipality, Chitwan, Nepal for a period of 205 days during November 2016 to July 2017. Replicated ponds with three different water depths (85 cm, 105 cm and 130 cm) were selected as treatments and named as shallow, moderate and deep pond, respectively. Carp fish with an average initial weight of 20.4±2.2 g was stocked at density 11500 fish/ha in all experimental ponds. The rate of water loss, change in water quality and fish growth was monitored monthly until harvest. Total ammonium (NH_4-N) concentration (0.8 mg/L) in shallow pond was significantly high compared to the concentration in moderate and deep ponds. Deeper pond had significantly higher nitrate concentration (14.3 mg/L) compared to that of ponds with shallow and moderate water depth. Phosphate level in shallow pond (0.43 mg/L) was significantly high in June compared to the phosphate concentration in ponds with moderate and deeper water depth. growth and yield of fish favored by an increase in water depth that the low water depth in pond. The study concluded that low water depth in shallow pond (85 cm) had negative impact and deeper pond (130 cm) had positive impact on water quality, growth and fish yield. High phosphorus content was evident in shallow pond.

Keywords: Water depth, Water quality, Fish productivity, Water loss

Introduction

Nepal has plenty of seasonal and shallow ponds facing the problem of enough water for growing fish in annual production cycle. Many fish farming facilities are rain fed ponds constructed to reverse water for other purposes have brought in to aquaculture. They are seasonal by nature, water dried up during summer season. Carp polyculture technology developed for a pond having specific depth of water, such technology undermines the production from low water depth ponds. Low productivity of fish (<3 t/ha) with the carp polyculture from shallow and seasonal ponds have been reported (FRD, 2012). Depth as a factor in pond ecosystem management has been given little experimental attention, despite its theoretical importance in autotrophic production.Over the past few years, rising global temperatures have received much attention because of their worldwide impact on ecosystems. Climate is an environmental factor that is strongly associated to aquaculture productivity (Hamdan *et al.*, 2011). Being coldblooded animal, fish is affected by the temperature of the surrounding water which influences the body temperature, growth rate, food consumption, feed conversion, and other body functions (Britz *et al.*, 1997; Azevedo, 1998). In temperate and some sub-tropical regions, fish culture is highly affected by sensitivity to low and high water temperatures leading to poor growth (Charo-Karisa *et al.*, 2005). Recent survey conducted in Terai region of Nepal has also reported that most of the disease outbreak and associated fish mortality occurred during winter season as a consequence of low temperature and sub-optimal environmental condition. Shallow and rainfed ponds are more prone to these climate change related phenomena. Emerging trend in climate change would also play role further examine the water crisis. Studies are pertinent to optimize fish farming operation to exploit the production potential of ponds having different water depth and water deficit pond as well studies are needed to develop water conservation and reuse technology in aquaculture. This report attempted to examine the relationship among water temperature, water loss and the productivity of fish to provide a basis of water management in fish pond of Terai region of Nepal.

Materials and Methods

An experiment was carried out to investigate the trend of water loss and associated change in water quality and fish productivity in earthen ponds having different water depths in Ratna Nagar Municipality, Chitwan, Nepal for a period of 205 days from November 2016 to July 2017. Replicated ponds with three different water depths, viz. 85 cm, 105 cm and 130 cm were selected as treatments and named as shallow, moderate and deep pond, respectively. Carp fish with an average initial weight of 20.4±2.2 g was stocked at density 11500 fish/ha in all experimental ponds in November 2016. The proportion of stocked fish was 1:1.5:1.5:3.5:1.5:1, respectively, for Silver Carp, Bighead Carp, Grass Carp, Common Carp, Rohu and Naini. Fish were fed with farm made feed approximately comprised of 22% crude protein at the rate of 3-4% of fish biomass daily. The rate of water loss, change in water quality and fish growth was monitored monthly until harvest in July 2017. Water depth, water quality and fish yield data were subject to ANOVA to infer any significant differences among water depths of fish ponds.

Results

Water level of all categories of ponds decreased constantly with significantly ($p<0.05$) high water loss in shallow pond (44.5%) followed by moderate pond (35.3%) and the least loss in deep pond (27.8%) from November to June (Figure 1). All the experimental ponds started to regain water depth during July due to intense rainfall prevail in this month. An increase in water temperature from March and onward resulted in sharp decline in water level in all ponds with varying water depth. A decrease in water depth in shallow ponds was severe that of moderate and deeper ponds (Figure 1).

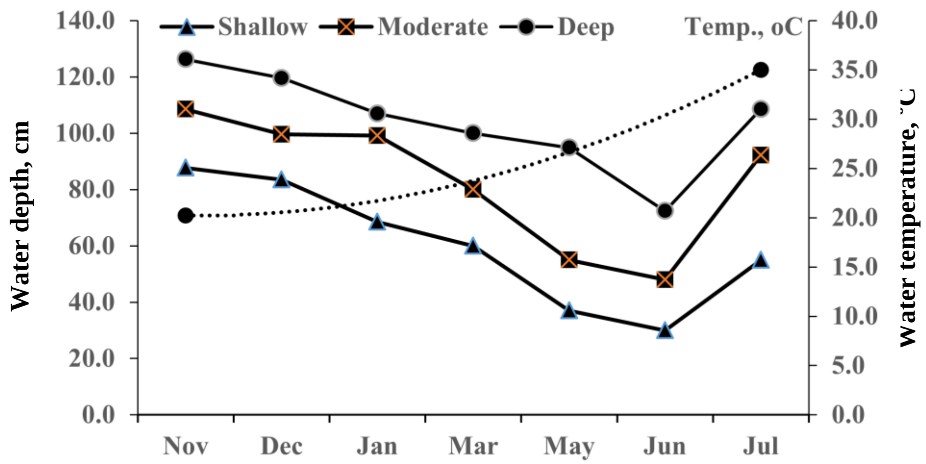

Figure 1. Water depth and corresponding water temperature in earthen fish ponds in Chitwan district.

Comparison of water quality, at the end of study, revealed that ammonium (NH_4-N) concentration (0.8 mg/L) in shallow pond was significantly ($p<0.05$) high compared to the concentration in moderate and deep ponds (Table 1). Deeper pond had significantly higher ($p<0.05$) nitrate concentration (14.3 mg/L) compared to that of ponds with shallow and moderate water depth. Phosphate level in shallow pond (0.43 mg/L) was significantly high ($p<0.05$) in June compared to the phosphate concentration in ponds with moderate and deeper water depth. Sechi disc visibility, as an indicator of plankton turbidity was significantly low ($p<0.05$) in shallow pond (22.7 cm) and gradually high in ponds with moderate and deeper water depth.

Table 1. Water quality of fish ponds with different water depth at harvest (June 2017). Different superscripted letter represents significant different at $p<0.05$.

Parameters	Shallow	Moderate	Deep
Temp. ^{o}C	29.7	30.0	30.8
DO, mg/L	7.7	7.9	8.1
pH	7.4	7.8	7.2
Ammonium, NH_4-N, mg/L	0.8^{a}	0.7^{ab}	0.06^{b}
Nitrite, NO_2-N, mg/L	0.07^{a}	0.65^{b}	0.05^{a}
Nitrate, NO_3-N, mg/L	6.5^{a}	7.9^{a}	14.3^{b}
Phosphate, PO_4-P, mg/L	0.43^{a}	0.39^{b}	0.28^{c}
Total alkalinity as $CaCO_3$, mg/L	79.0	79.3	75.7
Total hardness as $CaCO_3$, mg/L	92.3^{a}	124.7^{b}	85.0^{a}
Turbidity, NTU	29.6^{a}	22.0^{b}	26.4^{a}
Conductivity, µs/sec	300.0	287.3	306.0
Sechi disc depth, cm	22.7^{a}	31.7^{b}	56.7^{c}

Pooled data of water loss and fish yield irrespective of categories of pond have shown that the fish yield decreased exponentially with high correlation coefficient (R^2=0.88) with the increase in water loss from the fish-pond (Figure 2). In other word, growth and yield of fish favored by an increase in water depth that the low water depth in pond. Figure 3 revealed that higher water depth at the beginning of fish farming could provide better environment for higher fish yield despite the natural water loss due to evaporation resulting from an increased water temperature occurred at the later stage of fish farming.

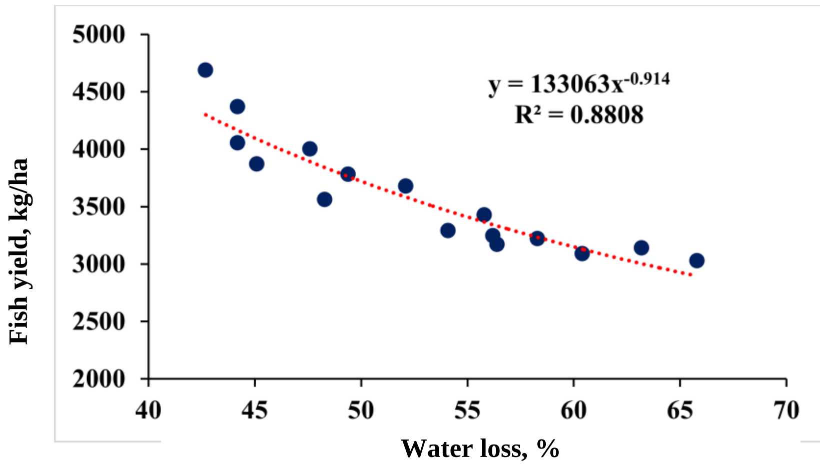

Figure 2. Relationship between water loss from pond and fish yield.

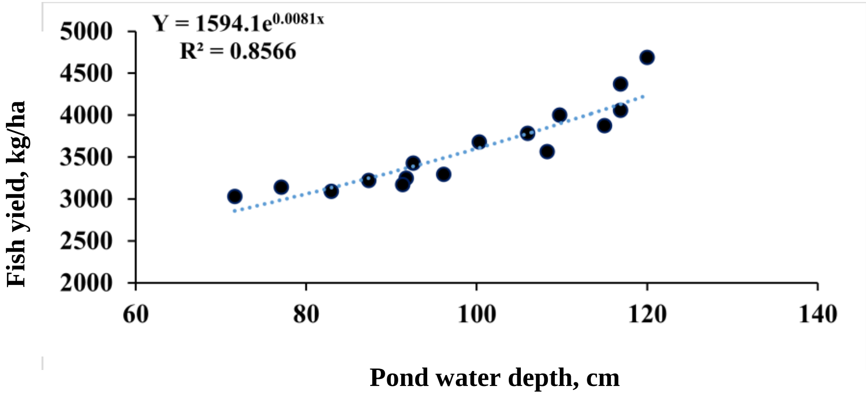

Figure 3. Relationship between pond water depth and fish yield.

