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STATUS AND DEVELOPMENT TREND OF AQUACULTURE AND FISHERIES IN NEPAL

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ABSTRACT
Nepal's fisheries sector, deeply rooted in historical practices, constitutes crucial component of the nation's natural wealth, buoyed by its diverse geographical features and ample water resources. While traditional capture fisheries have historically played a significant role in fish production and employment, maintaining production levels presents challenges necessitating thorough reassessment. The landlocked geography compels a focus on inland finfish farming in the aquaculture subsector, supported by governmental initiatives such as the Fish Mission and Prime Minister Agriculture Modernization Project. The Terai belt, with its favorable climate, serves as a pivotal area for fish production, covering 89% of the total pond area. Nepal's total fish production stands at 113,736 mt, with capture fisheries contributing 18% and aquaculture 82%, collectively employing around 0.52 million individuals. Fish consumption in Nepal is on the rise, with per capita availability increasing from 330 g to 3.92 kg between 1981/82 and 2022/23. Efforts from both the government and private sector aim to commercialize the sector, addressing issues of food security, poverty reduction, and creation of income and employment opportunities. Despite challenges, targeted endeavors through governmental initiatives, private sector involvement, and sustainable practices are vital to unlocking the full economic, employment, and food security potential of fisheries sector.

Key words: Productivity, Production, Capture fishery, Aquaculture, Fish seed

INTRODUCTION
Nepal boasts rich geographical diversity and abundant water resources, making it a land of significant natural wealth. In addition to its diverse geography, Nepal boasts a substantial biodiversity profile, particularly in the realm of fish, harboring a total of 252 fish species (Shrestha 2019), constituting approximately 1.6% of the global freshwater aquaculture diversity. Within this diverse array, Nepal is home to 16 endemic fish species. Concurrently, the inhabitants of Nepal have an enduring engagement in capture fisheries, a historical practice deeply rooted in ancient times. This historical significance of fish in Nepal's diverse cultures and traditions is substantiated by archeological evidence and documentation, which underscore the presence of fishing gear and the cohabitation of indigenous communities (such as Majhi, Malah, Bote, Danuwar, etc.) in proximity to natural water bodies (Adhikari and Thapa 2016). Despite the historical significance of fish in Nepal, the modern era of fish farming commenced with the establishment of the Fisheries section under the Agriculture Council in 1956 AD.

Aquaculture has emerged as a rapidly advancing agricultural subsector in Nepal, driven by its landlocked geography, necessitating exclusive reliance on inland finfish farming. The diverse climatic conditions in Nepal allow for the cultivation of both warm and cold-water species, including indigenous and exotic carps, pangasius, tilapia, catfish, and rainbow trout. Despite the slow progress in institutional aquaculture development over the past seven decades, the sector witnessed significant growth in the previous decade (Kunwar and Adhikari 2017). Government initiatives such as the Fish Mission, One Village One Product, resource center establishment, and the Prime Minister Agriculture

Modernization Project (PMAMP) played a crucial role in this advancement (Chaudhary and Jha 2018).The Terai belt of Nepal holds a prominent position in fish production, accounting for 88% of the overall pond area(Zhuang and Ghimire 2017). The leading districts in terms of fish production are Bara, Dhanusha, Saptari, Rupandehi, Siraha, Morang, Parsa, Rautahat, Sarlahi, and Chitwan (CFPCC 2019).

The primary objective of Nepal's fisheries development program is to enhance the production and productivity of fish by utilizing the diverse water resources available in the country. The program aims to commercialize the fisheries sector, contributing to food and nutrition security, poverty reduction, and increased income and employment opportunities. Private sector involvement, along with technical support for the conservation of fishery resources and the development of an eco-friendly fisheries sector, is crucial for the success of these initiatives. These programs operate within the framework defined by the Constitution of Nepal, which delegates responsibilities to local, provincial, and federal levels. There exist diverse institutional frameworks dedicated to advancing fisheries. The Central Fisheries Promotion and Conservation Center (CFPCC), along with its three subordinate offices under the central government, each equipped with specific mandates play a pivotal role in this regard. Additionally, fish development centers, Veterinary Hospitals, and Animal Service Expert Centers operate under provincial government jurisdiction, while livestock section under local government, contribute significantly to the execution of fisheries development programs. Fishery extension is mostly carried out by local government. Development of fish market and large investment is done by provincial government and Central government mostly works on policy, quarantine issue and is responsible for coordination with national and international organization. To bolster research initiatives in fisheries development, institutions like the Nepal Agricultural Research Council are actively engaged in overseeing various fisheries research activities. Furthermore, the human resources involved in fisheries activities are nurtured and developed through academic institutions such as the Agriculture and Forestry University (AFU) and Tribhuvan University (TU). These universities play a key role in shaping the skill sets and knowledge base of individuals contributing to the fisheries sector in Nepal.

While fish consumption in Nepal lags behind poultry, pork, buffalo, and mutton, a growing awareness of the health benefits associated with fish consumption has fueled increased demand for aquaculture products (Rijal and Jha 2020). The government actively supports the establishment of commercial fish farms to boost employment and income in rural areas. Notably, many of these newly established farms are managed by returning youths with experience abroad, contributing to a reduction in youth migration and aligning with the broader goal of promoting economic development and sustainability in Nepal's aquaculture sector (Rijal and Jha 2020).

PRODUCTION AND PRODUCTIVITY TREND

Capture Fisheries

Nepal is rich in natural water resources, with rivers, lakes, reservoirs, swamps, and lowland irrigated paddy fields standing out as significant sources of fresh water (Figure 1). Rivers and lowland irrigated paddy fields emerge as the primary and most influential natural water resources. Moreover, lakes marginal swamps and reservoirs cover a smaller water surface area compared to other natural water resources. In addition to these water bodies, there are extensive network of 7,900 kilometers of irrigation canals throughout the country (Gurung2014).

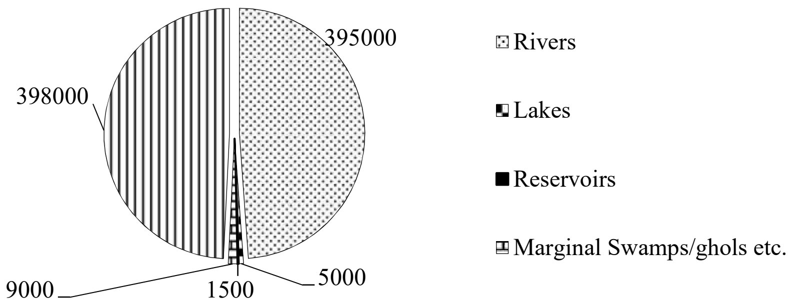

Figure 1: Natural water resources (ha) in Nepal

The capture fisheries sector is vital for its dual role in fish production and employment generation. Fish production from capture fisheries has remained stable at 21,000 tons over recent years. Noteworthy contributors to capture fish production include irrigated paddy fields, rivers, and swamps, while lakes and reservoirs play a minimal role (Figure 2). The preeminent contributors to the natural water area in Nepal are rivers and lowland irrigated paddy fields, collectively constituting 98% of the total natural water surface. Despite their expansive coverage, these areas demonstrate comparatively low productivity, yielding 18 kg of fish per hectare. In contrast, lakes and reservoirs, encompassing a smaller portion of the natural water landscape, exhibit higher productivity at 200 kg and 350 kg per hectare, respectively. Notably, marginal swamps, constituting a mere 1.1% of the total natural water body, stand out for their remarkably elevated productivity, producing 577 kg of fish per hectare. This datahighlights the complex interplay between the extent of natural water areas and their corresponding productivity levels, providing valuable insights for fisheries management strategies in Nepal.

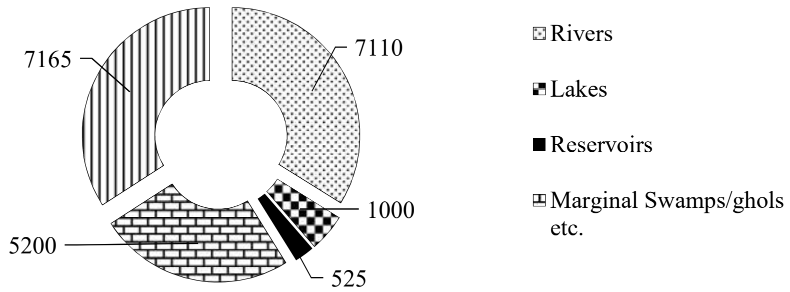

Figure 2: Fish capture (mt) from diverse natural aquatic sources.

While capture fisheries initially exhibited an increasing trend, it has remained almost constant since the year 2000, posing a significant challenge to maintain this capture at a standstill. Therefore, there is a need for a comprehensive reevaluation of the status and potential of capture fisheries, including an assessment of freshwater snail, crab, shrimp, water chestnut, and makhana collection and yield. It is also essential to estimate the communities and population that consume these products, along with assessing the farming perspectives of these fisheries commodities.

Aquaculture

Pond fish culture emerges as the predominant practice among various fish culture methods, experiencing rapid growth while other aquaculture activities have largely remained stagnant over the past decades. Since the inception of aquaculture development in Nepal, finfish culture has been a major focus, with a significant portion dedicated to major carps, particularly common carp and

Chinese carps, utilized in carp poly-culture. This approach has made substantial contributions to aquaculture production in Nepal. The introduction of new technologies and species has seemingly led to an increase in aquaculture production in the country.

The introduction of rainbow trout has brought a fresh perspective to cold-water aquaculture, marking a paradigm shift in Nepal's aquaculture landscape. Similarly, the introduction of tilapia and pangasius has opened up new avenues for promoting monoculture systems, offering significant production potential for aquaculture in Nepal. The innovation of the 'chhadi fish' pond aquaculture technology has proven to be a lucrative investment for fish farmers, enabling faster returns and increasing national production. In this technology, fry/hatchlings are densely stocked in ponds, and multiple harvests (at least 3-4 times a year) is done to capture fish that have reached sizes less than 50 gcontributing 35% of total fish production (Pathak et al. 2023). Local consumers show a preference for smaller, single-piece fish from head to tail, referred to as 'chhadi' fish (Rijal and Jha 2020). The national fish production has witnessed a remarkable increase, surging more than four fold over the last 20 years, from 17,665 mt in 2002/03 to 97,385 mt in 2021/22 (Figure 3).

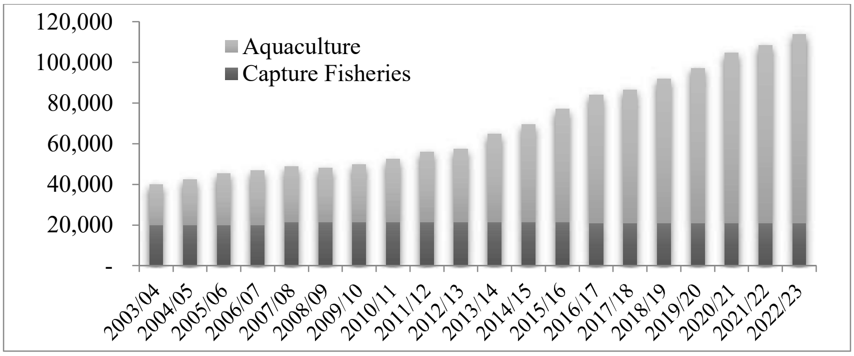

Figure 3: Fish production trend capture fisheries and aquaculture.

The state of aquaculture in Nepal is currently in a phase of growth, and although the fish production levels are comparatively lower than those of larger countries worldwide, recent advancements in the sector are highly promising. Pond aquaculture, particularly with common carps, Chinese, and Indigenous Major Carps, significantly dominates the overall fish production, boasting an average productivity of 5.41mt per hectare. Monoculture of common carp, tilapia, and especially catfish is also practiced in various locations across the country. The interest in aquaculture is growing swiftly among young farmers, reaching 55 districts out of 75 in 2017, a significant jump from 30 districts a decade ago (Chaudhary and Jha 2018). Remarkably, fish farming has expanded to 76 districts out of 77 in the current scenario. This surge in interest is especially notable following the successful implementation of rainbow trout farming technologies in the colder regions of hills and mountains.

Currently, the poly-culture technology of carp fish farming in ponds has been widely disseminated in the southern plain areas and mid-hill regions of the country, emerging as a viable and common aquaculture activity. However, monoculture of pangasius and tilapia are also gaining popularity contributing about 10% of fish production from pond. Pond culture alone contributed to 72.23% (82,161 mt) of the total fish production in 2022/23 (CFPCC 2022/23) (Table 1).

Following pond aquaculture, swamps represent the second-largest contributor to fish production, encompassing an area of 3,670 ha and yielding 9,125 mt of fish in 2022/23. These swamps are predominantly concentrated in the mid-western and far-western Terai region of Nepal. To enhance the productivity of these swamps for various purposes, their restoration, maintenance, and management are imperative for the sustainability of natural resources and the well-being of marginalized communities relying on them for food, nutrition, livelihood, and employment opportunities.

Cage culture practices in lakes and reservoirs contributed approximately 370 mt of fish in the fiscal year 2022/23. The introduction of cage fish culture technology in Nepal dates back to 1972 in Lake Phewa, initially used for raising brood fish of common carp. Presently, the estimated fish culture coverage area has reached nearly 71,205 m^3 with an average fish productivity of 5.19 kg/m^3. Cage fish cultivation primarily involves plankton-feeding fish, relying on naturally available phytoplankton, zooplankton, detritus, and some aquatic vegetation for growth. While external feed is generally not applied, there is a possibility that this practice may evolve in the future due to the potentially increased profitability of adopting feeding practices in cages.

Rice cum fish culture, a successful farming technique in countries like India, Indonesia, China, and Bangladesh, has gained limited attention in Nepal.The extent of land dedicated to rice cum fish farming has experienced a substantial reduction, declining from 300 ha in the fiscal year 2007/08 to a mere 49 ha in 2022/23, accompanied by a decline in productivity as well. The notable reduction in area can be attributed to limited availability of fish seed in mid hills and increased pesticide usage in rice fields. However, to promote this integrated farming system, especially in the Terai and lower mid-hill regions, special long-term projects should be prioritized in the future.

The introduction of rainbow trout, a cold-water species, began in 1969 from India and later from England and Japan in 1988 (Rai 2010). After the successful breeding program of trout commercial trout farming initiated in Rasuwa and Nuwakot districts under collaborative efforts of Nepal Agricultural Research Council, Japan International Cooperative Agency, and the Directorate of Fisheries Development Program contributing a significant growth of trout farming in Nepal. Then, trout has become a distinctive and valuable fish species in the Nepali market, known for its taste and high nutritional value. The production of trout has gained popularity and witnessed a substantial increase, reaching 1007 mt tons in the fiscal year 2022/23. This is a remarkable surge from a mere 192 mt a decade ago. Furthermore, integrating trout farms with restaurants has become a successful practice, particularly beneficial for small-scale farmers to sustain their businesses.

Table 1: Status of aquaculture production in 2022/23 (CFPCC 2022/23)

Particulars	Pond (Nos.)	Total Area (ha)	Fish Production (mt)	Productivity kg/ha
1. Aquaculture production			**92,736**	
1.1 Pond culture	49,862	14,745	82,161	5,572
1.1.1 Mountain	670	48.96	82	1,674
1.1.2 Hill	8,874	1,452.85	6,787	4,671
1.1.3 Terai	40,318	12,635.2	75,292	5,958
1.2 Marginal Swamps (ghols)		3,670	9,125	2,486
1.3 Rice cum fish culture		49	17	346
1.4 Cage culture (m^3)		71,205	370	
1.5 Enclosure (Pen) culture		35	48	1,371
1.6 Trout culture in Raceway		6.8	1,007	148,088
1.7 Fish Production in Public Sector			8	
2. Capture Fisheries production			**21,000**	
3. Total Fish Production			**113,736**	

The productivity of pond fish was only 0.8 mt/ha in 1981/82 but has seen a significant increase, reaching 5.57 mt/ha by 2022/23. This notable increase in pond fish productivity is attributed by various factors, including improved availability of fry, effective fertilization practices, strategic feeding regimes, and enhanced management practices such as the introduction of aeration technology, effective control measures for fish diseases, comprehensive training programs, and adherence to Good Management Practices (GMP). Furthermore, the Government of Nepal consistently underscores the importance use of improved technology, advocating for the utilization of pellet machines to produce cost-effective, high-quality feed on farms and the incorporation of aerators to enhance water quality. These measures are intended to achieve higher productivity in pond fish culture.

