

Annual Report

वार्षिक प्रतिवेदन
2080/81 (2023/24)



Government of Nepal

Nepal Agricultural Research Council

National Animal Science Research Institute

National Fishery Research Centre

Godawari, Lalitpur, Nepal

2024





Breeding activities and seed distributed of carp species at NFRC, Godawari, Lalitpur



NASRI Director Neena Amatya Gorkhali, Ph.D., Senior Scientist Shree Prasad Bista, Ph.D. of NARC and Binaya Kumar Chakraborty, Ph.D., Former Director, Department of Fisheries and Culture of Mud-Eel, Mud-Crab, Aquaculture and Management Centre, Bangladesh, visited at NFRC, Godawari, Lalitpur

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Citation:

NFRC, 2024. Annual Report 2080/81 (2023/24), National Fishery Research Centre, Godawari, Lalitpur, Nepal.

NARC Publication Serial No.: 060/081/82

Cover Photo: Office Building of National Fishery Research Centre

FOREWORD

The National Fishery Research Centre (NFRC), Godawari, Lalitpur (formerly the Fisheries Research Division, Godawari, Lalitpur) was established in 1993 following the creation of NARC in 1991. The primary goal of the Centre is to advance research, build capacity, and foster linkages and partnerships in the fisheries sector. This annual report provides a compilation summary results of the activities completed of both on-station and on-farm research works and production programs during the fiscal year 2080/81 (2023/24). The major research activities of centre was stock improvement of cultivable asala (*Schizothorax richardsonii*) based on morphological and genetic studies, fish species characterization and domestication efforts, inventory and macrohabitat studies of wetland fish fauna, evaluation of drugs for controlling pathogenic bacteria and parasites, disease surveillance and management, integrated bedding materials using nitrogen-fixing and phosphorus-solubilizing bacterial consortia to enhance carp culture production and productivity and quality assessment of fish product (physicochemical, microbial and sensory changes). Additionally, the report highlights the diversification of quality fish products and summarizes outreach research activities undertaken by the centre. The participatory research programs implemented are playing an increasingly significant role in supporting the growth of carp and trout farming across the country. It is anticipated that the findings and information in this report will assist in addressing various challenges faced by the fish farming community.

I eagerly welcome constructive feedback and valuable suggestions from relevant institutions and individuals to help us improve the quality of our work. This annual report will be very useful to policy makers, extension officers, farmers and persons who directly and indirectly involved in agriculture.

I extend my gratitude to the Executive Director and Directors of Nepal Agriculture Research Council (NARC) and continuous support to all staff members of NFRC, Godawari, Lalitpur who directly or indirectly contributed to the research and preparation of this annual report. On behalf of the National Fishery Research Centre, I also thank the farmers, associated institutions, and stakeholders for their support, provision of experimental sites, and the necessary information required for our studies.

Umita Sah
A. Chief
National Fisheries Research Centre
Godawari, Lalitpur
December 2024

ABBREVIATIONS

AFU	Agriculture and Forestry University
ANOVA	Analysis of variance
AOAC	Analysis of office association of chemist
oC	Degree centigrade
cfu	Colony forming unit
CF	Crude fiber
cm	Centimeter
CP	Crude protein
CRD	Complete randomized design
D	Depth
DF	Discriminant function
DGC	Daily growth co-efficient
DO	Dissolve oxygen
DoFD	Directorate of Fisheries Development
DOAR	Directorate of Agriculturl Research
CFPCC	Central Fisheries Promotion and Conservation Centre
e	Exponential
FAO	Food and Agriculture Organization
FCR	Feed conversion ratio
FE	Feed efficiency
FGD	Focus group discussion
FRC	Fisheries Research Centre
FY	FiscalYear
g	gram
GSI	Gastro somatic index
GPS	Geographic Positioning System
GLRP	Grain Legume Research Program
ha	Hectare
HICAST	Himalayan College of Agriculture Science& Technology
IAAA	Integrated Aquaculture-Agriculture-Animal Husbandry
IAAS	Institute of Agriculture & Animal Science
kg	Kilogram

km	Kilometer
KU	Kathmandu University
L	Liter
m	Meter
meq	Milli equivalent
ml	Milliliter
mg	Milligram
msl	Mean sea level
Mt/ton	Metrickton
NABGRC	National Animal Breeding and Genetics Research Centre
NAHRC	National Animal Health Research Centre
NANRC	National Animal Nutrition Research Centre
NFRC	National Fishery Research Centre
NS	Number of samples
NARC	Nepal Agricultural Research Council
NARI	National Agriculture Research Institute
NASRI	National Animal Science Research Institute
Ne	Effective population size
NEA	Nepal Electricity Authority
NGOs	Non-government organizations
No	Number
NTU	Nephelometric Turbidity Unit
NTFP	Non-timber forest product
O ₂	Oxygen gas
OR	Outreach Research
P	Probability
PCA	Principal Component Analysis
PER	Protein efficiency ratio
P& FD	Pasture and Forage Research Division
PPP	Public-Private-Partnership
PRA	Participatory rural appraisal
r	Correlation
R ²	Correlation coefficient
RTFRS	Tainboe Trout Fisheries Research Station

RLG	Relative length of gut
Rs	Nepali Rupees
SAA	Synthetic amino acid
SD	Standard deviation
SE	Standard Error
Sec	Second
SGR	Specific Growth Rate
SPSS	Statistical Package for Social Sciences
SWP	Silk worm pupae
TGC	Thermal growth coefficient
TPC	Total plate count
TL	Total length
TSS	Total suspended solid
TU	Tribhuvan University
µg	Microgram
µl	Microliter
µs	MicroSiemens
W	Width
Wt.	Weight
Y	Yield

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संक्षिप्त वार्षिक प्रतिवेदन

आर्थिक वर्ष २०८०/०८१ मा राष्ट्रिय मत्स्य अनुसन्धान केन्द्र, गोदावरीले ५ वटा अनुसन्धान, एक बाह्य अनुसन्धान, एक फार्म व्यवस्थापन र उत्पादन कार्यक्रमहरू सफलतापूर्वक संचालन गरेको थियो। केन्द्रको पोखरी लगायत मत्स्य पालनका भौतिक सुविधाहरूमा पानीको टङ्कारो समस्या रहेको भयतापनि स्थानीय माछाको घरेलुकरण, आनुवांशिक अध्ययन, माछामा लाग्न रोग व्याधि सम्बन्धी अध्ययन, रोगव्याधी नियन्त्रणको अध्ययन, तालतलैयामा हुने रैथाने माछाहरू र प्राणीजन्य जीवाणु अध्ययन, बायोचार अनुसन्धानबाट कार्प जातको माछाको उत्पादन बढाउने सम्बन्धी अध्ययन, पंगास र कार्प माछाबाट तयारी म.म. र फिस बलको पोषण विश्लेषण अध्ययन अनुसन्धान क्रियाकलापहरूको प्रमाणिकरण अध्ययनका साथसाथै गोदावरी स्थित केन्द्रको हाताभित्र उत्पादन कार्यक्रमहरू संचालन गरिएको थियो।

जलीय जैविक विविधता

झापा, मोरङ र सुनसरी जिल्लामा १६११ देखि २०२७ मिटर उचाइमा रहेका पाँचवटा सिमसार क्षेत्रमा माछाको जातीय विविधता Ichthyofauna र Macrohabitat को अध्ययन, साथै पानीको गुणस्तर सम्बन्धी अध्ययन गरिएको थियो। माछाको पहिचानका लागि नमुना संकलन तथा स्थानीय समुदायहरूसँगको छलफल मार्फत ती सिमसार क्षेत्रमा माछाको अवस्था र माछा पालनको प्रचुरता सम्बन्धी जानकारी लिईयो। स्वदेशी माछा प्रजातिहरूको जैविक विविधता र जल गुणस्तर सम्बन्धी अध्ययन निम्न पाँच तालहरूमा गरिएको थियो: झापा जिल्ला: बिराटपोखरी (वितर्तामोड नगरपालिका-३) र भीमसेनपोखरी (अर्जुनधारा नगरपालिका-५), मोरङ जिल्ला: वेतिनी सिमसार (उर्लाबारी-२, मङ्गलबारे) र शान्ति भुल्के सिमसार (शनिश्चरे-१, पथरी), सुनसरी जिल्ला: राष्ट्रिय सम्पदा संरक्षण ताल (रामधुनी-७)। अध्ययन गरिएका सिमसार क्षेत्रहरूमा ६ वंश, ६ परिवार र ६ वर्गका ११ माछा प्रजातिहरू पाइएका थिए। Cypriniformes वर्गका ६ प्रजातिहरू (११ प्रजातिको ५४.५५%) सबैभन्दा धेरै पाइयो। Channiformes, Gobiiformes, Anabantiformes, Synbranchiformes, Siluriformes वर्गका १/१ प्रजातिहरू (९.०९%) फेला परे। यस अध्ययन गरिएका सबै सिमसार क्षेत्रहरूमा CARP-SIS बहुसंस्कृति (CARP-SIS Polyculture) प्रणालीका लागि उच्च सम्भावना पाइएको थियो। साथै यी क्षेत्रहरूले पर्यटन प्रवर्द्धनका लागि पनि उच्च सम्भावना देखाएका छन्।

माछा संरक्षणमा स्थानीय समुदायहरूको सक्रिय सहभागिता देखिएकोले कृषिमा आधारित पर्यटन प्रवर्द्धनका लागि ठूलो सम्भावना देखिएको छ। भीमसेनपोखरी (अर्जुनधारा नगरपालिका-५, झापा) मा केज कल्चर फार्मिङ (Cage Culture Farming) को उत्कृष्ट सम्भावना पाइएको छ। यस अध्ययनले सिमसार क्षेत्रहरूको जैविक विविधता, दिगो मत्स्य पालन र पर्यटन प्रवर्द्धनका लागि महत्वलाई उजागर गर्दै स्थानीय समुदायलाई संरक्षण कार्यमा संलग्न गराउन आवश्यकतामा जोड दिएको छ।

आनुवंशिक अध्ययन

यस अध्ययनले नेपालका हिमाली नदीहरूमा पाइने चिसो पानीको महत्त्वपूर्ण माछा प्रजाति ‘स्नो ट्राउट’ ‘असला’ (*Schizothorax richardsonii*) को आकारगत र आनुवंशिक विविधता

केन्द्रित अध्ययन गरेको छ । अध्ययनका लागि माछाका नमूना म्याग्दी खोला, चेपे खोला, तादी खोला, किम्टाङ खोला र उफ्रा खोलाबाट सङ्कलन गरिएका थिए । पाँच विभिन्न खोलाबाट सङ्कलित माछाका नमूनाहरूमा गरिएको डिस्क्रिमिनेन्ट विश्लेषणले DF1 (५४.४२%) ले म्याग्दी खोला र चेपे खोलाका माछाका प्रजातिहरूलाई छुट्यायो, जबकि DF2 (४५.५८%) ले तादी खोला र उफ्रा खोलाका माछाका प्रजातिहरूलाई अलग पार्‍यो । डेनडोग्रामले म्याग्दी खोलाका माछाका प्रजातिलाई सबैभन्दा भिन्न आनुवंशिक प्रजाति देखायो, जबकि तादी खोला र उफ्रा खोलाका माछाका प्रजातिहरू एकअर्कासँग नजिकको सम्बन्ध भएका देखिए । माइक्रोसेटेलाइट मार्करहरूको प्रयोगबाट गरिएको आनुवंशिक परीक्षणले म्याग्दी खोलाका प्रजातिमा सबैभन्दा उच्च आनुवंशिक विविधता (७१.४३% पोलिमोर्फिज्म) देखाएको छ भने किम्टाङ खोलामा सबैभन्दा कम (५३.५७%) पाइएको छ । डीएनए बारकोडिङबाट प्रजाति पहिचान र संरक्षणका लागि जनसंख्या संरचनाको जानकारी प्रदान गरियो । केही प्रजातिमा आनुवंशिक विविधताको कमीले इन्ब्रिडिङको जोखिम देखाएको छ । यस अनुसन्धानले *Schizothorax* प्रजातिको जैविक विविधता संरक्षणको महत्त्वलाई जोड दिँदै, देशव्यापी दिगो व्यवस्थापन योजनाद्वारा मुख्य बासस्थान क्षेत्र (प्रजनन र पालनस्थल) को पहिचान आवश्यकतालाई औलाएको छ तथा छनोटान्मक प्रजनन योजनाहरूको माध्यमबाट यस माछाको मत्स्यपालनलाई प्रवर्द्धन गर्नुपर्ने महत्त्वलाई जोड दिएको छ ।

बायो-चारको प्रयोग गरी कार्प माछाको बृद्धि अध्ययन र बायो-चारको बहुस्थान परिक्षण
बायोचार अनुसन्धानबाट कार्प जातको माछाको उत्पादन बढाउनको लागि राष्ट्रिय मत्स्य अनुसन्धान केन्द्र, गोदावरी स्थित पोखरीहरूमा मत्स्य उत्पादक व्यवसायीले प्रयोग गर्दै आएको माछाको दानासंग बायो-चार एकीकृत बेडिंग (बायोचार+हुंगाको धुलो+हड्डीको धुलो) का साथ विभिन्न उपचार विधिमा ब्याक्टेरियल कन्सोर्टियाको प्रयोग गरी तुलनात्मक अनुसन्धान गरिएको थियो । जसमा औसत 41.09 ± 0.60 ग्रामका कार्प जातका माछाका भुराहरू विभिन्न भण्डारण घनत्व ($30,000$ र $40,000$ गोटा/हेक्टर) स्टक गरी ९० दिनसम्म बृद्धि अध्ययन गरिएको थियो ।

माछा बृद्धि नतिजा अनुसार $30,000$ गोटा/हेक्टरका साथमा बायो-चार एकीकृत बेडिंग (बायोचार+हुंगाकोधुलो+हड्डीको धुलो) सहित ब्याक्टेरियल कन्सोर्टियाको प्रयोग गरिएको उपचार बिधि ३ मा उच्चतम दैनिक बृद्धि दर (1.62 ± 0.01 ग्राम/दिन) र सबैभन्दा कम फिड रुपान्तरण अनुपात (FCR) (1.47 ± 0.02) पाइएको छ । जबकि तल्लो मानहरू (lower values) क्रमशः उपचार-1 (0.73 ± 0.01), उपचार-२ (0.91 ± 0.02), उपचार-५ (1 ± 0.01), उपचार-६ (1.05 ± 0.00), र उपचार-४ (1.38 ± 0.01) मा दैनिक बृद्धि दरका लागि थिए । त्यस्तै, उच्च मानहरू क्रमशः उपचार-1 (2.90 ± 0.07), उपचार-२ (2.50 ± 0.11), उपचार-६ (1.98 ± 0.02), उपचार-५ (1.85 ± 0.04) र उपचार-४ (1.64 ± 0.05) मा फिड रुपान्तरण अनुपातको लागि थिए । अध्ययन अवधिमा उपचारहरू बीच कुनै अक्सिजन कमि र अमोनिया विषाक्तता पाइएन ।

मत्स्य रोग अध्ययन

यस अध्ययनमा नेपालका ५ वटा जिल्लाहरू (झापा, मोरंग, सुनसरी, चितवन र नवलपरासी) का सरकारी तथा निजी हयाचरीमा पालिएको *Pangasius hypophthalmus* मा ब्याक्टेरियल रोगजनक विश्लेषण गर्न १३१ कलेजो तथा मृगौलाहरूको नमूना संकलन गरी पहिचान, लक्षण

निर्धारण र एन्टिबायोटिक प्रतिरोध क्षमता विश्लेषण गरिएको छ । चार प्रमुख ब्याक्टेरिया प्रजातिहरू (*Aeromonas* spp., *Pseudomonas* spp., *Edwardsiella* spp., र *Vibrio* spp.) पहिचान गरिएका छन्, जसमा *Aeromonas* spp. सबैभन्दा बढी देखा परेको छ । चयनात्मक र विभेदकारी अगारमा कल्चर गरेपछि, *Aeromonas* spp. (४४.४४%), *Edwardsiella* spp. (३६.३६%), *Pseudomonas* spp. (३३.३३%), र *Vibrio* spp. (२८.५७%) पत्ता लागेका छन्, साथै *Salmonella* spp. र *Citrobacter freundii* पनि फेला परेका छन् । कलेजो (liver), मिर्गौला (kidney) को तुलनामा बढी ब्याक्टेरियल संक्रमणको शिकार भएको थियो । ८ विभिन्न एन्टिबायोटिक संवेदनशीलता परीक्षण (disc diffusion) बाट Ciprofloxacin (5µg), Gentamycin (10µg), Doxycycline (20µg), र Nitrofurantoin (300µg) १००% प्रभावकारी antibiotics देखिएको थियो, तर Novobiocin लाई ९५.८३% ब्याक्टेरिया प्रजातिहरूले प्रतिरोध (resistance) देखाएका छन् । *Vibrio*, *Edwardsiella*, *Aeromonas* र *Pseudomonas* spp. विरुद्ध सबैभन्दा प्रभावकारी एन्टिबायोटिक औषधिहरू Ciprofloxacin (0.065–4 µg/ml) र Gentamycin (0.25–8 µg/ml) भएको न्यूनतम अवरोध सांद्रता Minimum Inhibitory Concentration (MIC) परीक्षणबाट पुष्टि भएको छ । एन्टिबायोटिक प्रतिरोध परीक्षणले Gentamycin र Ciprofloxacin लाई सबैभन्दा प्रभावकारी एन्टिबायोटिकका रूपमा देखायो, जबकि Doxycycline र Nitrofurantoin प्रति प्रतिरोध, विशेष गरी *Pseudomonas* spp. मा, प्रभावकारी थियो । मिनिमम इनहिबिटरी कन्सन्ट्रेशन (MIC) परीक्षणले Ciprofloxacin र Gentamycin लाई प्रभावकारी देखाए पनि विभिन्न प्रतिरोध ढाँचाहरूले एन्टिबायोटिकहरूको सावधानीपूर्वक प्रयोग आवश्यक भएको देखिएको छ ।

यस अनुसन्धानले लक्षित रोग निदान र अनुकूलित एन्टिबायोटिक उपायहरूको महत्वलाई स्पष्ट पार्दछ, जसले नेपालमा पांगासियस माछापालनलाई दिगो र प्रभावकारी बनाउन मद्दत गर्दछ ।

सहभागी प्रविधि विकास, प्रमाणीकरण र प्रवर्धन परियोजना

रातो थोप्ले रोगको रोकथाम र नियन्त्रण उपायहरूको प्रमाणीकरण गर्न र उक्त रोग नियन्त्रण गर्न पांगासियस माछाका भुरा व्यवस्थापन गरेर एक औषधि परीक्षण कार्यक्रममा पांगासियस माछा फार्म चितवन रत्ननगर-२, पञ्चकन्यामा परिक्षण गरिएको थियो ।

राष्ट्रिय मत्स्य अनुसन्धान केन्द्र, गोदावरी, ललितपुर द्वारा संचालित एक सहभागी अनुसन्धान अध्ययनमा पांगासियस माछामा रातो थोप्ले रोग नियन्त्रण गर्न प्रभावकारी पाइएका दुई उपचारहरू: सिप्रोफ्लोक्सासिन र जेन्टामाइसिन (५० मि. ग्रा/किलो माछा) तथा किसानहरूले प्रयोग गर्ने उपचार नुन (३%) + बेसार (०.५%) ।

यसरी परीक्षण गर्दा, सिप्रोफ्लोक्सासिन रोगी पंगास माछाका नमूनामा औषधि प्रयोग गरेपछि एक हप्तामा चयनात्मक माध्यममा कुनै ब्याक्टेरिया वृद्धि देखिएन । तर, जेन्टामाइसिन र नुन-बेसार उपचारमा भने ब्याक्टेरिया वृद्धि देखियो । यो वृद्धि ती उपचारहरूलाई पाँच दिनको ब्रेकपछि पुनः अर्को हप्ता प्रयोग गर्दा पनि देखिएको थियो ।