Mean weight gain of fish increased over the time in all categories of pond albeit at higher growth rate in pond with high water depth (Figure 4). Intermediate fish growth in moderate and deeper ponds was higher than the growth in shallow pond. Survival rate (82.8%) of fish in shallow pond was significantly low ($p<0.05$) than the survival rate in moderate and deep ponds (Table 2). Mean harvest size of fish was significantly high ($p<0.05$) in deeper pond (472.3 g) than the mean size of fish obtained from moderate (372.8 g) and shallow pond (348.7 g). Absolute growth rate and yield of fish were significantly different ($p<0.05$) among depth group of ponds. Net yield (4720 kg/ha) obtained from deep pond was 54.7% and 36.8% higher than the net yield obtained from shallow and moderately deep ponds, respectively and the fish yields were significantly different ($p<0.05$) .

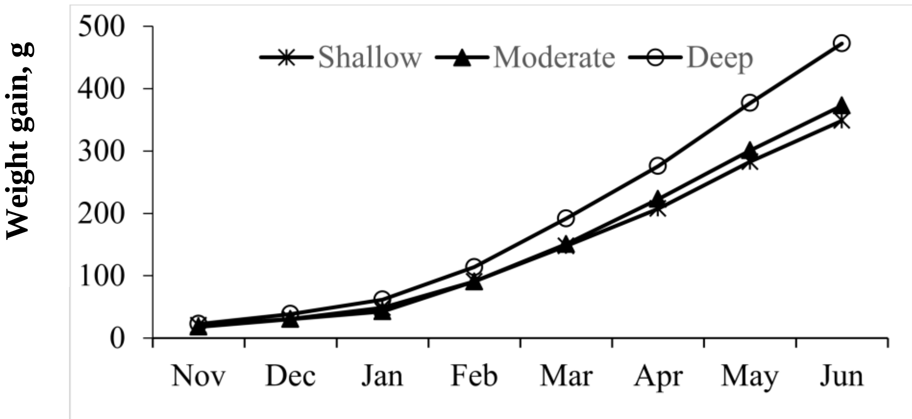

Figure 4. Monthly weight gains of fish in ponds with varying water depth.

Table 2. Production characteristics of ponds of varying water depth. Different superscripted letter represents significant different at $p<0.05$.

Production characteristics	Fish growing period: 190 days		
	Shallow	Moderate	Deep
Mean water surface area, m^2	1555	2111	2333
Mean water depth at beginning, cm	87.8	108.5	126.3
Mean water depth at harvest, cm	30.0	48.0	72.4
Total water loss, %	65.8	55.8	42.7
Fish stocking density, No./ha	11500	11500	11500
Stocking size, g	20.2	18.3	22.7
Biomass at stocking, kg	232.3	210.5	261.1
Harvest size, g	318.2[a]	337.1[ab]	444.6[b]
Survival rate, %	82.8[a]	88.4[b]	91.7[b]
Growth rate, g/day	1.57[a]	1.68[b]	2.22[c]
Yield, kg/ha	3029.9 [a]	3426.9 [b]	4688.5 [c]
Net yield, kg/ha	2797.6 [a]	3216.5 [b]	4427.4 [c]

Discussion

Fish culture is a water-intensive endeavor and requires much more water than conventional agriculture (Boyd, 1982). Despite of this the value of aquacultural production per unit of water used greatly exceeds that of irrigated agriculture (Boyd and Gross, 2000). However, fish production from shallow ponds (low water depth) with respect to water use is questionable because of low productivity and lack of appropriate farming technology.

High concentration of nutrients, e.g. ammonium, phosphate and plankton turbidity, was measured from the shallow pond in this study (Table 1), The shallow depth of the ponds allowed constant nutrient recycling coupled with greater heat absorption from the surrounding hot humid air (Ssanyu and Schagerl, 2010). High temperature and permanent mixing are essential in promoting recycling and availability of nutrients for growing algae (Erikson *et al.*, 1998). Remineralisation of settled organic matter is a common occurrence in shallow ponds. This leads to creation of anaerobic pockets which favour denitrification, releasing ammonia in response to a concentration gradient extending from the sediment layer into the water column. High phosphorus is attributed to the release from the sediments which is enhanced especially in shallow water bodies with high turbulence and small anaerobic bottom zones (Ssanyu and Schagerl, 2010).

There is a strong relationship between algal net productivity and the net yield of fish whose diets consist of food produced within the pond system. These advantages of rapid nutrient recycling and algal bloom in shallow ponds, which subsequently become shallow pond over the time before onset of monsoon, has not been properly utilized for optimizing fish production. Lack of technology is apparent, specially the choice of fish species and their density, and synchronizing production cycle with fish farming operation for ponds having <80 cm water depth only for about 6-7 months including low-temperature winter month.

Water loss (42.7 to 65.8%) was apparent in all categories of pond and more severe for ponds with low water level in a fish-growing period of 205 days in the present study. Present study also showed that high water level in pond has an impact on stabilizing water quality, fish growth and yield. Elevated temperature and prolonged drought due to possible climate change impact and irregular weather conditions in annual cycle might results in water crisis for aquaculture (Sharma *et al.*, 2013). In such conditions many fish ponds become vulnerable to low water depth. Water conservation measures such as maintaining storage capacity in ponds equal to the normal, maximum daily precipitation, reduction in seepage beneath dams and through pond bottoms, fish harvest without draining ponds, and water re-use as means of preparedness to climate change impact have been suggested by (Sharma *et al.*, 2013; Pimolrat *et al.*, 2013). Reduction in effluent volume is the most effective water saving means, and not only reduces water consumption but also reduces the pollution potential of pond aquaculture (Boyd and Gross, 2000). The water productivity of aquaculture can be increased through improving system design, good water quality, and or using a combination of non-competing species that fill different niches in the aquatic ecosystem. The determination appropriate species structure of carp in varying pond water depth would enhance fish productivity, which is generally lacking. The focus should be on using intensive culture practices for aquaculture production. The main water consumption in pond aquaculture is in the form of evaporation and seepage losses. The seepage loss may be controlled by adopting different control measures in pond aquaculture (Jayanthi *et al.*, 2004). In intensive culture systems, focus should be on assuring the reuse of water so that these should no longer be considered as a loss.

Conclusion

The study concluded that low water depth in shallow pond (85 cm) had negative impact and deeper pond (130 cm) had positive impact on water quality, growth and fish yield.High phosphorus content was evident in shallow pond in the present study which is supposed to enhance by high turbulence and small anaerobic bottom zones. This advantages of rapid nutrient recycling and algal bloom in shallow ponds was not properly utilized for optimizing fish production. Lack of technology is apparent, specially the choice of fish species and their density, and synchronizing production cycle with fish farming operation for ponds having <50 to 85 cm water depth including low-temperature winter month. The water productivity of aquaculture can be increased through improving system design, good water quality, and or using a combination of non-competing species that fill different niches in the aquatic ecosystem. In this context, research on the determination of appropriate species structure of carp in varying pond water depth would be necessary.

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Freshwater pearl culture: an initiation in Nepal

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Abstract

Freshwater pearl culture in pond is environmental friendly, and economic for fish growing farmers with additional item as pearl. Nepal is rich in water resources with abundant species of mussel's which could be utilized for pearl culture. *Lamillidens marginalis* was identified for the pearl culture and in the preliminary study, the acceptance of foreign and materials and development of glister on the inserted bead observed. Its further research with compatible beads needed. The prospects of pearl farming is tremendous and it could be remarkable industry in Nepal that will improve the welfare of fish growing farmers with extra income.

Keywords: Freshwater pearl, *Lamillidens marginalis*, Glister, Beads, Implantation

Introduction

Pearl is natural gem produced by mussels. For millennia, pearl have fascinated humanity the world over. Pearls are used for decorative and jewelry purposes. In addition, they have also been used as medicine to cure diseases. Recent trends show India, Bangladesh, China, Japan, Vietnam and several other countries has developed freshwater pearl cultivation technologies abroad (Miah *et al.*, 2000; Misra *et al.*, 2009). Freshwater pearl culture is growing as a source of employment and income in many South-East Asian countries (Misra *et al.*, 2009). Pearl culture technologies involving different implantation methods have been developed with different mussel species (Janakiram, 1989; Janakiram and Tripathi, 1992; Janakiram *et al.*, 1994; Sakpal and Singh, 2000). Freshwater pearl culture in pond is environmental friendly, and economic for fish growing farmers with additional item as pearl (Misra *et al.*, 2009). Nepal is rich in water resources with abundant species of mussel's which could be utilized for pearl culture (Husen *et al.*, 2017). More than 18 species of Freshwater mussels have been used for pearl farming around the world (Husen *et al.*, 2017). As fresh water pearl culture is new frontier of aquaculture in Nepal, Nepal Agricultural Research

Council (NARC) has been started the freshwater pearl culture at Fishery research Station, Pokhara, Begnas since 2017. Development of freshwater pearl cultivation farming practices in Nepal is the main objective of this study.

Development of pearl farming technologies

Selection of mussel's species

The species of mussels which were identified for freshwater pearl culture from Nepal are listed in Table 1. Among these species *Lamillidens marginalis* (Figure 1) was selected for the implantation and culture of the pearl in pond fish farming.

Figure 1. *Lamillidens marginalis*.

Table 1. List of mussel's species to be utilized for pearl culture and their distribution in Nepal.

SN.	Species	Distributions in Nepal	Sources
1	*Lamillidens corrianus*	Rauthat, Kamdai Nadi and Dodi Nadi near Gaur	Nesemann *et al.* 2007
2	*Lamillidens marginalis*	Terai region and Mid mountains region, Khudikhola at Begnastal	Nesemann *et al.* 2007
3	*Parreysia corrugata*	Terai region in central Nepal.	Nesemann *et al.* 2007
4	*Lamillidens jenkinsianus*	Terai region in central Nepal	Nesemann *et al.* 2007
5	*Parreysia favidens*	Western zones of Nepal	Nesemann *et al.* 2007

Collection and rearing of bivalves in captivity

The mussels were collected from drainage canal and reared in ponds, tank of FRS, Pokhara. Bivalves are feed by natural phytoplankton development in ponds. In tank artificial feed as powdered feed were fed. The ponds selected for pearl culture was manured by compost, urea and DAP frequently to maintain phytoplankton level. Water quality parameter maintained in the ponds are presented in Table 2. Mussels are also maintained in the drainage canals.