The prevalent fish culture technique, carp poly-culture, is predominantly characterized by the dominance of Chinese carps, Indian carps, and common carps in production. Within this context, silver carp holds the highest contribution to production, accounting for 20% of the total, followed by common carp at 18% and naini at 16%. In contrast, rainbow trout and tilapia exhibit minimal contributions, each making up around 10 of the overall production.

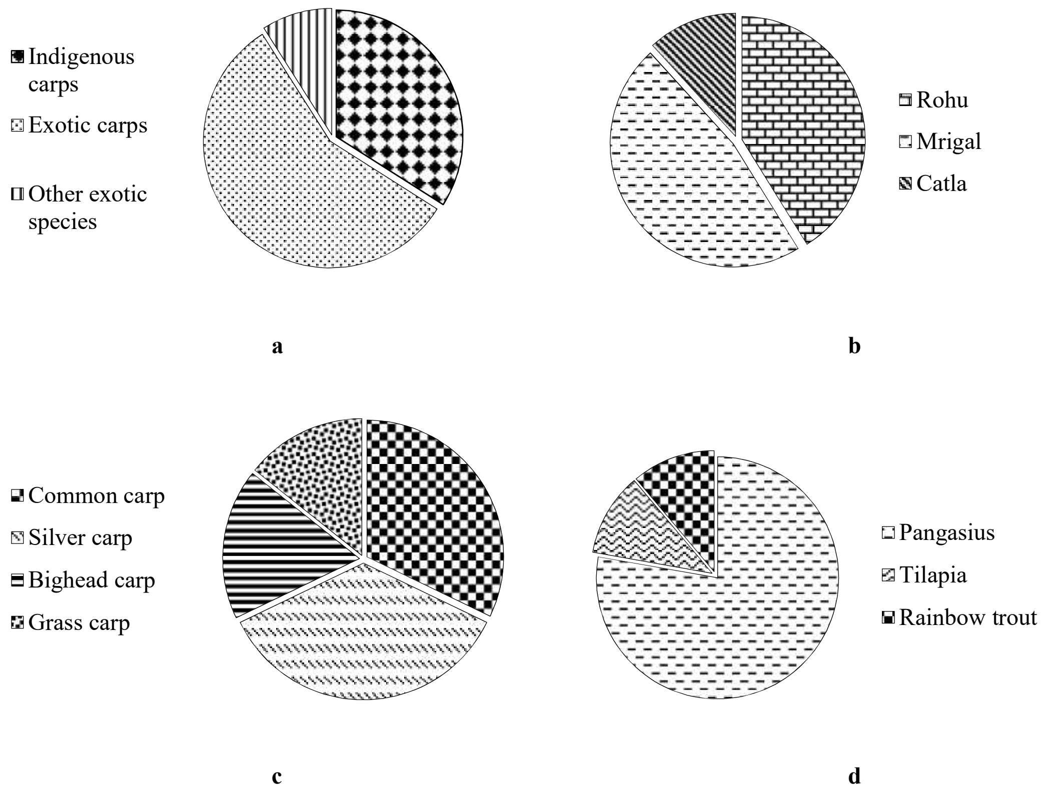

Figure 4: Species wise contribution in aquaculture production (**a.** Group wise contribution; **b.** Contribution of indigenous carps; **c.** Contribution of exotic carps; **d.** Contribution of other exotic species)

FISH SEED PRODUCTION

Seed is a critical input for aquaculture production, and ensuring the quality of seed is essential for enhancing the productivity of aqua farms (Kunwar and Adhikari 2017). In Nepal, fish seed is distributed in three forms: hatchlings (4-5 days old), fry (2-3 cm or more than 1 g), and fingerlings (more than 5 g body weight on average).

Table 2: Status of fish seed production in 2021/22 (CFPCC 2021/22)

A. Fish seed(Fry) Production/Distribution (No. in '000)	569,070
A[1] Public Sector	131,239
a. Hatchling*	335,600
b. Fry	17,158
c. Fingerling	16,869
A[2] Private Sector (Fry)	437,831

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

In the context of Nepal, while self-sufficiency has been attained in the production of fish seed of carp species and rainbow trout, there persists a requirement for importing (around 90% of total demand) of pangasius fingerling to sustain pangasius production. The fish seed sector in the country involves the participation of seven government institution and five research centers, 99 private hatcheries and 232 nurseries. The aggregate fish seed production in Nepal has reached 569 million, with private sector contributing around 77%, and public sector contributing the remaining 23%.

Over the past decade, seed supply by both private and public sector has experienced a substantial increase producing 5.7 million in 2001/02 to 569 million in 2022/23 (Table 3). This growth can be attributed to the government's emphasis on encouraging private sector participation in seed supply. Various supportive programs have been initiated to empower the private sector, including the establishment of fish seed resource centers under private ownership.

AQUACULTURE EXPANSION

The aquaculture sector in Nepal has evolved into a lucrative sub-sector of agriculture, providing favorable returns on investment. In the Terai region of Nepal, the consumer base for fish is expanding, driven by increased availability in the market. Aquaculture practices in the country encompass diverse areas, including pond culture, rainbow trout culture in raceways, rice-fish farming, fish culture in swamps and enclosure, cage culture with innovative and modern techniques like aquaponic, re-circulating aquaculture.

Despite the diverse array of aquaculture practices, pond culture has gained the highest popularity, witnessing substantial growth from 6,093 ha in 2003/04 to 14,756 ha in 2022/23.This is closely followed by trout culture in raceways and cage culture, while rice-fish farming and enclosure fish farming are on a declining trend. Initially, the adoption of pond aquaculture was limited due to high initial investments and insufficient technologies and expertise, coupled with infrastructural challenges. However, the scenario changed with the initiation of a government subsidy program for pond construction, leading to a notable increase in the popularity of pond fish farming.

The Government of Nepal has continued its support for aquaculture businesses through various subsidy programs, contributing to the widespread adoption of pond fish aquaculture. The number and water surface area of fish cultivating ponds have seen a marked increase (Figure 4). The fiscal year 2015/16 witnessed the highest achievement in pond construction, reaching 734 ha (CFPCC 2017).

While pond fish culture remains dominant in the Terai belt, its expansion into hill regions has gained momentum, particularly after the government implemented a pond expansion program in mid-hill districts starting from the fiscal year 2011/12.

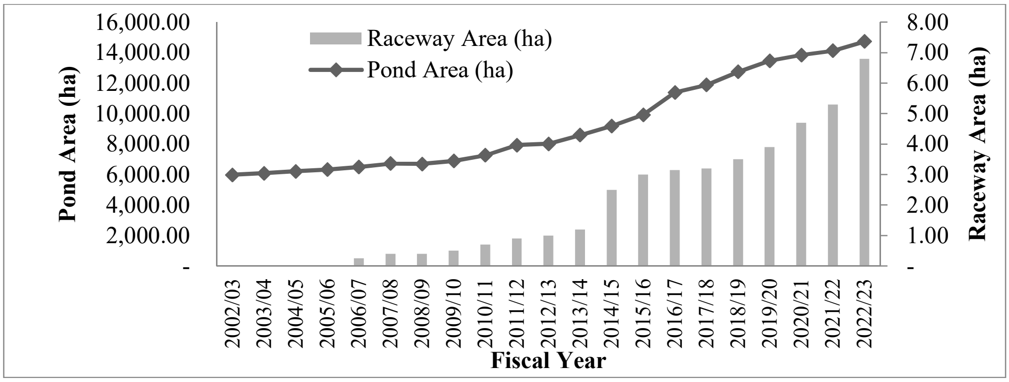

Figure 5: Pond and raceway area expansion trend.

The expansion of areas was primarily concentrated in the Terai region of Nepal, attributed to its favorable climate for warm water fish culture. Conversely, significant growth in raceway areas was observed in the middle hills of the central part of the country. Considering that the Terai region was a major production area, Madhesh province exhibits the highest number of ponds and water areas, followed by the Lumbini province. In contrast, Karnali Province demonstrates the least contribution to the aquaculture sector, contributing less than 1% in both pond area and raceways.

Table 3: Contribution of different province in pond water surface area.

S.N.	Province	Pond Water Surface Area (ha)
1.	Koshi	2,019
2.	Madesh	7,745
3.	Bagmati	1,263
4.	Gandaki	391
5.	Lumbini	2,866
6.	Karnali	32
7.	Sudur Paschim	429
	Total	14,745

ECONOMIC CONTRIBUTIONAND EMPLOYMENT GENERATION

In Nepal, the pervasive issue of unemployment, driving a significant annual migration of youths in search of jobs, poses a serious challenge and heavy reliance on remittance in the Nepali economy makes it vulnerable and unstable (ILO 2015). To address the outmigration problem and foster national development, the expansion of aquaculture emerges as a viable option to create jobs domestically and attract young talent within the nation. Given this context, the fisheries sub-sector holds potential as an alternative, offering employment opportunities in various fisheries and aquaculture-related activities.

The combined efforts of capture fisheries and aquaculture, including related activities such as harvesting, processing, marketing, and other industries associated with aquaculture, are generating substantial employment opportunities for a significant portion of the population. Currently, the aquaculture and fisheries sector contribute 0.44% to GDP and 1.83% to AGDP. Notably, the sector's contribution to the economy is on a consistent upward trajectory.

Capture fisheries contribution in employment generation

Natural water bodies, particularly rivers, swamps, and lakes, play a vital role in sustaining the economy of numerous fishing communities. Approximately twelve distinct ethnic communitiesare directly or indirectly involved in fisheries (Gurung 2005). These communities reside in close proximity to water resources, relying on fisheries and aquatic resources for their livelihoods across generations. Engaging around 362,000 individuals in capture fisheries (CFPCC 2023) with 60% being female, these communities actively participate in various aspects of the fishing industry (Rijal and Jha 2020). Female members contribute not only to the fishing activities but also play roles in the preparation of fishing gears, nets, and other equipment, as well as in the selling of fish in the market.While capture fisheries have been a traditional source of livelihood for many, the number of individuals relying on natural water bodies for their livelihoods through capture fisheries is gradually diminishing. This decline is attributed to the comparatively low income generated from capture fisheries, prompting individuals to seek alternative and more lucrative income-generating opportunities.

Aquaculture contribution in employment generation

Over the past few decades, aquaculture has evolved into a more intensive and diversified practice, offering faster returns on investment. Consequently, there has been a substantial increase in the participation of individuals in the aquaculture business, and the number of households engaged in the aquaculture sector has witnessed significant growth. In the last 20 years, these figures have doubled, with the number of people involved reaching 158,000, and the number of households engaged in aquaculture rising to 62,000. In the realm of aquaculture and related businesses, there is a predominant male presence, making up 68% of the workforce (Rijal and Jha 2020). This stands in contrast to the opposite scenario observed in capture fisheries.

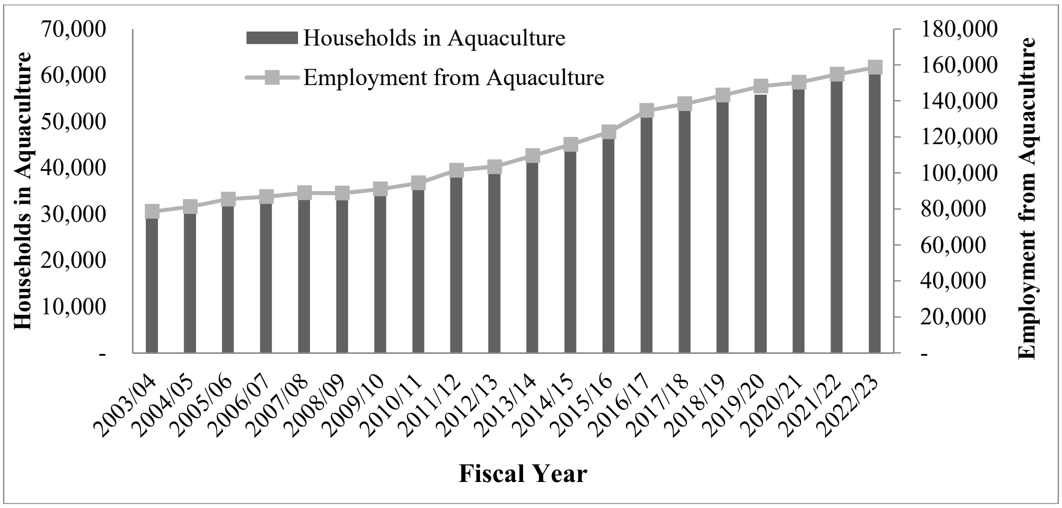

Figure 6: Trend of employment (no) from aquaculture and household (no) in aquaculture.

FISH MARKET AND MARKETING SYSTEM

Fish marketing system in Nepal is not well developed andmarketing strategies exhibit variability across regions, lacking a singular approach. The various functions or services within fish marketing encompass collecting small quantities from numerous producers, grading, packing, transporting to distant city-based wholesale markets, and distributing to retailers. Small produce is directly dispatch to local markets by farmers themselves andfor substantial productions, contractors typically handle marketing activities. Farmer organizations often operate through cooperatives, exemplified by the success of Harpan Phewa Matsya Sahakari in Kaski district and similar cooperatives in Nawalparasi, Rupandehi, and Kanchanpur districts (KBNPK 2010).

A recent trend in the emergence of live fish marketing systems is observed, reflected in the increasing number of live fish shops. The government supports this by providing financial assistance to establish fish marketing stalls and collection centers. Concentrated mainly in the capital and other major cities where demand is high, there are currently stalls in Nepal. Over the last decade, the prices of agricultural commodities, including fish, have seen a substantial increase. In 2001/02, fresh fish was priced at NRs 100 per kg, and was 300 per kg in 2018 (Chaudhary and Jha 2020) which has now reached an average of NRs 350 per kg. Despite the rise in price, fish remains a more affordable source of animal protein for lower and middle-class citizens compared to other meat products.

Fish prices vary across locations, with metropolitan and capital cities experiencing higher costs. The demand for fish fluctuates monthly, with a study revealing increased fish demand during winter and lower consumption reported in Asadh, Shrawan, and Bhadra (KBNPK 2010). While national production falls short of meeting the entire demand, Nepal imports a certain quantity of fish. India stands as the major fish exporter, followed by Vietnam, China, and Bangladesh, contributing to fish imports in Nepal.

Table 4: Price of fish in Kalimati market (Source: Kalimati Fruit and Vegetable Market Development Board. November 13, 2023)

Fish Species	Minimum Price	Maximum Price	Average Price
Fresh Rohu	360	370	365
Fresh Pangasius	260	270	265
Fresh Chhadi	240	280	260

Table 5: Price of farm gate and consumer price in Chitwan (Source: Fishermen's Association Nepal, Chitwan. November 13, 2023)

Fish Species	Farm gate price	Consumers price in market
Silver carp, Tilapia	300	400
Other carps	350	450
Pangasius, Chhadi	280	350-400

Most fish markets in Nepal portray local, unregistered, and unmanaged characteristics, frequently lacking adequate sanitary maintenance.However, in urban areas, a few well-managed markets stand out. The principal challenges faced by fish markets in Nepal include issues related to the preservation of fish quality, inadequate waste management practices, lack of market accessories like collection center, chilling center, road connectivity to the production site and the use of unscientific and unsophisticated methods for fish transportation, and a limited emphasis on product diversification and value addition.

Post-harvest management
Post-harvest loss in the fish industry pertains to the disposal or sale of fish at a reduced value due to either a decline in quality or market-related factors (Dhakal et al. 2020).Fish, being a highly perishable agricultural commodity, should be preserved promptly following harvest.However, Nepal is at an early stage in post-harvest management and most of the harvested fish are sold within a day in local market witha significantly low amount of post-harvest loss accounting only 3-5%.

In Nepal, typical post-harvest management practices include grading of fish based on size and species at the harvest site. These graded fish are then transferred to local markets by local vendors. For those fish destined for distant markets, middleman collect them at a centralized collection center. Subsequently, the fish are graded and packed in styrofoam with a 2:1 ratio of fish to ice. Various types of vehicles are employed to transport the packaged fish to distant markets. Additionally, given the popularity of selling live fish in the country, live fish are collected and transported to distant markets in tanks equipped with aeration facilities.The primary obstacles in post-harvest management include insufficient awareness regarding its significance, a dearth of resources and infrastructure, elevated costs associated with post-harvest management, insufficient training, and gaps in existing policies.

Value addition of fishery products is gaining popularity in Nepal. The primary motivation behind this shift towards value-added products is to enhance the product's price, make it convenient or ready-to-consume, and secure better prices during periods of surplus production. The predominant methods of value addition in Nepal have traditionally been drying and smoking. However, contemporary techniques such as freezing and vacuum packaging, pickling, and filleting are becoming increasingly popular in the current scenario.