प्रमाणित प्रविधि हस्तान्तरण

प्रधानमन्त्री कृषि आधुनिकीकरण परियोजना (पीएमएमपी) कार्यान्वयन इकाईसँगको समन्वयमा रुपन्देही जिल्लाका विभिन्न स्थानहरूमा कार्प र पांगासियस माछा पालन गर्ने २५ जना माछा किसानहरूलाई राष्ट्रिय मत्स्य अनुसन्धान केन्द्र, गोदावरी, ललितपुर द्वारा अनुसन्धान र परजीवी जुकाको रोग र मुख्य रूपमा माछामा देखिने फङ्गल इयुस रोगका विभिन्न चरणहरूबारे छलफल गरी विकास गरिएको रोग व्यवस्थापन प्रविधिबारे सियारी गाउँपालिका-५, बनघुसरीमा रहेको जलदेवी सहकारीको सभा कक्षमा अभिमुखीकरण र छलफल गराइयो। सोही समयमा रोगका लागि कम्तिमा एउटा उपचार विधिमा आवश्यक पर्ने औषधिहरू १ किटमा (१० यम एल आइभरमेक्टिन, २०० ग्राम सेफालेक्सिन, २०० ग्राम भिटामिन सी, र १ लिटर बेन्जाल्कोनियम क्लोराइड (डिसइन्फेक्टेन्ट)) वितरण गरिएको थियो।

पोष्ट हार्भेष्ट

गोदावरीस्थित NFRC प्रयोगशालामा Plate Count Agar, Mannitol Salt Agar, EMB Agar, TCBS Agar र Salmonella-Shigella Agar प्रयोग गरेर सूक्ष्मजीव परीक्षण गरिएको थियो। Plate Count Agar मा ब्याक्टेरियाको संख्या ३० दिनसम्म बढेर ९० दिनमा स्थिर रहयो। Mannitol Salt Agar मा Treatment-1 र 5 मा हलोटोलरन्ट ब्याक्टेरियाको बृद्धि देखियो। EMB Agar मा Gram-negative ब्याक्टेरिया घटेको पाइयो। TCBS Agar मा Vibrio spp. Treatment-3 र 5 मा प्रभावकारी रूपमा दबाइएको देखियो। Salmonella र Shigella को जीवित रहन क्षमता मध्यम पाइयो, जहाँ Treatment-4 मा यसको उपस्थिति सबैभन्दा धेरै थियो। ३० औं दिनसम्म ब्याक्टेरियाको संख्या बढ्दै गयो र ९० औं दिनमा स्थिर रहयो, जसमा Treatment-5 मा सबैभन्दा धेरै संख्या पाइयो। Mannitol Salt Agar मा, Treatment-1 र 5 मा हलोटोलरन्ट ब्याक्टेरियाको बृद्धि ९० औं दिनसम्म महत्वपूर्ण रूपमा देखियो। EMB Agar मा सबै Treatment मा Gram-negative ब्याक्टेरियाको संख्या घटेको देखियो। TCBS Agar मा Vibrio spp. Treatment-3 र 5 मा प्रभावकारी पाइयो। Salmonella र Shigella मध्यम रूपमा जीवित रह्यो र Treatment-4 मा यसको सबैभन्दा धेरै उपस्थिति देखियो।

यस केन्द्रले आ.व. ०८०/०८१ मा कार्प जातका २१४५४५ गोटा माछा भुरा उत्पादन गरी बागमती प्रदेशको मध्य पहाडी भेगका जिल्लाहरूका कृषकहरूलाई वितरण गरिएको थियो। आ.व. ०८०/०८१ मा मत्स्य व्यवसायका विभिन्न विधामा (माछा पालन तथा पानीको गुणस्तर सम्बन्धी जानकारी) २३ जना कृषकहरूलाई सल्लाह र सुझाव दिइएको थियो।

राष्ट्रिय मत्स्य अनुसन्धान केन्द्रले व्यवसायीक माछापालन सम्बन्धी जानकारी कृषि टिभिद्वारा र मत्स्य उत्पादनमा आत्मनिर्भरता बारे अन्तर्वार्ता प्रसारण गरिएको थियो। विभिन्न जिल्लाहरूका २५७२ जना मत्स्य पालक किसान, शिक्षक र विद्यार्थीहरूले मत्स्य पालनका विभिन्न पक्ष, जैविक विविधता तथा एकिकृत मत्स्य पालन सम्बन्धी जिज्ञासा लिई यस केन्द्रमा भ्रमण गरेका थिए।

आ.व. ०८०/०८१ मा केन्द्रको कार्यक्रम संचालन गर्न रु.३,३१,९०,०००/- स्वीकृत बजेट मध्ये रु.२,९९,१९,९००.७३ खर्च भएको थियो। उक्त आ.व. मा अनुसन्धान उप उत्पादन, भुरा तथा खाने माछा, प्रशासनिक आम्दानीबाट जम्मा रु.२,१६,०६०/- राजश्व संकलन गरिएको थियो।

EXECUTIVE SUMMARY

National Fishery Research Centre (NFRC) has successfully implemented five research projects, one of each outreach, FMP and production program during fiscal year 2080/81. NFRC has executed its most of the research activities in participation with target clients. Despite of water supply problem at NFRC, Genetic study of different population of Asala, fish species characterization and domestication, inventory of fish fauna of wetlands and its macrohabitat study, study on drugs to control pathogenic bacteria and parasites, surveillance of fish disease and its management, integrated bedding with consortia of nitrogen fixing and phosphorus solubilizing bacteria on carp culture production and productivity and quality fish product diversification and breeding activities related to seed production of carps were carried out within the centre complex.

Aquatic biodiversity

Study on fish ichthyofaunal and macrohabitat including water quality was carried out in five wetlands situated in Jhapa, Morang and Sunsari districts at elevation from 1611 to 2027 msl . Fish sampling for identification as well as discussion with local communities was carried out to know the fish fauna status and its abundance in corresponding wetlands. A study was conducted on the biological diversity of indigenous fish species and aquatic qualities in a total of five lakes: Jhapa District: Biratpokhari (Birtamod Municipality-3) and Bhimsenpokhari (Arjundhara Municipality-5), Morang District: Betini Wetland (Urlabari-2, Mangalbare) and Shanti Bhulke Wetland (Shanishchare-1, Pathari), Sunsari District: National Heritage Conservation Pond (Ramdhuni-7).

As a fish fauna study, eleven species were represented by six genera, six families and six orders in the surveyed wetlands. Order Cypriniformes was constituted the species number six (54.55% out of 11 species) followed by Channiformes, Gobiiformes, Anabantiformes, Synbranchiformes, Siluriformes were constituted species number 1 (9.09%) in the assemblage. Almost all surveyed wetlands lake were found to have highest potential for “CARP-SIS Polyculture” including have tourism potential high and these lakes needs promotional activities. Additionally, the active involvement of local communities in fish conservation was observed, highlighting a high potential for promoting agriculture-based tourism. Among the surveyed lakes, Bhimsenpokhari (Arjundhara Municipality-5, Jhapa) was identified as having potential for cage culture farming.

Genetic study

The study focuses on the phenotypic and genetic diversity of snow trout, Asala, (*Schizothorax richardsonii*), a vital cold-water fish species in Nepal. Samples were collected from Five rivers: Myagdi Khola, Chepe Khola, Tadi Khola, Kimtang Khola, and Ufra Khola. Discriminant analysis of samples collected from five rivers showed DF1 (54.42%) separating Myagdi and Chepe populations, while DF2 (45.58%) differentiated Tadi and Ufra. The dendrogram highlighted Myagdi as

the most genetically distinct population, with Tadi and Ufra fish populations being closely related. DNA profiling using microsatellite markers showed the highest genetic diversity (71.43% polymorphism) in Myagdi Khola populations, while Kimtang Khola showed the lowest (53.57%), indicating varied adaptability. DNA barcoding confirmed the species' identity and provided insights into population structures, aiding conservation strategies. However, reduced genetic diversity in some populations indicates risks of inbreeding. The research highlights the need for selective breeding and conservation programs, focusing on genetic traits and stock improvement. The findings emphasize the importance of conservation of Schizothorax Species diversity, nationwide sustainable management plan need for key habitat characterization (i.e. breeding and nursing ground) identification, and and advancing aquaculture of this species through selective breeding program.

Fish production, using Bio-char

Integrated bedding with consortia of nitrogen fixing and phosphorus solubilizing bacteria on carp culture production and productivity.

The result of effect of Bio-char Integrated bedding with consortia of nitrogen fixing and phosphorus solubilizing bacteria on carp polyculture showed that the fishes of treatment 3 that is supplementary feeding + bedding (biochar, bone meal and stone dust) + Microbial consortia showed highest daily growth rate (DGR) (1.82 ± 0.01 g/day) and lowest feed conversion ratio (FCR) (1.47 ± 0.02). While, lower values were for DGR in T1(0.73 ± 0.01), T2 (0.91 ± 0.02), T5(1 ± 0.01), T6(1.05 ± 0.00) and T4(1.38 ± 0.01) respectively. Similarly, higher values were for FCR in T1 (2.90 ± 0.07), T2 (2.50 ± 0.11), T6 (1.98 ± 0.02), T5(1.85 ± 0.04) and T4(1.64 ± 0.05) respectively. No oxygen depletion and ammonia toxicity problem was recorded among treatments during study period.

The free-living nitrogen fixing bacteria could have provided nitrogen source to phytoplankton at bottom. Biological nitrogen fixing bacteria transform toxic nitrogenous product to less toxic product via nitrogen cycle. Phosphorus solubilizing bacteria mineralize the embedded phosphorus, thus utilized by planktons and fish itself. Hydroxyl apatite provides the calcium supplement. Although, Preliminary results using bio-char bedding with consortia of nitrogen fixing and phosphorus solubilizing bacteria amendment had better performance on fish growth and pond health. Further research work on different polyculture fish species combination and extended culture period should be carried out thus promising results could be upscale in farmer's level.

Fish disease study

Identification, characterization and antimicrobial susceptibility pattern of pathogenic bacteria isolated from (*Pangasius hypophthalmus*).

The study investigates the bacterial pathogens affecting cultured *Pangasius hypophthalmus* in Nepal's terai regions (Jhapa, Morang, Sunsari, Chitwan and Nawalparasi) of government and private hatchery and 131 samples were collected

from liver and kidney of pangas, highlighting their identification, characterization, and antimicrobial susceptibility. Following culturing on selective and differential agar, *Aeromonas* sps. (44.44%), *Edwardsiella* sps. (36.36%), *Pseudomonas* sps. (33.33%), and *Vibrio* sps. (28.57%) were found along with *Salmonella* sps. and *Citrobacter freundii*. Results also shows that Liver was more exposed to bacterial infection as compared to kidney. 8 different Antibiotic sensitivity tests done through disc diffusion revealed that Ciprofloxacin (5µg), Gentamycin (10µg), Doxycycline (20µg) and Nitrofurantoin (300µg) were 100% effective drugs but Novobiocin was resisted by (95.83%) strains. The most efficient antibiotics against strains of *Vibrio*, *Edwardsiella*, *Aeromonas* and *Pseudomonas* sps. were found to be Ciprofloxacin (0.065–4 µg/ml) and Gentamycin (0.25–8 µg/ml) according to Minimum Inhibitory Concentration (MIC) assays. Four major bacterial species (*Aeromonas* sps., *Pseudomonas* sps., *Edwardsiella* sps., and *Vibrio* sps.) were identified, with *Aeromonas* sps. being the most prevalent. Antimicrobial susceptibility tests revealed Gentamycin and Ciprofloxacin as the most effective antibiotics, while resistance to Doxycycline and Nitrofurantoin was notable, especially in *Pseudomonas* sps.

The results showed a key for the targeted use of or less widespread implementation of antimicrobial intervention programs in aquaculture, while stressing regular monitoring as an essential measure to efficiently combat infections caused by these pathogenic microbes.

Participatory technology development, verification and dissemination project

Verification of red spot disease prevention and control measures in Pangasius fish. A drug response trial has been set up by managing fry to control red spot disease in pangasius fish at Ramesh Wagle, a pangasius fish farmer located in Ratnanagar-2, Panchakanya, Chitwan district.

In a participatory research study conducted by NFRC Godawari, two treatments that were found to be effective in controlling red spot disease in pangasius fish were ciprofloxacin and Gentamycin 50 mg/kg fish and the treatment used by the farmers, salt (3%) + turmeric (0.5%). When tested in fish farming farms, no bacterial growth was found in the samples of the ciprofloxacin group after one week of drug treatment on selective media, but bacterial growth was found in the Gentamycin and salt-turmeric treatments and this growth was observed when these treatments were used again after a five-day break for the next week.

Hand over the verified technology

In coordination with the Prime Minister's Agricultural Modernization Project (PMAMP) Implementation Unit, twenty-five fish farmers rearing carp and pangasius fish in different places in Rupandehi district were given orientation and discussion on the disease management technology developed by the National

Fisheries Research Centre, Godawari, Lalitpur after researching and discussing various stages of parasitic lice diseases and fungal Eus diseases that mainly appear in fish at the meeting hall of Jaldevi Cooperative located in Siyari Rural Municipality-5, Banghusari. At the same time, medicines required for at least one treatment method for the disease were distributed in 1 kit (10 ml of Ivermectin, 200 grams of Cephalixin, 200 grams of Vitamin C and 1 liter of Benzalkonium Chloride (disinfectant)).

Post Harvest

Microbial tests were conducted at NFRC Laboratory, Godawari, lalitpur, Nepal, using Plate Count Agar, Mannitol Salt Agar, EMB Agar, TCBS Agar, and Salmonella-Shigella Agar. Bacterial counts on Plate Count Agar increased until Day 30 and plateaued by Day 90, with Treatment-5 showing the highest counts. On Mannitol Salt Agar, halotolerant bacteria grew significantly in Treatments 1 and 5 by Day 90. EMB Agar showed a decline in gram-negative bacteria across all treatments. *Vibrio* sps. was effectively suppressed in Treatments 3 and 5 on TCBS Agar. *Salmonella* and *Shigella* showed moderate survival, with Treatment-4 exhibiting the highest persistence. Fish momo prepared with 100% and 80% pangasius fish (T-1 and T-2) demonstrated better sensory and microbiological stability and maintained high quality during 30 days of frozen storage at -20°C.

1. WORKING CONTEXT

National Fishery Research Centre, Godawari is located in Godawari -3, Lalitpur District, Bagmati Zone at 1500 msl. The centre expands from east to west in foothill terrain surrounded by community forest from three sides. Spring water flowing to downstream is the sole source of irrigation to the aquaculture facilities of the division. Aquaculture research and development works are mostly implemented in division's own complex. Technology verification and outreach research are conducted across eco-regions and development regions in collaboration with Fisheries Research Stations located in different parts of the country and target groups (Annex 1.1)

The water quality, particularly the water temperature, of fish ponds and raceways of the centre is suitable for raising cold water fish species (Annex 1.2). Although suitability of water quality, the research and development work in aquaculture has been severely constrained by the volume of water available during dry months. At present the division is facing its hard time with respect to availability of irrigation water. Most of the spring water sources have been diverted by the local people for drinking water purpose. The centre has relied on rearing facilities of FRCs and farmers for conducting research in aquaculture.

Approximately 5% of the total area of the country is occupied by different freshwater aquatic habitats. Rivers in Nepal cover an estimated area of 395,000 hectares. Similarly, a number of small to medium sized lakes in various parts of the country cover 5,000 hectares and about 1,500 hectare of small reservoirs have been constructed in the country. In addition, there is a considerable amount of surface area present in village ponds (8700 hectare) and irrigated paddy field covering about 398,000 hectares. It is estimated that about 4-5% of the irrigated area in Terai region are low lying, generally unsuitable for crops cultivation, and can suitably be developed into fish ponds. Major aquaculture systems adopted are carp polyculture in ponds, lakes and enclosures. Cage culture of herbivorous carp species and common carp in rice- fish culture are common practices. A change from extensive systems to intensive-farming methods is currently occurring in aquaculture system in the country. At present, technology of carp farming in ponds has been widely disseminated in the southern part of the country because warm climatic conditions in these areas. Trout farming in hilly region is slowly expanding among the farmers.

The National Fishery Research Centre and Fisheries Research Stations of Nepal Agricultural Research Council (NARC) have developed a number of technologies for culture of carps, tilapia, catfishes and trout through their research and development efforts. While some of these technologies are meant for small farmers and medium entrepreneurs, some others can be adopted for large scale operations, possibly with a focus on exports. Intensive trout fish culture technologies developed in other countries have been successfully adapted to

suitable national besides these centres works on conservation of indigenous fishes conditions and are now readily available for use.

Despite of these efforts, average per capita fish consumption is only 3.9 kg in Nepal (2022/023). Wide variation exists in fish protein consumption among people living in different ecological regions because of regional imbalance in fish production and poor accessibility of technology. National average fish consumption completely masks the poor fish availability to people living in hills (0.473 kg/caput) and mountains (0.186 kg/ caput). Terai people have higher accessibility to fish (3.474 kg/caput), since major portion of aquaculture production (approx. 95%) comes from this warm region. Low productivity of fish from all aquaculture systems. The major emerging problems and key researchable issues identified in fisheries across eco-region are as follows:

Challenges

- ◆ Water resources underutilized/unutilized
- ◆ Limited fish species diversity in aquaculture
- ◆ Inadequate fish farming technologies suited to different ecological/climatic region
- ◆ Aquaculture commercialization
- ◆ Aquaculture practices in natural waters
- ◆ Genetic degradation of aqua-cultured fish species
- ◆ Climate change

Research needs in fisheries

- ◆ Finding the breeding ground of cultured species in the natural waters of Nepal
- ◆ Studying the genetic quality of cultured indigenous species collected from natural water bodies
- ◆ Study the impact of climate change on fish and fisheries
- ◆ Study the impact of Damming on major rivers
- ◆ Study the impact of Other human interventions on major river system
- ◆ Develop breeding techniques for economically important and threatened fish species for restocking/ stock enhancement (Sahar, Asla, Katle etc.)
- ◆ Appropriate harvesting technique for different types of water bodies

Research needs in aquaculture

- ◆ More intensive technical package for warm water fish production.
- ◆ Species diversification in different culture system.
- ◆ Genetic improvement of cultured species
- ◆ Technique to increase seed survival rate.

- ◆ Year-round management of fish seed to meet the demand of farmers
- ◆ Cage culture technique for various types waterbodies
- ◆ Developing fish packing technique with local material for transportation/ marketing
- ◆ Use of local herbs for disease control
- ◆ Substitution of fish meal with plant material
- ◆ Development of better management practice for aquaculture in local environment.

2. INTRODUCTION

2.1 Introduction

National Fishery Research Centre previously known as Fisheries Research Division became mandatory since 1991 after establishment of the Nepal Agricultural Research Council (NARC). Its primary mission was to enhance fish production, preserve aquatic resources for food security, nutritional needs, and to create avenues for employment and income generation. In 1993, the National Fishery Research Centre (NFRC) was officially established under the National Animal Science Research Institute (NASRI) within NARC to further these objectives.

Working area of NFRC covers stock improvement of Asala for aquaculture, identification of fish health problem and their control measures, formulation of low cost feed for better economic returns, participatory research, recommendation of suitable species for both cold water and warmwater fish culture and technological package development for improvement of warm and cold water aquaculture. Within the country, its major working partners are AFU, IAAS, TU, KU, CFPCC, NEA, HICAST, CMDN, and GFRI. Fisheries Research Centre has the mandate to develop, implement and co-ordinate multidisciplinary and multi-commodity adaptive research in collaboration with national and international institutions and organizations to achieve the goals, purpose and objectives mentioned below.

2.2 Goal

- ◆ Enhance food security and livelihood of the Nepalese nationals without undermining natural resources.

2.3 Objectives

- ◆ To improve performance of commercially exploited stocks and, identify and evaluate suitable fish species for major agro-ecological zones.
- ◆ To develop sustainable aquaculture, resource management and production technologies.
- ◆ To identify, evaluate and conserve native fish germplasm for future use.
- ◆ To introduce, evaluate and promote productive aquatic animal and plants.
- ◆ To enhance participation of artisanal fishers and other communities in aquatic biodiversity conservation.
- ◆ To establish national and international linkages for strengthening fisheries management and aquaculture research.