Table 2. Water quality parameters recorded in pearl culture ponds.

Parameters	Range
Temperature(°C)	15.0-30.0
Dissolved oxygen(mg/L)	5.1-10.2
pH	7.0-8.5
Total alkalinity(mg/L)	35.0-55.0
Total hardness(mg/L)	62.0-76.0
Secchidisc reading (cm)	30-40

Lamellidens marginalis

Figure 2. *Lamellidens marginalis m*aintained in the outlet canals of Fishery research Station.

Preparations of image and circular bead

Materials required: Shells of mussels, motor and pestle, bleaching powder or lime, sieve, araldite (epoxy adhesive), molds, forceps, needles.

Procedure

The shells of dead mussels were soaked in to lime and water solution for 20-25 days and stirred periodically. To decrease the time period bleaching powder could be used. After the desired soaking period when the shells become whitish, the shells are taken out, wash with water and sun dried. The sun dried shells are grind into mortar and pastel to make powder. The powder is sieved. To make the dough of fine shell powder, the araldite adhesive is taken equal part, mixed equally and add powder and mixed again. The small quantity of dough is taken and put on molds to from the images and the circular bead is made by use of finger and palm (Figure 3).

Figure 3. Image beads.

Implantations of beads

Pre-operative conditioning

The collected mussels were kept for pre-operative conditioning for 2 to 3 days by keeping them in crowded condition in plastic tanks with supply of tap water at a stocking density of 1 mussel/liter

Mantle cavity implantation

The key materials used for surgical implantations were beads or nuclei and a pieces of mantle taken from mussels. Mantle cavity implantation were done in the 500 number of mussels (100 in each batch) average weight (Mean±SD, 65.9±14.0) by inserting beads into the mantle cavity region of mussel after opening the two valves (without causing injury to mussels at both ends) of animal and separating carefully the mantles of anterior sides from the shell with the help of surgical set(Figure 4). Implantation were done in mantle cavities of both the valves. After placing the beads in desired place the gaps created during implantation are closed just by pushing the mantle into the shell.

Figure 4. Beads placement in the mantle cavity of mussel *Lamillidens marganalis*.

Post-operative care

Implanted mussels were kept in post-operative care unit in plastic tanks for 10 days with antibiotic treatment and supply of natural food. The plastic tanks were examined daily. At the time of checking, dead mussels were removed.

Pond culture of pearl mussels

After post-operative care the implanted mussels were stocked in the pond. The mussels were kept in nylon bags (10mm mesh, 20cm×20cm) (Figure 5) at the rate of 2 mussels per bag and were hung in ropes fixed in iron pipes and placed in ponds at 0.80 cm depth. The mussels were cultured at stocking density of 20,000 /ha. The fresh water pearl culture pond environment were managed as employed for the aquaculture of the carps. Periodical checking of mussels were done and dead ones were removed. Frequently bags were cleaned to avoid fouling.

Figure 5. Mussels placed in nylons bags hanged in pond for culture.

Preliminary achievements

The present study showed that in the six months culture, the survival percentage of mental cavity implanted mussels were 50-75% varied in batch wise. Maximum survival (85%) of implanted mussels was recorded with mantle cavity implantation method (Pandey and Singh, 2015). Sakpal and Singh (2000) reported 70% survival of *L. marginalis* and 0.071 to 0.106 mm thick nacreous layer around pearl nuclei in mantle cavity implantation method. The methods of mental cavity implantation and rearing of implanted mussels in nylon bag is the most suitable combination for producing freshwater pearls in *L. marginalis* (Pandey and Singh, 2015).

In the present study, nacre development on implanted beads were observed in the *Lamillidens marginalis* and it was white shining (Figure 6). This species have been successfully implanted and cultured in the others countries (Miah *et al*. 2000 and Misra *et al.*, 2009). On the basis of present study, it can be said that the mussels used for pearl farming should be above 100 gm size and implanted mussels should be reared for 12-18 months in the ponds with high density of plankton for the good results. Previous studies also reported that half round pearl could be obtained from the *Lamillidens marginalis* and image pearl could be obtained easily in 12-18 months (Miah *et al.*, 2000; Misra *et al.*, 2009). This acceptance and attachment of beads and development luster on the attached beads is the clear indication that this species could be used as the pearl culture in Nepal also. More species of mussels should be tested and its further research should be carried for implantation methods, beads preparation, propagation and nursing's of mussels to develop the pearl culture technology.

Figure 6. Attached beads in the mantle cavity of mussel *Lamillidens marganalis*.

Way forward

Diversification of aquaculture products is necessary to introduce new aquaculture area to increase profitability of farming systems in Nepal. Freshwater pearl culture is one of the emerging areas in aquaculture sector. In the preliminary study, the mussels *Lamillidens* marginalis is identified as potential candidate for the pearl culture. The indication of acceptance of foreign materials and some coating on the inserted bead seems to be a success towards the pearl culture innovation in Nepal. The larger size mussels' fresh water mussels species *Hyriopsis cumingii* which is successfully cultured in china (Yan *et al.,* 2009) should be imported for the addition of new pearl species in research work and recommendation in culture practices.

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Fisheries management models implemented in theLakes of Pokhara valley for fish biodiversity conservation and to obtain sustainable fish yield

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Abstract

Fisheries managementaims to improve the welfares of the society from harvesting fish. The lakes environment and its fish biodiversity should be managed properly to get sustainable yields. Two fisheries managements and utilizations model have been developed and implemented in the Pokhara valley lakes for fish conservations and lake environment management with participation of fisher community to obtain sustainable fish yield. First model is participatory fisheries management and second model is cooperative based fisheries management. These models are found successful to protect the fish biodiversity and enhanced the fish production from lakes. Due to successful implementation of these model, fisher community livelihood have been improved. To make active involvement of local people to protect these type of natural resources, equal sharing of resources needed with the community living around.

Key words: Phewa Lake, Begnas Lake, Rupa Lake, Fisheries management, Participatory, Fish biodiversity

Introduction

Fisheries managementisthe integrated process that aims to improve the benefits that society receives from harvesting fish (adopted from FAO, 1997). It is a complex task, however, many countries in the world have managed to develop fisheries management regimes that can improve the economic efficiency of the fisheries. To manage and conserve fisheries effectively in the long term requires an understanding of the resources, resource users and also of the wider ecosystem that supports fisheries. Property rights-based fisheries management regimes have shown promising results in the management of fisheries resources (MRAG, 2010). Community-based cooperative fisheries management is one of the property rights based fisheries management systems, which has received attention in recent years particularly in the developing countries (Brown, 1998). In the longer-term fisheries management plan, ecological considerations including ecological economics are essential for maintaining productivity and resilience (Garcia and Cochrane, 2007). Co-management, the only realistic solution for the majority of the world's fisheries, can solve many of the problems facing global fisheries (Gutiérrez *et al.*, 2011).

The capture fisheries contribute only 25% to total fish production of Nepal in 2016/17. The estimated area of lakes of Nepal is 5,000 ha occupying 0.6% of water resource of the country (DOFD, 2016/17). Phewa lake is the largest (523 ha) followed by Begnas Lake (328 ha) and Rupa Lake (around100 ha) of Pokhara valley, Kaski, Nepal. These lakes have been providing multipurpose services to local communities. Approximately 200 families of Jalaris, a deprived ethnic fisher community livelihood is dependent on the fisheries of these lakes. Fishing is the traditional occupation of Pode or Jalari in Pokhara valley, capture fishery using gill nets was widely adopted during the 1960s (Swar and Gurung, 1988). There are 23 fish species have been reported from lakes of Pokhara valley. This paper highlighted the fisheries management practices which have been done for fish biodiversity conservation to get sustainable yield from the lakes of Pokhara valley.

Characteristics of lakes

The Pokhara valley lakes are situated in the central part of Nepal: Phewa lake at 28.1°N and 82.5°E, Begnas lake at 28°10'26.2″N and 84°05'50.4″E, and Rupa lake at 28°08'N to 28°10'N and 84°06'E to 84°07'E (Figure 1).

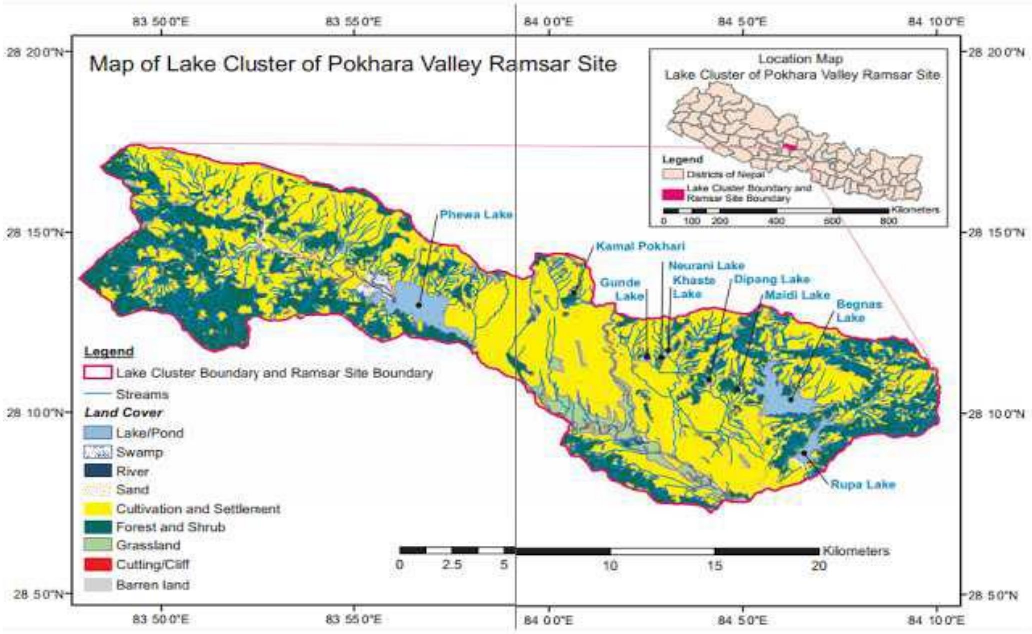

Figure 1. Lakes of Pokhara valley (Source: Ramsar websites).

Phewa, Begnas and Rupa lakes are different in their physical characteristics. Phewa Lake is largest lake in the Pokhara valley and its catchments area includes urban area like Pokhara City and dense population of people. Comparatively, Begnas and Rupa lakes includes only rural area and having less populations of people. Rupa Lake is very shallow and narrow width. All these lakes have multiples uses such as fisheries, irrigation, tourism, boating and electricity. Physical parameters of three lakes of Pokhara valley are presents in Table1

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Table 1. Physical parameters of three lakes of Pokhara valley (FRC, 2010/11).