Import and export of fish and fishery products
Nepal is at an early stage in post-harvest management, resulting in approximately a 3-5% loss. Typical post-harvest management practices in Nepal involve grading based on fish size and species, followed by preservation in a ratio of 2:1 for fish and ice. Post-harvest loss in fish refers to fish that is either discarded or sold at a relatively low price because of quality deterioration or owing to market dynamics.
The import of fish in Nepal shows decreasing trend, with less than 3% of the total fish consumption in the fiscal year 2022/23 attributed to imports from various countries. This marks a substantial decline from the approximately 20% observed a decade ago. The primary contributors to the import volume are fresh rohu and pangasius, followed by notable quantities of dried fish and pangasius fillets. The import portfolio also encompasses comparatively smaller quantities of species such as salmon, shrimp, scallops, mussels, octopus, and marine arachnids.The export value is notably lower compared to the import figures in Nepal's fishery trade. A substantial portion of the exports is directed towards India, primarily comprising fresh fish sourced from ponds located in close proximity to the border.

Table 6: Import and Export value of fish and fishery product in Nepal (Source: Costume Department)

Fiscal Year	Import (thousand, NRs)	Export (thousand, NRs)
2017/18	1,853,570	1,911
2018/19	1,894,018	1,459
2019/20	1,765,136	334
2020/21	1,698,061	0
2021/22	1,346,580	1
2022/23	1,030,417	449

MAJOR ISSUES OF THE SECTOR

Despite its vast scope and potential, the sector has not experienced the anticipated growth due to various challenges. Issues related to policies, technical aspects, human resources, and organizational matters persist. Essential institutions such as Central and provincial Fish labs, Rainbow Trout and Aquarium Fish Development Centers are notably absent. Additionally, Karnali province lacks a dedicated Fish Research and Development Center.

The absence of a specific Aquaculture and Fisheries Act to regulate the sector is a significant drawback. The current regulation under the Animal Health and Livestock Service Act 2055 does not adequately address the concerns of the fishery sector. The sector also grapples with the unavailability of sufficient and quality inputs such as seeds, feed, and machinery. The lack of a well-established marketing system contributes to high losses and safety issues.

Furthermore, fish farmers face challenges, including high electricity charges and the unmet demand for Krishi Meters in Aquaculture. Access to insurance and concessional loans is limited, and the sector remains highly vulnerable to climate change. Nevertheless, the most pressing challenge remains the scarcity of human resources, as only 312 technicians cater to the sector under central, provincial, and local government jurisdictions. This shortage of personnel makes it difficult for farmers to access technical services, compounded by a low ratio of extension workers to farmers, standing at approximately 1:192.

CONCLUSION AND RECOMMENDATION

Aquaculture stands as a thriving sector within Nepal's food industry, boasting an annual growth rate of approximately 10% in recent decade, the highest among SAARC nations. Recognizing its significance and potential, both federal and provincial governments are increasingly directing attention towards aquaculture. This focus suggests a potential substantial increase in fish production within the country. The escalating demand for fish has created market opportunities, attracting investments in commercial fish farms. However, newly established farms require technical support to enhance competitiveness in local, regional, and global markets.

The current state of technical expertise among human resources in aquaculture is insufficient to represent the advancements of the 21st century due to limited exposure to study and training programs. Specialized hands-on training and studies in fields such as fish breeding, disease management, nutrition, genetics, and water quality are essential aspects that need attention from relevant authorities in the near future. Establishing a robust coordination mechanism among development, research, and educational institutions is imperative for effective and efficient implementation of aquaculture and fisheries programs.

Pond aquaculture currently dominates the fish farming landscape and is a prioritized practice. However, marginal swamps, covering an area of 12,500 ha, should not be neglected. Only 30% of these swamps are currently utilized for aquaculture, highlighting the need for proper planning and management to optimize fish production. This will not only generate employment opportunities but also provide income to many landless individuals. Nepal's abundant natural water resources, including lakes, reservoirs, and swamps, make the nation highly potential for culture-based fisheries, an area that is still underutilized for fish production.

The favorable water resources and climatic conditions in Nepal also support cold-water fisheries, particularly in trout farming. Promoting trout culture requires minimizing production costs to attract more farmers in the future, making trout accessible to middle-class consumers. Similarly, for sustainable development of fishery sector, our indigenous fish breed should be conserved and promoted. Fish like Asala, Sahar, Katle having unique taste and features should be promoted for commercial culture developing package of production. For sustainable utilization and promotion of the sector, effective implementation of Fishery Development Policy, 2079 is crucial. All the institution involved in the development of this sector, should plan and implement program according to the objective envisioned by Policy.

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PLANKTON DIVERSITY AS MIRRORED BY SILVER CARP (*Hypophthalmichthys molitrix*) GUT CONTENTS IN RUPA LAKE, POKHARA, NEPAL

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ABSTRACT

A study was conducted in Rupa Lake from February to April of 2023 to investigate plankton diversity and gut content in silver carp (*Hypophthalmichthys molitrix*) in the lake. Samples were collected from 25 locations using a <75 µm mesh plankton net. The samples were preserved in Lugol's solution, and quantitatively analyzed using a Sedgewick Rafter Counting Chamber. Environmental parameters like temperature, pH, dissolved oxygen, and water transparency were examined. Silver carp samples were collected from daily catch of the lake and gut sample was preserved in 70% ethanol. The gut contents were analysed qualitatively using microscope. Altogether 28 genera, 13 belonging to phytoplankton and 15 belonging to zooplankton were recorded in the lake. The phytoplankton belonged to 4 groups with Chlorophyceae, Bacillariophyceae, Cyanophyceae, Zygnematophyceae based on dominance. The zooplankton belonged to 3 groups with Rotifera, Copepoda, Cladocera based on dominance. The highest number of phytoplankton and zooplankton were recorded in the deeper region, and lowest in the outlet area. The dominant phytoplankton in the gut of silver carp was Chlorophyceae, Cyanophyceae and Bacillariophyceae. The dominant zooplankton in the gut of silver carp was Cladocera, Copepoda and Rotifera. Diet of silver carp mostly corresponded with phytoplankton and zooplankton available in the Lake.

Keywords: Silver carp, phytoplankton, zooplankton

INTRODUCTION

A large number and high diversity of plankton including zooplankton and phytoplankton exhibit in water bodies, despite the fact that competitive species require the same types of resources (Hutchinson 1961). Thus, lakes are highly productive environments, characterized by complex food web networks and high levels of diversity (Barbier et al. 1997). Planktons are drifting aquatic organisms along the current in water bodies. They directly or indirectly support fish populations and other various aquatic organisms including nektons. They are of two types: phytoplankton and zooplankton. Phytoplankton are minute, microscopic chlorophyll-bearing organisms (Kushwaha 2012) that live suspended in the water column. One of the major consumers of phytoplankton is zooplankton, which is comprised of animals in the origin of the planktonic community. In the complex food web network, phytoplankton serves as the foundation of the food chain and contributes significantly to the productivity of the water body (Mahaseth 2013). They produces oxygen through photosynthesis, which improves habitat quality for fish growth and production (Gurung et al. 2019). However, sudden excessive growth limits the penetration of sunlight into the deeper regions, inhibiting photosynthesis by submerged and emergent plants (Gurung et al. 2019; Bastakoti and Timilsina 2020).

Phytoplankton standing abundance in aquatic environments represents a balance between production and loss. The loss factor of phytoplankton in lakes is highly associated with predation by several types of grazers (microplankton and metaplankton) including Pisces, silver carp (*Hypophthalmichthys molitrix*). Silver carp are surface feeders, they collect suspended food items from the water mechanically rather than selectively (Spataru and Gophen 1985). They do not breed naturally, but occur in Rupa Lake due to annual stocking by local fishers as they subsist on plankton and grow fast and bigger with no additional feed from outside, instead by consuming planktons, especially phytoplanktons (Gurung 2007). However, some studies have suggested that silver carp may be an opportunistic consumer and may consume small zooplanktons as well (Spataru 1977; Spataru and Gophen 1985; Wu et al. 1997; Smith 1989; Tang et al. 2002). The gill rakers of silver carp are known to be capable and suitable for filtering phytoplankton and zooplankton (Spataru 1977; Spataru and Gophen 1985; Xiao et al. 2010). The silver carp are captured using gill nets almost every day by fishers as food, to sell in the local market. The silver carp fishery has been supporting the local livelihood of hundreds of fishers since last early 1980 in Rupa Lake (Gurung 2007; Chaudhary et al. 2015; FAO 2019; Husen et al. 2019; Pant 2019).

Recent studies have shown that the water quality in Rupa Lake is poor with a Water Quality Index (WQI) score of Grade C (Sahadev et al. 2020). Such changes in water quality affect aquatic life (Biro and Vörös 1982; Verma et al. 2020). Phytoplankton has short carbon turnover rates and is sensitive to water quality conditions (Makarewicz 1993). So, it has been recognized as a significant bio-indicator of water quality, reflecting the pollution levels in aquatic ecosystems, as well as indicating the ecological quality of water through its biomass (Fakioglu 2013; Mahaseth 2016; DC et al. 2022). Since, it is empirically not well examined which plankton are abundantly consumed by silver carp and what could be the possible implication of plankton grazing in subtropical Lake Rupa. Therefore, this study attempted to analyse what types of plankton might be preferred by silver carp (*Hypophthalmichthys molitrix*) and how the plankton consumption might be impacting plankton diversity and lake water quality. The types of phytoplankton and zooplankton consumed has been attempted to be mirrored through examining the gut contents of silver carp in Rupa Lake.

MATERIALS AND METHODS

Study area

The study was conducted in Rupa Lake (28°08'58.61" N and 84°06'39.90" E), located in the Kaski District of Gandaki Province Nepal. It is the third largest lake after Phewa and Begnas of Pokhara Valley which is located at an altitude of 600 masl. The lake has an area of 1.35 km^2, an average depth of 3 m, and a maximum depth of 6 m (Rai et al. 1995). The lake has a catchment area of 28 km^2.

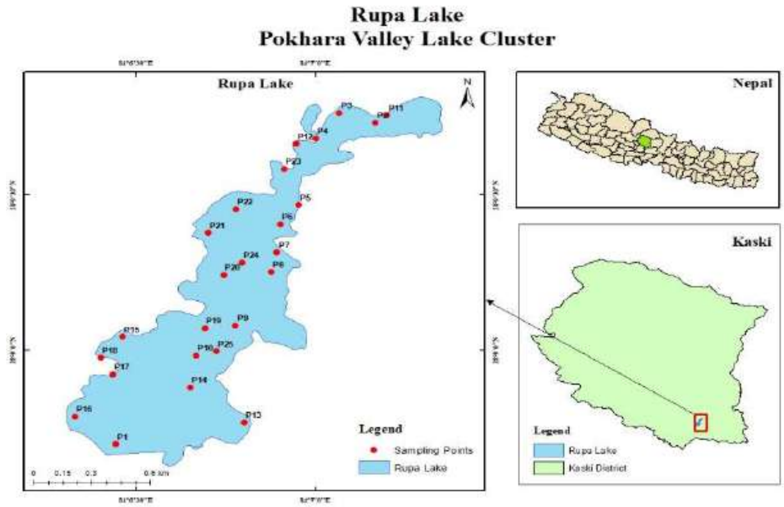

Figure 1: Map showing study area of Rupa Lake, Pokhara, Kaski

Rupa Lake is irregularly populated by several plankton species (Rai 2000). Therefore, five sampling sites (Site 1 to Site 5) were selected strategically including outlet area, settlement area, deeper region, shallow region and inlet area as shown in (Table 1). Total of 25 sampling points (P1 to P25) as shown in (Figure 1) were selected from the periphery of sampling sites, to perform a comprehensive understanding of the phytoplankton distribution and diversity across the different parts of the Lake Rupa.

Table 1 : Sampling location with geographical coordinates and site descriptions

Sample sites	Coordinates	Site descriptions
Site 1	28°08'41.712" N and 84°06'26.676" E	An outlet area with minimal human intervention.
Site 2	28°08'45.924" N and 84°06'48.204" E	The settlement area with the highest level of human activity.
Site 3	28°09'4.68" N and 84°06'46.728" E	The deeper region of the lake near Devithan temple.
Site 4	28°09'39.852" N and 84°06'56.916" E	The shallow region of the lake with barren area.
Site 5	28°09'40.932" N and 84°07'0.12" E	An inlet area characterized by cattle grazing and agriculture practices.

Water quality analysis

Among various water quality parameters, Temperature, pH, Dissolved Oxygen (DO) were measured using multi-parameter probes. Transparency was measured using Secchi disc in different sampling points. The parameters were analysed during the month of February, 2023.

Study of planktons

A plankton sample was collected using a plankton net with a mesh size of <75 µm. The samples were preserved with Lugol's solution upon collection, and brought to the lab for analysis. The sampling was done in the month of February, 2023. The quantitative analysis of plankton was done using Sedgewick Rafter Counting Chamber. The UNESCO phytoplankton manual was referred for counting and freshwater algae key and image based key was used for the identification (Caspers 1980; APHA 1998; Haney 2013). Similarly, the silver carp gut samples were obtained from the daily catch of fishes by RLRFC (Rupa Lake Restoration and Fishery Cooperative) in the month of April, 2023. A dead specimen of silver carp was randomly selected from the entire catch early in the morning, and this process was repeated daily for a week. Then, with a single incision at ventral side in between the pectoral fins at the anterior end, the gut was removed. The foregut (from the anterior part of the oesophagus to the first bend) was sampled (Yu et al. 2019; Tumolo and Flinn 2019). The dataset for the sampled fish was recorded on site (weight of fish, weight of full and empty gastrointestinal tract, and weight of the gut contents). The sample was preserved in 70% ethanol solution, stored in an ice box and carried to lab for examining the contents eaten by the fish. The gut contents were examined qualitatively by recording and identifying the features of remaining body parts, as have been reported in several earlier studies (Yu et al. 2019; Tumolo and Flinn 2019; Rai 2000). Plankton species were observed under 10X and 40X magnifications using OLYMPUS CX 41 microscope. Likewise, the plankton biomass was determined by using Ash Free Dry Weight Method (AFDW) following APHA (1998).

Dry weight, mg/L = [(*A*-*B*) × 1000] / (sample volume, mL)
Where,
A = weight of filter + dried residue, mg
B = weight of filter, mg

AFDW, mg/L = [(*C*-*D*) × 1000] / (sample volume, mL)

Where,
C = weight of filter + residue before ignition, mg
D = weight of filter, mg

RESULTS AND DISCUSSION

Water quality analysis

Some of the water quality parameters measured on various sampling points during February, 2023 has been shown in (Table 2). Water temperature varied between 18.4 ºC to 24.6 ºC during the study period, with high and low temperatures both in the outlet area. Temperature appears to be relatively stable, with only a few sites showing variations of more than 2º C. The highest value of pH i.e. 8.5 was recorded in sampling point (P10) at the settlement area and lowest value of pH i.e. 7.5 in sampling point (P11) at the inlet area. Such difference in the pH may be due to human activities such as agriculture or sewage discharge, which may contain alkaline substances in the settlement area. Moreover, the pH values align with previous studies by Jones (1989) that have noted the neutral to alkaline nature of Nepalese lakes. Similarly, the findings for DO concentrations are consistent with the findings of Gautam et al. (2016) i.e. 4.0 mg/L to 9.8 mg/L. The highest value of DO i.e. 9.5 mg/L was recorded in the deeper region and lowest value of DO i.e. 4.1 mg/L was recorded in the outlet area. The Secchi disc transparency was observed to be lowest in the outlet area i.e. 45 cm and highest in the settlement area i.e. 1.0 meter. The low transparency in the outlet area could be due to the sediments runoff from upstream. The maximum Secchi depth was recorded as 3.5 meter in this study.

Table 2: Mean and range of water quality parameters measured during February 2023 in Rupa Lake

Parameter	Mean ± SD	Range
Temperature (℃)	20.4 ± 1.2	18.4 - 24.6
pH	8.0 ± 0.2	7.5 - 8.5
Dissolved Oxygen (mg/L)	8.4 ± 1.3	4.1 - 9.5
Transparency (cm)	73 ± 13	45 - 100

Phytoplankton composition and distribution

A total of 1161 phytoplankton cells were enumerated, which comprised of 13 genera of 4 groups (i.e. Chlorophyceae, Bacillariophyceae, Cyanophyceae and Zygnematophyceae). The maximum number was counted for *Staurastrum spp.* (262 cells) and a minimum for *Tetraedrongracile* (2 cells). The highest number (88 cells) was recorded in the deeper region (Site 3), whereas a poor number (17 cells) was recorded in the inlet area (Site 5) as shown in (Figure 2).