2.4 Strategies

- ◆ Research on aquaculture system intensification
- ◆ Verification of trout in cold water region

- ◆ Enhancement of aquaculture product diversity (introduction and evaluation of cultivable species)
- ◆ Use of biotechnological tools in fish breeding and stock improvement
- ◆ Fish base inventory for rational use of aquatic resources
- ◆ Integration of Aquaculture-Agriculture-Animal Husbandry (IAAA) for resilience of farming system
- ◆ Research on key emerging issues (adaptation to climate change)
- ◆ Public-Private-Partnership (PPP) for technology generation and dissemination & aquatic resource conservation
- ◆ Strong linkage and networking to national and international organizations

2.5 Current thrust areas for research

- ◆ Aquaculture intensification through aeration
- ◆ Production package of tilapia and Asian River Catfish
- ◆ Genetic improvement of carp and trout
- ◆ Parasites control strategies for carps and trout
- ◆ Fish preservation and value addition
- ◆ Farming system resilience
- ◆ Resilience of climate change
- ◆ Feed improvement and substitution on animal protein for trout feed
- ◆ Domestication of native fish species
- ◆ Impact assessment of intensive aquaculture

2.6 Infrastructure and facilities

NFRC premises cover an area of 6.5 ha. It is comprised of office building, laboratories, feed house, hatchery, residential quarters occupying approximately 2500 m²; 26 ponds and 19 cemented raceways approx. 2.6 ha; feeding canal 1000 m and black topped road 700 m. spring water running down from the community forest foot hills is the sole source of irrigation in the farm. It has water quality and disease diagnostic lab to support its clients (Annex 2.1 & 2.2).

2.7 Organization structure and human resource

The Centre has 28 approved employees of which 18 staffs are working in the place. At present three scientist, four technical officers, two administrative and nine supporting staffs are serving the NFRC, Godawari (Annex 2.3).

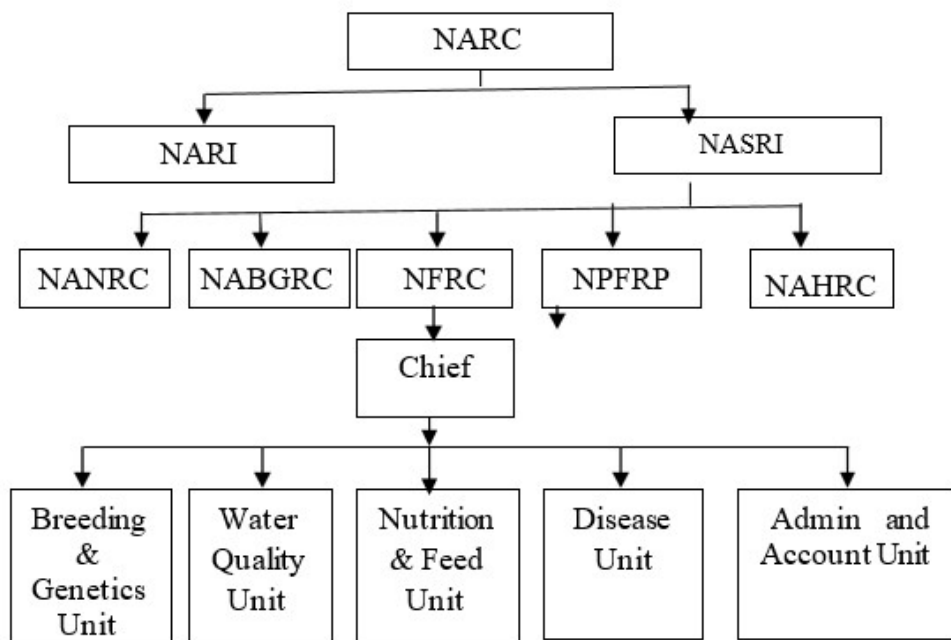


Figure 1: Organization structure with Organogram of NFRC

3. RESEARCH HIGHLIGHTS

3.1. Aquatic Biodiversity

3.1.1 Correlation in fish assemblage, zooplankton and water quality variables of the wetlands situated in Koshi Province at Jhapa, Morang, and Sunsari districts of Nepal

Assessing the suitability of aquatic fish fauna relies on factors such as macrohabitat, plankton diversity, and physico-chemical water parameters within wetland ecosystems. As the deterioration of habitats continues to escalate globally, preserving species richness and biodiversity has emerged as a pivotal concern within conservation biology (Jones et al., 2004). Many shallow lakes are situated in lowland regions, often accompanied by dense human populations, leading to substantial environmental impacts. Numerous studies underline this issue (Giller et al., 2004). In Nepal, approximately 5.5% of the land area, totaling 743,563 ha, is occupied by wetlands encompassing diverse types such as rivers, lakes, reservoirs, paddy fields, and more (Bhandari, 1992; DOAD, 1992). Ranging from glacial lakes at high altitudes to river floodplains and ox-bow lakes, Nepal's wetlands play a significant role. These solitarily scattered lakes constitute roughly 3% of the available water (Sharma, 1997). A survey of the wetlands in the terai region of Nepal identified 163 wetlands across five development regions, with the far-western region hosting the most (38%), followed by the central (23%), western (21%), eastern (11%), and mid-western (7%) development regions (Bhandari, 1998). While wetlands hold vital importance for biodiversity, comprehensive examinations of fish fauna and zooplankton within Nepalese wetlands, notable on a global scale, remain scarce. Utilizing patch-based methodologies becomes imperative to gather up-to-date insights within wetland systems. These approaches offer the potential to enhance comprehension and facilitate conservation efforts for wetland fish fauna, concurrently supporting local communities through potential aquaculture ventures. Generally, untangling the intricate relationship between fish species diversity, zooplankton, and wetland habitats poses challenges (Gorman and Karr, 1978; Schlosser, 1982). To fathom the underlying intricacies, this study employs a macro habitat approach to scrutinize the habitat prerequisites of freshwater fish fauna and zooplankton diversity. Collaborative investigations focusing on fish assemblage structure, zooplankton populations, and their habitat requisites in Nepalese wetlands have been lacking. Therefore, the main objective of this present study was to assess the relationship of variable among fish species in relation to physico-chemical parameters of five wetlands.

Materials and methods

Study on fish ichthyofaunal and macrohabitat including water quality was carried out in five wetlands situated in Jhapa, Morang and Sunsari districts at elevation from 1611 to 2027 msl . Fish sampling for identification as well as discussion with local communities was carried out to know the fish fauna status and its abundance

in corresponding wetlands. Besides nature induced and anthropogenic related threats to native fish species was also assessed. Fish fauna and macrohabitat study Fish assemblage and macrohabitat were recorded at each surveyed site. Macro habitat features, such as lake depth, lake width, riparian cover, in-wetland cover, habitat types and substrates were assessed. Fishes were photographed in the field and then preserved in 10% formalin for identification using morphometric and meristic characteristics in Laboratory of National Fisheries Research Centre, Godawari. The meristic and morphometric measurements are based on the methods by Day, 1875-78; Talwar and Jhingran, 1991. Preserved fish specimens were displayed in Fish Museum of National Fishery Research Centre under Nepal Agricultural Research Council, Kathmandu, Nepal. A study was conducted on the biological diversity of indigenous fish species and aquatic qualities in a total of five lakes:

1. National Heritage Conservation Pond (Ramdhuni-7)

Fish fauna and macrohabitat study

Fish assemblage and macrohabitat were recorded at each surveyed site. Macro habitat features, such as lake depth, lake width, riparian cover, in-wetland cover, habitat types and substrates were assessed. Fishes were photographed in the field and then preserved in 10% formalin for identification using morphometric and meristic characteristics in Laboratory of National Fishery Research Centre, Godawari. The meristic and morphometric measurements are based on the methods by Day, 1875-78; Talwar and Jhingran, 1991. Preserved fish specimens were displayed in Fish Museum of National Fishery Research Centre under Nepal Agricultural Research Council, Kathmandu, Nepal.

Results

Diversity of fish fauna

As a fish fauna study, eleven species were represented by six genera, six families and six orders in the surveyed wetlands. Order Cypriniformes was constituted the species number six (54.55% out of 11 species) followed by Channiformes, Gobiiformes, Anabantiformes, Synbranchiformes, Siluriformes were constituted species number 1 (9.09%) in the assemblage. Almost all surveyed wetlands except lake were found to have highest potential for “CARP-SIS Polyculture” including have tourism potential high and these lakes needs promotional activities. Additionally, the active involvement of local communities in fish conservation was observed, highlighting a high potential for promoting agriculture-based tourism. Among the surveyed lakes, Bhimsenpokhari (Arjundhara Municipality-5, Jhapa) was identified as having potential for cage culture farming.

Table 1: Water quality parameters of surveyed wetlands in Jhapa, Morang and Sunsari districts, Nepal

Water quality parameters	Birat Pokhari, Jhapa	Bhimsen Pokhari, Jhapa	Betani Simshar, Morang	Shanti Bhulke Simshar, Morang	Rastriye Sampada Lake, Sunsari
Water temperature, °c	22.6	23	22.5	22	20.6
Dissolve oxygen, mg/L	8.4	8.4	8.1	9.3	7.2
pH	8.6	8.9	8.3	8.5	8.2
Ammonia, mg/L	0.01	0.4	0.01	0.02	0.1
Total alkalinity, mg/L	104	Low	85	152	163
Total hardness, mg/L	99	132	232	261	198
Total phosphate, mg/L	0.02	0.02	0.02	0.02	0.03
Total Nitrite (No ₂) mg/L	0.01	0.01	0.02	0.01	0.01
Total Nitrate (No ₃) mg/L	0.02	0.01	0.02	0.01	0.01
Transparency (cm)	20-25	30-35	38	17-18	20

Discussion

In the present study, a comprehensive inventory of aquatic fish fauna and their respective macrohabitat of the five wetlands of Jhapa, Morang and Sunsari districts were initiated. An assessment of fish biodiversity unveiled that human-induced activities did not appear to impact the native fish habitats within the examined wetland areas. These surveyed wetlands hold significant cultural and religious value, and discussions with local communities revealed that these wetlands do not serve as sources of food production. Notably, the surveyed wetlands remain undisturbed, with no discernible disruptions in the fish fauna attributed to agricultural runoff containing pesticides. The relationship between habitat and fish fauna revealed that cyprinid species displayed broad preferences for the available habitats. Macrohabitat approaches have the potential to significantly increase understanding and ability to safeguard the fish fauna of wetlands. Safeguarding the conservation of wetland resources can be effectively achieved through community engagement. The global recognition of involving communities in wetland resource conservation and management is well-established (Williams, 2002). Within the context of Nepal, the foremost concerns pertaining to wetland conservation revolve around the deficiency and lack of enforcement of pertinent legislation. The absence of a dedicated wetland-specific policy within the country is also notable. To ensure forthcoming advancements, the adoption of a macrohabitat approach stands as a promising means to characterize fish assemblage structure. The formulation of National Wetland Policies directed towards water resources and the preservation of aquatic life should prioritize granting local and traditional communities rights over resource

conservation and sustainable utilization. The Agriculture Development Strategy (ADS, 2013) should acknowledge the potential of wetlands for environmentally friendly, dynamic fish farming, while safeguarding the native fish fauna within their habitats to contribute to rural livelihoods and the economy. Enlisting community participation is pivotal for effective future conservation initiatives and sustainable management practices. At the grassroots level, youth groups specialized in biodiversity monitoring should be established to oversee wetland conditions effectively. Additionally, it is imperative that benefits accrued from Nepalese lakes are channeled towards educating impoverished communities in the vicinity. Elevating awareness and understanding about the significance of wetland conservation on a global scale should be actively promoted among individuals, planners, and policy-makers.

3.2. Genetic Resources

3.2.1 Phenotypic variance of snow trout (*Schizothorax richardsonii*) collected from different rivers of Nepal

Asala, *Schizothorax* species, is highly prevalent in Nepal's mountain waters, demonstrating its strong adaptability to reproduce and thrive in cold, fast-flowing rivers. However, in recent years, populations have declined in many areas due to factors such as the introduction of exotic species, dam construction, overfishing, pollution, habitat loss, and climate change, posing a constant threat to their survival. Therefore, it is imperative for government bodies to initiate conservation efforts and action plans. Nepal hosts around 10 species of Asala, with *Schizothorax raraensis*, *S. macrophthalmus* and *S. nepalensis* being endemic to Lake Rara in the Karnali River drainage. Efforts to domesticate and breed indigenous Asala species (*Schizothorax* spp.) have begun at Fishery Research Centre under NARC. However, attempts to artificially breed Asala in controlled farm conditions have been sporadic, possibly due to challenges in achieving proper growth and maturity synchronization with breeding seasons. Previous studies on Asala domestication indicate a slow growth rate (15 to 17 grams per year), which currently limits its economic viability for farming with existing technological knowledge (FRD, 2012). Establishing initial stocks with greater genetic diversity and desirable traits, followed by selective breeding programs, could enhance their performance. Studies using landmark-based truss measurements have identified significant morphometric variations among *S. richardsonii* populations in different rivers of Nepal, such as those in the Indrawati and Khudi rivers, the Melamchi and Phalaku rivers, and the Sabha and Tadi rivers (FRD, 2016; Wagle et al., 2015). These findings underscore the potential for exploiting this species in cold-water aquaculture, warranting further investigation and follow-up.

Consequently, a recent study aimed to identify stocks of snow trout (*S. richardsonii*) collected from five different rivers of Nepal by analyzing morphometric measurements related to size and shape. This research represents a crucial step towards effectively developing and managing this species. A total of 145 samples of Asala were gathered, and their sample size, average total length, and body weight are detailed in Table 1.

Table 1: Collection of Asala samples from different rivers

Location of fish samples	Sample size	Mean total length (mm)±SD	Mean body weight (g)±SD
Myagdi Khola, Myagdi (MKM)	30	186±27.72	63.07±44.12
Lumjung, Chepe Khola, (LCK)	30	214.13±28.89	84.64±30.86
Taadi Khola, Nuwakot (TKN)	30	125.21±27.72	19.56±16.88
Kimtang Khola, Nuwakot (KKKN)	25	122.48±26.55	17.2±10.79
Ufra Khola, Nuwakot (UKN)	30	137.53±20.03	23.21±13.06

Morphometric and Truss Analysis

Each specimen underwent detailed morphometric measurements using dial and Vernier calipers, where 18 point-to-point measurements (Fig 3.2.1.1) and 11 landmarks defining 22 distances on the body (Fig 3.2.1.2) were recorded. To assess body size differences across habitats, univariate analyses (ANOVA) were initially conducted. Given the considerable overlap in size distributions between habitats, the collected data were then compiled into a database for subsequent factor analysis. Standardization of all measurements followed methods outlined by Elliott et al. (1995) to remove any effects from allometric growth. Size-adjusted measurements (Madj) were computed using the formula,

$$\text{Madj} = M (L_s / L_o)^b;$$

where M represents the original measurement, Madj the adjusted measurement, Lo the total length (TL) of the fish, and Ls the overall mean TL across all fish samples. Parameter b, specific to each character, was derived from observed data as the slope of the log-transformed M plotted against log-transformed Lo, utilizing data from all fish groups.

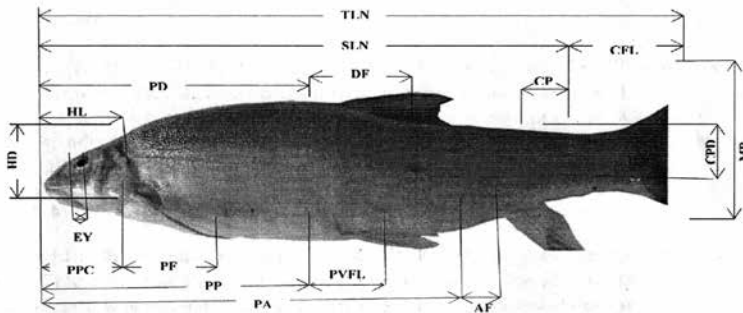
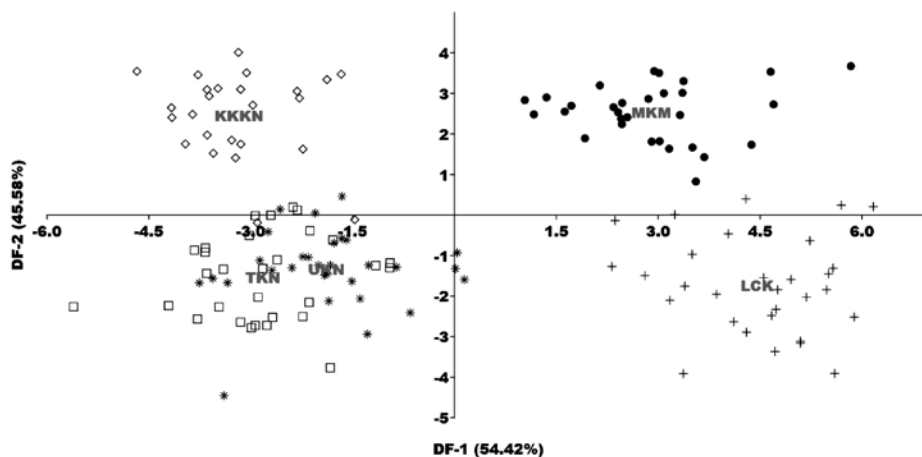


Figure 3.2.1.1. External morphology of *Schizothorax richardsonii* showing the in the texts.

Discriminant Functional Analysis

The transformed data underwent discriminant analysis to investigate phenotypic differences among populations. This analysis aimed to predict the habitat of origin for each individual. Principal Component Analysis (PCA) and Discriminant Function (DF) were computed using PAST version 4.03. In traditional morphological measurements, DF1 explained 54.42% of the total variance, effectively separating the Myagdi Khola, Myagdi (MKM) and Lumjung, Chepe Khola (LCK) populations from the remaining populations. DF2, accounting for 45.58% of the total variance, distinguished the Taadi Khola, Nuwakot (TKN) and Ufra Khola, Nuwakot (UKN) populations from the other three populations



Pop	Notations		
MKM	Dot	Pop 1=	Myagdi Khola, Myagdi (MKM)
LCK	Plus	Pop 2=	Lumjung Chepe Khola (LCK)
TKN	Square	Pop 3=	Taadi Khola, Nuwakot (TKN)
KKKN	diamond	Pop 4=	Kimtang Khola, Nuwakot (KKKN)
UKN	star	Pop 5=	Ufra Khola, Nuwakot (UKN)

Figure 3.2.1.2. Discriminant function analysis scores (DF) of conventional morphometric characters of *S. richardsonii*.

Discussion

Observations of morphometric characteristics have revealed distinct differences in *S. richardsonii* from Myagdi Khola, Myagdi (MKM), and Kimtang Khola, Nuwakot (KKKN), compared to specimens from three other rivers in Nepal. These variations in morphometry could be attributed to geographic and topographic disparities among these river systems. These morphological differences observed in *S. richardsonii* are crucial for understanding its biodiversity and represent an initial step towards utilizing this species in aquaculture and other stock enhancement programs. Morphometric variations within species are significant for assessing population structure and identifying distinct stocks.

Truss Network Analysis offers a refined approach compared to traditional morphometric datasets, providing a more systematic geometric characterization of fish shape and demonstrating enhanced capability in describing inter-specific shape differences. However, additional studies employing molecular markers are needed to further investigate genetic differentiation among these populations, which would contribute to a more comprehensive understanding of the observed morphological variations.

3.2.2 Population Structure and Genetic Variation of *Schizothorax richardsonii* in Nepal Using COI Barcoding and Microsatellite Analysis

Schizothorax richardsonii, also known as snow trout or Asala, is a vital cold-water fish species found in the Himalayan River systems, including those in Nepal. It thrives in fast-flowing rivers at altitudes between 300 and 3,000 meters, making these environments rich in biodiversity. The fish plays a key role in the local economy and is important for the livelihoods of communities relying on fisheries for sustenance.

However, the population of *S. richardsonii* has been declining due to factors like overfishing, habitat loss, pollution, and infrastructure projects, particularly hydropower developments. These pressures have altered the natural flow of rivers, directly affecting this species. Additionally, climate change is exacerbating the situation by impacting river ecosystems, which further threatens the survival of *S. richardsonii*. Therefore, efforts to conserve this species are becoming increasingly necessary.