Characteristics	Lake Phewa	Lake Begnas	Lake Rupa
Elevation (msl)	742	650	600
Area (ha)	523	328	100
Catchment area (km^2)	110	20	30
Water depth (m)			
Maximum	24.0	10.0	6.0
Average	7.5	6.6	3.0
Volume of water (m^3)	393.2×10^5	179.6×10^5	32.5×10^5
Inlet stream	Harpan Khola & Sedi Khola	Syankhudi Khola	Tal Bensi Khola
Outlet Stream	Phusre Khola	Khudi Khola	Sistani Ghat

Water quality parameters of lakes

Water quality parameters of lakes are presented in Table 2.

Table 2. Water quality variablesofPhewa, Begnas and Rupa Lake water during the year 2015/16.

Parameters	Lake Phewa	Lake Begnas	Lake Rupa
Water temperature(^{o}C)	16.3-30.0	16.1-29.5	15.0-29.3
D.O. (mg/L)	7.4-8.4	5.5-6.8	7.7-8.6
pH	6.8-8.3	6.7-9.1	8.3-8.7
Transparency(m)	2.1-2.5	2.2-2.5	0.4-0.7
Chlorophyll a (mg/m^3)	8.3-10.8	7.2-8.5	34.3-52.5
Ammonium(mg/L)	0.002-0.015	0.002-0.012	0.002-0.023
Nitrate+Nitrite(mg/L)	0.03-0.043	0.014-0.067	0.037-0.065
Phosphorus(mg/L)	0.002-0.006	0.002-0.004	0.003-0.005

On the basis of chlorophyll-a concentration, status of Phewa Lake fluctuated between mesotrophic- eutrophic condition while Begnas Lake fluctuated between oligo-mesotrophic and Rupa Lake considered eutrophic condition (Husen *et al.*, 2012a).

Capture fishery of lakes

Fish species diversity

In the FY2016/2017, 24 fish species were captured; 18 were native fish and 6 were exotic in the catches of Pokhara Valley Lakes Phewa, Begnas, and Rupa and it was found same was reported by Husen *et al.* (2016). Total nineteen native fish species belonging to five order and five families were collected from Begnas and Rupa lakes, among which 17 and 16 native fish species were recorded from Begnas and Rupa respectively with four exotic fish species (Pokharel, 1999). There are 23 fish species reported from lakes of Pokhara valley (Gurung *et al.*, 2005).

Fishing gear

The major types of fishing gears used by Jalari fishers in the lakes of Pokhara valley were gill nets, cast nets, and fish hooks. Gill nets 350-450 m^2were the most common fishing gear, with different mesh sizes to capture small to large fish (Husen *et al.*, 2016).

Fish species contributions to fish production from lakes

Total fish harvested from thethreelakesofPokharaValley in FY 2016/17 was 118.5metric ton (Figure 2A). Seasonal variations in the catches from three lakes showed that highest catch was obtained during winter months. Species contribution to the total catch of each lake was varied greatly. The percentcontributionbyexoticfish speciestototalcatchwere88.9.18, 78.9, 86.1 while native species contributed only 11.1%, 21.1 %, 13.9 % in Phewa, Begnas and Rupa lakesrespectively. Nile Tilapia (*Oreochromis niloticus*) species was the major species contributed alone 71.3%, 51.9% and 42.8% of total exotic fish catch from Phewa, Begnas and Rupa respectively. Nile Tilapia has now established in these lakes (Husen *et al.*, 2016). Now due to thepeculiar adaption ability of Nile tilapia, its populations and harvest has increased in these lakes. Other major species in these lakes were bighead carp, silver carp, common carp and grass carp. Among native species, Bhitta (*Puntius* spp.) contribution was highest in lakes Phewa and Begnas while Naini (*Cirrhinus mrigala*) species dominated in catch for Lake Rupa.The productivity (yield, kg/ha) of Phewa, Begnas and Rupa lakes were 132.1, 34.8 and 489.1 respectively (Figure 2B). The productivity of fish directly related to trophic status of lakes. The water quality of Rupa Lake is much productive followed by Phewa and Rupa lakes (Table 2).

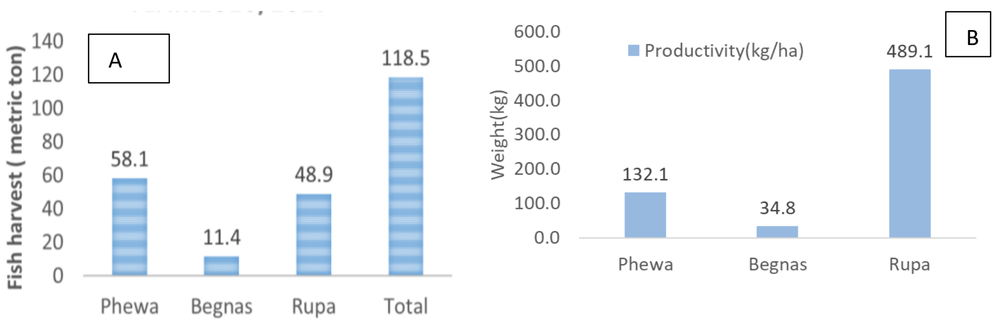

Figure 2. Fish harvest (A) and productivity (B) of lakes of Pokhara valley in 2016/2017.

Major ethnic communities

InPhewa and Begnas Lakes, mostly traditional man and women fishers known as Pode or Jalari, while in Rupa Lake many ethnic groups such as Kami, Damai, Sarki, Magar, Gurung, Chhetri and Brahmin including Pode/Jalari are engaged in lake fisheries for their livelihood.

Model 1: Participatory fisheries management in Phewa and Begnas Lake

Formation of fish entrepreneurs committee and their roles

For the participatory fisheries management in Phewa and Begnas Lake, Jalari fisher community were organized in to fish entrepreneurs committee: Begnas Fish Entrepreneurs Committee (BFEC), Phewa Fish Entrepreneurs Committee (PFEC) (Gurung *et al.*, 2005; Wagle *et al.*, 2007; Husen *et al.*, 2012). These fisher group were sensitized for the conservation of native fish species and uses of fisheries resources wisely. Women group of Jalari community (Machhapuchhre Women Group (MWG), Phewa, Piple Women Group (PWG), Begnas) were mobilized for protection of native fish by patrolling the breeding ground during the spawning season (Gurung *et al.*, 2005; Nepal *et al.*, 2011; Husen *et al.*, 2012b). Mesh size limit of gill net and catch size limit were regulated in each lake to protect the native fish juveniles and to protect from vulnerability of native fish species. Members of the fish entrepreneurs committee have been chasing outsider with illegal fishing devices to protect the native fish population decline.

Fish biodiversity conservations and Lake Environment management

For fish sanctuary in the Pokhara valley lakes, area for demarcation were identified and regulated to protect the native fish species to avoid over exploitation and conservation fish biodiversity. Monthly monitoring of water quality of lakes were carried out from each lake to know the trophic status of lakes and to make its further management plan. On the basis of water quality and survey of antropogenic activities in the catchments area of each lakes, Lake Environment management plan was prepared periodically and it was shared in the stakeholder meeting (Local government, INGO, NGO, government offices, fisheries community etc. related to lakes) and make awareness. Every year, manual removal of water hyacinth *(Eichhornia crassipes)* have been done to check the growth and make the lake clean (Husen *et al.*, 2012b). Campaign for the conservation of native fish on the wetland day were organized to aware the people about the importance of lake environment and fish diversity protection. Hording board were placed in different protected places of lakes and feeding river of lakes for mass awareness. Mass awareness materials such as pamphlets were distributed to local people for the sensitization about fisheries conservation and Lake Environment safeguard. Stakeholders (Local government, INGO, NGO, government offices, fisheries community etc. related to lakes) meetings were organized to discuss and find a way for the lake environment management and fish biodiversity conservation. To check the escape fish from outlets, galvanized iron net screen have been installed in each lake.

Stock enhancement

Daily fish catch monitoring and record from each lakes fish landing sites were done to regulate the stocking plan. Stock enhancement of native fish species were done every year to increase the population of native fish species. For the stock enhancement, native fish species were stocked at the rate of 500-600 fry/ha/yr, 1000-1500 fry/ha/yr and 1500-2000 fry/ha/yr for Phewa, Begnas and Rupa lakes, respectively. The native fish species used for stocking are: sahar (*Tor putitora*), rohu (*Labeo rohita*), naini (*Cirrhinus mrigala*), bhakur (*Catla catla*), and gardi (*Labeo dero*). In addition, bighead carp (*Aristichthys nobilis*), silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*) are also stocked in these lakes.

Model-2: Community-based cooperative management in Lake Rupa

Till 1999, the lake Rupa was in degraded condition and heavily encroached by weeds to such an extent that even boating was not possible. To tackle these problems leading to the reduced fisheries production, a cooperative of local villagers was established in 2002 (Gurung, 2007). The cooperative's major goal was to conserve, manage, and enhance the lake's fisheries to welfare the community. Grass carp (*Ctenopharyngodon idella*) to control submerged vegetation, common carp (*Cyprinus carpio*) uprooted submerged plants and Silver (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) to control plankton blooms were introduced since year 2002 by cooperatives as a strategic approach for restoration (Gurung, 2007). Net installation was placed at outlet to check the fish escape. Degraded lake was managed by cleaning the lake with uprooting the unwanted plants. The lake became clean, restored and fish harvest increased. The local communities have gained more awareness of the importance of water, the lake, and related resources. Capture fishery with regular fish stocking program was initiated for financial sources (Gurung, 2007).

Annually, profits have been distributed to members of the cooperative. The cooperative was started with only 329 member's member and now reached to 840 members. The cooperative has established their own hatchery nursery for fish seed production and stock enhancement in the Rupa Lake. The cooperative now doing initiation for land erosion management through coordinating with different NGO'S and INGO'S, Government offices and also linking the Ministry and Planning Commission for Rupa Lake Management. The cooperative has drafted fisheries business plan to double the fish production and also they have plan to increase the lake depth by damming of lake. Co-operative helps for biodiversity conservation of fish as well as other aquatic organisms and flora and fauna of Rupa lakes and serves for society welfare. Cooperative management approaches to restore ecological function and economically profitable fisheries in the Lake Rupa an ecofriendly model to apply in other lakes of world.