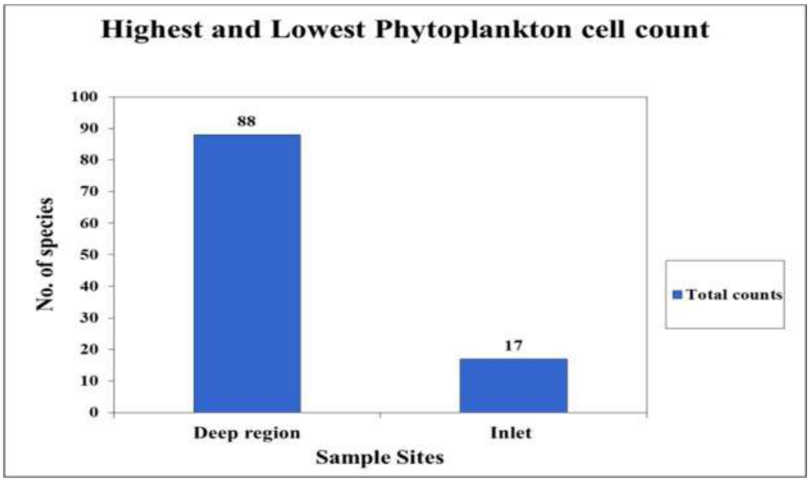

Figure 2: Sampling sites with highest and lowest phytoplankton cell count

The most dominating group was Chlorophyceae (677 cells), consisting of *Staurastrum spp*., *Ankistrodesmus falcatus*, *Pediastrum duplex*, *Scenedesmus spp*., *Sphaerocystis schroeteri* and *Tetraedrongracile* followed by Bacillariophyceae (396 cells), comprising of *Melosira granulate*, *Thalassionema*, *Rhizosolenia erienis*, *Surirella sp*. and *Fragilaria sp*. and by Cyanophyceae (72 cells), comprising of *Merismopedia tennuissima* and by Zygnematophyceae (16 cells), comprising of *Spirogyra sp*. In previous studies, *Tabellaria fenestrate* and *Melosira granulate* were dominant species within Bacillariophyceae (Rai, 2000; Dhakal et al., 2011); whereas *Staurastrum spp*. was within Chlorophyceae dominated throughout this study. Similarly, *Cyclotella spp*., *Melosira granulate*, and *Synedra ulna* were identified as major species by Dhakal et al. (2011), while this study identified *Staurastrum spp*., *Ankistrodesmus falcatus*, and *Pediastrum duplex* as the most dominant species. Also, this study identified *Spirogyra sp.* within Zygnematophyceae as a group of phytoplankton present, whereas it was absent in the previous study by Dhakal et al. (2011). The *Staurastrum spp.* was present during the month of March-April but was not recorded during February by Dhakal et al. (2011). But in this study, *Staurastrum spp*. was present with maximum phytoplankton count (262 cells) in the month of February, 2023. Such an interesting shift in species may be due to environmental variables and seasonal differences in sample collection.

Zooplankton composition and distribution

A total of 424 zooplankton individuals were enumerated in this study, which comprised of 15 genera of 3 groups (i.e. Rotifera, Copepoda and Cladocera). The maximum zooplankton was counted for *Bosmina longirostris* (73 individuals) and a minimum for *Leptodiaptomus tyrrelli* (3 individuals). The highest count (42 individuals) was recorded in the deeper region (Site 3) throughout the study period, whereas the lowest count (3 individuals) recorded in the outlet area (Site 1) as shown in (Figure 3).

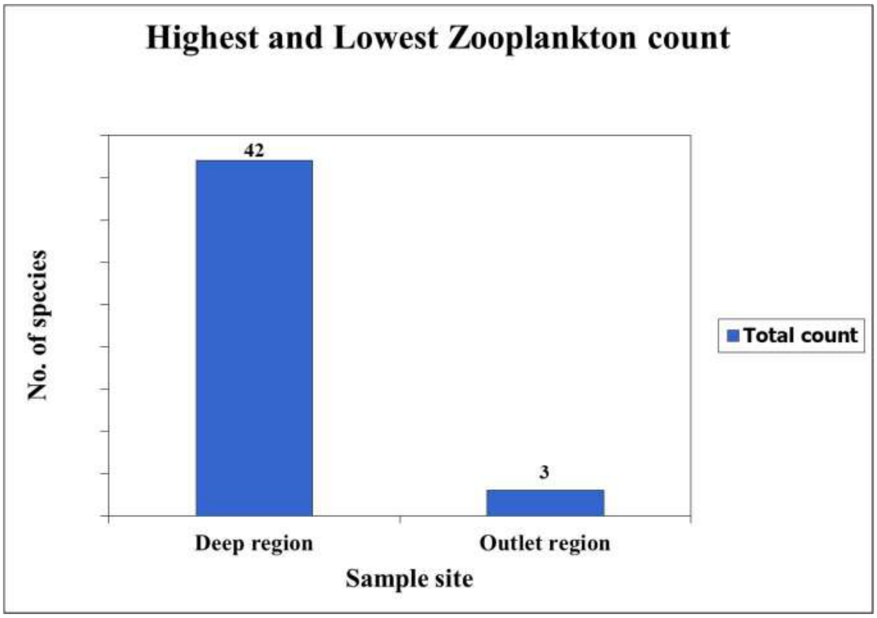

Figure 3: Sampling site with highest and lowest zooplankton count

The most dominating group Rotifera (199 individuals), consisted of *Trichocerca cylindrica, Branchionus havanaensis, Polyarthra trigala, Keratella cohleans, Keratella valga, Keratella tecta,* and *Branchionus angularis* followed by Copepoda (139 individuals), comprising of *Cyclopoid nauplius, Nauplius, Microcyclops rubellus, Mesocyclops leukarti, Leptodiaptomus tyrrelli* and *Cyclops vicinus* and by Cladocera (86 individuals) comprising of *Bosmina longirostris* and *Eubosmina tubicen*. In previous study, Copepoda were the most abundant species in Rupa Lake, followed by Rotifera (Rai 2000). However, Rotifera was most dominant followed by Copepoda including species like: *Keratella cohlearis*, *Polyartha trigala*, and *Nauplius* (Husen et al. 2011; Gautam et al. 2016). In this study, Rotifera were dominant over Copepoda and Cladocera including species like: *Bosmina longirostris*, *Keratella valga*, *Cyclopoid nauplius*, and *Branchionus havanaensis* that were present abundantly. Genus *Branchionus* is linked to eutrophic water, while *Trichocerca* is almost exclusively found in oligotrophic water (Sládeček 1983). So, establishing a *Branchionus*: *Trichocerca* quotient $Q_{B/T}$.

$Q_{B/T}$ = Number of species of *Branchionus* / Number of species of *Trichocerca*
(Oligotrophic if $Q_{B/T} < 1$, Mesotrophic if $\leq Q_{B/T} < 2$, Eutrophic if $Q_{B/T} \geq 2$)

The number of species of *Branchionus* species is 2 (i.e. *Branchionus havanaensis* and *Branchionus angularis*). Similarly, the number of species of *Trichocerca* is 1 (i.e. *Trichocerca cylindrica*). Therefore, the $Q_{B/T}$ quotient is (2/1) which gives 2. So, based on this quotient, the trophic status of Rupa Lake may rank as eutrophic water body. The differences in number of zooplankton species reported by different authors may be due to seasonal differences in sampling and locations.

Biomass of plankton

The mean biomass of plankton in the lake was found to be 0.18 ± 0.27 mg/L (Figure 4). The highest biomass was found to be 1.0 mg/L in sampling point (P25) at settlement area and lowest was found to be 0.02 mg/L in sampling points (P6, P8 P10, P24 and P23) at shallow, deep, settlement and inlet region. From the result it can be inferred that the change in biomass may be associated with several factors, such as predation, food availability, environmental stressors, and the presence of submerged macrophytes etc. (Dembowska et al. 2018; Gurung et al. 2019; Takamura 2003). During the field visit, there was absence of submerged macrophytes in the sampling point (P25) at settlement area and (P18) at outlet area whereas, submerged macrophytes were observed in the sampling point (P6) at shallow region and (P23) at inlet area. According to Takamura (2003) and Gurung et al. (2019), plankton biomass decreases may show poor value if lakes are dominantly covered by submerged macrophytes in lakes. This is because submerged macrophytes provide refugee for zooplankton, which increases their survival and grazing pressure on phytoplankton, ultimately leading to a reduction in biomass. The other reasons explained is that in shallow lakes the rooted vegetation can outcompete phytoplankton in nutrients uptake, as rooted vegetation has the advantages to uptake nutrients directly from sediments (Gurung 2007).

Plankton species mirrored in gut of silver carp

In this study, the major phytoplankton species in the gut of the silver carp were *Pediastrum duplex*, *Ankistrodesmus falcatus*, *Staurastrum sp.* of Chlorophyceae; *Melosira granulate* of Bacillariophyceae and *Merismopedia tennuissima* of Cyanophyceae. Among these species *Pediastrum duplex*, *Ankistrodesmus falcatus*, and *Merismopedia tennuissima* was observed in almost all the gut samples. The dominant phytoplankton group observed in the gut of silver carp was in the order, Chlorophyceae > Cyanophyceae > Bacillariophyceae.

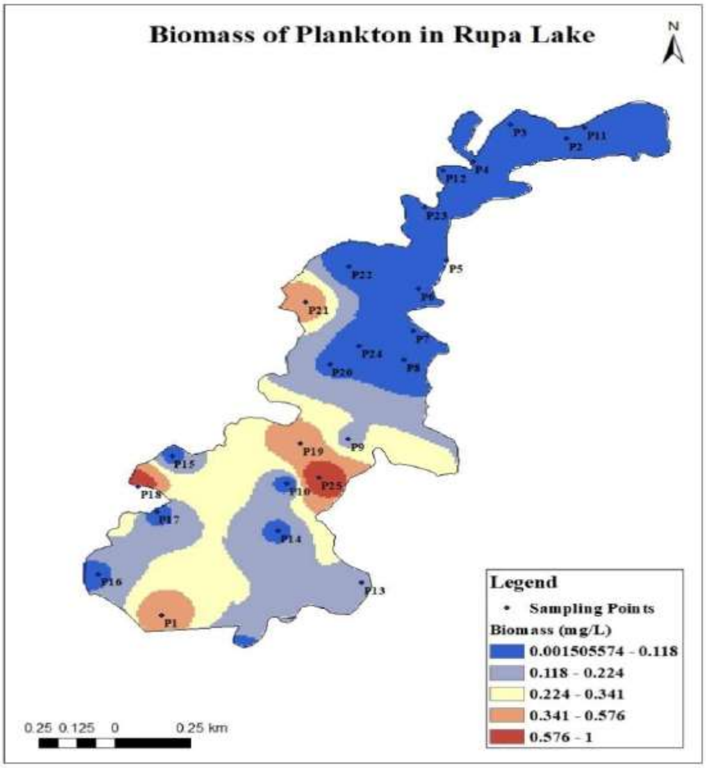

Figure 4: Biomass of plankton in Rupa Lake

The major zooplankton species in the gut contents of the silver carp were *Bosmina longirostris*, *Eubosmina tubicen*, of Cladocera and *Cyclopoida spp.* (*Microcyclops rubellus* and *Cyclops vicinus*) of Copepoda. Among these species *Bosmina longirostris* was found to be dominant throughout the study followed by *Cyclopoida spp.* and *Eubosmina tubicen.* The dominant zooplankton group in the gut of silver carp was in the order, Cladocera > Copepoda > Rotifera.

Pediastrum duplex of (Chlorophyceae) which was dominant in gut was recorded with (82 cells) in the lake whereas; *Staurastrum spp.* of (Chlorophyceae) which was least in gut was recorded with (262 cells) in the lake. So, it may be assumed that the quantity of food available for silver carp in the lake as limited. Also, many of the *Pediastrum duplex* was found undigested in the gut i.e. the species were identified distinctly. We can also say that the phytoplankton fed by silver carp may be compromised in quality due to their poor digestibility. The cause of *Staurastrum sp.* for being least in the gut may be due to better digestibility or least preference by the silver carp because *Staurastrum spp*. was dominant in the lake.

Bosmina longirostris (Cladocera) which was dominant in the gut was recorded with (73 individuals) in the lake whereas; *Cyclopoida spp*. (*Microcyclops rubellus* and *Cyclops vicinus*) of (Copepoda) which was least in the gut was recorded with (30 individuals) in the lake. *Eubosmina tubicen* of (Cladocera) was also consumed dominantly by silver carp, which was recorded with (13 individuals) in the lake. So, the least species of *Eubosmina tubicen* in the lake can be inferred to its high consumption by silver carp.

CONCLUSION

The dominant phytoplankton species were *Staurastrum spp.* of (Chlorophyceae) and zooplankton species was *Bosmina longirostris* of (Cladocera) in the lake. Among phytoplankton species, *Pediastrum duplex* of (Chlorophyceae) was considered dominant and preferred food of silver carp. Among zooplankton species, *Bosmina longirostris* of (Cladocera) was considered dominant and preferred food of silver carp. During the study, similar groups of plankton species were observed in lake as well as in gut of silver carp. Therefore, the gut contents of silver carp (*Hypophthalmichthys molitrix*) corresponded with the plankton in the Lake Rupa. The feeding preference of silver carp corresponded with the plankton diversity in the lake. So, it implies that feeding preference of silver carp directly as well as indirectly impact over the water quality of the lake. As silver carp are harvested every day by RLRFC, the removal helps to remove N, P and organic carbon in the form of phytoplankton and zooplankton from the lake. That means the removal of phytoplankton feeder fish controlling the lake to be overloaded with inorganic nutrients such as N, P and organic carbon in the form of planktons from the lake.

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EFFECTS OF GINGER (*Zingiber officinale*) AND GARLIC (*Allium sativum*) SUPPLEMENTED DIETS ON GROWTH AND FEED UTILIZATION OF RAINBOW TROUT (*Oncorhynchus mykiss*) JUVENILE

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ABSTRACT
Incorporation of herbal additives into fish diets has emerged as a promising strategy to boost growth and bolster immunity in aquaculture. This study aimed to evaluate the impact of two such additives, ginger (*Zingiber officinale*) and garlic (*Allium sativum*), on the growth and feed efficiency of rainbow trout (*Oncorhynchus mykiss*). For this, 3600 trout averaging 3.52 ± 0.15 g, allocated to 15 raceway tanks, housing 240 fish per tank were fed diets containing varying levels of ginger (1 and 2%) and garlic (1 and 2%), alongside a control group with no additives over a 60-days period at 5% of their body weight. Results revealed that the final weight (22.0±0.8g), weight gain (18.3±0.8g), specific growth rate (SGR) (2.97±0.04%/day) and condition factor (2.39±0.36) were significantly higher ($p< 0.05$) in 1% ginger-supplemented groups compared to the control and garlic-supplemented groups. Similarly, fish fed with 2% ginger exhibited significantly higher SGR than control and 1% garlic fed groups. However, there was no significant difference between 2% ginger, 1 and 2% garlic, and control group in terms of weight gain. The survival rates remained consistent across the treatments. Consequently, this study advocates for the inclusion of 1% ginger in rainbow trout diets as a viable, cost-efficient, and eco-friendly means of enhancing growth.

Keywords: Herbal additives, medicinal plants, immunostimulant

INTRODUCTION
Rainbow trout (*Oncorhynchus mykiss*) is a popular species to grow in hilly and mountainous regions of Nepal due to the availability of cold-water resources, which is considered as one of the most important aspects for the successful cultivation of this species (Nepal et al. 2021). At higher altitudes, warm water species have not yet flourished as biologically and economically sustainable ventures in Nepal (Gurung et al. 2017). After comprehensive research and development of rainbow trout farming technological packages suitable for mountainous region, rainbow trout farming offers substantial opportunities for commercial culture in hilly areas of Nepal. In addition, several job and income opportunities have been generated by the promotion of trout farming in the highlands of Nepal. It has been made possible because farmers can now prepare cost-effective feed using locally available ingredients (Voorhees 2011; Mahato et al. 2023). With the advancement in the technology of breeding and fry production coupled with the popularity of rainbow trout among consumers and motivated farmers, rainbow trout farming in the private sector is increasing trend. Moreover, interest in growing rainbow trout is increasing in the hilly parts of the country resulting in a total of 120 farms throughout the country, producing more than 550 metric tons of rainbow trout (NFRC 2021).