Genetic Diversity of *Schizothorax richardsonii*

The genetic diversity of *Schizothorax richardsonii* is crucial for its ability to adapt to environmental changes. Studies using molecular markers like RAPD and microsatellites have shown that this species has a moderate to high level of genetic variation, which is key to its survival in fluctuating environments. Genetic diversity allows populations to be more resilient to threats like habitat fragmentation and climate change. International research has demonstrated that preserving genetic diversity is essential for the long-term conservation of species. A loss in genetic diversity can lead to reduced adaptability and increased vulnerability to diseases or environmental stresses. In Nepal, maintaining the genetic diversity of *S. richardsonii* is critical to its continued survival. Conservation strategies should focus on genetic monitoring to prevent inbreeding and preserve this valuable resource for future generations.

Fish sample collection

Specimens of *S. richardsonii* were collected from Taadi Khola, Nuwakot (TKN), Ufra Khola, Nuwakot (UKN), Lumjung, Chepe Khola (LCK), Myagdi Khola, Myagdi (MKM), and Kimtang Khola, Nuwakot (KKKN) of Nepal using gill nets, cast nets, and local traps. A minimum of thirty specimens were collected from each habitat for body measurements, except for Kimtang Khola, Nuwakot (KKKN), where 25 specimens were used.

DNA extraction and Purity

About 50 mg of fin tissue was sterilized using ethanol and then dried on tissue paper. DNA extraction was performed using the Wizard Genomic DNA Purification Kit with minor adjustments. The purity of the extracted DNA was evaluated spectrophotometrically (Eppendorf Bio Photometer plus) at 260/280

nm. DNA samples with OD (optical density) values ranging from 1.7 to 1.9 were chosen for PCR analysis, while samples with OD values outside this range were processed to address contamination by RNA or proteins.

Table 3.2.2.1. List of Fish genotypes used for DNA profiling.

S.N.	Locations	Total Sample	DNA extracted sample
1	Myagdi Khola, Myagdi	30	25
2	Lumjung, Chepe Khola, (LCK)	30	25
3	Taadi Khola, Nuwakot (TKN)	30	24
4	Kimtang Khola, Nuwakot (KKKN)	25	24
5	Ufra Khola, Nuwakot (UKN)	30	25

PCR Amplification

Amplification of each DNA samples was performed in a 15µl reaction mixture on a Master Cycler Gradient (Bio-Rad, USA). One negative control was performed for each set of amplifications with the following parameters: 4 min of initial denaturation at 94°C followed by 35 cycles of 45s at 94°C, 45s at locus specific annealing temperatures and 60s at 72°C and final extension at 72°C for 5 min. Finally, PCR products were separated through 1.5% (w/v) Agarose gel (Promega) stained with molecular grade ethidium bromide (Promega) in 1X TBE buffer (Promega) for 1hr 45 mins, while 100bp DNA ladder (Solis Biodyne) used as standard marker. The gel image was documented and analyzed in UV Trans-illuminator unit. (Geldoc, Major Science).

For species identification, genic variation and sequencing, 30 different SSR markers were adapted from different literature as well as designed manually. Among them 14 markers were successfully amplified.

Table 3.2.2.2 List of successfully amplified primers:

S. N	Primers name	Forward primer	Reverse primer
1	SRLP-004	GCACCTGTTACCATCTCTCA	GGAAACGCTTGGTATGCAGG
2	SROP-010	CAAAGCTTCTCCCCTACTGCT	AGGTCTGGTCCATGCCAAATCC
3	SRM-017	TGACAATCTCGTTTACAAGGCTC	GCTGCGGCTTCATTACGATG
4	SRM-134	GATGATGAGGAGGAGGAGGA	CCCTGTTTCTGCCAGTCATT
5	Tt1 B01	CATGGACCAAATTACAAGGATTT	AACCTGTGAGGGATGTCCAG
6	Tt1 F02	GAGGGGCATTTTGTCTTGA	GCTTCCCCTCATAAGCCTTC
7	Tt2 F04	ATGCCAGCTACAGGTCCAAT	CGTGTGTATGATGCCACCTC
8	Tt2 F07	GAGACGACTCTAGTCGCTGACA	GTGTGGCCAGTGTAGCTGAA
9	Tt1 C10	GCTGAAGCAGGTGAATCTGA	TGATGCCTGTCAAACCTGTG
10	Cytb	TGACTTGAAAAACCACCGTTG	CTCCGATCTCCGATTACAAGAC
11	ATPase6/8	AAAGCRTYRGCCTTTAAAGC	GTTAGTGGTCAKGGGCTTGRTC
12	CO-II	AAAGGAAGGAATCGAACCCCC	GCTCATCAGTGGAGGACGTCTT
13	COX1-F1R1	AACACAAAAGACATTGGTAC	GGTGTCCAAAGAATCAGAAT
14	COX1-F2R2	ATTCTGATTCTTTGACACC	TTAATTGATTGAATTGACAA

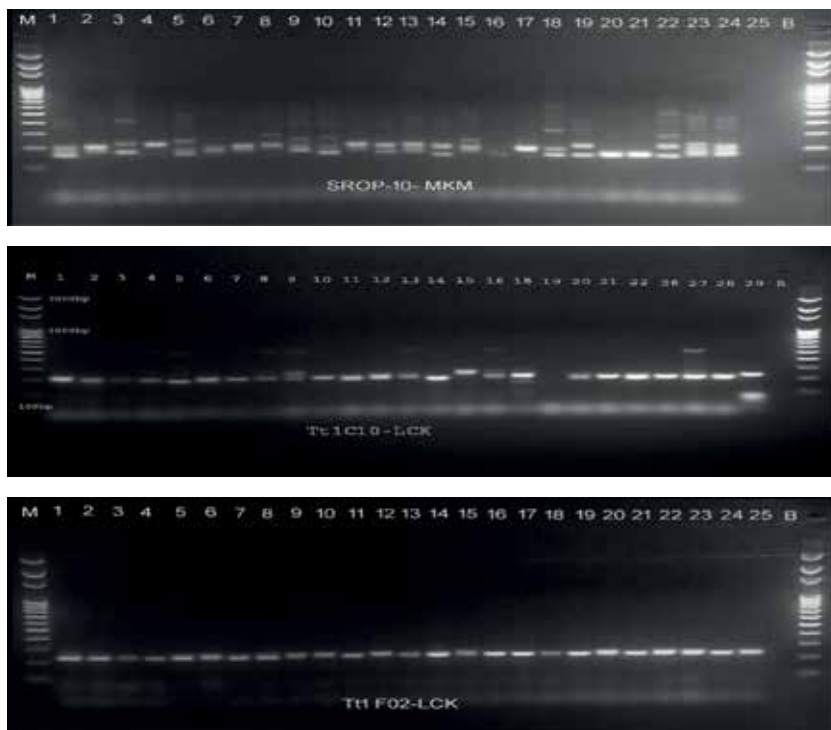


Figure 3.2.2.1 Gel images of Asala DNA samples

Table 3.2.2.3. Summary of Genetic variation statistics analyzed from POPGENE32 software

S. N	Locus	Sam- ple size	Na*	Ne*	h	I	Polymor- phism (%)
1	LCK	25	1.7143 (0.9512)	1.3987 (0.5288)	0.203 (0.2571)	0.3205 (0.4084)	57.17
2	MKM	25	2.1429 (1.069)	1.8827 (0.9767)	0.3438 (0.2976)	0.5643 (0.5108)	71.43
3	KKN	25	1.4286 (0.5345)	1.3563 (0.4631)	0.192 (0.2431)	0.2738 (0.3443)	53.57
4	TKN	25	1.7143 (0.9512)	1.5195 (0.6833)	0.2313 (0.2915)	0.3681 (0.4701)	57.14
5	UKN	25	1.8571 (1.069)	1.4492 (0.6062)	0.2144 (0.2722)	0.3614 (0.4574)	57.14

* Na = Observed number of alleles

* Ne = Effective number of alleles [Kimura and Crow (1964)]

* h = Nei's (1973) gene diversity

* I = Shannon's Information index [Lewontin (1972)]

Nei's unbiased measures of genetic identity and genetic distance, See Nei (1978) Genetics 89:583-590

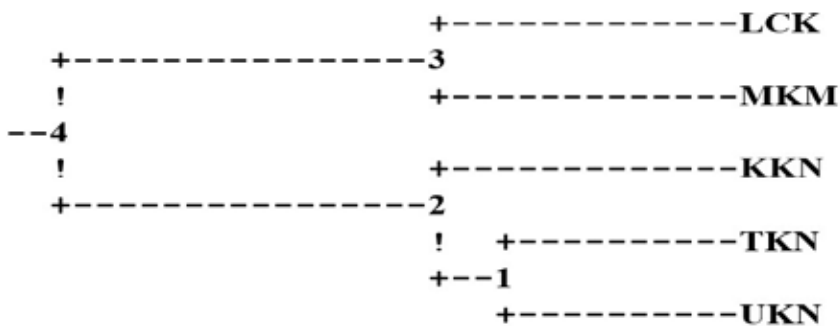
Higher diversity at loci like MKM, with a polymorphism of 71.43%, indicates a robust population capable of responding to environmental changes, while lower diversity at loci like KKN suggests a reduced capacity for adaptation and a potential risk of inbreeding or genetic drift. These findings are significant for conservation efforts, as populations with greater genetic variation are more resilient to extinction, and for breeding programs, where maximizing genetic diversity ensures healthier and more adaptable offspring. Understanding the variation across loci helps guide efforts to maintain genetic health in both natural and managed populations.

Table 3.2.2.4. Nei's Unbiased Measures of Genetic Identity and Genetic distance

Pop ID	LCK	MKM	KKN	TKN	UKN
LCK	***	0.8204	0.6344	0.5603	0.5817
MKM	0.198	***	0.4192	0.3025	0.311
KKN	0.4451	0.8695	***	0.9335	0.9329
TKN	0.5792	1.1957	0.0688	***	0.9536
UKN	0.5418	1.168	0.0694	0.0475	***

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Populations with high genetic identity, such as TKN and UKN, are closely related and share a large portion of their genetic material, indicating low differentiation, which can be important for maintaining genetic integrity in conservation or breeding programs. In contrast, the high genetic distance between MKM and other populations, especially TKN and UKN, suggests significant genetic divergence. This can signal historical isolation or adaptation to different environments, which is crucial for species conservation, as highly divergent populations may harbor unique genetic traits that are valuable for biodiversity. Understanding these relationships helps guide strategies to maintain genetic diversity and manage population health.



The dendrogram illustrates the genetic relationships among five populations (LCK, MKM, KKN, TKN, UKN), showing that TKN and UKN are the most genetically similar, followed by KKN, which clusters closely with them. LCK and MKM are more genetically distinct, with the greatest divergence seen between them and the TKN-UKN-KKN group. The significance of this clustering lies in its implications for conservation and genetic management. Populations with closer genetic relationships, like TKN and UKN, may share important adaptive traits, while the divergence of LCK and MKM highlights unique genetic differences that could be valuable for preserving overall genetic diversity within the population. The insights gained from the genetic analysis using Microsatellite markers and Nei's gene diversity index are pivotal for understanding population dynamics and guiding conservation strategies. These markers are highly polymorphic and provide a detailed view of genetic variation within and among populations, making them particularly useful for assessing genetic diversity. The high polymorphism seen quantified through these markers, aligns with a robust Nei's gene diversity value, indicating a population capable of adapting to environmental changes. Conversely, the lower diversity observed reflects reduced Nei's gene diversity, signaling a higher risk of inbreeding and decreased adaptive potential. low Nei's gene diversity, suggests a shared genetic background that may facilitate adaptive traits crucial for survival. Meanwhile, the significant genetic distance emphasizes unique genetic characteristics that contribute to biodiversity.

By identifying populations with high diversity and unique genetic traits, conservationists can prioritize their management and breeding programs accordingly. These findings emphasize the need for targeted conservation strategies that preserve distinct genetic lineages and promote overall genetic health, ultimately ensuring the resilience and long-term survival of species in changing environments.

DNA Barcoding of *Schizothorax richardsonii*

DNA barcoding is a valuable technique for identifying species and aiding in conservation efforts. It uses genetic markers, particularly from the mitochondrial COI gene, to create a unique "barcode" for each species. For *Schizothorax richardsonii*, DNA barcoding has been instrumental in confirming its identity and distinguishing it from closely related species in the Himalayan region. Barcoding has also helped clarify the population structure of *S. richardsonii* across various river systems. This information is essential for designing targeted conservation measures to protect genetically distinct populations. Moreover, barcoding provides a baseline for ongoing research, making it easier to monitor population changes over time, thereby contributing to effective conservation management.

1. PCR and Sequencing Outcomes: Predicted fragment sizes:

- COX1-F1R1: 690 bp (observed: 630–690 bp)
- COX1-F2R2: 850 bp (observed: 820–890 bp)

The study had employed Sanger Sequencing to analyze the mitochondrial COI gene (Cytochrome Oxidase Subunit I) for species identification. DNA sequences had been obtained from amplified PCR products, which had been sent to BGI Genomics, Hong Kong, for sequencing. Raw sequences had been examined using Chromas to trim low-quality regions, and alignment had been performed using Clustal W to ensure consistency. Species identification had been confirmed through NCBI BLAST analysis by comparing sequences with the database. A phylogenetic tree had been constructed using MEGA software to determine relationships among the samples, and DNA barcodes had been generated to provide unique identifiers for each species. This methodology had established a robust framework for accurate molecular authentication of *Schizothorax* species.

Results and Discussion

1. Species Identified: All samples belonged to the *Schizothorax* genus. Most frequently identified species:

- ◆ *Schizothorax esocinus*
- ◆ *Schizothorax plogastomos*
- ◆ *Schizothorax nepalensis*

3.3. Bio-char and its Multilocation trial

3.3.1 Integrated Bedding with Consortia of Nitrogen fixing and Phosphorus Solubilizing Bacteria on Carp co-culture production and productivity

Aquaculture necessitates the fine-tuning of nutritional strategies to effectively enhance the health, growth, and reproductive capabilities of fish, ensuring a dependable supply of aquatic sustenance in the upcoming years (M Hixson, 2014). Given that fish are monogastric creatures, their growth heavily relies on a well-balanced nutrient intake. While this is typically achieved through feed supplements, the growing demand for both feed and food due to the expanding population calls for innovative approaches to enhance fishery output. An alternative avenue involves bolstering phytoplankton and zooplankton populations. However, fostering the growth of these planktonic organisms also hinges on nutrient availability. For instance, the application of nitrogen sources, such as urea, to stimulate phytoplankton growth results in the release of ammonia as part of the nitrogen cycle. Nonetheless, this process also leads to elevated nitrous oxide emissions—an influential greenhouse gas. Counteracting this, the utilization of biochar has been observed to curtail nitrous oxide production (Harter et al., 2016), thereby mitigating its adverse impact on pond water quality. Biochar serves to capture fecal matter, while the ensuing bacterial proliferation competes with pathogens and establishes an anoxic environment in their niche. Conversely, deficiencies in calcium and phosphorous pose significant challenges within the realm of commercial aquaculture. These deficiencies give rise to stunted growth, skeletal anomalies, inefficient feed conversion, and inadequate bone mineralization (Sugiura, Hardy, & Roberts, 2004). Furthermore, the phosphate present in feed or other sources often assumes an insoluble form, rendering it inaccessible to planktonic organisms. Enhancing the presence of nitrogen-fixing bacteria within pond substrates not only augments the nitrogen supply to plankton (Karlson et al., 2015), but also facilitates the delivery of readily absorbable nitrogen to fish through intestinal absorption. Additionally, the introduction of phosphate solubilizing bacteria heightens phosphate intake. The increased solubility of phosphate, combined with the availability of reduced nitrogen in water, extends these benefits to zooplankton as well. Consequently, an optimized feed intake is projected to expedite fish weight gain over a shorter span, driven by these enhanced nutritional dynamics.

Materials and Methods

For the study, bio-char, i.e., byproduct of industries (beer, rice mill, brick industry etc) was procured from the Iceberg Beer factory located at Godawari, stone dust from local stone crusher factory and bone meal from supplier from Chitwan. The isolated nitrogen fixing and phosphate solubilizing bacteria consortia was procured from ministry of social development, Young Scientist Encouragement Fund, Bagmati Province. The consortia of nitrogen fixing and phosphorus

solubilizing bacteria was amplified in disease laboratory using Luria and Nutrient Broth supplemented with sodium acetate and other minerals. After collection of bio-char, stone dust and bone dust, bacteria consortia amplification bio-char bedding was set in CRD design. The treatments were

T1: (Supplementary feeding +Fertilization (Farm yard manure 3000 kg/ha, Urea 23 kg/ha, DAP 15 kg/ha: 30,000/hac)

T2: (Supplementary feeding + Bedding (Biochar, Bone Meal and Stone Dust) + Fertilization: 30,000/hac)

T3: (Supplementary feeding +Bedding + Microbial consortia: 30,000/hac)

T4: (Supplementary feeding +Bedding +Fertilization+ Microbial consortia: 30,000/hac)

T5: (Supplementary feeding +Bedding + Microbial consortia: 40,000/hac)

T6: (Supplementary feeding +Bedding +Fertilization+ Microbial consortia: 40,000/hac)

Bedding material stocking Ratio

Stone Dust: Biochar = 2:1

Bone Meal (20% P approx.) = 18% P of final harvest weight

Bacteria consortia stock solution: Molasses 0.05 g/L and Probiotic 0.004 g/L

Fish species: Common carp 35%, Rohu 20%, Naini 20%. Silver carp 15%, and Bighead carp 10%

Feeds and Feeding: Daily hand feeding were provided on the basis of morning pond water temperature monitored using a digital pen thermometer and feeding rate be adjusted according to (Miyatake, 1997). The total daily ration of feed was divided into three equal portions and provided at 8:00 am; 12:00 noon and 4:00 pm. Feed was adjusted according to growth checkup performed biweekly by measuring total body weight and the total length of fish of 10% of the fish sample (Fig. 3.3.1.1)



Figure 3.3.1.1. Fish growth biweekly during research period.

The preliminary result of effect of bio-char integrated bedding with consortia of nitrogen fixing and phosphorus solubilizing bacteria on carp polyculture showed that the fishes of treatment 3 that is supplementary feeding + bedding (biochar, bone meal and stone dust) + microbial consortia showed highest daily growth rate (DGR) (1.82 ± 0.01 g/day) and lowest feed conversion ratio (FCR) (1.47 ± 0.02). While, lower values were for DGR in T1 (0.73 ± 0.01), T2 (0.91 ± 0.02), T5 (1 ± 0.01), T6 (1.05 ± 0.00) and T4 (1.38 ± 0.01) respectively. Similarly, higher values were for FCR in T1 (2.90 ± 0.07), T2 (2.50 ± 0.11), T6 (1.98 ± 0.02), T5 (1.85 ± 0.04) and T4 (1.64 ± 0.05) respectively. No oxygen depletion and ammonia toxicity problem was recorded among treatments during study period. The growth trend of fishes during study is shown in fig. 3.3.1.2.

The free-living nitrogen fixing bacteria could have provided nitrogen source to phytoplankton at bottom. Biological nitrogen fixing bacteria transform toxic nitrogenous product to less toxic product via nitrogen cycle. Phosphorus solubilizing bacteria mineralize the embedded phosphorus, thus utilized by planktons and fish itself. Hydroxyl appetite provides the calcium supplement. Although, Preliminary results using bio-char bedding with consortia of nitrogen fixing and phosphorus solubilizing bacteria amendment had better performance on fish growth and pond health. Further research work on different polyculture fish species combination and extended culture period should be carried out thus promising results could be upscale in farmer's level.