Conclusions

The lake fisheries management is continuous process. The fish production from lakes depends on the lake environments. Lake Environment and fisheries should be managed with the changing context. Participatory fisheries management in Phewa and Begnas lakes have been proved to be a successful model for lake fisheries management in the Pokhara valley lakes. Lake fisheries management of Lake Rupa by forming a cooperative is a successful another model for the management of degraded lake and conservation of native fish species. These two successful models could be applied to other lakes of world.

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Effect of eyed egg density and transportation period on survivability, hatchability and deformed number of rainbow trout (*Oncorhynchus mykisss*) fry

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Abstract

One of the major challenges for wider adoption of trout aquaculture throughout the country is to supply quality fingerling in adequate quantity to growing areas. The effect of stocking density of eyed eggs and transportation period on eyed egg survivability, hatching rate and deformed number of rainbow trout were examined in successive two years at Rainbow Trout Fishery Research Station, Dhunche, Rasuwa. Eyed eggs were treated with polyvinyl pyrrolidone at 100 mg/L for 15 minutes before transportation. Temperature of treated eggs (3.06±0.09 & 3.91±0.09 mm diameter) of the same broods in respective years were gradually acclimatized at the rate of 1.0ºC/hour to attain final temperature of 4°C for transportation. Eggs were packed at density 500, 1000 and 1500 egg/plastic packet having dimension of 323.01 x 151.06 x 0.05 mm in alternate layers with ice at egg: ice ratio of 1:1.5. Plastic bags with eggs were placed in cooler box and transported for 24, 48 and 72 hours. Each density of eggs and transportation period was replicate three times. Density of 500 eggs/packet and transportation duration of 24 hours were prominent being safest with lower mean egg mortality, hatching rate and deformed ratio in first year. Similarly, density of 1000 eggs/packet and transportation duration of 24 hours were superior being safest with lower mean egg mortality, hatching rate and deformed ratio in second year. In overall, eggs from second year breeding of the rainbow trout had larger egg size, lower mortality, higher hatching rate and lower deformed ratio.

Keywords: Eyed egg, Hatching rate, Mortality, Stocking density, Transportation

Introduction

Abundancy of the inland water with over 6000 rivers and rivulets flowing from north to south, carry out vast potentiality for the farming of the rainbow trout in Nepal. For this, third attempt for the introduction of the rainbow trout (*Onchorynchus mykiss* Walbum.) was done in 1988 with 50,000 eyed eggs of Donaldson strain which was brought from the Kobayashi Fisheries Experimental Station, Miyazaki Prefecture, Japan. These eyed eggs were received at the Godawari Fish Farm in Lalitpur (Gurung and Basnet, 2003). For the expansion of the aquaculture in the hills of the country, Rainbow trout; a cold water fish has been introduced utilizing the readily available cold water resources. With the development of the complete cultural package the rainbow trout production was started in 1995 on government- run farms and in 1998 on private farms (Rai *et al.,* 2005). From then the trout culture in Nepal is booming as now there has been more than 120 farms out of which 18 private farms are involved in trout seed production in 26 districts. In present context, private hatcheries produced more than 90% of the total seed production (FRD, 2017).

One of the major challenges for wider adoption of trout aquaculture throughout the country is to supply quality fingerling in adequate quantity to growing areas (FRD, 2013).Most prominent incubation temperature for the rainbow trout eggs has been found in between 7°C and 12°C; inflated fatality with increase in temperature above 15°C and near 2°C (Kwain, 1975; Raleigh *et al.,* 1984; Velsen, 1987; Myrick and Cech, 2001; Carter, 2005). Breeding and nursing of the fry can be considered as important and crucial part of trout farming. For maximizing production and profit by grow-out farmers either the government or private sector or both must consider brood maintenance as a top priority which ensure the receiving of the quality seed. But the government and private farms working in fry and fingerlings production in Nepal are very less, skewed and scattered which needs to be transported as economically as possible ultimately causing the price of fry and fingerlings to swell up. The price of the trout fingerling varies high with size, season and availability ranging from NRs. 7-25 per piece.

The traditional method of transport uses open containers and aerate with oxygen cylinder on long journey on trucks and jeeps which is still prevalent in many parts of Nepal which actually had laid the foundation of the trout farming in the country which is very expensive. In this process, the seeds gets heavily stressed which suffers mortality either during transportation or soon after stocking. Being weak, it easily falls prey to parasites and predators. Open containers have been eliminated in the modern ways which were very tedious and heavy which needed continuous attendants, water exchange and oxygenation. The fingerlings is now packed in the plastic bags filled with 1/3 water and 2/3 oxygen. Plastic bags are kept in the light containers or cardboard cartons and transported long distances by road or air. Hatching rate (61%) and fry survival rate (67.8%) are still low to meet current seed demand (FRD, 2017). Also, mortality during and post transportation of fish seed is very high in Nepal as many potential stressors such as seizing, loading, transport, unloading, temperature difference, water quality change and stocking outcome in immuno-suppression, physical injury, shorten growth and even mortality (Azambuja *et al.,* 2011; Becker *et al.,* 2012;Husen and Sharma; 2015;Inoue *et al.,* 2005;Iversen *et al.,* 2005).

For the formation of the eyed egg stages of rainbow trout it takes about 250 degree days (Ballard, 1973), which are the summation of the average daily water temperature in degree celsius. This 250 degree days timeframe is convenient for hatcheries in that it takes place well after the embryos are resistant to mechanical shock (Jensen, 2003; Jensen and Alderdice, 1989) and is several days before hatching, which allows time for eyed eggs to be sorted to remove dead and sub-viable eggs, and then be shipped to production facilities. After the eyed egg formation of the rainbow trout, there is very less chances of mortality (Nagler *et al.*, 2000). The present study focuses on egg transport and subsequent effect on yolk-sac larval development of *O. mykiss* in relation to stocking density and period of transport. Different stocking densities were tested to determine optimum number of egg per packet during transportation for higher viability. Additionally, in the case of transportation, different period were tested to discriminate safe transport period and higher viability of eyed eggs at different stocking densities. For trout larvae the survival, hatchability and deformed number were investigated in relation to stocking density and transportation period.

Materials and methods

Experimental design

This experiment was conducted for two successive years. The same broods were used for the successive year for this experiment. This experiment was conducted in three cooler box of capacity 25 kg (each dimensions 0.47 x 0.29 x 0.35 m^3) at the Rainbow Trout Fisheries Research Station located in Rasuwa, Nepal. The experiment was conducted in 3 x 3 factorial completely randomized design (CRD) having three stocking densities, density 1 (500 number per Packet); density 2 (1000 number per Packet) and density 3 (1500 number per Packet) and three transportation period, duration 1 (24h) duration 2 (48 h) and duration 3 (72h). Altogether there were nine treatments replicated three times. T_1= Density1 Duration 1; T_2=Density 1 Duration 2; T_3=Density 1 Duration 3; T_4=Density 2 Duration 1; T_5=Density 2 Duration 2; T_6=Density 2 Duration 3; T_7= Density3 Duration 1; T_8=Density 3 Duration 2; T_9=Density 3 Duration 3.

Fish breeding and egg collection

Seven trout female broods and same number of male broods reared at Rainbow Trout Fisheries Research Station, Dhunche were bred in captivity for consecutive two years by stripping and dry fertilization. Mean length and weight of female and male; size of eggs, calculated from representative sample of each brood is given Table 1.Trout eyed eggs were ready to transport after 220–250 degree days or 18-20 days post spawn at 9-11 ºC.

Table 1. Mean weight and length of male and female and size of 10 eggs of broods of consecutive years.

Length of Female (cm)	Weight of Female (g)	Length of Male (cm)	Weight of Female (g)	Size of 10 eggs (mm)
First Year				
33.26±0.95	529±19.28	33.43±1.43	569±19.38	3.06±0.09
Second Year				
Length of Female (cm)	Weight of Female (g)	Length of Male (cm)	Weight of Female (g)	Size of 10 eggs (mm)
40.26±0.95	735±59.57	39.43±0.83	769±29.36	3.91±0.09

Tempering and disinfection during stocking and transportation of the eyed eggs

Three stocking densities 500, 1000 and 1500 number per packet were tested at three different transportation period 24, 48 and 72 h, respectively as shown in Table 2. Homogeneity was maintained by mixing eggs of all broods at the eyed egg stage. The eyed egg were then divided into three sub-samples and placed in smooth muslin cloth hung on 20L plastic buckets containing incubation water. For each transportation period there were three replicates, corresponding to the three stocking densities, each replicate consisting of a polythene packet with size 32.3 x 15.1x 0.005 cm. Tempering of the eggs at incubation was gradually decreased with iced water as 1°C per hour until it reaches to 4ºC. Continuous aeration of eyed egg through-out the process was done with help of YL-10000 aerator. Then, eyed eggs prior to transport were treated with iodophore: polyvinyl-pyrrolidone (PVP) iodine, at rate of 100 ppm for 15 minutes. These eggs were placed in a purpose made special racked wooden tray which were stocked in cooler box, one box for each experimental transportation period at 1: 1.5: egg: ice ratio. Foam soaked in iced water was used as intermittent filler to prevent eyed eggs directly contact with ice. Eyed egg survival was determined at 24, 48 and 72 h by counting the dead eggs on each packet of each stocking density group in relation to transportation period.

Eyed eggs were opened at guided transportation period and tempering of the packed eggs was gradually increased as 1°C per hour to match the incubation temperature of the hatchery. Then after, eyed eggs were treated with iodophore as stated above. Each treatment eyed eggs were poured separately on locally wooden tray of size 33 x 33 cm^2 and thickness 2.5 cm with clear marking of treatment type. Then, these trays of same transportation period groups were tied-in and stake together irrespective of stocking density to stock into the cemented Atkins. Where, uniform, flow through system was maintained at the rate 2 litre/

minute for 10,000 eggs. For all eyed egg incubations mixed water source of spring as well as riverine water, locally called Ghatte Khola was used.

The mass hatching was completed in 11, 8 and 5 days after incubation in first year whereas it was completed in 10, 8 and 4 days in second year of 24h, 48 hand 72 h transportation period groups, respectively. The hatched out larvae were taken from the trays and were put to the 75 x 55 x 30 cm size net cages for nursing. Hatchability of each treatment group was recorded. Similarly, spinal and cephalic anomalies larvae of each treatment group were recorded as deformed larvae.

Table 2. Stocking densities and transportation period of the different treatments.