The success of rainbow trout farming is related to the high-quality protein feed required by carnivorous fish while lowering the FCR (Barimani et al. 2013) and promoting the immunity of the fish, which otherwise can be severely suppressed at higher stocking density in raceway tanks, a commonly practiced production method for rainbow trout (Nepal et al. 2021). In this context, especially when public perception is changing and attention has been increased towards the farming of the trout, the welfare of the farmed trout is a critical aspect to be considered. A study has shown that fish can have impaired immunity, making them susceptible to bacterial infections even at lower stocking densities when they are fed inadequately (Winfree et al. 1998). Therefore, researchers have constantly focused on the development of a feed strategy that can promote the immune response and growth of rainbow trout, regardless of the stocking density. At higher stocking densities, bacterial infection causing tail and fin rot is one of the major causes of production halts, making this condition an operational and welfare indicator for trout farms (Ellis et al. 2009). Due to the intensive farming of rainbow trout and the common occurrence of bacterial infections in such farms, farmers often resort to indiscriminate antibiotic use without fully understanding the potential consequences such as antibiotic resistance in bacteria, as highlighted by Timalsina et al. (2022). Therefore, to address this issue, the development of functional feed containing plant-based ingredients that serve as growth promoters, immunostimulants, antioxidants, and anti-pathogenic agents should be explored to support the intensive culture of rainbow trout while minimizing antibiotic usage. Plant extract-based functional feed has ameliorated the immune response in Atlantic salmon (*Salmo salar*), thereby improving the health status of the fish (Reyes-Cerpa et al. 2018). Moreover, studies have proved that natural plant-derived products can be used in aquaculture as substitutes for vaccines and synthetic chemicals in order to prevent disease and enhance growth and immune responses due to their richness in bioactive compounds (Citarasu 2010; Reverter et al. 2017). Garlic (*Allium sativum*) and ginger (*Zingiber officinale*) are the most commonly used spices and additives in aquaculture for their nutritional, physiological, and pharmacological properties. Hence, the use of these compounds as growth promoters and immunostimulants in the feed for rainbow trout might be a promising way to improve the performance of rainbow trout.

Garlic (*Allium satium*), a plant that grows as a bulb, is a popular member of Liliace family and has been used for decades as a flavoring agent and traditional medicine to improve physical and mental being of humans (Paulin et al. 2021). Earlier studies have shown that garlic, as a functional feed additive in fish feed, promoted growth and improved antioxidant, immunological, and hematological parameters (Diab et al. 2008; Yılmaz and Ergün 2012). The garlic-supplemented feed, either in aqueous or dried powder form contains several organic sulfur compounds like ajoene, allicin, diallyl disulfide, and S-allylcysteine (Chi et al. 1982). It also contains minerals like calcium and phosphorous, vitamin A, C, and B complexes, linolenic acid, and other important compounds like iodine and silicate, which makes it a suitable candidate for incorporating as a functional additive in fish feed (Chi et al. 1982; Corzomartinez et al. 2007). Similarly, ginger (*Zingiber officinale*), a plant that grows as an underground stem or rhizome, is a widely used food as a spice around the world. It is one of the most studied and common members of the Zingiberaceae family. The biologically active compounds like alkaloids, polyphenols, flavonoids, saponin, carotenoids, and nutrients such as carbohydrates, vitamins, and minerals, along with the potent antioxidant component Zingerone, are highly abundant in this plant (Paulin et al. 2021). Therefore, several earlier studies have successfully incorporated ginger in the diet of rearing fish to promote growth, immune response, digestion, metabolism, and antimicrobial and antiparasitic properties (Mohammadi et al. 2020; Shakya 2015). Although these studies have shown that garlic and ginger can be a potential functional additive for promoting fish growth and overall health, such information on the effect of ginger and garlic in the context of rainbow trout farming in flow-through systems in Nepal is limited. Hence, the aim of the present study was to evaluate the effect of garlic and ginger powders as feed additive on growth performance and feed efficiency of juvenile rainbow trout. Ultimately, the findings of this study can be applicable in intensive rainbow trout culture to ensure food and nutritional security while enhancing the livelihood of the farmers.

MATERIALS AND METHODS

Study location and experimental conditions

The study was conducted at the Fishery Research Station (FRS), Trishuli from August to October 2022. A total of 4000 healthy rainbow trout juveniles were purchased from the private hatchery and were acclimatized in two raceway tanks at FRS for two weeks. Fish were fed a farm-made basal diet with 34.9% crude protein (CP) at a rate of 5% of body weight during acclimation. After the acclimation, 3600 rainbow trout juveniles (3.52±0.15 g) were randomly distributed into 15 nursery raceway tanks (3m × 0.5m × 0.2m) at a stocking density of 240 fish per tank. Fish were randomly divided into 5 experimental groups as follows: fish fed with basal diet without ginger and garlic (control) (T1), fish fed with 1% ginger in the basal diet (T2), fish fed with 1% garlic in the basal diet (T3), fish fed with 2% ginger in the basal diet (T4), and fish fed with 2% garlic in the basal diet (T5). The experiment was performed in triplicate. Fish were fed at 5% body weight twice a day for 60 days. The water source was the Trishuli River, from which the water was first collected in a siltation tank then drawn to the experimental tanks. The water quality parameters, including temperature, pH, and dissolved oxygen, were monitored daily using multimeter probes.

Acquisition of feed ingredients and preparation of experimental diets

All feed ingredients used to prepare the experimental diets were obtained from the local market, including raw ginger and garlic. Raw ginger and garlic were first dried and crushed into powdered form mechanically, followed by sieving with fine mesh to obtain powdered ingredients, which were then stored in an airtight container until the formulation and preparation of the experimental diets. Five isonitrogenous (45% CP) diets were formulated using the Pearson's square method. Feed ingredients used for the preparation of experimental diets were ginger powder, garlic powder, soya full, small shrimp meal (jwala), wheat flour, rice bran, vitamin and mineral premix, and additional materials. The formulation was based on the percentage composition of the ingredients, as shown in Table 1. To prepare the formulated experimental diets, all the dry ingredients in their powder form were weighed and mixed thoroughly, put into the electric feed pelleting machine to make pellets. The pellets were dried for 24 hours and stored in an airtight container at room temperature until used. The proximate composition of experimental diets was determined following the standard method of AOAC (1990). Dry matter was analyzed by drying the sample feed in a hot-air oven at 105 °C overnight. Crude protein was analyzed by following the Kjeldahl method after acid digestion (% crude protein = % nitrogen × 6.25). Crude lipid was determined by the Soxhlet extraction method using petroleum ether. The total ash content in the experimental diet was determined by the combustion of samples in a muffle furnace at 600 °C for 6 hours (Table 1).

Table 1: Formulation and proximate composition (%) of experimental diets.

Ingredient (%)	T1 (Control)	T2 (1% Ginger)	T3 (1% Garlic)	T4 (2% Ginger)	T5 (2% Garlic)
Soya full	30	30	30	30	30
Jwala (small shrimp)	44	44	44	44	44
Wheat flour	10	9	9	9	9
Rice bran	7	7	7	6	6
Vitamin premix[a]	1	1	1	1	1
Mineral mix[b]	1.45	1.45	1.45	1.45	1.45
Yeast	3	3	3	3	3
Additional					
Liver tonic	2.5	2.5	2.5	2.5	2.5

Phytase	0.03	0.03	0.03	0.03	0.03
Butylated hydroxyl toluene	0.02	0.02	0.02	0.02	0.02
Salt	1	1	1	1	1
L-Lysine	0.2	0.2	0.2	0.2	0.2
Ginger (%)	0	1	0	2	0
Garlic (%)	0	0	1	0	2
Proximate composition (%)					
Dry matter	94.69±0.31	96.48±0.01	96.21±0.12	96.27±0.22	95.55±0.33
Crude protein	45.8±0.34	45.07±0.01	45.38±0.14	45.75±0.52	45.19±0.31
Crude lipid	6.67±0.4	6.31±0.11	6.45±0.21	6.45±0.41	6.66±0.29
Total Ash	10.6±0.28	10.8±0.04	10.05±0.11	10.46±0.17	10.19±0.04
Energy (MJ/Kg)	19.88±0.13	19.71±0.21	19.89±0.01	19.85±0.18	19.90±0.64

[a] Vitamin mixture/kg premix containing the following: 33000IU vitamin A, 3300IU, vitamin D3, 410IU vitamin E, 2660mg Vitamin BI, 133mg vitamin B2, 580mg vitamin B6, 41mg vitamin B12, 50mg biotin, 9330mg choline chloride, 4000mg vitamin C, 2660mg Inositol, 330mg para-amino benzoic acid, 9330mg niacin, 26.60mg pantothenic acid. [b] Mineral mixture/kg premix containing the following: 325mg Manganese, 200mg Iron, 25mg Copper, 5mg Iodine, 5mg Cobalt.

Growth, survival rate and feed utilization parameters

On every 15-day interval from stocking, fish were starved for 24 hours prior to the sampling. Sampling was done by netting 20% of the fish from each tank to determine their growth and daily ration needs. Fish were anesthetized with clove powder (200 mg/L) before each sampling (Naderi et al. 2017). At the end of the experiment, on the 60th day, all fish were sampled, anesthetized, and measured for their individual weight and length to calculate the weight gain (WG), specific growth rate (SGR), condition factor (K), feed conversion ratio (FCR), and survival rate (SR) according to Aqmasjed et al. (2023):

WG (g) = Final weight (g) - Initial weight (g)
SGR (%/day) = 100 × [ln (final weight) – ln (initial weight)]/days
K = 100 × [final weight/ (final length)^3]
FCR = Dry feed intake (g)/ weight gain (g)
SR (%) = 100 × (final number of fish/initial number of fish)

Statistical analysis

All the results (mean ± SE) were analyzed by using SPSS software (Version 25, IMB, Armonk, NY, USA). Data were checked for normality and homogeneity of variance with the Shapiro-Wilk and Levene tests, respectively before analysis. Data collected throughout the experiment were subjected to one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test to compare the means among treatments. Means were regarded as significantly different when $p < 0.05$.

RESULTS

Water quality parameters

Throughout the experiment, the natural light-dark cycle was maintained, and daily monitoring of water quality parameters such as temperature, pH, and dissolved oxygen was conducted. These parameters were recorded as 18.2±1.4 °C for temperature, 7.4±0.1 for pH, and 7.7±0.8 mg/L for dissolved oxygen, respectively.

Fish growth, feed utilization and survival

As shown in table 2, inclusion of ginger and garlic induced a significant change in both weight body and length in the juvenile rainbow trout. Final weight was significantly higher in 1% ginger group (T2) than in Control (T1), 1% garlic (T3), and 2% garlic (T5) fed group (p = 0.003). Weight gain was significantly higher in the 1% ginger group (T2) group than control (T1), 1% garlic (T3), and 2% garlic (T5) (p = 0.004) group. Final length was significantly higher in the 2% garlic group (T3) than 1% and 2% ginger (T2 and T4) (p = 0.01) fed group. Specific growth rate (SGR) was significantly higher in 1% ginger group (T2) than in control (T1), 1% garlic (T3), and 2% garlic (T5) (p = 0.001) group. Average daily weight gain (ADWG) was significantly higher in 1% ginger group (T2) than in control (T1), 1% garlic (T3), and 2% garlic (T5) (p =0.004) (Figure 1). The condition factor in 1% ginger group was significantly higher than control (T1), 1% garlic (T3), and 2% garlic (T5) (p = 0.003). Similarly, feed conversion ratio was significantly lower in 1% ginger group than control (T1), 1% garlic (T3) (p=0.002). In this study, the survival rate was not affected by the inclusion of dietary garlic or ginger at any level (p > 0.05).

Table 2: Growth performance, feed utilization, and survival of *O. mykiss* in different treatment at the end of the feeding trial. T1 (Control), T2 (1 % Ginger), T3 (1% Garlic), T4 (2% Ginger), and T5 (2% Garlic)

Treatment	T1	T2	T3	T4	T5	
Parameters	Control	1% Ginger	1% Garlic	2% Ginger	2% Garlic	*p*
Initial weight (g)	3.34±0.11	3.69±0.09	3.65±0.05	3.50±0.14	3.41±0.11	0.14
Final weight (g)	15.13±0.84^{b}	22.00±0.84^{a}	17.17±0.58^{b}	19.80±0.80^{ab}	17.00±0.95^{b}	0.003
Final length (cm)	11.56±0.30^{ab}	9.83±0.56^{b}	12.90±0.58^{a}	10.20±0.71^{b}	10.97±0.04^{ab}	0.01
Weight gain (g)	12.80±1.09^{b}	18.31±0.76^{a}	13.52±0.60^{b}	16.31±0.82^{ab}	13.59±0.87^{b}	0.004
Specific growth rate (%/day)	2.41±0.05^{c}	2.97±0.04^{a}	2.58±0.05^{c}	2.89±0.10^{ab}	2.67±0.07^{bc}	0.001
Condition factor	1.05±0.11^{bc}	2.39±0.36^{a}	0.82±0.12^{c}	1.94±0.31^{ab}	1.29±0.07^{bc}	0.003
Feed conversion ratio	2.04±0.25^{a}	1.07±0.11^{c}	1.67±0.12^{ab}	1.29±0.14^{bc}	1.49±0.16^{bc}	0.002
Survival rate (%)	79.33±5.20	84.33±7.37	79.32±7.94	79.00±4.19	83.83±4.76	0.94

Values in each row with different superscripts letters are significantly different at p < 0.05 (Mean ± SE).

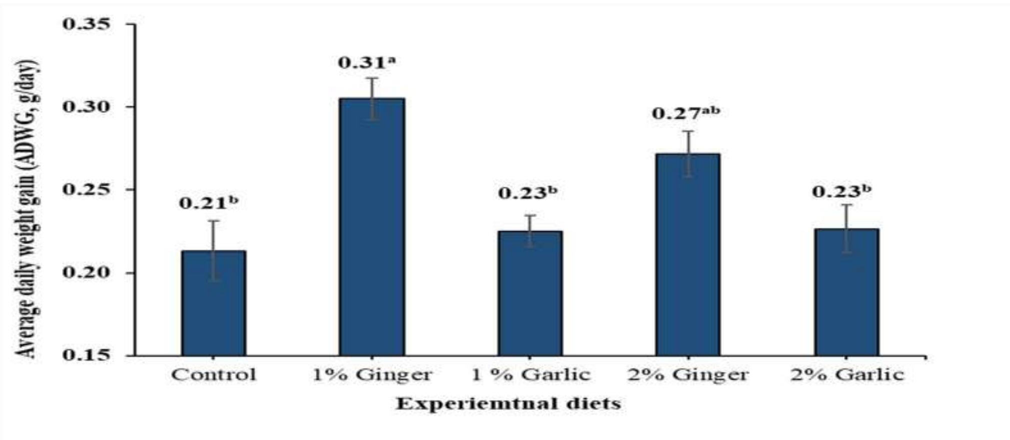

Figure 1: Average daily weight gain (ADWG, g/day) of *O. mykiss* fed diet supplemented with different levels of ginger and garlic. Values with different superscripts letters are significantly different at p < 0.05. Values are mean ± SE of three replications.

DISCUSSION

In the present study, it was found that the growth performance of rainbow trout was significantly higher in 1% ginger-supplemented groups compared to the garlic-supplemented groups and control group. These results were consistent with findings from previous studies conducted in other fish species, which also demonstrated that body weight gain, SGR, and condition factors were significantly increased in the fish fed with 1% ginger supplemented feed compared to the 1% garlic supplemented feed and control groups in juvenile *Huso huso* fish after 60 days of experiment (Gholipour et al. 2014). Sukumaran et al. (2016) also reported the positive effect of 0.6-1% inclusion of ginger on the growth performance of Rohu (*Labeo rohita*) when fed for 60 days. However, the present results were in contradiction with those obtained by Aqmasjed et al. (2023), who observed no improvement in the growth rate, condition factor, or feed utilization in rainbow trout fed with a diet containing 0.5% ginger after 60 days of culture in a fiberglass tank as compared to the control. The conflicting results of the present study with those of Aqmasjed et al. (2023) could therefore be attributed to the difference in the inclusion level of ginger in the diet or the culture condition. Studies had reported the beneficial effects of ginger on the growth performance, immune system, blood parameters, and antioxidant status of several other fish species (Ahmadifar et al. 2019; Nya and Austin 2009; Sukumaran et al. 2016; Talpur et al. 2013) as it is a medicinal herb and an excellent source of nutrients (Thanikachalam et al. 2010). The ginger in a dry powder form generally contains around 60-70% carbohydrates, 9% protein, 3-6% lipid, 3-8% fibers, protease about 2-6%, 1-3% volatile compounds such as zingiberol, gingerol, zingiberene, shogaol, and vitamin A, C and B3 (Mohammadi et al. 2020). Therefore, in the present study, the growth enhancement and lower fed conversion ration after the supplementation of ginger, especially at 1%, have been attributed to the nutrient richness of ginger rhizomes, which may have enhanced digestion and nutrient absorption in the gastrointestinal tract. Ginger can also positively affect the intestinal morphology and bacterial micro flora, which further enhances nutrient availability, thereby promoting the overall performance of the fish (Ali et al. 2008).