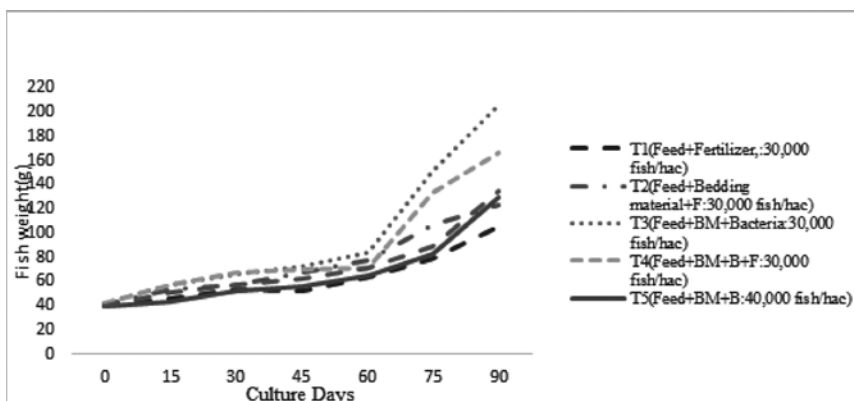


Figure 3.3.1.2: Growth trend of fishes during study period

3.3.2 integrated bedding with consortia of nitrogen fixing and phosphorus solubilizing bacteria on carp co-culture production and productivity multilocation trial at DoAR Khajura, Banke

For the study, bio-char, i.e., byproduct of industries (beer, rice mill, brick industry etc) was procured from the beverage factory located at Khajura Banke, Nepalgunj, stone dust from local stone crusher factory and bone dust from supplier from Banke. The isolated nitrogen fixing and phosphate solubilizing bacteria

Consortia was procured from Ministry of social Development, Young Scientist Encouragement Fund, Bagmati Province. The Consortia of nitrogen fixing and phosphorus solubilizing bacteria was amplified in disease laboratory using Luria and Nutrient Broth supplemented with sodium acetate and other minerals. After collection of bio-char, stone dust and bone dust, bacteria consortia amplification bio char bedding was set in CRD design. The treatments were

T1: (Supplementary feeding +Fertilization (Farm yard manure 3000 kg/ha, Urea 23 kg/ha, DAP 15 kg/ha: 30,000/hac)

T2: (Supplementary feeding + Bedding (Biochar, Bone Meal and Stone Dust) + Fertilization: 30,000/hac)

T3: (Supplementary feeding +Bedding + Microbial consortia: 30,000/hac)

T4: (Supplementary feeding +Bedding +Fertilization+ Microbial consortia: 30,000/hac)

T5: (Supplementary feeding +Bedding + Microbial consortia: 40,000/hac)

T6: (Supplementary feeding +Bedding +Fertilization+ Microbial consortia: 40,000/ha)

Bedding material stocking Ratio

Stone dust: Biochar = 2:1

Bone meal (20% P approx.) = 18% P of final harvest weight

Bacteria consortia stock solution: Molasses 0.05 g/L and Probiotic 0.004 g/L

Fish species: Common carp 35%, Silver carp 35%, Rohu 20% and Naini 10%.

Feeds and feeding: Daily hand feeding were provided on the basis of morning pond water temperature monitored using a digital pen thermometer and feeding rate be adjusted according to (Miyatake, 1997). The total daily ration of feed was divided into three equal portions and provided at 8:00 am; 12:00 noon and 4:00 pm. Feed was adjusted according to growth checkup performed biweekly by measuring total body weight and the total length of fish of 10% of the fish sample (Fig. 3.3.2.1)



Figure 3.3.2.1. Fish growth at monthly basis during research period at DoAR Khajura, Banke

Result

The preliminary multilocation trial result of effect of bio-char integrated bedding with consortia of nitrogen fixing and phosphorus solubilizing bacteria on carp polyculture showed that the fishes of treatment 3 that is supplementary feeding +bedding (biochar, bone meal and stone dust) + fertilization (FYM, Urea and DAP) + microbial consortia showed highest growth 315.69 ± 16.74 g during 90 days research period at DoAR Khajura, Banke, Nepalgunj. No oxygen depletion and ammonia toxicity problem was recorded among treatments during study period. The growth trend of fishes during study is shown in fig. (3.3.2.2.)

The free living nitrogen fixing bacteria could have provided nitrogen source to phytoplankton at bottom. Biological nitrogen fixing bacteria transform toxic nitrogenous product to less toxic product via nitrogen cycle. Phosphorus solubilizing bacteria mineralize the embedded phosphorus, thus utilized by planktons and fish itself. Hydroxyl appetite provides the calcium supplement.

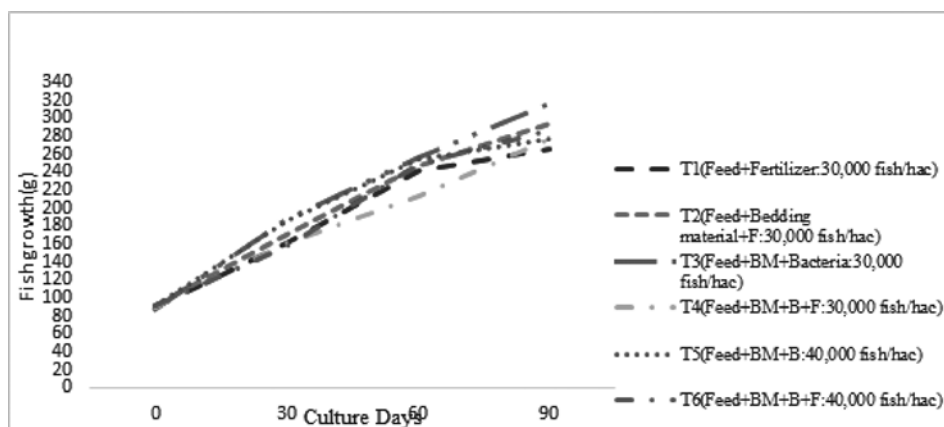


Figure 3.3.2.2 Growth trend of fishes during study period at DoAR Khajura, Banke.

Although, Preliminary results using bio-char bedding with consortia of nitrogen fixing and phosphorus solubilizing bacteria and fertilizer amendment had better performance on fish growth and pond health. Further research work on different polyculture fish species combination and extended culture period should be carried out thus promising results could be upscale in farmer's level.

3.4 Fish Disease

3.4.1. Identification, Characterization and Antimicrobial Susceptibility Pattern of Pathogenic Bacteria Isolated from Cultured *Pangasius hypophthalmus* of different Terai regions (Chitwan, Nawalparasi, Sunsari, Jhapa and Siraha) of Nepal.

Introduction

Pangasius hypophthalmus, also known as the striped catfish or pangasius, is extensively cultured across Asia, including Nepal, due to its omnivorous diet, rapid growth, high stocking density tolerance, ease of culture, disease resistance, and strong market demand. Pangas fish is capable of surviving in various environmental conditions, aided by a unique respiratory system allowing it to tolerate low oxygen levels. However, the intensive farming practices associated with pangasius often lead to outbreaks of bacterial infections, posing significant economic challenges. Common bacterial pathogens affecting Pangas include *Aeromonas* sps., *Pseudomonas* sps., *Streptococcus* sps., *Flavobacterium* sps., *Edwardsiella* sps., and *Vibrio* sps.

This study understands the bacterial threats affecting pangasius aquaculture which involves the identification of the specific types of bacterial presence, characterizing their biological and genetic traits and determining their responsiveness to different antibiotics. These efforts aim to provide insights that can guide effective disease management strategies, ensuring the health and sustainability of pangasius farming and aquaculture industry.

Material and methods

Sample collection, isolation & screening of pathogenic bacteria

A total of one hundred thirty-one bacterial swab samples (symptomatic and asymptomatic) of liver and kidney of *P. hypophthalmus* were taken for isolation and identification of the target pathogen causing the diseases. Fish sample were collected from eight different farms located in Nawalparasi, Chitwan, Siraha, Jhapa and Sunsari (Table 3.4.1.1). After which the fishes were taken for anesthesia using 50-100 mg/L clove oil (Javahery et al., 2012) then subsequently sacrificed for the sample collection. In accordance to the guideline manual for the fish sampling, in an aseptic condition using a sterile cotton swab, sample were collected from the kidney and liver section of the sacrificed fishes. These cottons swabs were then transferred in screw capped test tube and store in 4°C.

Table 3.4.1.1 Fish swab sample collection

S.N	Sample name location	Kidney		Liver		Total samples
		Symptomatic	Asymptomatic	Symptom- atic	Asymptom- atic	
1	Nawalparasi (RS)	5		5		10
2	Nawalparasi (AP)	5	5	5	5	20
3	Bhandara (B), Chitwan	5		5		10
4	Chitwan (S)	5	5	5	5	20
5	Farm ponds					7
6	Siraha (RC)	5	5	5	5	20
7	Jhapa	3	5	3	5	16
8	Sunsari (Tarahara)	5	5	5	5	20
9	Chitwan private farm	5		4		9

Swab samples were enriched in Brain Heart Infusion Broth (BHI) as a master culture at 37°C for 24 hrs. For the selective isolation of suspected pathogens, the master culture was streaked on different selective media which include *Aeromonas* Selective Agar (M1890-500G) at 35°C for 24 hrs., Cetrimide Agar (MH024-500G) at 37°C for 24 hrs., *Edwardsiella ictaluri* Medium (KM0026-500G) at 32°C for (24-48) hrs., Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS) (M89-500G) at 35°C for 24 hrs., and Yersinia Isolation Agar (M564- 500G) at 30°C for (24-48) hrs.

**Figure 3.4.1.1** swab sample collection

Biochemical test for bacteria identification

The isolated bacteria were primarily characterized based on their colony morphology (shape, margin, color, size, texture, and elevation). Subsequently, the suspected colony was further subculture on BHI agar for 18-24 hours at 37°C, resulting in a pure culture. Following this step, biochemical testing was performed to definitively identify the specific bacteria.



Figure 3.4.1.2 Analytical Profile Index test results for *Aeromonas* spp. (A), *Pseudomonas* spp. (B), *Vibrio* spp. (C) & *Edwardsiella* spp. (D).

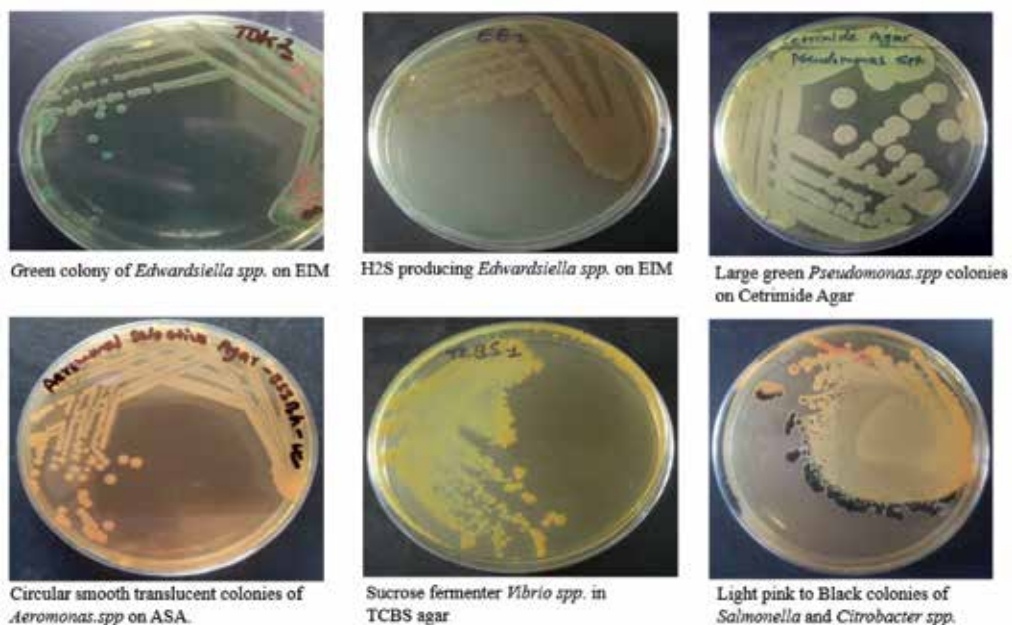


Figure 3.4.1.3 Identified bacterial pathogens in different selective medium

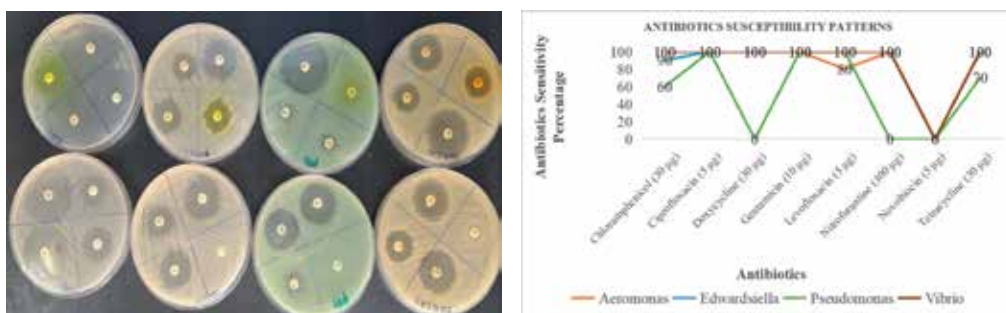


Figure 3.4.1.5 AST of different pathogens against different antimicrobial agents.

Table 3.4.1.2 Analytical profile index test results of different isolated pathogens

S.N	Test	<i>A. hydrophila</i>	<i>P. aeruginosa</i>	<i>Vibrio</i> sps.	<i>Edwardsiella</i> sps.
1	ONPG	-ve	-ve	-ve	-ve
2	Lysine utilization	-ve	-ve	-ve	+ve
3	Ornithine utilization	-ve	-ve	-ve	+ve
4	Urease	+ve	-ve	+ve	+ve
5	Phenylalanine De-amination	-ve	+ve	-ve	D
6	Nitrate reduction	+ve	-ve	+ve	D
7	H ₂ S production	-ve	D	D	-ve
8	Citrate utilization	-ve	+ve	+ve	+ve
9	Voges Proskauer's	-ve	-ve	+ve	-ve
10	Methyl red	+ve	+ve	-ve	-ve
11	Indole	+ve	-ve	+ve	-ve
12	Malonate utilization	+ve	+ve	+ve	-ve
13	Esculin hydrolysis	+ve	+ve	+ve	+ve
14	Arabinose	-ve	-ve	+ve	-ve
15	Xylose	-ve	+ve	-ve	-ve
16	Adonitol	+ve	-ve	-ve	-ve
17	Rhamnose	+ve	-ve	-ve	-ve
18	Cellobiose	-ve	-ve	-ve	-ve
19	Melibiose	-ve	-ve	-ve	-ve
20	Saccharose	-ve	-ve	+ve	-ve
21	Raffinose	-ve	-ve	-ve	-ve
22	Trehalose	-ve	-ve	+ve	-ve
23	Glucose	+ve	+ve	+ve	-ve
24	Lactose	-ve	-ve	-ve	-ve
25	Oxidase	+ve	+ve	+ve	-ve
26	Catalase	+ve	+ve	+ve	+ve
27	O/F	F	O	F	F

Antibiotic sensitivity test (AST) Profiling

After the identification and characterization of the bacteria, antimicrobial susceptibility testing (AST) was performed using the disk diffusion method. The antibiotics that were administered for the AST include Chloramphenicol (30µg/disc), Novobiocin (30µg/disc), Tetracycline (30µg/disc), Ciprofloxacin (5µg/disc), Gentamicin (10µg/disc), Doxycycline Hydrochloride (30µg/disc), Levofloxacin (5µg/disc) and Nitrofurantoin (300µg/disc). The antibiotic-impregnated discs were placed on an agar plate inoculated with the bacteria. After 24 hrs of incubation at 37°C, the antibiotics diffused outward and created zones of inhibition around the discs where bacterial growth was prevented.

The diameter of the inhibition zone was measured in millimeters (mm) using a ruler, and the results were compared with reference to the standard provided by the Clinical Laboratory Standards Institute to determine whether the bacteria's susceptibility was sensitive, intermediate, or resistant to each antibiotic tested.

Table 3.4.1.3 Antibiotic sensitivity test on isolated bacteria from Pangas fish.

Antibiotic Disc	<i>Pseudomonas aeruginosa</i>	<i>Vibrio</i> sps.	<i>Aeromonas hydrophila</i>	<i>Edwardsiella</i> sps.
Ciprofloxacin(5mcg)	+++	++	+++	+++
Gentamycin (10mcg)	+++	+++	+++	+++
Doxycycline (30mcg)	+++	+++	+++	+++
Tetracycline (30mcg)	+	+++	+++	+++
Novobiocin (5mcg)	-	-	-	+
Levofloxacin (35mcg)	++	++	+++	++
Nitrofurantoin (300mcg)	-	+	+	+
Chloramphenicol (30mcg)	+	+++	+++	++

-: no inhibition, +: inhibitory zone less than 14mm, ++: inhibitory zone between 12-15 mm, +++: inhibitory zone equal 18 mm or above

Among the antibiotics used, Gentamycin (100%) and Ciprofloxacin (100%) showcased the broad-spectrum activity against all the pathogens. While Chloramphenicol (87.5%), Levofloxacin (95%) and Tetracycline (92.5%) displayed different range of sensitivity depending upon the bacterial genus whereas, Doxycycline hydrochloride (75%) and Nitrofurantoin (75%) shows moderate sensitivity. On the other hand, Novobiocin showed the resistance against all the pathogens tested. Some pathogens particularly *Pseudomonas* sps. shows resistance to certain antibiotics like Doxycyclin and Nitrofurantoin.

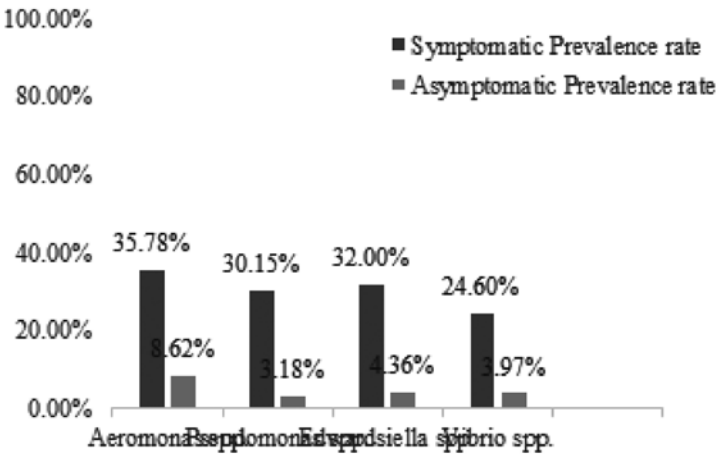


Figure 3.4.1.6 Prevalence rate of pathogens.

Based on the morphological and biochemical analysis, the presences of four different pathogenic bacteria had been found, where, *Aeromonas* sps. (44.44%) had the highest prevalence rate in both symptomatic and asymptomatic sample. Which is followed by the *Edwardsiella* sps. (36.36%) and *Pseudomonas* sps. (33.33%) having the similar prevalence patterns while, *Vibrio* sps. (28.57%) had the lowest prevalence rate.

Minimum Inhibitory concentration (CLSI)

Minimum iInhibitory Concentration (MIC) is the lowest concentration of an antimicrobial agent required to inhibit the visible growth of a microorganism, and it is essential for guiding effective antibiotic therapy. Determined through methods like broth or agar dilution, MIC values help identify whether a pathogen is susceptible, intermediate, or resistant to specific antibiotics, thus influencing treatment decisions, especially in the context of multidrug-resistant infections. The results can vary based on testing methods and conditions, making standardization important for accurate interpretation.

The MIC was determined following the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI). The MIC tests were performed using three antibiotics i.e., Ciprofloxacin, Gentamycin and Doxycycline to assess the distribution of Minimum Inhibitory Concentration (MIC) for major pathogenic bacteria isolated from infected pangas fish.

The Minimum Inhibitory Concentration (MIC) results for the bacterial isolates were assessed using three antibiotics: Ciprofloxacin, Gentamicin, and Doxycycline. Ciprofloxacin and Gentamicin exhibited greater antimicrobial activity in comparison to Doxycycline.

