TADPOLES WITH A TRICK: OVERWINTERING ECOLOGY OF TADPOLES IN A HIMALAYAN STREAM, UTTARAKHAND, INDIA

A THESIS
Submitted by

V. JITHIN

For the award of the Degree of

MASTER OF SCIENCE
IN
WILDLIFE SCIENCE

Under the guidance of

ABHIJIT DAS, Ph.D. & J.A. JOHNSON, Ph.D.

भारतीय वन्यजीव संस्थान
Wildlife Institute of India

Wildlife Institute of India, Dehradun

Saurashtra University
Rajkot- 360 005

JULY 2021

© Original illustrations: K.M. Adila Farisa; Digital Designing: V. Jithin

Other illustrations in the derivative works are either available under Pixabay License (2020) (OR) used with permission from
"Stream Corridor Restoration: Principles, Processes, and Practices, 10/98, by the Federal Interagency Stream Restoration Working Group (FISRWG)"

Citation:
Jithin, V. (2021) Tadpoles with a trick: Overwintering Ecology of tadpoles in a Himalayan stream, Uttarakhand, India. M.Sc. Thesis. Wildlife Institute of India, Dehradun, India and Saurashtra University, Rajkot, India. pp. 1-107

DEDICATION

This thesis is dedicated, with love, to my parents,
for being with me always in the tougher times.

भारतीय वन्यजीव संस्थान
Wildlife Institute of India

DECLARATION

I, **V. Jithin**, hereby declare that the research work entitled "**Tadpoles with a trick: Overwintering Ecology of tadpoles in a Himalayan stream, Uttarakhand, India**", carried out in partial fulfilment of M.Sc. (Wildlife Science) degree of Saurashtra University, Rajkot is an original piece of research. Information derived from the published or unpublished work of others have been acknowledged in the text as well as in the references list. Fieldwork for this study was conducted after receiving necessary research permission from the Chief Wildlife Warden, Forests Department, Government of Uttarakhand (No: 2144/5-6, 21 January 2021). This research work was carried out under the supervision of **Dr. Abhijit Das** and co-supervision of **Dr. J.A. Johnson**, at the Wildlife Institute of India from December 2020 to July 2021. I hereby declare that this work has not been submitted in any form for any other degree or diploma at any university or other institutions.

Date: 12 August 2021
Place: Dehradun

12/08/21

V. Jithin
Post-Graduate Programme in Wildlife Science
Wildlife Institute of India
Chandrabani, Dehradun
Uttarakhand, India -248 001

पत्रपेटी सं. 18, चन्द्रबनी, देहरादून-248 001, उत्तराखण्ड, भारत
Post Box No. 18, Chandrabani, Dehradun - 248 001, Uttarakhand, INDIA
ई.पी.ए.बी.एक्स : +91-135-2640100, 2640114, 2640115, फैक्स : 0135-2640117
EPABX : +91-135-2640100, 2640114, 2640115, फैक्स : 0135-2640117
ई-मेल/E-mail : wii@wii.gov.in, वेब/Website : www.wii.gov.in

भारतीय वन्यजीव संस्थान
Wildlife Institute of India

CERTIFICATE

This is to certify that Mr. **V. Jithin** has carried out an original piece of research in partial fulfilment of Master's Degree in Wildlife Science of the Saurashtra University, Rajkot, Gujarat. The topic of his dissertation was "**Tadpoles with a trick: Overwintering Ecology of tadpoles in a Himalayan stream, Uttarakhand, India**". The study was carried out under our supervision from December 2020 to July 2021. We hereby certify that this work has not been submitted for any degree to any university.

Abhijit Das

Abhijit Das, PhD
(Supervisor)
Scientist 'D'
Department of Endangered Species Management
Wildlife Institute of India
Chandrabani, Dehradun
Uttarakhand, India -248 001

J.A. Johnson, PhD
(Co-supervisor)
Scientist 'E'
Department of Habitat Ecology
Wildlife Institute of India
Chandrabani, Dehradun
Uttarakhand, India -248 001

Date: 12 August 2021
Place: Dehradun

पत्रपेटी सं. 18, चन्द्रबनी, देहरादून-248 001, उत्तराखण्ड, भारत
Post Box No. 18, Chandrabani, Dehradun - 248 001, Uttarakhand, INDIA
ई.पी.ए.बी.एक्स : +91-135-2640100, 2640114, 2640115, फैक्स : 0135-2640117
EPABX : +91-135-2640100, 2640114, 2640115, फैक्स : 0135-2640117
ई-मेल/E-mail : wii@wii.gov.in, वेब/Website : www.wii.gov.in