On the other hand, in this study, garlic fed trout performed apparently better yet statistically similar to the control group in terms of growth performance, feed utilization, and survival. However, in contrast to these results, Nya and Austin (2009) indicated that rainbow trout fingerlings (15g average body weight) when fed with dietary garlic at 0.1-1% significantly improved the growth and feed conversion rate. Similarly, at the end of 90-day feeding trail, the specific growth rate and feed conversion efficiency were significantly higher than the control group when rainbow trout (64.1±0.3g) were fed with garlic at a rate of 1-2% for 120 days (Öz and Dikel 2022). They found these results when the rainbow trout used in their experiment was either bigger in average size or the number of culture days was longer than the present study. However, in slight agreement with the results of present study, garlic has been linked previously with growth suppression in fish as shown in juvenile tilapia at 1% level with no significant improvement in growth performance (Ndong and Fall 2007). Similarly, Nwabueze (2012) and Thanikachalam et al. (2010) found no significant effect on the weight gain, SGR, and FCR of African catfish (*Clarias gariepinus*) when fed garlic at the rate of 0.5-15%. Likewise, when garlic powder was fed at 2-4% for 60 days, blood parameters such as red blood cell count (RBC), hemoglobin (Hb) concentration, hematocrit (%), and mean corpuscular hemoglobin were significantly lowered in European Sea Bass (*Dicentrarchus labrax*) as reported by Irkin et al. (2014). Based on the findings in different fish species, it may be concluded that the dose of garlic, size of fish, and duration of exposure to garlic may alter the effects of garlic on the growth performance, feed utilization, and physiology of fish. Moreover, the differences in the results may also be due to the differences in diet formulation, purity of the plant, form of the garlic (powders, oils, or extracts) used in the diet, physiological characteristics, and culture condition of the fish (Dadgar et al. 2017). Despite the constant controversy about the effects of garlic as growth enhancer for fish, few studies have shown that garlic is an important medicinal herb with organosulfur compounds, such as allicin, widely known to improve digestion and promote the growth of fish (Büyükdeveci et al. 2018; Lee et al. 2012). Therefore, the present study suggests that dietary garlic does not decrease the growth of rainbow trout and may positively affect the growth of trout, taking into consideration the size and culture duration.

This study clearly indicated that 1% ginger supplement in the feed for juvenile rainbow trout elicited weight gain, specific growth rate, and condition factor. Ginger inclusion in rainbow trout feed at the 1% inclusion level is therefore suggested for juvenile rainbow trout to promote growth performance as a cost-effective and environmentally friendly feed additive. On the other hand, it is also concluded that garlic does not affect the growth of rainbow trout when included at 1-2% in feed. However, it remains for further research to consider the appropriate size of fish and dose for the application of garlic and the duration of culture for favorable garlic performance and to assess its impacts on growth performance. With the established history of garlic and ginger in dietary and medicinal applications as anti-infective agents, their potential to protect the rainbow trout against a specific pathogen is also another aspect that can be considered in future research to enhance the production of rainbow trout in the Nepalese context.

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ENHANCING THE SURVIVAL RATE IN LIVE FISH TRANSPORT BY UTILIZING NANOBUBBLE TECHNOLOGY

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ABSTRACT
Efficient transportation of live fish is crucial for aquaculture, necessitating methods to ensure that fish maximize survival rates. This study was carried out in March 2022 at the Rainbow Trout Fishery Research Center to evaluate various aeration systems and packing methods for transporting trout fingerlings for 12 hours, with a focus on nanobubble technology. Closed and open transport systems were tested using oxygenated, ozonized, and air nanobubbles, compared to conventional methods. In the closed system with 500-750 fish per bag, ozone nanobubbles achieved significantly ($P<0.05$) higher survival rates of 99.6±0.10 and 99.3±0.0 %, versus 64.5% with conventional oxygen packing. The ozone nanobubbles extended the duration until the dissolved oxygen reached 4 mg/L. For open transportation, oxygen nanobubbles with a droplet size of around 100-600 nm led to 99.0±0.10% survival, significantly ($P<0.05$) exceeding 76.7±5.20% with regular oxygen aeration. Crucially, nanobubble systems consistently maintained elevated dissolved oxygen (8-9 mg/L) and reduced ammonia concentrations (0.01-0.03 mg/L), indicating superior fish survival during transport. The findings highlight the transformative potential in optimizing fish transport for sustainable aquaculture. Refinement of nanobubble packaging and aeration could significantly increase survival rates and efficiency, promoting sustainable aquaculture practices.

Keywords: Live fish transport, nanobubble aeration, survival rates, sustainable practices

INTRODUCTION
The aquaculture industry in Nepal has been rapidly expanding in recent years, with a particular focus on the farming of trout in the hill regions of the country (Gurung 2008; Mulmi 2017). One of the major challenges faced by fish farmers is the high mortality rate of fingerlings during live transportation, often exceeding 50% due to factors such as low dissolved oxygen levels, accumulation of metabolic waste, and physical stress (Hargreaves and Steeby 1999). The live transport of fish presents several challenges that can compromise their health and survival. A primary concern is oxygen depletion in the water due to the respiration and bacterial activity of the fish, leading to stress, suffocation, and mortality (Guan et al. 2021). The accumulation of metabolic wastes such as ammonia and carbon dioxide create toxic conditions (Lekang 2013). Temperature fluctuations increase disease susceptibility and reduce appetite (Wedemeyer 1997). Physical injuries from improper handling, overcrowding, and poor water quality increase infection risks (Marino et al. 2016). Confined stressful conditions facilitate pathogen transmission between infected and healthy individuals (Hastein et al. 2005). Furthermore, the deterioration of water quality due to factors such as pH changes, ammonia, and the accumulation of organic matter negatively impact fish health (Lekang 2013). The combination of low oxygen, poor water quality, and injury induces significant stress, compromising immune function, growth, and survival rates (Wedemeyer 1997; Marino et al. 2016). Traditional transportation methods are limited by degradation of water quality over time, restricting safe transport distances (Guan et al. 2021), while conventional aeration is energy-intensive (Lekang 2013).

Nanobubbles (NB), gas bubbles ranging from a few nanometers to a few hundred nanometers in size (Edzwald 2010; Parmar and Majumder 2013), offer a potential solution for live fish transportation. Their high surface area-to-volume ratio enables efficient oxygen dissolution in water (Benstaali et al., 2013), crucial for meeting the respiratory needs to transport fish such as trout. Incorporating nanobubbles provides advantages such as improved oxygen transfer, longer duration of oxygen supply, reduced fish stress, and energy efficiency over traditional oxygenation methods (Hutagalung et al. 2023; Liu et al. 2016). Specialized nanobubble generators disperse nanobubbles in the water holding the fish, ensuring a continuous oxygenated supply during transit (Cheng et al. 2018; Yao et al. 2020). While ongoing research explores the optimal conditions and parameters for different species and scenarios (Guan et al. 2021; Shafiei et al. 2022), nanobubbles present a promising approach to address the challenges of live fish transportation.

The purpose of this study is to investigate the application of nanobubbles as an innovative approach to improve the conditions and survival rates of live trout during transportation. Nanobubbles are unique properties that may address several key challenges faced in live fish transportation. The findings could lead to improved practices and technologies that support the aquaculture industry by allowing long-distance transportation of live trout while minimizing losses and maintaining high fish health and quality. This introduction paragraph aims to provide an overview of the potential benefits of nanobubble technology in enhancing the survival rate of trout fingerlings during transportation in the context of Nepal's aquaculture industry.

MATERIALS AND METHODS

Study location

Pre-tests of aeration systems and fish packaging including conditioning were conducted at Trout Research Station, Dhunche (1900 masl), Rasuwa. The effectiveness of different aeration systems and gas diffusion in the transport of live trout fish was evaluated through round trip transport of fish from Dhunche to the lower part of Nuwakot district (600 m).

Experimental Design of Aeration System

This investigation focused primarily on evaluating the effectiveness of various aeration methods, specifically oxygen injection and diffuser systems, in maintaining adequate levels of dissolved oxygen to prevent hypoxia and improve the overall health of fish. This study used an EDON nanobubble generator and an ozone generator.

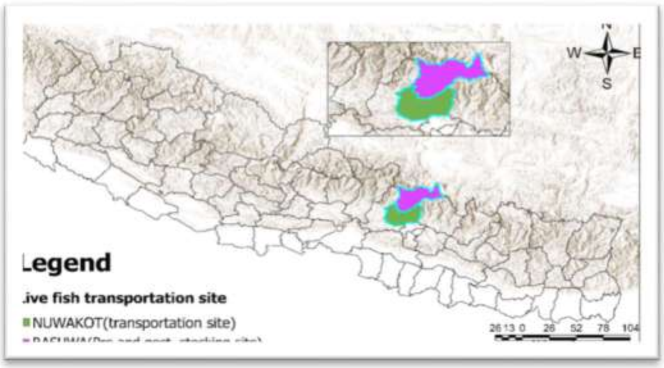

Figure : 1 Live the fish transport site of Nuwakot and Rasuwa

Closed system
In closed systems, trout fingerlings weighing 10 g each were stocked at two different densities: 500 and 750 fingerlings per plastic pack. These packs were subjected to various gas supply systems, including oxygenated water, oxygen nanobubble water, ozone nanobubble water, and air nanobubble water. The experiment followed a Factorial Completely Randomized Design (CRD) of 2 × 4 × 3 to assess the duration of survival under different gas supply conditions. A continuous dissolved oxygen (DO) sensor was placed inside each pack to monitor DO levels. The trial was terminated when the dissolved oxygen reached 4 mg/L due to the respiration of the fish.

Open system
In the open system, a 20-liter vessel housed 750 fingerlings for 8 h, with aeration methods including continuous infusion of four treatments: oxygen nanobubbles, normal air aeration, air nanobubble aeration, and ozone nanobubble aeration, after a 3-replication (CRD 4 × 3) design.

Transportation in an open system
In adapting transportation for open systems, a 100-liter vessel was utilized to house 3000 fingerlings for a duration of 12 hours. To ensure uninterrupted power supply during transportation, a separate electric backup generator was employed. This system implemented continuous aeration employing two treatments: oxygen nanobubbles and conventional oxygen aeration. The experiment followed a six-replication design with a completely randomized design (CRD) of 6 × 2. All vessels corresponding to the respective treatments were loaded onto a jeep/truck and transported from Dhunche to Trishuli and then back from Trishuli to Dhunche.

Sample/data collection
The process began by sampling the quality of the water to ensure precision. We recorded the number of trout fingerlings that were used in transportation and used a Pasco Scientific DO sensor meter for real-time monitoring with SparkVue using IOS software for water quality tracking factors such as temperature and dissolved oxygen. This approach focused on the well-being of fish and supported ongoing research. There is a carefully recorded time during the transportation of live fish when noting any changes in behavioral conditions are noted. As stress factors of ammonia and nitrite, the concentrations of transportation water were measured just before and after transportation.

Figure 2: Observation of survival and survival time with 500 fish per bag and 750 fish per bag in March 2022

Figure 3: Observation of the survival of trout fingerlings in an open tank fish transport system with oxygen aeration and nanobubble aeration in March 2022.

Figure 4: Observation of the survival of trout fingerlings in an open tank fish transport system with oxygen aeration and nanobubble aeration in March 2022.

Statistical analysis:
The differences between the group means of body weight gain, percent survival, survival time, loss, maintenance ratio, and water quality parameters were tested by analysis of variance (ANOVA). The post hoc test was applied to determine the significance of differences between the two means. All statistical tests were performed using the XLSTAT 2019 statistical package. Comparisons were made with 5% probability.

Instruments Validation and Quality Control
This study evaluated the performance of the EDON nanobubble generator, a specific nanobubble technology, at the Nepal Academy of Science and Technology (NAST) in Khumaltar, Lalitpur. To evaluate the efficiency of the generator, we employed a Zeta seizer for a comprehensive analysis of the nanobubbles produced. This assessment included measures of factors such as bubble size distribution, concentration, and stability over time.

Loss-maintain ratio
In the context of trout fish, the loss maintenance ratio refers to the efficiency of the transportation process in minimizing fish losses during transit. This ratio was calculated by comparing the number of fish initially loaded onto a transport vehicle or container with the number of fish that successfully reached their destination without perishing. The formula to calculate the loss maintenance ratio is as follows:

$$\text{Loss Maintain Ratio } = \frac{Total\ cost\ of\ loaded\ fish}{Cost\ of\ succesfully\ transported\ fish}\ x\ 100\%$$

In this formula:
The "Cost of Fish Successfully Transported" represents the number of loaded fish that reach their destination alive. The 'Total Cost of Fish Loaded' refers to the total quantity of fish initially loaded onto the transport vehicle. A higher loss maintenance ratio indicates better efficiency in ensuring safe and successful transportation of trout, which is crucial for maintaining profitability and economic viability.

RESULTS
In the study comparing closed packaging systems with different fish densities (500 fish/bag and 750 fish/bag), various parameters including survival rate, survival time, loss maintain ratio, DO levels, temperature, pH, ammonia and NO_2 concentrations were evaluated under different water conditions (normal water air NB water, oxygen NB water, and ozone NB water). For both fish densities, survival rates varied significantly among different water conditions 500 fish/bag: F 750 fish/bag: at $p < .05$. In the case of 500 fish/bag, oxygen, NB water showed the highest survival rates 99.6±0.1%, followed by ozone NB water, air NB water, and normal water. Similarly, for 750 fish/bag, oxygen NB water exhibited the highest survival rates 99.3±0.0%, followed by ozone NB water, air NB water, and normal water. Survival time also showed significant differences between conditions for both densities. Oxygen NB water consistently exhibited the longest survival time, followed by ozone NB water, air NB water, and normal water, regardless of fish density. Loss maintain ratio DO levels, temperature, pH, ammonia and NO_2 concentrations showed similar trends in different water conditions for both fish densities, with oxygen NB water generally providing the most favorable conditions, followed by ozone NB water, air NB water, and normal water.

Table 1 : Mean (± standard deviation) of survival rate, survival time, trout fingerlings, and water quality in plastic bags under the closed fish transport system in the Rainbow Trout Fishery Research Center, Dhunche Rasuwa.

Observations	Closed Packing system (500 fish/bag)			
	Normal water	Air NB water	Oxygen NB Water	Ozone NB water
Survival (%)	64.5±1.2[a]	88.7±1.0[b]	99.6±0.1[c]	99.3±0.1[c]
Survival time (min)	36.8±0.4[a]	73.5±1.5[b]	190.5±1.5[d]	151.5±5.1[c]
Loss Maintain Ratio (%)	57.4±1.4c	86.4±1.1b	99.5±0.1a	99.2±0.2a
DO (mg/L)	7.6±0.1[a]	8.4±0.1[b]	25.2±0.2[d]	21.0±0.2[c]
Temperature (°C)	15.4±0.0[a]	16.3±0.1[b]	17.3±0.1[c]	17.6±0.2[c]
PH	7.5±0.0[c]	6.8±0.0[a]	6.7±0.1[a]	7.1±0.0[b]
Ammonia (mg/L)	0.38±0.05[c]	0.10±0.00[b]	0.00±0.00[a]	0.00±0.00[a]
NO_2 (mg/L)	0.24±0.01[b]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	Closed Packing system (750 fish/bag)			
Survival (%)	53.9±2.7[a]	87.7±0.1[b]	99.3±0.0[c]	99.0±0.2[c]
Survival time (min)	30.0±0.0[a]	48.9±0.4[b]	142.5±2.9[d]	132.0±2.4[c]
Loss Maintain Ratio (%)	44.7±3.2c	85.2±0.1b	99.1±0.1a	98.8±0.2a
DO (mg/L)	7.6±0.1[a]	8.4±0.1[b]	25.2±0.2[d]	21.0±0.2[c]
Temperature (°C)	15.4±0.1[a]	17.4±0.1[b]	18.4±0.1[c]	18.7±0.1[d]
PH	7.4±0.0[b]	6.6±0.1[a]	6.6±0.1[a]	6.6±0.2[a]
Ammonia (mg/L)	0.45±0.03[c]	0.10±0.00[b]	0.10±0.00[b]	0.00±0.00[a]
NO_2 (mg/L)	0.24±0.00[b]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]

Note: Different superscript letters within the row are significantly different at α0.05.