Factors	Treatments								
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
Stocking density (number of eggs/packet)	500	500	500	1000	1000	1000	1500	1500	1500
Transportation Period (h)	24	48	72	24	48	72	24	48	72

Statistical Analysis

The data entry was done through MS Excel 2016. For data analysis ANOVA was used with the eyed egg and larvae viability variables as dependent variables and the stocking density and transportation period as the independent variable. Tukey test was used as a multiple comparison post hoc test to determine which treatments differed significantly where, $p<0.05$ was considered significant. Calculations were performed with the R-Stat version 3.6.3.

Results and Discussion

Egg mortality

During the first year, there was significant difference in mortality percentage between the different density/packet. 500 eggs/packet (0.0092±0.002) has significantly lowest mortality percentage than 1000 eggs/packet (0.0107±0.004) and 1500 eggs/packet (0.0114±0.004) ($p>0.05$), whereas 1000 eggs/packet and 1500 eggs/packet were not significantly different with each other. Whereas, there was no significant difference in mortality percentage in second year among 500 eggs/packet (0.006±0.002), 1000 eggs/packet (0.005±0.003) and 1500 eggs/packet (0.008±0.004) as shown in Table 3 and Table 4. Similarly, there was significant difference in mortality percentage of the eyed eggs in different duration of transportation. Mortality percentage of 24 hours (0.005±0.001) was significantly lower than that of 48 hours (0.009±0.19) and 72 hours

(0.017±0.003)) in first year whereas in second year mortality percentage of 24 hours (0.003±0.001) was not significantly different with 48 hours (0.004±0.001) but both have significantly lower mortality percentage than 72 hours (0.13±0.02) which is shown in Table 3 and Table 4 ($p>0.05$).

Table 3. Mean mortality % of eyed eggs in different treatments in first year.

Density/ Packet	Duration (Hours)			Mean
	24	48	72	
500	0.0036±0.0004	0.009±0.0005	0.0152±0.0003	**0.0092±0.002^{a}**
1000	0.0046±0.0003	0.01±0.0004	0.02±0.0005	**0.0107±0.004^{b}**
1500	0.01±0.0001	0.011±0.0003	0.02±0.0004	**0.0114±0.004^{b}**
Mean	**0.0046±0.001^{a}**	**0.0097±0.001^{b}**	**0.017±0.003^{c}**	

Table 4. Mean Mortality % of eyed eggs in different treatments in second year.

Density/Packet	Duration(Hours)			Mean
	24	48	72	
500	0.005±0.000	0.003±0.001	0.011±0.001	**0.006±0.002^{a}**
1000	0.002±0.001	0.003±0.001	0.011±0.003	**0.005±0.003^{a}**
1500	0.003±0.001	0.005±0.0004	0.016±0.002	**0.008±0.004^{a}**
Mean	**0.0033±0.001^{a}**	**0.004±0.001^{a}**	**0.013±0.02^{b}**	

There was significant increase in the mortality percentage with the increase in the transportation duration whereas slight decrease in the hatchability percentage with the increase in the transportation duration in the first year whereas no significant difference was seen up to 48 hours in second year in the both cases which was similar to the findings of the Tensen *et al.* (2009) in their work on storage of Atlantic salmon gametes. Similar trends were also reported in chum salmon gametes where decline in the viability was seen with the increase in the storage duration (Jensen and Alderdice, 1984). In the present study, both groups of parents were raised in similar conditions. Therefore, the difference in mortality are attributed to parental age and egg size. Some possible advantages of using older females that yield larger eggs in a rainbow trout production program include lower mortality of eggs, fry, and fingerlings (Pitman, 1979).

Hatching rate

In the results of first year, there was significant difference in hatching percentage between the different density/packet. 500 eggs/packet (98.84±0.22) has highest hatching percentage than 1000 eggs/packet (98.53±0.23) and 1500 eggs/packet

(98.28±0.29) which were significantly different with each other at p>0.05. However, 1000 eggs/packet was not significantly different with both 500 eggs/packet and 1500 eggs/packet. Likewise, similar results of hatching percentage was seen in second year in which 500 eggs/packet (99.41±0.15) has highest hatching percentage than 1000 eggs/packet (99.34±0.26) and 1500 eggs/packet (99.26±0.38) which were significantly different at p>0.05 as shown in Table 5 and Table 6. However, 1000 eggs/packet was not significantly different with 1500 eggs/packet. Similarly, there was significant difference in hatching percentage of the eyed eggs during the different duration of transportation. Hatching percentage of 24 hours (99.5±0.04) transportation duration was significantly higher than that of 48 hours (98.79±0.07) and 72 hours (97.35±0.12)) in first year whereas in second year hatching percentage of 24 hours (99.79±0.07) was not significantly different with 48 hours (99.34±0.04) but both have significantly higher hatching percentage than 72 hours (98.88±0.16) which is shown in Table 5 and Table 6(p>0.05).

Table 5. Mean hatching % of eyed eggs in different treatments in first year.

Density/Packet	Duration (Hours)			Mean
	24	48	72	
500	99.64±0.04	99.12±0.05	97.76±0.12	**98.84±0.22**[a]
1000	99.54±0.02	98.62±0.07	97.44±0.14	**98.53±0.23**[ab]
1500	99.25±0.14	78.91±19.69	96.84±0.09	**98.28±0.29**[b]
Mean	**99.50±0.04**[a]	**98.79±0.07**[b]	**97.35±0.12**[c]	

Table 6. Mean hatching percentage of eyed eggs in different treatments in second year.

Density/Packet	Duration(Hours)			Mean
	24	48	72	
500	99.68±0.07	99.41±0.13	99.15±0.13	**99.41±0.15**[a]
1000	99.79±0.09	99.34±0.09	98.88±0.58	**99.34±0.26**[b]
1500	99.91±0.06	99.26±0.04	98.61±0.27	**99.26±0.38**[b]
Mean	**99.79±0.07**[a]	**99.34±0.04**[a]	**98.88±0.16**[b]	

During the eyed stages, eggs can be cleaned and shipped. The stressed given to eggs also known as mechanical shock due to transportation of eggs. Hatching percentage has been significantly high in 24 and 48 hours but low in 72 hours.

The eyed eggs can be successfully transported up to 48 hours as shown in the work of Nash and Novotny (1995) which has similar findings as our work.

Deformed rate

In the results of first year, there was significant difference in deformed number of fries between the different density/packet. 500 eggs/packet (1.2±0.38) has lowest deformed number of fries than 1000 eggs/packet (3.93±2.5) and 1500 eggs/packet (9.1±5.69) which were significantly different with each other (p>0.05). Likewise, similar results of deformed number of fries was seen in second year in which 500 eggs/packet (0.0±0.0) has lowest number of deformed fries than 1000 eggs/packet (0.56±0.29) and 1500 eggs/packet (1.55±1.39) which were significantly different at p>0.05 as shown in Table 7 and Table 8. Similarly, there was significant difference in deformed number of the fries during the different duration of transportation. Number of deformed fries of 24 hours (99.5±0.04) transportation duration was significantly lower than that of 48 hours (98.79±0.07) and 72 hours (97.35±0.12) in first year whereas in second year number of deformed fries of 24 hours (99.79±0.07) was not significantly different with 48 hours (99.34±0.04) but both were significantly lower than 72 hours (98.88±0.16) which is shown in Table 7 and Table 8 (p>0.05).

Table 7. Mean deformed number of fries in different treatments in first year.

Density/Packet	Duration (Hours) 24	48	72	Mean
500	0±0.00	0±0.00	3.6±0.4	**1.2±0.38**[a]
1000	0±0.00	4±0.32	7.8±0.86	**3.93±2.5**[b]
1500	2±0.00	5±0.32	20.4±0.74	**9.1±5.69**[c]
Mean	**0.4±0.38**[a]	**3±0.1.52**[b]	**10.6±5.04**[c]	

Table 8. Mean deformed number of fries in different treatments in second year.

Density/Packet	Duration(Hours) 24	48	72	Mean
500	0.00±0.0	0.00±0.0	0.00±0.0	**0.00±0.0**[a]
1000	0.00±0.0	0.67±0.67	1.0±0.58	**0.56±0.29**[b]
1500	0.00±0.0	0.33±0.33	4.33±3.84	**1.55±1.39**[c]
Mean	**0.00±0.0**[a]	**0.73±0.43**[b]	**1.68±0.99**[c]	

Deformity of the fries is also seen very high in case 72hours in both years. Limited work has been done in case of the stocking density of the eyed eggs of rainbow trout. High change in temperature was found in the highest stocking density i.e. 1500 eyed eggs/packet in both years. Temperature difference of a few degree during the egg incubation and early larval stages have a profound influence on the muscle differentiation (Chang *et al.* 2004; Itzkowitz *et al.* 1983; Johnston, 1993). Similar work has been found in Baeverfjord *et al.*(2009), where they have stated that long exposure of the high number of eggs below 8°C are likely to induce skeletal malformations.

The mortality percentage was lower, hatchability was higher and deformed number was lower in the second year eyed eggs which were hatched from the large size female with large sized eggs. As suggested from the Pitman (1979), large size female produce large size eggs which has less mortality and hatchability than that of the smaller sized female. Therefore larger sized female of older age must be considered for the breeding purposes.

Conclusion

Tempering and disinfection is very important in rainbow trout eyed eggs. Tempering helps eyed eggs from shocks of the sudden temperature drop in the water as well as to replace the water loss in the shipping. Disinfecting the eyed eggs helps to avoid possible contamination of the hatchery as wells as eyed eggs with the disease organisms. Density of 500 eggs/packet and transportation duration of 24 hours were prominent being safest with lower mean egg mortality, hatching rate and deformed fry ratio in first year. Similarly, density of 1000 eggs/packet and transportation duration of 24 hours were superior being safest with lower mean egg mortality, hatching rate and deformed ratio in second year. In overall, eggs from second year breeding of the rainbow trout had larger egg size, lower mortality, higher hatching rate and lower deformed ratio.