भारतीय वन्यजीव संस्थान
Wildlife Institute of India

CERTIFICATE OF PLAGIARISM CHECK

It is certified that the M.Sc. thesis titled "**Tadpoles with a trick: Overwintering Ecology of tadpoles in a Himalayan stream, Uttarakhand, India**" submitted by Mr. **V. Jithin** has been examined by us for plagiarism check as per UGC (Promotion of Academic Integrity and Prevention of Plagiarism in Higher Educational Institutions) regulations. The following inferences are drawn from this check:

1 Thesis has significant new work/ knowledge as compared to already published work or work under consideration for publication elsewhere.

2 No sentence, equation, diagram, table, paragraph or section is found to have been copied verbatim from previous work unless it was placed under quotation marks and the source was duly cited.

3 The work presented is original work of the author (*i.e.* there is no plagiarism) and there is no fabrication of data or result by manipulating research material, equipment or processors or by changing or by omitting data or results such that the research is not accurately represented.

The similarity indices for the individual chapters as reported by the software iThenticate® are as follows:

Sl. No.	**Chapter**	**iThenticate® similarity index**
1.	Background	6 %
2.	Materials and Methods	8 %
3.	Results	5 %
4.	Discussion	2 %
5.	Conclusions	0 %
	Overall	**5 %**

Smt. Sunita Agarwal
(Librarian)
Wildlife Institute of India
Chandrabani, Dehradun
Uttarakhand, India -248 001

पुस्तकालयाध्यक्ष
Librarian
भारतीय वन्यजीव [illegible], देहरादून
Wildlife Institute [illegible], D.Dun

J.A. Johnson, PhD
(Co-supervisor)
Scientist 'E'
Department of Habitat Ecology
Wildlife Institute of India
Chandrabani, Dehradun
Uttarakhand, India -248 001

Date: 12 August 2021
Place: Dehradun

पत्रपेटी सं. 18, चन्द्रबनी, देहरादून-248 001, उत्तराखण्ड, भारत
Post Box No. 18, Chandrabani, Dehradun - 248 001, Uttarakhand, INDIA
ई.पी.ए.बी.एक्स : +91-135-2640100, 2640114, 2640115, फैक्स : 0135-2640117
EPABX : +91-135-2640100, 2640114, 2640115, फैक्स : 0135-2640117
ई-मेल/E-mail : wii@wii.gov.in, वेब/Website : www.wii.gov.in

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisors, for believing in me and giving the freedom to ask endless questions on overwintering tadpoles. Their encouragement and support were the crucial part of this study, for sure. I cannot thank enough Dr. Abhijit Das and Dr. J.A. Johnson for introducing me to the world of tadpoles, and stream ecology respectively, and that too at my field site during one of the course field trips. All further field-visits as part of the 'Torrent frog project' of the Wildlife Institute of India made me strong enough to choose this topic for my thesis. Another important step which I cannot skip mentioning is the term paper on global review of overwintering larval anurans I have done with Dr. Abhijit Das. I fondly remember the encouragement and support I received from him during the work, in the first COVID-19 lockdown. All the discussions I had with Dr. Abhijit Das and Dr. J.A. Johnson gave brighter ideas, and improved the study significantly. The theoretical and practical knowledge on stream ecology provided by Dr. J.A. Johnson was of immense help during the planning and fieldwork, which are invaluable. The joy of exploring the field site, understanding the area, and learning the field techniques from them made my field days easy and comfortable. I thank them for the patience in going through each revision of the manuscripts starting from the research proposal to this thesis, and several online and offline meetings we had. Without the expertise and equipment provided by Dr. J.A. Johnson and Dr. Abhijit Das, I would not have been able to do the data collection in the desired manner.

I thank Dr. Dhananjai Mohan IFS (Director), Dr. Y.V. Jhala (Dean, Faculty of Wildlife Science), Dr. Monali Sen IFS (Registrar), and Dr. Bitapi C. Sinha (Research Coordinator) of the Wildlife Institute of India, Dehradun for their support during the study.