In examining the open-live fish transportation performance of oxygen nanobubble (NB) water versus oxygen-aerated water for live fish transportation, several critical parameters were evaluated, shedding light on their respective effectiveness and implications for fish survival and water quality. The oxygen NB water demonstrated significantly higher survival rates 99.0±0.1% compared to oxygen aerated water 76.7±5.2%, emphasizing its potential as a superior transport medium to maintain fish viability. The oxygen NB water exhibited substantially higher initial concentrations 18.0±0.3 mg/L and final 13.0±0.2 mg/L DO compared to oxygen-aerated water 8.6±0.3 and 6.5±0.7 mg/L, respectively), indicating better oxygenation and potentially reduced risk of hypoxia during transport. Both oxygen NB water and oxygen aerated water maintained similar temperatures 18.0±0.1 and 16.8±0.3°C, respectively, suggesting a minimal impact on temperature regulation between the two treatments. The pH levels in the oxygen NB water pH 6.4±0.0 were slightly lower compared to the oxygen aerated water pH 6.5± 0.1, although the difference is relatively small and may have minimal physiological effects on the transported fish. The oxygen NB water showed lower NO_2 0.10±0.00 mg/L and ammonia 0.10±0.00 mg/L compared to oxygen-aerated water 0.23±0.01 mg/L for NO_2 and 0.20±0.04 mg/L for ammonia), indicating a potentially reduced accumulation of nitrogenous wastes and associated stress on the fish. The oxygen NB water exhibited a higher loss maintaining ratio 88.6±0.1% compared to oxygen aerated water 72.0±6.2%, indicating better maintenance of fish health and viability during transportation.

Table 2: Mean (± standard deviation) of survival rate, survival time, trout fingerlings and water quality in 20 L vessels under the open fish transport system at the Rainbow Trout Fishery Research Center, Dhunche, Rasuwa

Observation	Oxygen NB water	Oxygen-aerated water
Survival (%)	99.0±0.1[b]	76.7±5.2[a]
Initial DO (mg/L)	18.0±0.3[b]	8.6±0.3[a]
Final DO (mg/L)	13.0±0.2[b]	6.5±0.7[a]
Temperature (°C)	18.0±0.1	16.8±0.3
pH	6.4±0.0	6.5±0.8
NO_2 (mg/L)	0.10±00[a]	0.23±0.01[b]
Ammonia (mg/L)	0.10±00[a]	0.20±0.04[b]
Loss Maintain Ratio (%)	88.6±0.1[a]	72.0±6.2[b]

Note: Different superscript letters within the row are significantly different at α0.05.

The descriptive statistics compared with live fish transportation practices; oxygen nanobubbles, aerated water, and oxygen-aerated water for live fish transportation reveal notable differences across various parameters, providing insights into their effectiveness and implications for fish survival and water quality. The nanobubble water of open system demonstrated the highest survival rate 97.3±0.1%, followed by aerated water 90.3±1.0% and oxygen aerated water, indicating the potential benefits of oxygen nanobubbles in maintaining fish viability during transport (Table 3). The water exhibited the highest initial DO concentration 19.0±0.2 mg/L, followed by aerated water 9.6±0.1 mg/L and oxygen aerated water, suggesting superior oxygenation capacity in the water treated with nanobubbles. Similarly, water treated with oxygen nanobubbles maintained the highest final DO concentration 13.1±0.3 mg/L, followed by aerated water 7.1±0.2 mg/L and oxygen-aerated water, reinforcing its effectiveness in maintaining oxygen levels during transportation. Both the oxygen nanobubble treated water and oxygen-aerated water maintained similar temperatures 19.6±0.0 and 20.1±0.0 °C, respectively, indicating consistent thermal conditions in all treatments. Oxygen nanobubble-treated water exhibited a lower pH 6.5± 0.1compared to aerated water 7.2±0.0, suggesting potential differences in the water chemistry between the two treatments. The water displayed lower levels of NO_2 0.00±0.00 mg/L and ammonia (0.00±0.00 mg/L) compared to aerated water 0.02±0.00 mg/L for NO_2 and 0.01±0.00 mg/L for ammonia, indicating improved water quality and reduced accumulation of nitrogenous waste accumulation. Oxygen nanobubble-treated water demonstrated a higher loss maintenance ratio 96.8±0.1% compared to aerated water 88.4±1.2%, indicating better preservation of fish health and viability during transportation.

Table 3: Mean (± standard deviation) of survival rate, survival time, trout fingerlings, and water quality in 100L vessels with open fish transported from Dhunche to Trishuli, and then back from Trishuli to Dhunche

Descriptive	Oxygen NB water	Oxygen-aerated water
Survival (%)	97.3±0.1[b]	90.3±1.0[a]
Initial DO (mg/L)	19.0±0.2[b]	9.6±0.1[a]
Final DO (mg/L)	13.1±0.3[b]	7.1±0.2[a]
Temperature (°C)	19.6±0.0	20.1±0.0
pH	6.5±0.1[a]	7.2±0.0[b]
NO_2 (mg/L)	0.00±0.00[a]	0.02±0.00[b]
Ammonia (mg/L)	0.00±0.00[a]	0.01±0.00[b]
Loss Maintain Ratio (%)	96.8±0.1[b]	88.4±1.2[a]

Note: Different superscript letters within the row are significantly different at α0.05.

DISCUSSION

The present study underscores the critical importance of water quality in closed packing systems for fish, with profound implications for their survival rates, longevity and overall well-being (Ahmed et al. 2023; Ashley 2007; Miranda-Filho 2019; Hoseini et al. 2022; Joshy et al. 2022; Kamalam et al. 2017; Owen 2023; Shabani et al. 2016; Singh et al. 2004). Irrespective of fish density (500 fish/bag or 750 fish/bag), oxygen nanobubble (NB) water consistently emerged as the most favorable environment, facilitating higher survival rates and extended survival times. This finding highlights the crucial role of maintaining optimal dissolved oxygen levels in aquaculture settings, corroborating previous research (Hao 2021; Mahasri et al. 2018).

Furthermore, the study delineates a distinct hierarchy among the tested water conditions, with oxygen NB water demonstrating superior conditions, followed by ozone NB water air NB water, and normal water. The observed trends in the loss maintenance ratio and other water quality parameters, such as lower pH levels, NO_2 and ammonia concentrations, corroborate the superiority of oxygen NB water in preserving fish health and viability (Hao 2021; Owen 2023). In particular, although increasing fish density resulted in lower survival rates, shortened survival times, and compromised water quality under all conditions, the relative ranking among water conditions remained consistent. This observation aligns with previous findings that highlight the detrimental effects of high stocking density on fish welfare during transportation (Kamalam et al. 2017; Muzaddadi et al. 2017; Singh et al. 2004).

The study findings underscore the paramount importance of vigilant water quality management to ensure the prosperity and sustainability of closed packing systems in aquaculture operations. Specifically, the superior performance of oxygen NB water compared to oxygen-aerated water and aerated water in maintaining fish viability during transportation is a significant outcome (Joshy et al. 2022; Lima et al. 2020; Suryadi et al. 2020). This conclusion is supported by higher survival rates, better oxygenation indicated by higher dissolved oxygen concentrations, lower concentrations of nitrogenous wastes, and a higher loss maintaining ratio observed in NB oxygen water treatment (Barton and Peter, 1982; Miranda-Filho 2019; Hao 2021; Sadek and Ching 2019).

The study's findings suggest that oxygen NB technology holds promise for enhancing live fish transportation outcomes by improving survival rates, maintaining optimal water quality, and minimizing stress on transported fish (Ahmed et al. 2023; Ashley 2007; (Barton and Peter, 1982; Miranda-Filho 2019; Hoseini et al. 2022; Joshy et al. 2022; Kamalam et al. 2017; Owen 2023). Further research and practical application of this technology could contribute to advances in aquaculture and fisheries management practices, addressing the challenges associated with ensuring fish survival and well-being during transportation (Ashley 2007).

Furthermore, the study emphasizes the importance of considering the duration of transport based on the packing system and examining how stock density, water quality, and packing systems affect vital water parameters such as dissolved oxygen, temperature, pH, and ammonia levels (Hao 2021; Owen 2023). These factors interact and can lead to stress, weakness of the immune system, and oxygen shortage, ultimately affecting fish survival (Kamalam et al. 2017; Owen 2023). The longer transport times of the oxygen NB water system can contribute to improving the endurance of the fish, as supported by previous findings (Lima et al. 2020; Suryadi et al. 2020). The integration of sensors and communication protocols for online monitoring of oxygen levels in tanks has proven beneficial in addressing mortality rates and providing early warning systems for oxygen management during fish transport (Zhang et al. 2020; 2021; 2023). These innovations have transformed aquaculture by improving the survival of fish during transit and enabling informed decision making by fish farmers to reduce aquaculture mortality.

CONCLUSION

The study emphasizes the crucial role of water quality in fish transport and closed packing systems. It highlights oxygen NB water as the most beneficial environment for fish survival and well-being, surpassing other water conditions such as ozone NB water, air NB water, and normal water. The high stock density negatively impacts fish welfare during transportation, necessitating careful water quality management. The oxygen NB water outperforms other aeration methods in maintaining fish viability during transit, indicating its potential to improve live fish transportation outcomes. The integration of sensors for online monitoring of oxygen levels in tanks proves beneficial in reducing mortality rates during transport. The study suggests that nanobubble aeration could be a more effective alternative to traditional methods, promising to improve fish welfare and survival rates during transit in aquaculture practices.

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A COMPARISON OF MONOCULTURE AND POLYCULTURE OF NILE TILAPIA (*Oreochromis niloticus*) WITH CARPS AND SAHAR (*Tor putitora*)

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ABSTRACT
Addition of Nile tilapia (*Oreochromis niloticus*) and sahar (*Tor putitora*) in carp-polyculture, and tilapia monoculture systems was tested. The experiment was conducted at the Agriculture and Forestry University, Chitwan, Nepal in 12 earthen ponds of 150 m^2 for 185 days. The experiment was conducted in a completely random design with four treatments in triplicate: a) Carp-polyculture (10,000/ha) + mixed-sex tilapia (3,000/ha) + sahar (1,000/ha) (T_1); b) Carp-polyculture + monosex tilapia (3,000/ha) (T_2); c) Monosex tilapia at 10,000/ha with fertilization only (T_3); and d) Monosex tilapia at 20,000/ha with fertilization and feeding (T_4). Silver carp, bighead carp, common carp, grass carp, rohu and mrigal were stocked in all ponds as of normal practice. The ponds were fertilized weekly with urea and DAP. Fish were fed daily with 26% CP feed at 2% body weight. Combined net fish yield was significantly higher ($p<0.05$) in T_4 (3.77±0.23 t/ha/crop) compared to T_3 (1.03±0.14 t/ha/crop); there was no significant difference among T_1 (2.82±0.23 t/ha/crop), T_2 (3.20±0.17 t/ha/crop) and T_4 (3.77±0.23 t/ha/crop). The mean harvest size, daily growth, GFY and NFY of monosex Nile tilapia in T_4 were significantly higher than in T_3. The gross profit was significantly higher in T_4 compared to T_3 without any significant difference between T_1 and T_2.

Keywords: Nile tilapia, Carps, Sahar, Polyculture

INTRODUCTION
Semi-intensive carp polyculture is an established fish culture system in tropical and subtropical regions of Nepal, using fertilized ponds with supplemental feed (Shrestha et al. 2022). The commonly cultured carp species include silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Hypophthalmichthys (Aristichthys) nobilis*), common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*). All six species are recommended in certain ratios with a combined density of 10,000-12,000 fish/ha, but fingerlings of all species are rarely available when needed for stocking, often due to variations in the breeding seasons of these species. The typical number of species cultured ranges from four to six. The annual pond yield of this semi-intensive carp polyculture system averages only 4.91 t/ha (Rijal and Jha 2020). The addition of other proven species (such as Nile tilapia, *Oreochromis niloticus* and Sahar, *Tor putitora*) with increased stocking density into the existing carp production system could increase productivity up to 57% and net returns by 61% (Shrestha et al. 2012; Shrestha et al. 2018) without any additional inputs. Since tilapia consume plankton, they also improve water quality in ponds and in effluents at harvest. Such improvements in water quality, larger economic gain, and production of fish with no further inputs all enhance the sustainability of an aquaculture system environmentally and economically. Sahar is a native endangered fish species (IUCN 2017). Addition of sahar into carp polyculture can aid in their conservation, utilization of pond resources and control of excessive Nile tilapia recruitment in carp ponds (Shrestha et al. 2011).

Previously, we conducted an experiment incorporating tilapia into carp polyculture. The results showed significant increases in yield (29%) and profit margin (81%) when tilapia and sahar were added to carp polyculture (Shrestha et al. 2018). Overall production was still relatively low, about four tons per ha annually. This production is lower than monosex tilapia culture in ponds in Thailand, where we have achieved annual yields of about 5 tons per ha with only fertilizer inputs and up to 20 tons per ha in fed ponds (Diana 2012). Monoculture of tilapia could possibly outperform polyculture with carps in Nepal, as well, both in terms of total production and economic returns. It is not possible to directly transfer results on monoculture of tilapia from Thailand to Nepal, given the generally cooler and more seasonal climate in Nepal. Therefore, the purpose of this experiment is to examine monoculture of tilapia along with inclusion of tilapia in polyculture as techniques to best incorporate tilapia into the aquaculture industry in Nepal. Since sahar is an endangered species (IUCN 2017), any success in rearing them could either relieve pressure on wild populations as a food source or could be used to supplement wild populations by stocking to improving sustainability of aquaculture in Nepal. The objectives of this study were to test a carp-tilapia-sahar polyculture and monosex tilapia culture system; to evaluate the culture potential of sahar and monosex tilapia to farmers; and to develop partial enterprise budgets of costs and values of fish crops among treatments.

MATERIALS AND METHODS

The experiment was conducted at the fish farm of Agriculture and Forestry University, Rampur, Chitwan, Nepal in twelve earthen ponds of 150 m^2 for 185 days (1 June to 3 December 2017). The experiment was conducted in a completely randomized design with four treatments in triplicate. The treatments were: a) Existing carp polyculture (10,000/ha) + mixed-sex tilapia (3,000/ha) + sahar (1,000/ha) (T_1); b) Existing carp polyculture and monosex tilapia at 3,000/ha (T_2); c) Monosex tilapia at 10,000/ha with fertilization only (T_3); and d) Monosex tilapia at 20,000/ha with fertilization and feeding (T_4). Silver carp, bighead carp, common carp, grass carp, rohu and mrigal of mean stocking size 3.2 and 3.3, 0.6 and 0.6, 13.6 and 12.5, 5.9 and 6.2, 21.7 and 20.6, 11.1 and 11.9 g, respectively were stocked in T_1 and T_2 at the ratio of 3.5:1:2.5:0.5:1.5:1. Mixed-sex Nile tilapia and sahar of 1.9 and 2.1 g size were added in T_1. Similarly, the stocking size for all-male tilapia in T_2, T_3, and T_4 were 0.9, 1.2, and 0.9 g, respectively. All experimental ponds were completely drained and treated with hydrated lime [$Ca(OH)_2$] at the rate of 450 kg per ha. The ponds were sun dried for 2-3 days then filled with canal water. Ponds were then fertilized at 4 kg N and 1 kg P/m^2/day with di-ammonium phosphate (DAP) (18% N and 46% P_2O_5) and urea (46% N). Fingerlings were stocked one week after pond fertilization. Subsequent fertilizations were done on a weekly basis. Feeding was done with commercial pellet feed (Machapuchhre Feed Industry, Kapilvastu, Nepal) at 2% of total carp biomass per day. The proximate composition of feed was 90.0% dry matter, 26.6% crude protein, 8.6% crude fiber, 2.4% ether extract and 5.4% total ash. Feeding was done once in the morning between 9 and 10 a.m. The quantity of feed was adjusted monthly based on fish sampling. Sampling of fish was done monthly from each pond starting 30 days after stocking. During sampling about 10% of the stocked population of each species was weighed to calculate feed quantity for next month, assuming 100% survival. For final harvest, all ponds were drained by pumping and all fish were harvested and weighed. Weekly and biweekly measurements of water quality parameters were conducted at 6-8 a.m. Water temperature, dissolved oxygen (DO), pH, and Secchi disk depth were measured in situ weekly using a dissolved oxygen meter (Lutron DO-5519), pH meter (Lutron pH-222) and Secchi disk, respectively. Water samples were collected biweekly from each pond using a plastic column sampler and analyzed for total alkalinity, total ammonium nitrogen (TAN), soluble reactive phosphorous (SRP), and chlorophyll a (APHA 1985). Proximate analysis of feed was done using methods provided in AOAC (1980). Simple economic analysis was done to determine economic returns from each treatment (Shang 1990). The economic analysis was mainly based on farm gate price for harvested fish and current local market prices for all other inputs in Nepal. Farm gate prices of sahar, tilapia and carps were 600, 250 and 250 NRs per kg, respectively. Prices for sahar, mixed-sex tilapia, and monosex tilapia fingerlings were 5, 1, and 2 NRs per piece, respectively. Prices for common carp, silver carp, bighead carp, grass carp, rohu and mrigal fingerlings were 5, 1, 0.5, 2, 5 and 3 NRs per piece, respectively. Prices for DAP, urea and feed were 50, 22 and 20 NRs per kg, respectively.