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Effect of dietary administration of stinging nettle (*Urticaparviflora* - Roxb.) and marigold (*Tagetus erecta* L.) on reproductive performance of rainbow trout, *Oncorhynchus mykiss*

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Abstract
Quality seed has been bottleneck to scale up commercial trout production in hills and mountains. Modern trout aquaculture and breeding operations have become vertically and horizontally intensified, necessitating a sustainable supply of nutritionally balanced, cost effective feed. The present experiment was designed to study the effect of stinging nettle and marigold flower as feed additives on the growth and reproductive performance of rainbow trout. There were three treatments having 3 diets viz., shrimp based, (control), shrimp+5% sisnu (stinking nettle) (SSN) and shrimp+5% marigold (SMG).The experiment was conducted for 60 days at Rainbow Trout Fisheries Research Station, Dhunche. Results showed that relative fecundity (eggs number/kg body weight of female), average size of eggs (mm) and average number of eggs per gram of fish, were not significantly different ($p>0.05$) between fish fed with control feed and sisnu containing feed, but fish fed with marigold containing feed was significantly different ($p<0.05$) from other two treatments.Total number of eyed eggs (58.7±0.01%) was significantly higher ($p<0.05$) in fish fed with sisnu containing feed followed by fish fed with control feed (56.2±0.02 %).Similarly, total number of hatching rate (54.9±0.04 %) was significantly higher ($p<0.05$) in fish fed with sisnu containing feed followed by fish fed with control feed (51.3±0.01 %). Significantly lowest values ($p<0.05$) of total eyed stage egg (53.36±0.02 %) and hatching rate (48.03±0.23 %) were observed in fish fed with marigold containing feed. Overripe eggs were obtained on fish fed with sisnu containing feed, suggests more frequent observation of the spawners is needed for recognizing of their appropriateness to ovulation.

Keywords: Rainbow trout, Stinging nettle, Eyed egg, Hatchability.

Introduction

Intensive rainbow trout culture in flow-through systems is common practice incold-water aquaculture of Nepal (Timalsina *et al.*, 2017) having annual fish production of 420 tons (CFPCC, 2019) and seed production of 2.7 million eyed eggs and 0.67 million fries (Singh, S.,2021 personal communication). The rapidly increasing number of newly developing trout farms of varying sizes within the country has increased demand for fish seed as stocking requirements throughout the year whereas availability of trout seed is seasonal. Moreover, fry produced from inferior brood stock (likely to be similar low quality) are easily sold as "premium quality"which becomes apparent to trout producer farmers many months later. In this context, the stress due to sub-optimal rearing conditions (Wedemeyer *et al.*, 1976) in intensive fish production could have been predisposing factor affecting welfare of brood fish. (Dawood & Koshio, 2016a, 2016b; Yan *et al.*, 2017).Previous studies, also showed that chronic and acute environmental stresses adversely affect the reproductive system (Pottinger & Pickering, 1990), and gamete quality of fish (Campbell *et al.*, 1992; Campbell *et al.*, 1994) as well as cortisol stress response had shown to vary among stocks of rainbow trout (Pottinger *et al.*, 1992; Fevolden *et al.*, 1993; Pottinger and Moran, 1993; Pottinger and Carrick, 1999). In most of cases, early cutback of stressful conditions in fishes may lead to self-cure (Meyer, 1991).

The use of immune-stimulant plants in aquaculture has increased world-wide as they are relatively cheap, easy to prepare, has multiple administration, and contains different natural organic compounds for potent growth promoters, as well as immune enhancers alternatives (Hai, 2015). Among the various sources of herbal supplements, the use of *Urtica spp.*(Stinging nettle; Urticaceae family) in aquaculture is gaining importance for its potent immune-stimulatory, anti-carcinogenic, anti-inflammatory, anti-oxidant, anti-analgesic, anti-ulcer activities; effective tolerance against various pathogenic microorganisms (Gülçin, *et al.*, 2004; Turker & Yıldırım, 2015)and widely accepted dietary accessory on the intensive farming (Vico *et al.*, 2018). *U. parviflora,* Nepalese tongue sisnu is one among 5 native nettle species reported to distributed all over the country (Bhatt & Chhetri, 2017), and commonly used for fodder, ethno-medicinal purpose and green vegetable (where, tender shoots and leaves are consumed as a vegetable or as a porridge). Regardless of great possibility as a useful crop still, it’s considered as a neglected and underutilized species often undervalued. Some research under NARC with promising results as feed additive in poultry and trout feed was shared by different authors, however limited information was found on reproductive performance of rainbow trout. Therefore, the aim of this study was to evaluate the effect of sisnu and marigold petal on reproductive performance of trout broods as feed additive.

Materials and Methods

Preparation of experimental diets

The experiment was conducted in Completely Randomized Design (CRD) with 3 experimental diets with same crude protein percentage (33%), diet-1or T_1: shrimp based (control, CON); diet-2 or T_2: shrimp +5% sisnu (SSN) and diet-3 or T_3= shrimp + 5% marigold (SMG). There were four replications for each treatment.

Sisnu tender tops were collected from periphery of Rainbow Trout Fishery Research Station, Rasuwa, Dhunche, Nepal. The tender tops (usually 4-6 flag leaves; in Nepalese tongue muna) were harvested with gloves or tongs. Caution was followed by not touching the plant with bare hands to avoid stinging sensation of stinging hairs called as trichomes. Authentication and identification of the plant was carried out at the National Herbarium and Plant Laboratories (NHPL), Godawari. The plants were cleaned and cut into small pieces, and then air dried in shade on plastic sheet. The dried samples were then pulverized into fine powder in a grinder and sieved through the 150 µm sized mesh, which was then stored at 4°C until use. Similarly, marigold flowers *Tagetus erecta* L., were collected from the Flower Development Centre, Godawari. The Floral parts namely sepals, bracts and flower stalks were removed and the remaining parts (ray florets and disc florets) dried under shade and powdered. Representative powder samples were separately stored in a plastic bag, air tied, well accessioned, and stored in refrigerator until pelletization. The proximate analysis of stinging nettle and marigold was carried out before including in the research diet. The proximate value of formulated feeds and their composition are given in Table 1.

Table 1. Composition and proximate value of formulated diets.

Parameter	Shrimp based diet (T1, Control)	Shrimp + 5% sisnu diet (T_2)	Shrimp + 5% marigold (T_3)
Organic matter (OM, %)	75.7	78.7	75..4
Crude Protein (CP, %)	33.8	33.2	32.5
Crude fat (EE, %)	2.1	1.4	1.4
Crude fiber (CF, %)	4.2	4.8	4.4
Total Ash (TA, %)	24.3	31.3	24.7
NFE (%)	11.3	8.0	12.3

NFE (%) = OM-CP-EE-CF-TA.

Research unit set-up, dietary treatment and management

This study was carried out in 12 head to head parallel raceways with flow through system having seven square meter surface area. These raceways were truss supported which in turn is overlapped by the higher metal roofing for easy working facilities. Water in the raceways was supplied from Ghatte Khola with backup reservoir passing through de-siltation raceway. In all raceways, water depth was maintained at 80 cm. Uniform inflow rates of at least 5 L/sec within raceways was determined by bucket test method, adjusted with inlet pipes sizes and monitored regularly.

Broods fishes were acclimatized for one week to research unit conditions prior to beginning of the experiment. During acclimatization process, fishes were fed ad libitum daily with control diet twice a day at 8 am and 5 pm. Rainbow trout 1^+ broods (approaching 2 years of age) with an average weight of 717.51±9.01 g were randomly stocked at the rate of $5kg/m^3$.The fish were fed with experimental diets for 60 days prior to natural spawning. Then after, the broods were fed with control diet. Generally, the mature trout individual spawns from early November to January at RTFRS farm conditions, principally in November and early December. Therefore, as the times of the natural spawning of the different treatments approached, state of ripeness was examined individually by stripping at lower abdomen once at a week during breeding period from last week of October. In the case of responded broods fertilization was carried out by dry method and the fertilized eggs were incubated placing the brood specific eggs on separate trays in cemented atkins where continuous and uniform water flow was maintained at the rate 2 L/minute for 10,000 eggs. All spent fish were placed into 3% salt solution before being returned to the experimental raceways.

The breeding parameters (Bromage, 1990): total number fish to show response to breeding, overripe broods, relative fecundity (number of eggs/kg of body weight) were determined. The size of eggs was determined by measuring 10 eggs by digital vernier calipers. The process was repeated three times and average size of eggs was calculated. Average number of eggs per gram and total eyed stage egg number and total hatchings percentage were recorded and analyzed. All weights were weighed by (±0.1 g, Phoenix instrument electronic balance Model SN-014739).

The outlet pipe of each raceway was removed daily for approximately 10 seconds before feeding to flush away the accumulated uneaten food and fecal matter on the drainage screens to reduce the risk of blockages. Monthly, raceways were cleaned scrubbing floor and its adjacent sides.

Proximate analysis of diet

Quadrant sampling was done at each lot of feed prepared during experiment period for proximate analysis. The proximate composition of the samples was determined following standard AOAC methods (1990) at the National Animal Nutrition Research Centre, Khumaltar. Moisture was determined by oven drying method, drying the sample at 105 °C to a constant weight. Nitrogen was estimated by the Kjeldahl method (2200 Kjeltec Auto distillation, Foss Tecator, Sweden) and crude protein was estimated by multiplying the percent nitrogen by 6.25. Crude fat content was determined by soxhelt ether extraction; Van Soest fiber analysis. Estimation of crude ash content was determined by heating the sample in a muffle furnace at 500 – 600 ^{o}C.

Water quality analysis

Dissolved oxygen (DO), pH and temperature were measured at out flow of each raceway.Temperature was measured using digital thermometer, daily at 7-7:15 am.Dissolve oxygen and pH value was measured weekly using Thermo Scientific Orion 1119000 5-Star Benchtop Multi-parameters Meter at 7-7:15 am.

Plasma cortisol analysis

Cortisol is the principal corticosteroid shows primary stress responses in teleosts (Donaldson, 1981).Response to stressful condition among species varies, salmonids being one of the species that respond almost immediately to handling and crowding stress (Carey & McCormick 1998). ELISA procedure, an accuracy and efficiency test was used for fish cortisol plasma determination (Cladwell *et al.*, 1990). 10% Spent Brood fish were anesthetized using clove oil (concentration 40 ppm) and blood sampling was done by caudal venous puncture. The blood samples drawn poured to lithium heparin coated tubes were transferred to Animal Health Division, Khumaltar for further analysis.

Statistical analysis

Statistical analyses of data were carried out with SPSS 16.0 software package (SPSS; Chicago, IL). Effect of treatments was carried out using one-way ANOVA, followed by a post-hoc Tukey HSD test whereP values less than 0.05 were considered as statistically significant. All means were given with ± standard error (S.E.).