I would like to thank J.S. Suhag IFS (Chief Wildlife Warden, Uttarakhand), Kahkashan Naseem IFS (Divisional Forest Officer, Mussoorie), Dr. Shipra Sharma (Range Officer, Mussoorie Wildlife Sanctuary) and Forest Department of Uttarakhand for facilitating my research by providing fieldwork permit and logistical support. The support I received from Dr. Shipra Sharma and her team at Binog WLS during my fieldwork was an essential component of the successful data collection. I thank her for the fruitful discussions, interactions and company during the fieldwork. I am thankful to Mr. Aman, for his kind help during the study.

I also would like to thank Shobha Bhargava, PhD. (Behavioral Neuroendocrinology and Developmental Biology Laboratory, Savitribai Phule Pune University), Balasubramanian Karthick, PhD. (Biodiversity & Paleobiology Group, Agharkar Research Institute, Pune), Sandeep Das (EDGE of Existence Programme, Conservation and Policy, Zoological Society of London, London and Kerala Forest Research Institute), Saanjbati Adhikari (School of Biological and Chemical Sciences, Queen Mary University of London), Swapnil Shewale (Bhavan's Hazarimal Somani College, Mumbai), Abhilash Damodaran IFS (CASFOS, Dehradun), Ninad Avinash Mungi, PhD., Ashish K. Jangid, Bhawna Dhawan (WII), P. Mohammed Jasil (Dept. of Chemical Science, The Gandhigram Rural Institute, Tamil Nadu), P. Afna (School of Environmental Sciences, Mahatma Gandhi University, Kottayam), Mohammed Abdus Shakur, Manu Mohan, Krishnendu Banerjee, Swati Nawani, Bitupan Boruah, and Naitik Patel (WII), Ayushi Jain (EDGE, ZSL), Kavya Jayarajan, and P.T. Hanan for insightful discussions during the study and their encouragement. I thank Rohit Jha (WII) for his encouraging comments on the earlier version of the thesis.

The fruitful discussions I had with Prof. Qamar Qureshi (Population Management, Capture & Rehabilitation Department, WII) prior to the study were encouraging and thought-provoking. I thank him for the discussions and the logistical support provided. I thank Dr. Y.V. Jhala (Dean, WII) and Dr. Bilal Habib (Animal Ecology and conservation Biology Department, WII) for their suggestions during the research proposal defense.

My heartfelt thanks to Dr. Ajith Kumar (Wildlife Conservation Society-India), Dr. Shomen Mukherjee (Azim Premji University, Bengaluru) and Dr. Soumya Prasad (Nature Science Initiative, Dehradun) for their encouraging comments and suggestions on the thesis.

My field work wouldn't have been possible without the generous help from Rishav Bhattarai, Shanul Nautial, Anjitha Devarajan, Swati Nawani and Krishnendu Banerjee. I am deeply indebted to Rishav and Shanul for accompanying me and ensuring my safety in the field during data collection and stream exploration in often very wet and difficult conditions. The long hours of stream inventory, night surveys, boat surveys and 24 hours monitoring were not possible without them, absolutely. A big thanks to Rishav for accompanying me with full of curiosity for frogs, fishes, and everything else. The unconditional love, support and care from Mr. Kuldeep Rawat and family, especially our 'aunty' made my field days beautiful and memorable. I miss Ayu, Aman, Anil Bhaiyya, Jagdeep Bhaiyya, Shobha Di, and Bala Di. Travelling frequently from campus to field station through the Dehradun-Mussoorie Road was not easy without the help from Pranav Kiran Ghokale, and Varchasva Dhunta.

I thank T.S. Rawat (Assistant Engineer, Uttarakhand Jal Sansthan, Mussoorie), and Mr. Arjun for providing insightful comments on the history of water management and extraction in the area. I am thankful to Mr. Arun Prasad Gaur (Devalsari Environment Protection and Technology Development Society) for immense support during the field-visit at Devalsari. I miss Devalsari, and feel sad for not being able to sample there due to logistical limitations especially due to COVID-19.

I thank Tammy Cheever (Information Technology Specialist, Natural Resources Conservation Service, United State Department of Agriculture) for his kind help in the usage of illustrations owned by the USDA in this thesis. I also thank Adila for drawing amazing tadpole illustrations for the thesis.