The data were analyzed by one-way ANOVA using SPSS (V 16.0). For significant differences in growth parameters among different treatments, LSD was used to compare the means. For testing different growth and production parameters of carps, a T-test was used. For all analysis alpha was set at 0.05.

RESULTS

The production of all carps was not significantly different between T_1 and T_2 ($p>0.05$; Table 1). The production of all-male monosex tilapia in T_4 was significantly higher than in T_3 ($p<0.05$). Similarly, the extrapolated gross fish yield (GFY) of tilapia in T_2 was significantly higher than T_1 ($p<0.05$). The combined extrapolated GFY of all species excluding and including tilapia recruits was significantly lower in T_3 than other treatments ($p<0.05$). Similarly, the combined extrapolated net fish yield (NFY) of all species excluding tilapia recruits was significantly lower in T_3 than T_4 ($p<0.05$). The gross and net fish yields for monosex tilapia without feed was significantly lower than monosex tilapia with feed and carp treatments. The apparent food conversion ratio (AFCR) was significantly lower in T_4 compared to T_1 and T_2 without any significant differences between T_1 and T_2 (Table 1).

Table 1. Production performance (mean ± SE) in different treatments. Mean values in a row with the same superscript are not significantly different ($\alpha = 0.05$).

Parameters	Treatments			
	T_1	T_2	T_3	T_4
Extrapolated GFY (t/ha/crop)				
Carps	2.42 ± 0.20^a	2.58 ± 0.14^a	-	-
Tilapia	0.49 ± 0.04^a	0.72 ± 0.09^b	1.04 ± 0.14^c	3.79 ± 0.12^d
Sahar	0.02 ± 0.00	-	-	-
Combined excluding tilapia recruits	2.93 ± 0.46^b	3.29 ± 0.17^b	1.04 ± 0.14^a	3.79 ± 0.12^b
Combined including tilapia recruits	3.00 ± 0.22^b	3.29 ± 0.17^b	1.04 ± 0.14^a	3.79 ± 0.12^b
Extrapolated NFY (t/ha/crop) excluding tilapia recruits	2.82 ± 0.23^{ab}	3.20 ± 0.17^{ab}	1.03 ± 0.14^a	3.77 ± 0.23^b
AFCR	2.42 ± 0.28^b	2.09 ± 0.14^b	-	1.86 ± 0.07^a

Each carp species showed similar production parameters in all treatments, indicating the addition of tilapia and sahar did not affect overall carp production (Table 2). There were no significant differences in mean harvest weight, total harvest weight, mean daily weight gain (DWG), survival rate, extrapolated GFY, and extrapolated NFY of different carp species among treatments. However, mean harvest size, daily weight gain, gross fish yield and net fish yield of monosex Nile tilapia in T_4 were significantly higher than in T_3 ($p<0.05$).

Table 2. Growth and production parameters (mean ±SE) in different treatments. Data based on a 150 m^2 pond for 185 days culture period. Mean values in a row with the same superscript are not significantly different ($\alpha = 0.05$).

Parameter	Treatment			
	T_1	T_2	T_3	T_4
Common Carp				
Mean stocking weight (g)	13.6 ± 1.1^a	12.5 ± 0.4^a	-	-
Mean harvest weight (g)	385.6 ± 46.8^a	397.0 ± 10.4^a	-	-
DWG (g/day)	2.01 ± 0.26^a	2.08 ± 0.05^a	-	-
Survival (%)	67.5 ± 8.9^a	71.1 ± 10.6^a	-	-
GFY (t/ha/crop)	0.64 ± 0.04^a	0.71 ± 0.09^a	-	-
NFY (t/ha/crop)	0.61 ± 0.04^a	0.68 ± 0.09^a	-	-
Silver Carp				
Mean stocking weight (g)	3.2 ± 0.2^a	3.3 ± 0.2^a	-	-
Mean harvest weight (g)	319.6 ± 15.8^a	370.3 ± 13.6^a	-	-
DWG (g/day)	1.71 ± 0.08^a	1.08 ± 0.07^a	-	-
Survival (%)	39.0 ± 2.3^a	29.6 ± 4.9^a	-	-
GFY (t/ha/crop)	0.44 ± 0.02^a	0.39 ± 0.08^a	-	-
NFY (t/ha/crop)	0.43 ± 0.02^a	0.38 ± 0.08^a	-	-
Bighead Carp				
Mean stocking weight (g)	0.6 ± 0.0^a	0.6 ± 0.0^a	-	-
Mean harvest weight (g)	470.4 ± 39.6^a	473.7 ± 47.0^a	-	-
DWG (g/day)	2.54 ± 0.21^a	2.56 ± 0.25^a	-	-
Survival (%)	68.9 ± 5.9^a	71.1 ± 8.9^a	-	-
GFY (t/ha/crop)	0.32 ± 0.00^a	0.33 ± 0.01^a	-	-
NFY (t/ha/crop)	0.32 ± 0.00^a	0.33 ± 0.01^a	-	-
Grass Carp				
Mean stocking weight (g)	5.9 ± 0.3^a	6.2 ± 0.4^a	-	-
Mean harvest weight (g)	446.0 ± 195.5^a	635.9 ± 47.7^a	-	-
DWG (g/day)	2.38 ± 1.06^a	3.40 ± 0.26^a	-	-
Survival (%)	79.2 ± 30.1^a	62.5 ± 12.5^a	-	-
GFY (t/ha/crop)	0.16 ± 0.06^a	0.21 ± 0.03^a	-	-
NFY (t/ha/crop)	0.16 ± 0.06^a	0.20 ± 0.04^a	-	-
Rohu				
Mean stocking weight (g)	21.7 ± 1.2^a	20.6 ± 0.7^a	-	-
Mean harvest weight (g)	396.3 ± 40.2^a	411.3 ± 62.5^a	-	-
DWG (g/day)	2.02 ± 0.22^a	2.11 ± 0.34^a	-	-
Survival (%)	94.2 ± 5.8^a	95.7 ± 9.1^a	-	-

Parameter	Treatment			
	T_1	T_2	T_3	T_4
GFY (t/ha/crop)	0.57±0.05[a]	0.59±0.05[a]	-	-
NFY (t/ha/crop)	0.53±0.05[a]	0.56±0.05[a]	-	-
Mrigal				
Mean stocking weight (g)	11.1±0.3[a]	11.9±0.4[a]	-	-
Mean harvest weight (g)	471.1±45.6[a]	480.0±42.1[a]	-	-
DWG (g/day)	2.49±0.25[a]	2.53±0.23[a]	-	-
Survival (%)	60.0±3.0[a]	73.3±3.9[a]	-	-
GFY (t/ha/crop)	0.29±0.04[a]	0.35±0.02[a]	-	-
NFY (t/ha/crop)	0.27±0.04[a]	0.34±0.02[a]	-	-
Nile tilapia				
Mean stocking weight (g)	1.9±0.1	0.9±0.0	1.2±0.0	0.9±0.1
Mean harvest weight (g)	267.1±28.4	356.5±33.6	152.1±29.2[a]	281.7±4.8[b]
DWG (g/day)	1.43±0.15	1.92±0.18	0.82±0.16[a]	1.52±0.03[b]
Survival (%)	62.2±2.3	66.7±2.6	70.0±5.1[a]	67.2±1.9[a]
GFY (t/ha/crop)	0.49±0.04	0.72±0.09	1.04±0.14[a]	3.79±0.12[b]
NFY (t/ha/crop)	0.49±0.04	0.71±0.09	1.03±0.14[a]	3.77±0.12[b]
Sahar				
Mean stocking weight (g)	12.1±1.1	-	-	-
Mean harvest weight (g)	35.7±1.9	-	-	-
DWG (g/day)	0.13±0.01	-	-	-
Survival (%)	68.9±5.9	-	-	-
GFY (t/ha/crop)	0.02±0.00	-	-	-
NFY (t/ha/crop)	0.01±0.00	-	-	-

There was no significant difference in average temperature, dissolved oxygen, total alkalinity, total ammonium nitrogen, soluble reactive phosphorous and chlorophyll-a among treatments during the experimental period (Table 3)

Table 3. Water quality parameters (mean ±SE with range in parentheses) in different treatments.

Parameters	Treatments			
	T_1	T_2	T_3	T_4
Water temperature (°C)	28.3±0.2 (21.2-32.2)	28.2±0.2 (21.7-31.6)	28.1±0.1 (21.5-31.6)	28.1±0.2 (21.5-31.6)
Dissolved oxygen (mg/L)	2.6±0.1 (0.7-7.1)	2.7±0.3 (0.7-6.3)	2.6±0.2 (0.5-5.7)	3.6±0.3 (0.7-8.3)
pH	7.2 (6.5-7.9)	7.1 (6.1-8.0)	7.2 (6.2-7.9)	7.2 (6.5-8.2)
Total alkalinity (mg/L as $CaCO_3$)	146.4±13.0 (86.8-178.5)	143.8±2.2 (107.5-179.4)	145.3±6.7 (104.1-199.7)	141.0±9.3 (104.6-180.9)
Soluble reactive phosphorous (mg/L)	0.43±0.00 [ab] (0.05-0.96)	0.46±0.00 [b] (0.01-1.08)	0.47±0.02 [b] (0.14-1.63)	0.37±0.03 [a] (0.06-0.96)
Total ammonium nitrogen (mg/L)	0.44±0.0 (0.05-1.27)	0.39±0.02 (0.08-1.20)	0.42±0.0 (0.04-1.10)	0.33±0.05 (0.04-0.88)
Chlorophyll-a (mg/m^3)	78.1±27.7 (15.9-216.6)	73.7±9.7 (19.0-210.2)	80.8±10.3 (12.7-206.3)	48.9±4.8 (15.1-103.1)

The gross margin for monosex tilapia with feed was significantly higher than monosex tilapia without feed, while carp treatments were intermediate in gross margin. The variable costs in all treatments consisted of seed, feed, lime, urea, and DAP (Table 4). Cost of seed was significantly different among treatments ($p<0.05$), whereas cost of feed was not significantly different among fed treatments ($p>0.05$). There was no significant difference in all other variable costs among different treatments ($p>0.05$). Total input cost and total output were significantly lower in T_3 than other treatments. The gross profit margin was significantly higher in T_4 (463,942±31,804 NPR/ha) compared to T_3 (174,940±35,836 NPR/ha) without any significant difference between T_1 and T_2 ($p<0.05$; Table 4).

Table 4. Economic analysis (NPR) for each treatment. Data based on a 150 m^2 pond area and culture period of 150 days. Mean values in a row with the same superscript are not significantly different ($\alpha = 0.05$).

Variable	Treatment			
	T_1	T_2	T_3	T_4
Seed	662±0.0^d	473±0.0^b	304±0.0^a	598±0.0^c
Feed	5440±326^a	5386±105^a	0.0±0.0	5660±74^a
Lime	136±0.0^a	136±0.0^a	136±0.0^a	136±0.0^a
Urea	306±0.0^a	306±0.0^a	306±0.0^a	306±0.0^a
DAP	536±0.0^a	536±0.0^a	536±0.0^a	536±0.0^a
Total Input	7088±326^b	6846±105^b	1281±0.0^a	7245±74^b
Total Output	11130±861^b	12348±630^{bc}	3906±536^a	14196±441^c
Gross Margin	4042±1071^{ab}	5502±704^{ab}	2625±536^a	6962±472^b
Gross Margin per ha	269756±71390^{ab}	366650±47198^{ab}	174940±35836^a	463942±31804^b

DISCUSSION

This study was carried out to expand the technology developed through AquaFish research on carps, tilapia and sahar production as well as technology of monosex tilapia production to farmers in order to demonstrate alternative fish production models (Shrestha et al. 2012; Shrestha et al. 2018). An on-station experiment on monoculture and polyculture systems, using carp with the addition of tilapia and sahar was conducted, to determine the most practical system for farm adoption. We observed that the addition of Nile tilapia and sahar in carp polyculture had no adverse effect on growth and production of all carp species, or in pond water quality. This results suggest that tilapia and sahar did not compete for pond resources with any carp species.

The daily weight gain of mixed-sex Nile tilapia and sahar were 1.43 and 0.13 g, respectively, which is comparable or slightly higher than in previous experiments. The daily weight gain of Nile tilapia in polyculture was higher than a grass carp-tilapia polyculture system (0.2-0.5 g; Pandit et al. 2004), carp-tilapia-sahar polyculture system (0.63-0.70 g; Bhandari et al. 2016), tilapia-sahar polyculture system (0.6-0.9 g; Shrestha et al. 2011), and tilapia-sahar polyculture system (1.15 g; Acharya et al. 2007). The daily weight gain of sahar was quite low in the present experiment. The quality of feed (low protein; 26.6% CP) may have contributed to slow growth of sahar. Sundar et al. (1998) reported better growth, survival, and FCR of sahar were achieved from feed with 45.4% crude protein among diets with 21.4% to 50.2% crude protein. In a similar study, Joshi et al. (1989) reported that 35% crude protein was best for growth and feed efficiency of sahar. Good growth rates of all carp species were achieved in all carp treatments. The average growth rate of carp species in all treatments was higher than reported by previous studies in carp polyculture (Rai et al. 2008; Jaiswal 2010) as well as in our previous on-farm and farmer's field trials (Bhandari et al. 2016).

The combined gross fish yield in carp treatments (5.8-6.5 t/ha/year) was higher than the national average of carp polyculture (4.91. t/ha/year) (Rijal and Jha 2020). The hypothesis that addition of tilapia and sahar would increase the yield and profit from polyculture ponds was supported by the results of this experiment. However, production and profit of monosex tilapia without feed was quite low. Diana (2012) achieved annual yields of monosex tilapia of about 5 tons/ha with only fertilizer inputs. Although the production of monosex tilapia with feed was higher than all carp treatments and monosex tilapia without feed treatment, this was still quite low than reported by Diana (2012). Although the growth rate was satisfactory (0.82-1.52 g/day), the poor production of monosex tilapia in both feed and non-feed systems in the present experiment was associated with poor survival of fish (67-70%, compared to over 90% in other systems). Mandal et al. (2020) reported a growth rate of 1.52 g/day of monosex Nile tilapia stocked at 2 fish/m^2 in a feeding system in hapa.

The number of tilapia recruits in the carp-tilapia-sahar system was quite low. This is due to the piscivorous nature of the stocked sahar. Shrestha et al. (2011) reported there was a significantly lower average recruit number and weight of Nile tilapia in treatments with sahar than in tilapia monoculture. Jaiswal (2010) also showed that the average number and weight of tilapia recruits in treatments with sahar was lower than with tilapia and carp only. Water quality was not significantly affected by stocking densities of fishes in species combination of carp-tilapia-sahar polyculture in ponds, as water quality parameters did not differ significantly among treatments. Most water quality parameters were within acceptable ranges for fish culture (Boyd 1990). Results of this experiment clearly indicates production, productivity and gross margin is better in tilapia and sahar incorporated carp-polyculture and tilapia monoculture with feed and fertilized system than only carp polyculture system.

CONCLUSION

The result of the present study demonstrates that three of the culture systems (polyculture of carps with mixed sex tilapia and sahar, carps with monosex tilapia, and monosex tilapia with fertilization and feeding) performed similarly and enhanced productivity and income compared to the currently used carp polyculture system in Nepal. Tilapia either in monoculture or in polyculture proved suitable additional species in the aquaculture for Nepal. It has been confirmed that incorporating tilapia can enhance the overall performance of the carp polyculture system in Nepal. As carp polyculture is the established culture system, adding species will be easier to adopt by fish farmers. While immediate adoption of a monoculture of tilapia be more challenging for farmers who have been practicing carp polyculture for decades, indications of higher production efficiency and profit will serve as initial steps in developing that system. Inclusion of sahar in polyculture will help to conserve sahar populations as well as in controlling tilapia recruitment in mixed sex tilapia culture where monosex fry is not available.

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