Table 3.4.1.4 MIC results of different pathogens against different antibiotics

Bacterial species	Antibiotics	MIC(µg/ml)	Breakpoint(µg/ml)	Interpretation
<i>Edwardsiella</i>	Ciprofloxacin	0.25	S:≤0.25, I: 0.5, R:≥1	Susceptible
	Gentamycin	0.25	S:≤4, I: 8, R:≥16	Susceptible
	Doxycycline	2	S:≤2, I:4, R:≥8	Susceptible
<i>Aeromonas</i>	Ciprofloxacin	0.5	S:≤0.25 , I:0.5 , R:≥1	Susceptible
	Gentamycin	0.25	S:≤4, I:8, R:≥16	Susceptible
	Doxycycline	4	S:≤2, I:4 , R:≥8	Intermediate
<i>Pseudomonas</i>	Ciprofloxacin	4	S:≤0.5, I: 1 , R:≥2	Resistance
	Gentamycin	2	S:≤4, I:8 , R:≥16	Susceptible
	Doxycycline	64	S:≤2, I:4 , R:≥8	Resistance
<i>Vibrio</i>	Ciprofloxacin	0.25	S:≤0.5, I: 1 , R:≥2	Susceptible
	Gentamycin	0.25	S:≤4, I: 8, R:≥16	Susceptible
	Doxycycline	2	S:≤2, I:4 , R:≥8	Susceptible

The MIC of Ciprofloxacin was determined to be 0.25 µg/ml for *Edwardsiella* and *Vibrio* species, 0.5 µg/ml for *Aeromonas*, and 4 µg/ml for *Pseudomonas*. Likewise, Gentamicin demonstrated an MIC of 0.25 µg/ml for *Edwardsiella*, *Vibrio* and *Aeromonas*, and 2 µg/ml for *Pseudomonas*. In contrast, Doxycycline exhibited higher MIC values, with 2 µg/ml for *Edwardsiella* and *Vibrio*, 4 µg/ml for *Aeromonas* and 64 µg/ml for *Pseudomonas*.

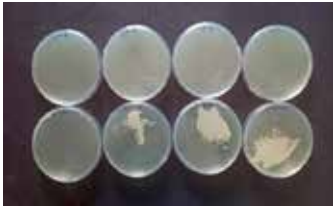


Fig: MIC of ciprofloxacin against *Aeromonas*



Fig: MIC of gentamycin against *Vibrio*

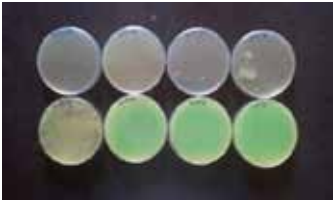
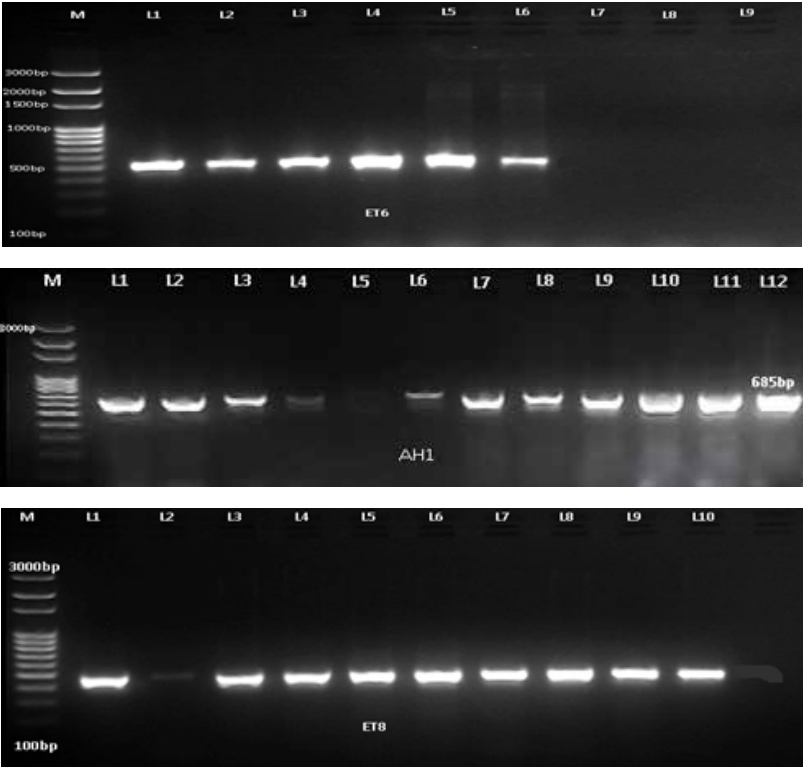


Fig: MIC of doxycycline against *Pseudomonas*

Figure 3.4.1.7 Minimum Inhibitory Concentration of Antibiotics

DNA Extraction

Genomic DNA of isolates was extracted using the Bacterial Genomic DNA kit (Pure Link Genomic DNA Mini Kit Thermo Fisher Scientific) following the manufacturer’s instructions. Using a spectrophotometer, quantification of the isolated bacterial DNA was performed subsequently the absorption ratio (A260/A280) of DNA samples were found to be of pure DNA.



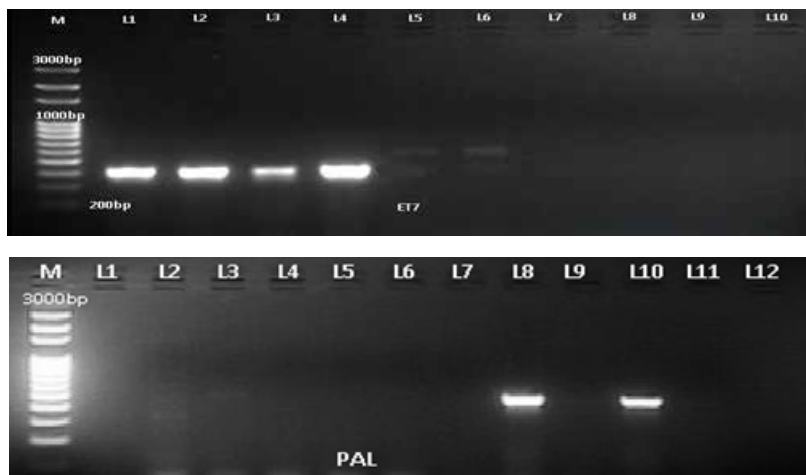


Figure 3.4.1.8 PCR amplicons produced with ET6, ET7, ET8, AH1 and PAL primer sets, were displayed on an Agarose gel under UV transilluminator.

PCR Profiling

Several sets of specific primer (Table 3.4.1.2) were used for the detection of *Aeromonas*, *E. ictaluri*, *Pseudomonas* and *Vibrio* sps., isolated from Pangas fish. Two microliters of the DNA template in a total volume of 15ul reaction mixture (Pro-mega master mix, Nuclease free water, forward and reverse primers) was reacted on a Master cycler gradient (Bio-Rad, USA) to the manufacturer's instructions. The PCR amplification consisted of an initial denaturation step of 95°C for 15min, followed by 35 cycles of 94°C for 30s, 59°C for 90s, and 72°C for 6s, and a final extension at 72°C for 10 min (AH1 and PAAS, Altinok et al. 2008). Also, another thermo-cycler was set as initial denaturation step at 94°C for 4min, followed by 35 cycles of 94°C for 1 min, 57°C for 1 min, 72°C for 1.5 min and a final extension at 72°C for 7 min (PsuA, Tripathy et al. 2006). Another thermo-cycler was set as initial denaturation step at 95°C for 10 min, followed by 40 cycles of 95°C for 15s, 60°C for 1 min, 72°C for 1 min and a final extension at 72°C for 5 min (ESC1 and ESC2, Griffin et al. 2011). Another PCR reaction was initiated with a denaturation of 5 min at 95°C, followed by 45 cycles of 95°C for 30s, 51°C for 30s, 72°C for 1 min and a final extension at 72°C for 10 min (AP4 and rpoN-ang, Pinto et al. 2017). PCR amplicons were analyzed by 2.5% agarose gel electrophoresis stained with ethidium bromide (Pro-mega), after which the gels were visualized and captured using Gel Doc, Major Science.

Under the UV trans-illuminator, in the gel image, “M” lane represents the 100bp DNA ladder while the lane “L” indicates the PCR amplicons. An expected 685 bp amplification product was observed in ten strains of *Aeromonas hydrophila*, while only two strains of *Pseudomonas aeruginosa* produced an amplicon of 500 bp. In *Edwardsiella* species, the ET6 and ET7 produced expected amplicons of 500 and 400 bp respectively, in 6 out of 9 and 4 out of 9 strains. Further, 9 out

of 10 strains of *Edwardsiella* yielded positive amplicons at 450bp with the ET8 primer set in this PCR study. The rest of the primers which includes rpoN-ang, AP4, ESC1, ESC2 and PAAS showcased a negative result.

Table 3.4.1.2 List of Bacterial primers for the amplification of Bacterial DNA samples.

S. N	Bacterial species	Primer's name	Forward primer	Reverse primer
1	<i>V.parahaemolyticus</i>	AP4	ATGAGTAACAATATAAAACATGAAAC	ACGATTTCGACGTTCGCCAA
2	<i>P. aeruginosa</i>	PAL	ATGGAAATGCTGAAATTCGGC	CTTCTTCAGCTCGACGCGACG
3	<i>A. salmonicida</i>	PAAS	CGTTGGATATGGCTCTTCCT	CTCAAAACGGCTGCGTACCA
4	<i>V. anguillarum</i>	rpoN-ang	GTTCATAGCATCAATGAGGAG	GAGCAGACAATATGTTGGATG
5	<i>A. hydrophila</i>	AH1	GAAAGGTTGATGCCTAATACGTA	CGTGCTGGCAACAAAGGACAG
6	<i>E. ictaluri</i>	ESC1	ACTTATCGCCCTCGCAACTC	CCTCTGATAAGTGTTCTCG
7	<i>E. ictaluri</i>	ESC2	ACTTATCGCCCTCGCAAC	GCCTCTGATAAGTGTTCTCG
8	<i>E. tarda</i>	ET6	ACGAGCGGAGGACGAGTAAGT	CTTAACAAACCGCCTGCGTGC
9	<i>E. tarda</i>	ET7	TTGGACGTGAAATCACCGGGC	CGCTGGATGTCAAGAGTAGGTA
10	<i>E. tarda</i>	ET8	CTGTAGAGATATGGGAGTGCCT	CTCCCGAAGGTTAAGCTAGCTA

Discussion

The study demonstrates the varying antimicrobial effectiveness of antibiotics against different bacterial pathogens, highlighting Gentamycin and Ciprofloxacin as the most potent agents, with broad-spectrum activity and low MIC breakpoints. The prevalence of *Aeromonas* sps. among the tested pathogens emphasizes its clinical significance, while the resistance observed in *Pseudomonas* sps. to certain antibiotics, including Doxycycline and Nitrofurantoin, raises concerns about treatment options. The discrepancies in MIC values and susceptibility breakpoints suggest potential issues with the formulation and availability of Ciprofloxacin, possibly impacting its efficacy in practice.

The molecular analysis reveals significant genetic diversity among the bacterial strains, indicating the presence of distinct genetic markers for each species. The reliable amplification in *Aeromonas hydrophila* suggests a robust method for its identification, while the limited amplification in *Pseudomonas aeruginosa* points to strain-specific variations that may complicate diagnostics. The varied results in *Edwardsiella* highlight the heterogeneity within this genus, suggesting multiple genetic variants. Overall, the findings emphasize the importance of using specific primers for accurate identification and understanding resistance mechanisms in these pathogenic bacteria.

3.5 Post Harvest

3.5.1. Study of the Safe storage period and Nutritional analysis of prepared momo from Pangasius fish Soyabeans.

Fish momo, a popular delicacy made from fish meat is consumed due to its unique flavor and nutritional value. The product's popularity has led to its commercialization, making it essential to ensure its safety and quality during storage. Fish momo is typically prepared with fish meat, vegetables, spices, and oil, offering a rich source of protein and essential nutrients. However, like many perishable products, it is susceptible to changes in quality over time, especially when stored under frozen conditions. To maintain its safety and appeal, it is crucial to assess its physicochemical properties, microbiological stability, and sensory attributes throughout its shelf life. This study investigates the changes in proximate composition, microbial load, and sensory qualities of fish momo stored at -20°C for 90-day period to determine the impact of different treatments on its overall quality.

Table 3.5.1.1 Composition of fish momo

Component	T-1	T-2	T-3	T-4	T-5
Fish Fillet	4000g	3200g	2800g	2400g	3200g
Onion	4000g	4000g	4000g	4000g	4000g
Ginger	60g	60g	60g	60g	60g
Garlic	200g	200g	200g	200g	200g
Fresh Corriander	1000g	1000g	1000g	1000g	1000g
Coriander powder	100g	100g	100g	100g	100g
Vinegar	100 ml	100 ml	100 ml	100 ml	100 ml
Black pepper powder	60g	60g	60g	60g	60g
Fish Oil	500g	500g	500g	500g	500g
Soyabeans	-	800g	1200g	800g	800g
Ajina moto	200g	200g	200g	200g	200g
Fish Spice	100g	100g	100g	100g	100g
Momo Spice	100g	100g	100g	100g	100g
Salt	200g	200g	200g	200g	200g
Wheat Flour	4000g	4000g	4000g	4000g	4000g

Methodology

Fish momo was prepared and stored at -20°C. Proximate analysis was done on days 0, 15, and 30, while organoleptic tests and microbial loads were conducted on days 0, 15, 30, 60, and 90. Proximate aAnalysis was analysed at ZEST Laboratories & Research Centre (P) Ltd. Bhaktapur, Nepal. Based on as per AOAC 21st edition.

Results and Discussion

Table 3.5.1.2 Proximate analysis of momo sample.

Sample Name	Moisture (%w/w)	Protein (%w/w)	Ash (%w/w)	Fat (%w/w)	pH	Thiobarbituric Acid (mg/kg)	Crude Fiber (%w/w)
Day-0 (T1)	53.806	8.193	1.772	3.166	6.17	1.369	0.734
Day-0 (T2)	56.23	7.891	1.991	3.013	6.19	1.706	1.244
Day-0 (T3)	54.501	9.577	1.742	2.762	5.98	1.501	1.17
Day-15 (T1)	51.213	8.322	1.689	2.542	6.14	1.196	0.743
Day-15 (T2)	56.221	9.379	2.057	3.001	6.22	1.593	1.43
Day-15 (T3)	56.313	8.583	1.768	2.751	6.2	1.364	1.396
Day-30 (T1)	52.7	8.15	2.243	2.72	6.37	2.064	2.049
Day-30 (T2)	54.274	8.286	1.596	2.808	6.13	1.43	1.893
Day-30 (T3)	57.248	9.08	2.052	2.701	6.11	1.203	2.015

Proximate analysis showed a decrease in moisture content over time, with Treatment-2 having the highest initial moisture (56.230%). Protein content remained stable, with Treatment-3 having the highest protein levels. Fat content slightly decreased, with Treatment-1 showing the highest initial fat (3.166%). Ash content was highest in Treatment-2 at Day 15 (2.057%), and crude fiber increased over time due to moisture loss. Thio barbituric acid (TBA) values increased, particularly in Treatments-1 and 5, indicating lipid oxidation.

Sensory evaluation: Assessed texture, taste, aroma, and overall acceptability by trained panelists in NFRC, Godawari Nepal.

Sensory evaluation showed a decline in texture, aroma and taste over the storage period. T-1 consistently received the highest sensory scores, indicating better stability. The decline in sensory attributes was attributed to moisture loss, lipid oxidation, and microbial growth.

Microbial loads: Using media such as plate count agar, mannitol salt agar, EMB agar, TCBS agar, and salmonella-shigella agar in NFRC laboratory, Godawari, Nepal.

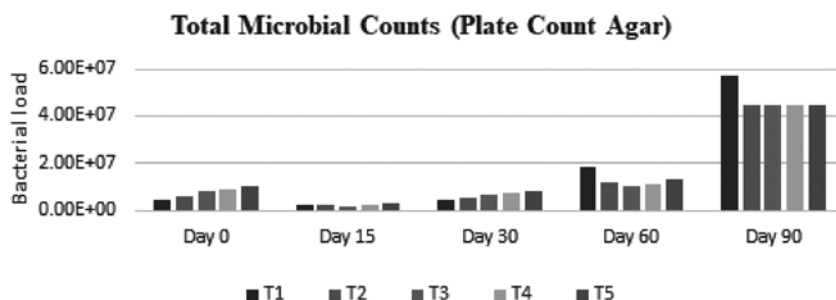


Figure 3.5.1.1 Total microbial counts in plate count agar

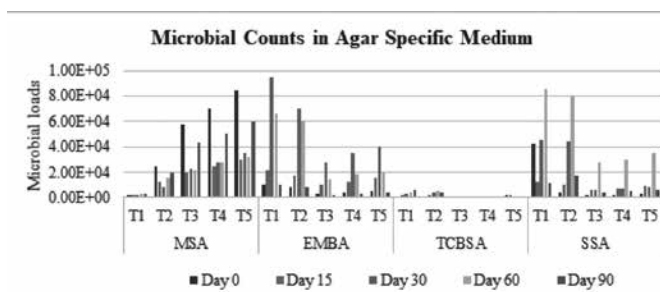


Figure 3.5.1.2 Microbial counts in agar specific medium

Microbial analysis showed that bacterial counts on plate count agar increased until day 30, plateauing by day 90, with treatment-5 having the highest counts. On mannitol salt agar, halotolerant bacteria grew significantly in treatments 1 and 5 by day 90. EMB agar showed a decline in gram-negative bacteria across treatments. *Vibrio* sps. were effectively suppressed in treatments 3 and 5 on TCBS Agar. *Salmonella* and *shigella* showed moderate survival, with treatment-4 exhibiting the highest persistence.

Conclusion

The study reveals that while fish momo retains significant nutritional and sensory qualities during frozen storage, there is a gradual decline due to moisture loss, lipid oxidation, and microbial growth. Treatment-3 emerged as the most stable, maintaining superior nutritional and sensory properties over the storage period. Future research could explore the use of natural antioxidants or modified atmosphere packaging to enhance shelf life.

If the optimized combination of *Pangasius* fish meat is further tested for extended period and optimized results are successfully upscaled among fish farmers, the extended the shelf life of sausages could have following implications:

- ◆ **Economic diversification:** The production of fish momo could open up new avenues for income generation and employment within the aquaculture industry. This diversification can improve the livelihoods of fish farmers and processors.
- ◆ **Year-round availability:** Longer shelf life of fish momo means that they can be stored and distributed even during periods of low fish availability, contributing to a more consistent supply of fish products throughout the year.
- ◆ **Improved food access:** The availability of preserved fish momo could reach remote and underserved areas, where fresh fish is hard to come by. This can enhance food access and contribute to better dietary diversity.
- ◆ **Food security resilience:** By reducing post-harvest losses and preserving fish nutrition, the study's outcomes could contribute to greater resilience in the face of environmental and economic shocks that might disrupt the food supply.

3.6 Participatory technology development, verification and dissemination project

3.6.1. Verification of red spot disease prevention and control measures in Pangasius fish.

Introduction

Pangasius hypophthalmus, also known as the striped catfish or pangasius, is extensively cultured across Asia, including Nepal, due to its omnivorous diet, rapid growth, and high stocking density tolerance, ease of culture, disease resistance, and strong market demand. Pangas fish is capable of surviving in various environmental conditions, aided by a unique respiratory system allowing it to tolerate low oxygen levels. However, the intensive farming practices associated with pangasius often lead to outbreaks of bacterial infections, posing significant economic challenges. Common bacterial (*Edwardsiella ictaluri*) pathogens injected in healthy fish.

This study understands the bacterial threats affecting pangasius aquaculture which involves the identification of the specific control drugs, characterizing their biological and genetic traits, and determining their responsiveness to different antibiotics. These efforts aim to provide insights that can guide effective disease management strategies, ensuring the health and sustainability of pangasius farming and aquaculture industry.

Experimental site

The experiment was conducted for 30 days at farmer's field at Ratnanagar-2, Panchakanya, Chitwan district. Fingerlings of *Pangasius* fish with an average weight of 10-15gm were stocked at density 15 fish/m² in 7m² in cemented pond. Two antibiotics (i) Gentamicin (ii) Ciprofloxacin were demonstrated.