Results and Discussion

Breeding Indices

The mean weight of broods responded to breeding, overripe brood fish number, relative fecundity, egg size, eggs number per gram, total eyed stage percentage, and hatching percentage in different treatments are presented in Table 2. Although overall responded females (including overripe) were higher in fish fed with sisnu supplemented diet (T_2), the total number of females brood participated in breeding was significantly higher in fish fed with shrimp based control diet T_1 (13±0.41) followed by sisnu supplemented diet T_2 (11±0.41). The female trout broods fed with marigold containing diet (T_3) showed very poor response to breeding(2±0.71).Relative fecundity (eggs number/kg body weight of female), average size of eggs (mm), average number of eggs per gram of fish, were not significantly different with each other ($p>0.05$) in fish fed with control feed (T_1) and sisnu containing feed (T_2) but significantly different ($p<0.05$) with fish fed with marigold containing feed.Significantly higher total eyed egg percentage (58.7±0.01%) and hatching rate (54.9±0.04 %) were observed in treatment T_2 (fish fed with sisnu containing feed) followed by T_1 (fish fed with control feed) (total eyed egg percentage,56.2±0.02 % &and hatching rate , 51.3±0.01 %). Significantly lowest values ($p<0.05$) of Total eyed stage egg (53.36±0.02 %) and hatching rate (48.03±0.23 %) were observed in fish fed with marigold containing feed (T_3). The increase in breeding performance in treatment groups supplemented with dietary inclusion of sisnu might be its beneficial effect on egg quality parameters. Supporting results were obtained with other species of *Urtica* in previous studies on laying performance of poultry birds (Melesse *et al.*, 2019; Zhang *et al.*, 2020). Moreover, restricted growth of many pathogenic and non-pathogenic group of bacteria in fish gut due to the herbal add might have also improved overall efficiency (Dawood *et al.*, 2014; Hai, 2015).

Table 2. Mean value (±SEM) of breeding parameters of trout during experimental period

Breeding parameters	**Treatments**		
	T_1	**T_2**	**T_3**
Breeding Month	Poush-Magh	Poush-Magh	Falgun
Female Stocking number	25	26	29
Average body weight female, g	715.6 ± 13.32^a	707.5 ± 22.58^a	687.53 ± 10.70^a
Average body weight male, g	738.65 ± 21.25^a	747.65 ± 13.02^a	708.23 ± 9.37^a
Hatchery temperature (°C)	9.1 ± 0.04^a	9.1 ± 0.041^a	10.5 ± 0.06^b
Responded female number and weight (in parentheses), Kg	13 ± 0.41^c(11.29)	11 ± 0.41^b (8.4)	2 ± 0.71^a(1.36)

Overripe female number	-	5	-
Relative fecundity (number of eggs/kg body weight of female)	2445.75±4.15^{b}	2513.75±15.3^{b}	2106.5±41.89^{a}
Average size of eggs, mm	3.98 ±0.07^{b}	3.96±0.01^{b}	2.79±0.05^{a}
Average number of eggs/gram	27.75±1.70^{a}	30.00±1.08^{a}	38±1.22^{b}
Total number of eggs on incubation	27613±1.47^{c}	21117±0.91^{b}	2865±1.87^{a}
Total eyed stage egg, %	56.21±0.02^{b}	58.71±0.01^{c}	53.36±0.02^{a}
Total hatching, %	51.27±0.01 ^{b}	54.9±0.04^{c}	48.03±0.23^{a}

Mean value with different superscript letter within same row are significantly different at $p < 0.05$

Water quality

Mean water quality parameters, measured during experimental period are presented in Figure 1 to Figure 3. All readings were within standard requirements of rainbow trout breeding (Gurung, 2008). Spatial variation of the temperature, dissolved oxygen, and pH readings implied that the hatchery conditions parameters (i.e. after 60D) were more critical compared to the brood management (0-60D).

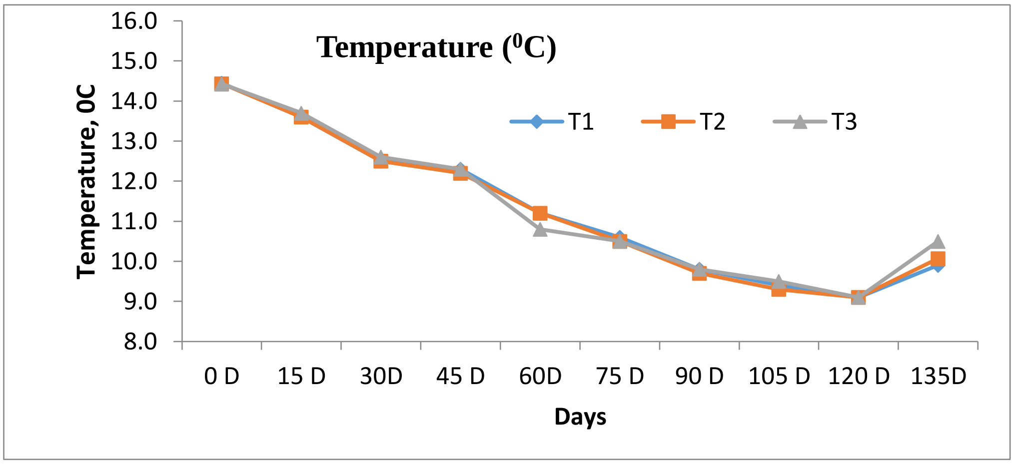

Figure 1. Temperature variation in different treatments during study, T_1= Shrimp based (Control, CON); T_2= Shrimp +5% sisnu (SSN) and T_3= Shrimp + 5% marigold (SMG).

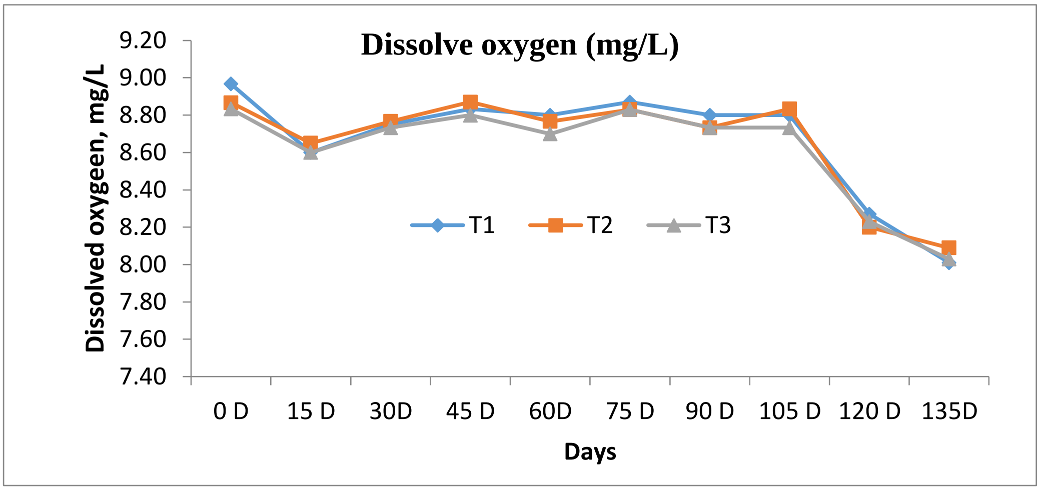

Figure 2. Dissolve oxygen (mg.L) variation in different treatments during study, T_1= Shrimp based (Control, CON); T_2= Shrimp +5% sisnu (SSN) and T_3= Shrimp + 5% marigold (SMG).

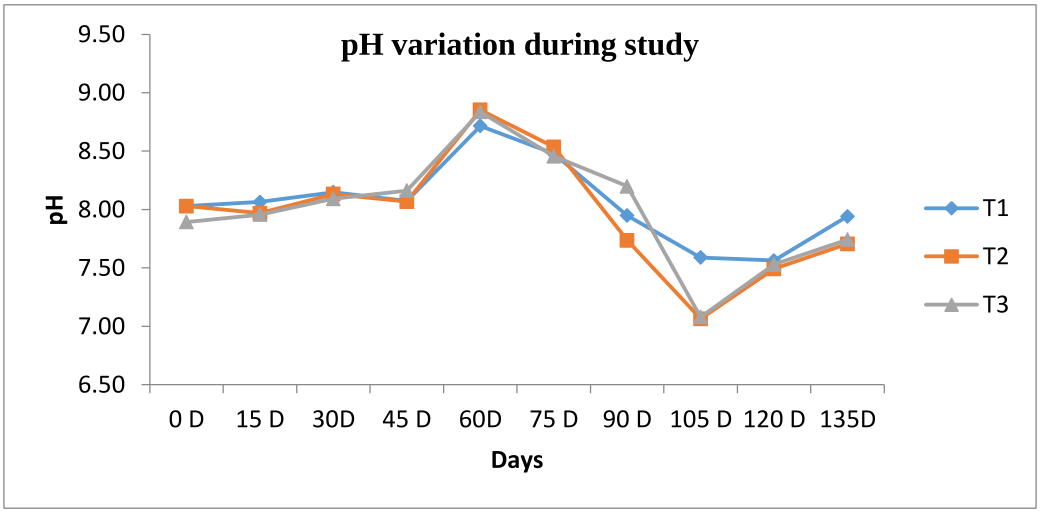

Figure 3. pH variation in different treatments during study, T1= Shrimp based (Control, CON); T2= Shrimp +5% sisnu (SSN) and T3= Shrimp + 5% marigold (SMG).

Plasma cortisol Concentration (ng/ml)

The increase in plasma cortisol in spent broods (Table 3) just after fertilization shows reduction in broods welfare or increase in stress sensitivity in current practice of brood management. The significant lower values of plasma cortisol in treatment groups fed with sisnu supplemented diet T_2 ($<0.18\pm0.04^a$) shows decease in induced stress level by dietary supplementation of *U. parviflora* due to suboptimal rearing conditions or handling stress during breeding activities(Pickering & Pottinger, 1990).

Table 3. Plasma cortical Concentration, ng/ml (±SEM) of spent trout broods during experimental period.

S.N.	Treatments	Concentration (ng/ml)
1	Control	0.786 ± 0.22^c
2	5% Sisnu	less than 0.18 ± 0.04^a
3	5% marigold petal	0.36 ± 0.05^b

Conclusion

The present study showed promising indications towards dietary inclusion rate of stinging nettle (*U. parviflora*) on immune stress responses and reproductive performance of rainbow trout and possibly contributed enhancement in trout broods welfare. The preliminary results demonstrated that reproduction performances of rainbow trout were significantly higher in fish fed with 5% sisnu containing feed compared to fish fed with shrimp based control feed and 5% marigold petal containing feed, However, further studies for longer duration is required to optimize inclusions level of those feed additives without any reduction in breeding performance as well as post-spawning effect i.e. growth and survival of rainbow trout fries. In the present study, marigold petal meal supplemented diet did not improve breeding performance of rainbow trout. Further studies will be needed to determine the causes of low immuno-stimulatory response and breeding performance of marigold based diet.

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