Methodology

The study evaluated the effects ciprofloxacin, and gentamicin on *Edwardsiella ictaluri* infection in *Pangasius* fish. Three experimental tanks, each containing 20 liters of water, were prepared. Salt (3%) + turmeric (0.5%) was dissolved in Tank 1, where fish were dipped for 5 minutes. Tanks 2 and 3 were treated with medicated feed containing Ciprofloxacin (50 mg/kg) and Gentamicin (50 mg/kg), respectively, provided for 15 days. A 10 ml suspension of *Edwardsiella ictaluri* (10⁸ CFU/ml) was added to each tank to induce infection. Liver and kidney swabs were collected from the fish before and after treatment, and bacterial loads were assessed by calculating CFU/ml using selective *Edwardsiella* media.

Results and discussion

Treatment	CFU/ml Before Treatment	CFU/ml After Treatment	Reduction (%)
Control (T1)	5×10^6	2.1×10^6	58%
Gentamicin (T2)	5×10^6	8.5×10^5	83%
Ciprofloxacin (T3)	5×10^6	6.2×10^5	87.5%

The study demonstrated the comparative effectiveness of turmeric and two antibiotics against *Edwardsiella ictaluri* in infected Pangasius fish. Ciprofloxacin showed the highest bacterial load reduction (87.5%), followed by Gentamicin (83%) salt and turmeric (58%). While salt and turmeric was less effective than antibiotics, it achieved a substantial reduction in bacterial load, indicating its potential as a natural antimicrobial treatment. The superior performance of Ciprofloxacin and Gentamicin aligns with their broad-spectrum antibacterial properties. Above reduction of cfu/ml was completely based on Total bacterial colonies presents in *Edwardsiella ictaluri* medium. However, the presence of *Edwardsiella ictaluri* are comparatively low than observed colonies. These findings highlight the effectiveness of antibiotics in controlling bacterial infections but also suggest the potential for integrating natural compounds like turmeric in aquaculture health management strategies.

Conclusion

Ciprofloxacin was the most effective treatment for reducing *Edwardsiella ictaluri* infection in Pangasius fish, achieving near-total bacterial clearance. Gentamicin also showed strong efficacy, while salt (3%) + turmeric (0.5%) offered a natural and moderately effective alternative. This study underscores the potential for combining natural and synthetic antimicrobial agents to manage bacterial diseases in aquaculture. Future research should explore the long-term effects and optimal dosages of these treatments for sustainable fish health management.

4. PRODUCTION

4.1 During FY 080/081, common carp fingerlings (214545), table fish of carp (305 kg) have been produced (Annex 4.1).

4.2 Common carp fingerlings (214545) were distributed to farmers of Bagmati Province. Similarly, 305 kg table fish produced and sold to consumers (Annex 4.2).

5. TECHNOLOGY TRANSFER AND SERVICES

5.1 Training/Workshops

In the FY 080/081, one day office level workshop has carried out at NFRC Godawari. In this workshop Dr. Neeta Pradhan (former senior scientist NARC) including scientist, technical officer and technician of NFRC Godawari has participated. The workshop mainly focused on upcoming research proposal of FY 2081/082 of NFRC Godawari.

Further, in the FY 081/082, two days Aquaculture Working Group Meeting Workshop has carried out at DoAR, Parwanipur, Bara. In this workshop Lumanidhi Pandey, Ph.D., (Planning Director), B.P. Yadav, Ph.D. (Director DoAR, Parwanipur), Chief NFRC Godawari, FRC Pokhara, FRC Trishuli, RTFRS Dhunche, DoAR Tarahara including scientist, technical officer and technician of fishery sector of NARC has participated. The workshop mainly focused on research proposal of FY 2080/081 and FY 2081/082 of NFRC Godawari (Annex 5.1).

5.2 Services

NFRC has provided counseling and technical services in the aspect of disease diagnostic and their control measures to 19 fish farmers. Water quality monitoring and related counseling services were provided to 5 fish farmers (Annex 5.2).

5.3 Information through media

NFRC Godawari has provided information about commercial fisheries and on self-reliance in fish production were telecasted through Krishi TV respectively (Annex 5.4).

5.4 Visits

During FY 080/081, 2572 farmers, students, entrepreneurs and extension officers visited in NFRC with different objectives of fish farming, commercialization, biodiversity, integrated fish farming etc. (Annex 5.5).

5.5 Fair and Exhibitions

NARC Day has celebrated by NFRC Godawari with fish releasing program in village pond at Godawari, Lalitpur and world water day celebrated CFPCC Balaju, fish releasing program in pond.

6. BUDGET AND EXPENDITURE

The centre has received annual budget Rs.3,31,90,000/- of which Rs. 2,99,19,900.73 was spent to accomplish the targeted activities (Annex 7.1). The revenue generated by the NFRC was Rs 2,16,060/- from the sales of fish (fry, table fish), research income and other administrative income (Annex 7.3).

7. KEY PROBLEMS

- ◆ Water supply (irrigation): Ground water source being used by the Centre in the past few decades has been diverted by the several water users group leading to acute water shortage for the smooth operation of farm activities. Application of rain water harvesting technology by the modification of ponds at higher elevation into reservoir would be the immediate option to solve the water problem to some extent. It requires adequate allocation of resources as early as possible.
- ◆ Seepage: Fish ponds in the division are characterized by bolder and rocks in the bottom topped by thin layer of soil which makes ponds highly prone to water loss through seepage and percolation. Construction of stone wall around each pond to prevent seepage loss, and clay soil ramming and plastic lining on the bottom of each pond to prevent percolation loss are necessary. Adequate resource allocation from NARC management to renovate the ponds is expected.
- ◆ Inadequate qualified human resource is the major obstacle to research needs of farmers. Recruitment of qualified human resource by creating additional posts is almost in different disciplines of fisheries, particularly in fish nutrition, pathology, breeding/genetics and limnology.
- ◆ Capacity building: Capacity enhancement of newly recruited and existing employee in fisheries research through short- and long-term training as well as higher education is essential. Such capacity building programs should be inbuilt in NARC regular program.

8. WAY FORWARD

A. Breeding and Genetics

- ◆ Brood stock improvement of carps and trout
- ◆ Promotion of economically important indigenous fish species including value addition
- ◆ Development of seed production technology and aquaculture practices of cold-water native species
- ◆ Assessment of genetic diversity of local germplasm and ex-situ establishment of elite gene pool
- ◆ Cryopreservation in fish showing sexual dimorphism and asymmetry in maturation

B. Fish feed and nutrition

- ◆ Production of extruded feed for high value fish species
- ◆ Feed formulation based on local resources for different development stages of carps, trout, tilapia, catfish and ornamental fish
- ◆ Substitution of animal protein source in feed
- ◆ Pond productivity assessment

C. Fish health management

- ◆ Identification of factors affecting susceptibility to disease on fish
- ◆ Parasite control
- ◆ Prevention of economically important diseases in fish
- ◆ Development of disease resistant strains through genetic selection/hybridization
- ◆ Development of eco-region-specific disease prevention and control protocol

D. Farming method/system

- ◆ Development of small-scale aquaculture model in hill and mountain areas
- ◆ Scaling up of trout farming technology in cold water region
- ◆ Development/standardization of aquaculture protocols in ghools and shallow wetlands
- ◆ Improvement of nursing and rearing of all aquacultured fish species
- ◆ Assessment of carrying capacity of fish production systems in different ecoregion
- ◆ Improvement and scale up of integrated aquaculture-animal husbandry-agriculture (AAA) farming system
- ◆ Development of package of practices for tilapia, catfish, prawn and ornamental fish
- ◆ Development of appropriate technology on culture practices of economically important indigenous fish species

E. Conservation

- ◆ Preparation of resource inventory of rivers, lakes and reservoirs including fishes therein in all geographic regions of the country.
- ◆ Threat categorization (vulnerability) of indigenous fish species
- ◆ Development of fish stock enhancement model in lakes and rivers
- ◆ Mitigation measures on threat imposed by development scheme in aquatic biodiversity
- ◆ Development/establishment of community managed river, lake basin

F. Biotechnology

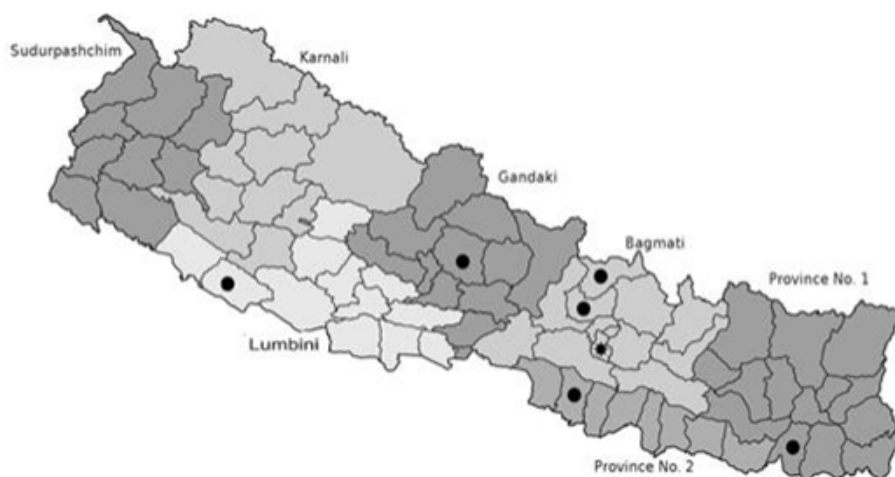
- ◆ Application of molecular marker (DNA) in genome mapping for marker assisted selection (MAS) in commercial fish species
- ◆ Genetic characterization and strain differentiation of indigenous fish species using biochemical and molecular marker
- ◆ Induction of polyploidy and cloning in exotic and native aquacultured fish species
- ◆ Sex reversal in tilapia

G. Other activities

- ◆ Nutrient analysis and value addition of small indigenous species
- ◆ Processing and packaging of high value commercial species
- ◆ Impact assessment and management of effluents from intensive aquaculture system

ANNEXES

Annex 1.1: Map of the Command Area



NFRC – National Fishery Research Centre, Godawari, Lalitpur

FRS – Fisheries Research Station, Begnas, Pokhara,

FRS- Fisheries Research Station, Trishuli, Nuwakot

DoAR, Madhesh Province, Parawanipur, Bara

DoAR, Koshi Province, Tarahara, Sunsari

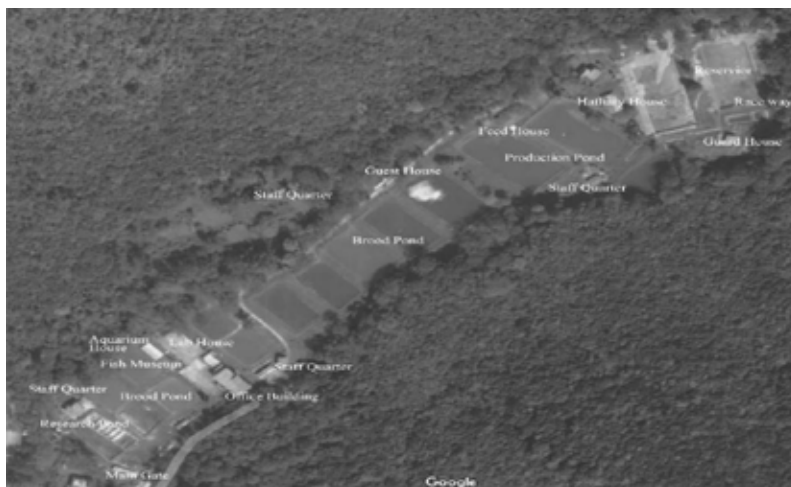
DoAR, Lumbini Province, Khajura, Banke

RTFRS, Dhunche, Rasuwa

Annex 1.2: Monthly Limnological Data of Ponds at National Fishery Research Centre, Godawari

Water Quality parameter				
Temperature, oC (range)			DO mg/L (range)	pH (range)
Month	Raceway	Pond		
January	8.1-9.2	8.8-10.4	6.6-7.4	6.8-7.4
February	9.0-10.2	9.8-14.8	7.1-8.4	7.1-7.3
March	10.1-13.7	12.0-18.5	6.5-8.3	7.2-7.6
April	12.4-14.8	18.2-22.1	6.3-7.2	6.8-6.9
May	15.7-18.6	21.4-29.5	3.9-5.3	6.5-7.0
June	18.3-18.5	22.5-28.2	3.7-8.1	6.5-6.8
July	16.4-17.8	21.4-24.4	6.4-8.2	7.0-7.1
August	16.4-17.3	22.8-28.3	5.9-8.9	6.5-7.0
September	16.5-15.4	22.4-25.6	6.1-7.8	6.6-7.6
October	16.2-16.6	17.2-23.3	6.7-8.3	6.8-7.2
November	12.1-14.2	12.4-20.5	7.3-8.8	7.0-7.3
December	9.4-12.6	10.3-13.3	6.6-8.3	6.9-7.2

Annex 2.1: Map of the Office/Station



National Fishery Research Centre, Godawari, Lalitpur at 27°36'03.42" N 85°23'19.03" E, 1901 feet (Source from Google Earth)

Annex 2.2: List of Laboratory Facilities

S N	Name of laboratory	Major instruments	Manpower in laboratory	Testing facilities
1	Water Quality	DO meter, pH meter, Photo Spectrometer, Autoclave, Weighing Machine, Refrigerator, Distillation Plant, Magnetic Stirrer with hot plate, Conductivity Meter, Altimeter, Water Analysis Kit, Water Sampler, Cooler box, Air pump, Electronic balance	None	Dissolve Oxygen, pH, ammonia, Nitrite, Nitrate, Phosphate, Zooplankton, Total, Dissolved Solids, Total Hardness, Total Alkalinity
2	Disease lab	Microscope, Dissecting microscope, Microtome, Tissue Processor, Oven, Refrigerator, Incubator, pH meter, Cooler box, Water bath, Centrifuge, Ice makers, biological hood, Micropipette, BOD incubator, Deep- freeze	None	Parasite, Bacteria
3	Genetics lab	Ice maker, Micro oven , Deep freezer, Jell doc, PCR machine, Volt guard, Refrigerator, Centrifuge machine, multi-channel micropipette, vertical electrophoresis system, Bio photometer, pH meter, Incubator, Air condition, laminar flow cabinet, Trinocular microscope	None	Genetic characterization, DNA amplification

Annex 2.3 Human Resource in 2080/081 (2023/24)

SN	Name	Position	Qualification	Specialization/ Working area
1	Ms. Asha Rayamajhi	Scientist (S-3)	M. S. (Aqua.)	Fish pathology and Aquaculture
2	Ms. Umita Saha	Scientist (S-2)	M. S. (Aqua.)	Aquaculture
3	Mr. Prem Timalsina	Scientist (S-1)	M. S. (Aqua.)	Aquaculture
4	Ms. Renu Aryal	Com. Officer T-6	B. A.	Computer
5	Mr. Churamani Bhusal	Tech. Officer T-6	B.Sc. Ag.	Aquaculture
6	Mr. Dhrubha Narayan Shrestha	Admin. Officer A-6	B. A.	Administration
7	Ms. Sumanna Shrestha	Account Officer A-6	Master	Finance
8	Ms Anita Gautam	Tech. Officer T-6	M. Sc. Fisheries	Aquaculture
9	Mr. Prakash Kunwor	Tech. Officer T-6	B.Sc. (Hons.) Ag.	Aquaculture
10	Mr. Jayaram K. C.	Tech. Asst, (L-5)	Literate	Technical support
11	Mr. Karna B. Rumba	Tech. Asst, (L-5)	Literate	Technical support
12	Mr. Tirtha Tamang	Tech. Asst, (L-5)	Literate	Technical support
13	Mr. Yam B. Limbu	Tech. Asst, (L-5)	S.L.C.	Technical support
14	Mr. Netra N. Sharma	Off. Asst. (L-5)	Literate	Office support
15	Ms. Maiya Koirala	Tech. Asst, (L-2)	B.A.	Technical support
16	Mr. Sanjaya Acharya	Tech. Asst, (L-2)	B.A	Technical support
17	Ms. Ambika Kafle	Tech. Asst, (L-2)	Literate	Technical support
18	Mr. Ram Krishna Maharjan	Light Driver	S.L.C.	Light Driver

Annex 3.1

Project code No/ Act	Name of project and activities	Project/ Act. Leader	Approved Budget (Rs.)'000	End Year	Major progress/ achievements
290	Genetic and phenotypic characterization of indigenous and aqua cultured fish species for stock improvement	P. Timal sina	2116		
Act 1	Collection of fish specimen from different habitats and geographical regions for morph and genetic assessment		243	4	Identification of suitable Buchche Asala fish stocks for selective breeding, 40 Asla samples each collected from Myagdi khola, Myagdi; Chepe Khola, Lamjung; Kailash khola, Achham; Tadi khola at Shikhabesi section, Nuwakot; Kimtang khola, Nuwakot and Ufraa khola, Nuwakot..
Act 2	Morphometric characterization for stock identification		61		Morphometric and Truss network analysis of buchhe asala Schizothorax richardsonii from Myagdi khola, Myagdi; Chepe Khola, Lamjung; Tadi khola at Shikhabesi section, Nuwakot; Kimtang khola, Nuwakot and Ufra khola, Nuwakot was completed. Asala samples collected from Kailash khola was distorted and damaged during transport so, Morphometric and Truss network analysis could not be done.
Act 3	Study of genetic profile of the fish by molecular variation assessment		1112	4	DNA extraction from pectoral fin clips of Buchche Asala samples collected from Myagdi khola, Myagdi; Chepe Khola, Lamjung; Kailash khola, Achham; Tadi khola at Shikhabesi section, Nuwakot; Kimtang khola, Nuwakot, Ufraa khola Nuwakot was done using DNA extraction kit Promega. Out of 30 primers, nine primers have been successfully optimized and PCR amplification completed. Data analysis software done using Ppgene32.
Act 4	DNA Sequencing		41	4	All together 120 PCR productes were processed for DNA sequencing:
Act 5	Rearing and domestication of potential species		369	4	Representative Asala fingerlings collected from Myagdi khola; Kailash khola; Kimtang khola and Thosne khola is being maintained at cemented cistern at NFRC godawari and multilocation reseach centre, FRS Trishuli
Act 6	Assessment of parent to be asala stock welfare and growth parameters.		290		Comparative growth trial of different river lines asala completed for 90 days. Paper published at Asian Journal of Fisheries and Aquatic Research 26(1):44-51 DOI: 10.9734/ajfar/2024/v26i1725
239	Characterization, documentation and assessment of aquatic biodiversity and environment impact for resource conservation and sustainable use	C.M. Bhusal	867		
Act 1	Survey, observation and identification of potential aquaculture site in wetlands		575	3	Study on fish ichthyofaunal and macrohabitat including water quality was carried out in five wetlands situated in Jhapa, Morang and Sunsari districts. All the five lakes investigated were found to be ideal for raising warm-water carp with conserving indigenous fish species, however Bhimsen pokhari is also suitable for cage fish farming. All surveyed lakes are oriented towards agro-tourism and promotion of fish conservation with the active involvement of the local community.
Act 2	Measurement of physico-chemical parameters of wetland		174	3	Jhapa District: Birtapokhari (Birtamod Municipality), Bhimsenpokhari (Arjundhara Municipality-5), Morang District: Betini Wetland (Urlabari-2, Mangalbare), Shanti Bhulke Wetland (Shanishchare-1, Pathari), Sunsari District: National Heritage Conservation Pond (Ramdhuni-

Project code No/ Act	Name of project and activities	Project/ Act. Leader	Approved Budget (Rs.)'000	End Year	Major progress/ achievements
Act 3	Survey on pesticides use and anthropogenic disturbances of wetlands		118	2	The survey and study on the effects of pesticides used in external farming and their human impact have been completed at Birat Pokhari, Birtamod-3, Jhapa; Bhimsen Pokhari, Arjundhara-5, Jhapa; Beteni Wetland, Uralbari-2, Morang; Shanti Bhulke Wetland, Shanishchare-1, Morang; and Rashtriya Sampada Lake, Ramdhuni-7, Sunsari.
259	Adaptation of Better Management Practices (BMP) and Developmen of Treatment Measures of Parasites & Disease in Aquaculture	U. Sah	1439		
Act 1	Study on occurrence of parasites and diseases in Pangasius hypophthalmus		355	2	The study investigates the bacterial pathogens affecting cultured Pangasius hypophthalmus in Nepal's Terai regions (Jhapa, Morang, Sunsari, Chitwan and Nawalparasi) of government and private hatchery and 131 samples were collected from liver and kidney of pangas, highlighting their identification, characterization, and antimicrobial susceptibility.
Act 2	Isolation, characterization of bacteria causing disease in Pangasius hypophthalmus		348	3	A total of 131 liver and kidney swab samples were enriched in Brain Heart Infusion Broth (BHI) and cultured on various selective media, including <i>Edwardsiella ictaluri</i> media, Bile Salt Brilliant Green Starch Agar, Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS), and Cetrimide Agar. Based on morphological characteristics and selective media, the presence of <i>Aeromonas</i> sps, <i>Vibrio</i> sps, <i>Pseudomonas</i> sps, <i>Edwardsiella ictaluri</i> , and <i>Salmonella</i> sps was identified. Further biochemical tests are being conducted to confirm the pathogenic bacteria. After biochemical analysis, the potential bacterial species identified include <i>Aeromonas hydrophila</i> , <i>Vibrio parahaemolyticus</i> , <i>Pseudomonas aeruginosa</i> and <i>Edwardsiella ictaluri</i> . The DNA extraction process has been completed, and PCR optimization is underway for genetic confirmation of the diseases. Further confirmation of <i>Edwardsiella ictaluri</i> and <i>Vibrio</i> sps is being performed using PCR assays with specific primers as described by Griffin et al. (2011) for <i>Edwardsiella ictaluri</i> and Dangtip et al. (2015) and Pinto et al. (2016) for <i>Vibrio</i> sps.
Act 3	Evaluation of different drugs/ herbs to control the parasites/ disease and Antibiotic Sensitivity Test (AST) of antibiotics		736	4	8 different Antibiotic sensitivity tests done through disc diffusion revealed that Ciprofloxacin(5µg), Gentamycin(10µg), Doxycycline(20µg) and Nitrofurantoin (300µg) were 100% effective drugs but Novobiocin was resisted by (95.83%) strains. The most efficient antibiotics against strains of <i>Vibrio</i> , <i>Edwardsiella</i> , <i>Aeromonas</i> , and <i>Pseudomonas</i> spp. were found to be Ciprofloxacin (0.065–4 µg/ml) and Gentamycin (0.25–8 µg/ml) according to Minimum Inhibitory Concentration (MIC) assays
724	Farm Management Research Support and Production Program	Chief	3477		
Act 1	Office support/maintenance e and beautification		717	4	Farm outlook and its maintenance are being maintained.

Project code No/ Act	Name of project and activities	Project/ Act. Leader	Approved Budget (Rs.)'000	End Year	Major progress/ achievements
Act 2	Working group meeting		260	2	Completed
Act 3	Office and farm security		29	4	Office and farm security maintained.
Act 4	Day celebration		100	1	Completed
Act 5	Field gene bank management		331	3	Field gene bank maintained.
Act 6	Source seed production		1551	4	214545 common carp fries has been sold to needy farmers.
Act 7	Aquaculture feasibility study		229	43	Completed
Act 8	Laboratory and technical services		195	4	Annual report 080/81 published.
Act 9	Technical information publication of research findings and newspaper expenses		55	4	
780	Bio-char bedding materials with stone dust, bone meal and microbial consortia for improved fish productivity and water quality	A. Gautam	1114		
Act 1	Collection of fish seed and bedding ingredients (biochar, stone and bone dust) etc.		306	2	Five Carp fish advanced fingerlings (namely Common carp, silver carp, Bighead carp, Rohu and Naini) and bedding ingredients (bio-char, stone and bone dust) were collected from local market and bought to NFRC Godawari
Act 2	Procurement of nitrogen fixing and phosphate solubilizing bacteria consortia		170	2	Nitrogen fixing and phosphate solubilizing bacteria consortia developed by Ministry of Social Development, Bagmati Province, Hetauda. Youth Scientist Encouragement Fund has been procured, and used in repeated in treatment groups @ Molasses 0.05 g/L and Probiotic 0.004 g/L
Act 3	Pond set up with bio-char, stone and bone dust with mixture of isolated bacteria		242	3	Six treatments allocated for study were namely: T1:(Supplementary feeding + fertilization (FYM 3000 kg/ha, Urea 23 kg/ha, DAP 15 kg/ha: 30,000/hac) T2: (Supplementary feeding + bedding (Biochar, bone meal and stone dust) + fertilization: 30,000/hac) T3: (Supplementary feeding + bedding + microbial consortia: 30,000/hac) T4: (Supplementary feeding + bedding + fertilization + microbial consortia: 30,000/hac) T5: (Supplementary feeding + bedding + microbial consortia: 40,000/hac) T6: (Supplementary feeding + bedding + fertilization + microbial consortia: 40,000/hac)
Act 4	Response study on production and productivity of fish in different treatments of biochar		165	4	The result of effect of Bio-char integrated bedding with consortia of nitrogen fixing and phosphorus solubilizing bacteria on carp polyculture showed that the fishes of treatment 3 that is supplementary feeding + bedding (Biochar, bone meal and stone dust) + microbial consortia showed highest daily growth rate (DGR) (1.82 ± 0.01 g/day) and lowest feed conversion ratio (FCR) (1.47 ± 0.02). While, lower values were for DGR in T1(0.73 ± 0.01), T2 (0.91 ± 0.02), T5(1 ± 0.01), T6(1.05 ± 0.00) and T4(1.38 ± 0.01) respectively. Similarly, higher values were for FCR in T1 (2.90 ± 0.07), T2 (2.50 ± 0.11), T6 (1.98 ± 0.02), T5(1.85 ± 0.04) and T4(1.64 ± 0.05) respectively. No oxygen depletion and ammonia toxicity problem was recorded among treatments during study period.
Act 5	Assessment of the effect of bio-char and consortia on zooplankton's growth		107	4	Zooplankton species have been counted in all treatments.

Project code No/ Act	Name of project and activities	Project/ Act. Leader	Approved Budget (Rs.)'000	End Year	Major progress/ achievements
Act 6	Water quality monitoring		124	4	Water quality parameters were measured during study. The observed ranges for these parameters were as follows: water temperature ranged from 23.8-24.9°C, dissolved oxygen levels between 8-8.3 mg/l, pH ranged from 8.4-9, phosphate concentrations varied between 0.01-3.5mg/l, unionized ammonia (NH ₃) concentrations ranged from 0.01-0.25mg/l, nitrite (NO ₂) concentrations were between 0.01-0.28 mg/l, nitrate (NO ₃) concentrations ranged from 0.09-0.12mg/l, total hardness levels ranged from 97-275 mg/l, and total alkalinity values were within the range of 131-169 mg/l.
3	Participatory Technology Development, Verification and Dissemination Project	Chief	850		
Act 1	Verification of red spot disease prevention and control measures in Pangasius		748	4	In a participatory research study conducted by NFRC Godawari, two treatments that were found to be effective in controlling red spot disease in pangasius fish were Ciprofloxacin and Gentamycin 50 mg/kg fish and the treatment used by the farmers, salt (3%) + turmeric (0.5%). When tested in fish farming farms, no bacterial growth was found in the samples of the ciprofloxacin group after one week of drug treatment on selective media, but bacterial growth was found in the Gentamycin and salt-turmeric treatments and this growth was observed when these treatments were used again after a five-day break for the next week.
Act 2	Hand over the verified technology		102	4	In coordination with the Prime Minister's Agricultural Modernization Project, Implementation Unit, twenty-five fish farmers rearing carp and pangasius fish in different places in Rupandehi district were given orientation and discussion on the disease management technology developed by the National Fisheries Research Centre, Godawari, Lalitpur after researching and discussing various stages of parasitic lice diseases and fungal Eus diseases that mainly appear in fish at the meeting hall of Jaldevi Cooperative located in Siyari Rural Municipality-5, Banghusari. At the same time, medicines required for at least one treatment method for the disease were distributed in 1 kit (10 ml of Ivermectin, 200 grams of Cephalixin, 200 grams of Vitamin C and 1 liter of Benzalkonium Chloride (disinfectant)).
844	Post harvest quality assessment for product diversification in Fish Marketing system	C.M. Bhusal	1091		
Act 1	Quality assessment of fresh fish available in the market Using Rapid Detection Test Kit (RDTK)		422	3	Regarding the testing of formalin adulteration in fish imported from Birgunj border (Raxaul) and local markets, samples were collected and brought to the laboratory of this centre for testing. When tested using HI-Media's Rapid Detection Test Kit (k137), no amount of formalin was found.
Act 2	Product diversification of pangasius (Fish Ball and Fish Mo:mo)		236	3	Fish momo samples were made in participatory research model at Chitwan fish house, Rijal chowk, Bharatpur-10. fish ball samples were made in NFRC, Godawari, Lalitpur.

Project code No/ Act	Name of project and activities	Project/ Act. Leader	Approved Budget (Rs.)'000	End Year	Major progress/ achievements
Act 3	Packaging and storage of fish Ball & momo		191	4	Fish momo were chilled freezed, vaccum packed and transferred to NFRC, Godawari for further analysis of shelf life fish balls were chilled freezed, vaccum packed and stored at NFRC, Godawari for further analysis of shelf life.
Act 4	Shelf-life of fish Ball & momo during storage		121	4	Sample preparation: Around 10gm of sample was mashed in autoclaved saline water and 10-fold serial dilutions were made. Media inoculation: For microbial count each sample are being tested in the variations of 0, 15, 30, 60 and 90 days with triplicate sample. For this samples are inoculated in Mannitol Salt Agar for <i>Staphylococcus</i> species, EMB Agar for <i>E. coli</i> , TCBS Agar for <i>Vibrio</i> sps, <i>Salmonella shigella</i> Agar for <i>salmonella</i> sps. and plate count agar for estimation of bacterial population. Based on microbial analysis and its 5-point likert scale for sensory evaluation (Appearance, texture, taste and touch) carried out at NFRC, Godawari. Treatment groups of 100% pangas include momos and 80% pangas + 20% soybean chunk include momo were safe as well as favoured by the sensory panellist involved.
Act 5	Proximate analysis of fish Ball & momo		93	4	In the nutritional study, key parameters such as crude protein (CP), fat, and ash exhibited significant variations among different groups.
Act 6	Microbial analysis of fish Ball & momo		28	4	Day-1: The momo was prepared (Day 0) and stored at -20 degrees celsius. Bacteria entered a dormant state immediately after freezing with minimal to no differences in growth. Bacterial counts were conducted on Day 1 using Plate Count Agar (for total viable bacterial load), Mannitol Salt Agar (for gram-positive bacteria, especially <i>Staphylococci</i>), EMB Agar (for gram-negative enteric bacteria), TCBS Agar (for <i>Vibrio</i> sps.), and <i>Salmonella-Shigella</i> Agar (for <i>Salmonella</i> and <i>Shigella</i> sps.). Day-15: Due to freezing, some bacteria may experience cellular damage leading to a reduction in viable count, especially sensitive species like gram-negative bacteria due to their thin cell walls, as observed in EMB agar, TCBS agar and <i>Salmonella-Shigella</i> Agar. Gram-positive bacteria, due to their thick cell walls and halotolerance, survive freezing, as seen in mannitol salt agar. Day-30: Prolonged freezing further reduces viable bacterial counts, especially among gram-negative and fastidious bacteria. Day 45 The centre experienced a power cut for 5 hours one day and intermittant power cut 3-4 times next day within this storage period. Momo samples when analyzed on 45 day had resilient bacteria still showing viability after thawing. Thus pangas momo prepared and stored at -20°C safe to consume within 30 days.

Annex 3.2 Summary Progresses of Special Research Projects and Activities in FY 2080/81 (2023/24)

Name of project/activity	Project/ Activity leader	Begin Year	End year	Budget allocated	Major progress

Annex 4.1 Production of products in FY 2080/81 (2023/24)

S N	Commodity	Species	Type of product	Unit	Target quantity	Produced quantity
1	Fish	Carp fish	Table fish	kg	300	305
	Fish	Trout	Table fish	kg	-	
	Total					305
2	Fingerling	Common carp	Seed	No.	2,50,000	214545

Annex 4.2 Distribution of (commodity/product) in FY 2080/81 (2023/24)

S N	Commodity/ product	Type	Quantity	Major stake holder	Distributed districts
1	Fish (carp)	Table fish (Kg)	305	General consumer	Kathmandu, Lalitpur, Bhaktapur
2	Fry (common carp)	Seed (No)	214545	Farmer	Kathmandu, Lalitpur, Bhaktapur, Kavre, Dhading, Dolakha, Ramechhap, Makawanpur

Annex 5.1 Training/Workshop/Seminar Organized in 2080/81 (2023/24)

SN	Name of participants	Position	Organization	Name of Training / Seminar/ Workshop
1				

Annex 5.2 Services Provided in FY 2080/81 (2023/24)

SN	Laboratory/field test/ counseling services provided	No	Major clients
1	Water quality monitoring	5	Fish farmers
2	Fish farming related counseling service	19	Fish farmers

Annex 5.3 Publications in FY 2080/81 (2023/24)

SN	Name of publication	Type	Language	Authors
1	Annual Technical Report	Book	English	NFRC Team
2				

Annex 5.4 Information Disseminated Through Media in 2080/81 (2023/24)

SN	Information disseminated/ media coverage	Type	Name/ Type of media	Date/Time
1	Fish farming	Interview	Krishi Television	Jan.19, 2024
2				

Annex 5.5 Visits of Office by Farmers, Extension Officials/Technicians, Entrepreneurs, Farmer Groups, NGO/CBO Officials in 2080/81 (2023/24)

SN	Category	No	Districts	Area of major interest
1	Extension officers, entrepreneurs & others	215	Different districts	Fish farming related knowledge
2	Students, teachers & farmers	2357	Different districts	General observation and educational tour

Annex 6.1 Training/Workshop/Seminar/Meetings Attended by Staff in FY 2080/81 (2023/24)

SN	Name of staff	Position	Name of Training / seminar/ workshop	Period	Place/Country	Organizer
1	Umita Sah	S-2	GIS Training	5 day	National Goat Research Program, Bandipur, Tanahu	NASRI, Khumaltar
2	Prem Timalisina	S-1	GIS Training	5 days	National Goat Research Program, Bandipur, Tanahu	NASRI, Khumaltar
3	Umita Sah	S-2	13 th National Workshop on Livestock & Fisheries Research in Nepal	2 days	Agriculture Research Station, Pakhribas, Dhankuta	NARC
4	Churamani Bhusal	T-6	13 th National Workshop on Livestock & Fisheries Research in Nepal	2 days	Agriculture Research Station, Pakhribas, Dhankuta	NARC
5	Anita Gautam	T-6	13 th National Workshop on Livestock & Fisheries Research in Nepal	2 days	Agriculture Research Station, Pakhribas, Dhankuta	NARC

Annex 6.2 Paper Published in FY 2080/81 (2023/24)

SN	Title of Paper	Authors	Name of proceedings, journal etc
1			

Annex 7.1 Regular Annual Budgets and Expenditure Record of FY 2080/081 (2023/24)

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फाउन्डेट टुप बीकी रेक्की	०,०००
गत आ व समको	०,०००
यस आ व को	
वरमा	०,०००
म्याद बापको रेक्की	
म्याद मनाएको रेक्की	

प्रमाणित गर्ने
नामा,
पद,
सिद्ध प्रमुख



वी को से वि.स.स. बलिपुल्लट ।
प्रा.स. २०७३/७४ को निर्देशन अनुसार
विषयगत र कार्य विज्ञानको इकाईमा कार्य ।

प्रमुख कोष नियन्त्रक

समाप्त गर्ने
नामा,
पद,
नेवा अधिकारी



मेषान् गणकार
भूमि जया गङ्गानदी विकास मन्त्रालय
परिषद्

राष्ट्रिय भाषा अनुवाधान केन्द्र, गोरखपुरी, लखनौपुर
 कार्यालय कोड नं. : ३१२४१३४४३

जार्जियन विवरण

आ.नं.: ३०८०/८९

डिप्टेड उपनिर्देशक भवन
आयोजन/कार्यक्रम भवन
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कृषि अनुसंधान कार्यालय

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स्रोतगत विवरण

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श्री को ले नि. का. सन्तिपुराद ।
सा. न. २०८५/०८५ को विनिर्देशन सर्वो
शिरसा र सब विन्दुको उपनिष्ठा गर्दा ।

प्रमुख कोष नियन्त्रक

प्राप्तकर्ता :
पता :
महानगर :
राज्य :

पैल नमै :
नामः
पाठः
मितिः

प्रमाणित करें:
 नाम: 
 पद: प्रमुख

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Annex 7.2 Special Project Budget and Expenditure Record of FY 2080/81 (2023/24) (in ‘000 Nepalese Rupees)

Name of the project	Funded by	Project period	Annual budget	Expenses

Annex 7.3 Revenue Status of FY 2080/81 (2023/24)

Source	Total	Remarks
Sale of fish seed and table fish	193930.00	
Research income		
Others administrative income	6500.00	
Other's source	15630.00	
Grand Total	216060.00	

Annex 7.4 Beruju Status of FY 2080/081 (2023/24)

Beruju	Amount	Remarks
Beruju till last year		
Remaining Beruju	5,08,034.77	
Clearance from the Auditor General		
Total Beruju clearance %		

Major past contribution of NFRC, Godawari in fisheries research in last five years

1. Praziquantel effectively reduced the intensity of the *Dactylogyrus vastator* parasite in common carp fingerlings, with the highest reduction at 15 mg/l concentration after 24 hours of treatment.
2. Study on antimicrobial resistance pattern of fin and tail rot causing bacterial pathogens were isolated from 15 different rainbow trout situated at Rasuwa, Nuwakot, Dhadhing, Sindhupalchowk, Lalitpur and Kaski districts as well as antibiotic sensitivity tests revealed Cephalexin was resistance in all the farms, while Doxycycline was found completely resistant in two farms and Moxifloxacin was effective in general (broad spectrum), emphasizing the importance of responsible antibiotic use in aquaculture.
3. Incorporating 1.5% sisnu meal and multienzyme into shrimp-based feed significantly improved growth, cortisol levels, profitability, and cost-benefit ratios for rainbow trout fingerlings.
4. Citric acid supplementation at 1.5% improved the growth of rainbow trout, showing potential as a dietary additive.
5. Replacing shrimp meal with 50% wheat gluten in rainbow trout fry diets led to better growth and feed conversion ratios, highlighting an economical alternative.

6. Sinking pellet diet and thrice-daily feeding resulted in higher growth, lower feed conversion ratios, and increased net fish yield for common carp in polyculture.
7. Using mustard cake in feeding gastropods and bivalves enhanced their growth compared to wheat flour, with water quality parameters within acceptable ranges.
8. Probiotic incorporation at 7.5% in feed improved the growth of sahar fish i.e., reached to 85.01 ± 10.3 g from 25.3 ± 10.8 at the stocking rate of 1 fish/ m^2 in 240 days culture period, offering potential benefits for aquaculture.
9. Frozen fillets of pangas fish exhibited lower microbial loads than fresh fish, and their texture and color and sensory evaluation showed first month of frozen storage is optimal. Fish fillets without skin had better quality in terms of texture and total volatile base nitrogen (TVBN) compared to fillets with skin.
10. The study on eyed egg transportation indicated that hatching rates 99.79 ± 0.07 , % were higher at lower stocking densities 500 eggs/pkt, with minimal mortality (0.005 ± 0.001) within 24 hours.
11. Clove oil at 0.025ml/L concentration was effective in inducing surgical anesthesia in bighead fry within three minutes. Packing bighead fry at a density of 50g/L resulted in the lowest mortality during transportation. Clove oil at 5 μ l/L concentration proved effective for the transportation of grass carp and common carp fry for 12 hr.

Phenotypic and genetic study of Asla (*Schizothorax richardsonii*) collected from different river of Nepal



Disease study on Pangas (*Pagasius hypophthalmus*) at different terai regions (Nawalparasi, Chitwan, Siraha, Sunsari and Jhapa) district of Nepal



Post harvest quality assessment for product diversification in fish marketing system of Nepal



Bio-char study on carp co-culture production and productivity at NFRC,
Godawari, Lalitpur





Participants attended the Aquaculture Working Group Meeting organized by NFRC, Godawari at DoAR, Parawanipur, Bara



Staff of National Fishery Research Centre, Godawari, Lalitpur