I thank Dr. Samrat Mondol and Dr. V. Navendu Page (Course Directors, XVII M.Sc. Wildlife Science) for all necessary help they have provided during the entire course period. I cannot thank enough our 'Negiji' (K.S. Negi, Course Assistant), for being with us always. I also thank all faculties and researchers of WII for providing insights on various topics during the course. Inputs from faculties during each of the tours we had as part of the curriculum, made huge impact in understanding various ecosystems, field conditions and ecology of animals. I thank Dr. Swanti Sharma, Arun (Analytical Lab, WII) and Dr. Panna Lal (IT and RS & GIS Cell, WII) for providing necessary logistical support during the dissertation period. I also thank the help from staff of the Old Hostel, New Guest House, Administrative, IT and RS & GIS Cell, WII, Analytical and Biology Labs and Finance Sections of the institute.

I thank the logistical support from the Wildlife Institute of India grant-in aid 'Torrent frog project', and Long-Term Ecological Observatories (LTEO) programme by the Ministry of Environment, Forest and Climate Change, Government of India and Centre for Ecological Sciences, Indian Institute of Science, Bengaluru.

The support from my family and friends, especially Aswathi Anil (School of Information and Communication Studies, University of Madras) and K.M. Adila Farisa (School of Biological Science, Central University of Kerala) kept me alive during the course and fieldwork. Special thanks to my companion Anjitha Devarajan, for her critical comments, ideas, suggestions, and patience for listening to me. I cannot thank enough my sister, Anjitha Vijayan for listening to all my odd conversations, accompanying me in the backyard explorations, and helping in literature collection and processing during my term paper work. I cannot forget the days I spent in the Old Hostel during the lockdown, between the fieldwork; thanks to Divya, Jason, Anubhuti, Pranav, Vishnu, and Swetha for making those days beautiful. I cannot forget the company and support given by Joonu, Vignesh and Yadu.

I am greatly thankful to all my mentors, especially Prof. T.N.C. Vidya (Associate Professor and Chair, Evolutionary and Integrative Biology Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru), for introducing me to the amazing world of ecology, evolution and behavior; teaching me basic statistical approaches. The Project Oriented Biology Education (POBE) programme of JNCASR, Bengaluru have huge influence in my scientific journey, and definitely on this thesis as well. I also thank Dr. A. Prasad (Dept. of Livestock Production Management, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy), Dr. Nisha Aravindan (VIT School of Agricultural Innovations and Advanced Learning, Vellore), Dr. K. Kishore Kumar (Dept. of Botany, Farook College, Kozhikode), Dr. S.V. Abdul hameed (Dept. of Zoology, Farook College, Kozhikode), Nushiba Naser (University of Calicut), Dr. S. Anjana (Govt. College, Madappally, Kozhikode), Manjusha and Resmi Panikker Prabhakar (PKMMHSS Edarikode, Malappuram), N. Nikhil Dev (School of Biology, IISER Thiruvananthapuram) for mentoring me at various stages of my schooling and college-life. I thank my teachers and friends at JNCASR, Farook College, PKMMHSS Edarikode, PMSAMUPS Pakkadappuraya and AMLPS Parappur Iringallur.

I thank the amazing people behind all free softwares and online utilities which were of immense use during the data analysis and thesis writing including Ubuntu OS, R, GIMP, Google Earth, Libre Office, QGIS, Zotero, Pixabay, and Google Scholar. Big thanks to the scientific community paved the way to my research; and the 'Robin Hood of Science'.

I owe a big thank you to my XVII M.Sc. friends for the company, discussions, time and memories in the field and classroom. Support from the XVI M.Sc. batch throughout the coursework was of great help. I fondly remember the time with Kushagra Meshram, Doli Borah, Abir Jain, and Bhavya Iyer at my field site.

Last, but the most important ones to thank is my parents for supporting and encouraging all my crazy ideas. I cannot comprehend their love and care during the tough times of my life, especially when I was tested positive for COVID-19 at the end of my fieldwork.

Thank you.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS[1]

EC	Electrical Conductivity
DO	Dissolved Oxygen
TDS	Total Dissolved Solids
BOD	Biological Oxygen Demand
LTRF	Labial Teeth Row Formula
IR	Infra-Red
PHABSIM	Physical Habitat Simulation
HSC	Habitat Suitability Curve
GLMM	Generalized Linear Mixed Model
PCA	Principal Component Analysis
ANOVA	Analysis of Variance
AIC	Akaike Information Criterion
UPGMA	Unweighted Pair Group Method with Arithmetic Mean

---

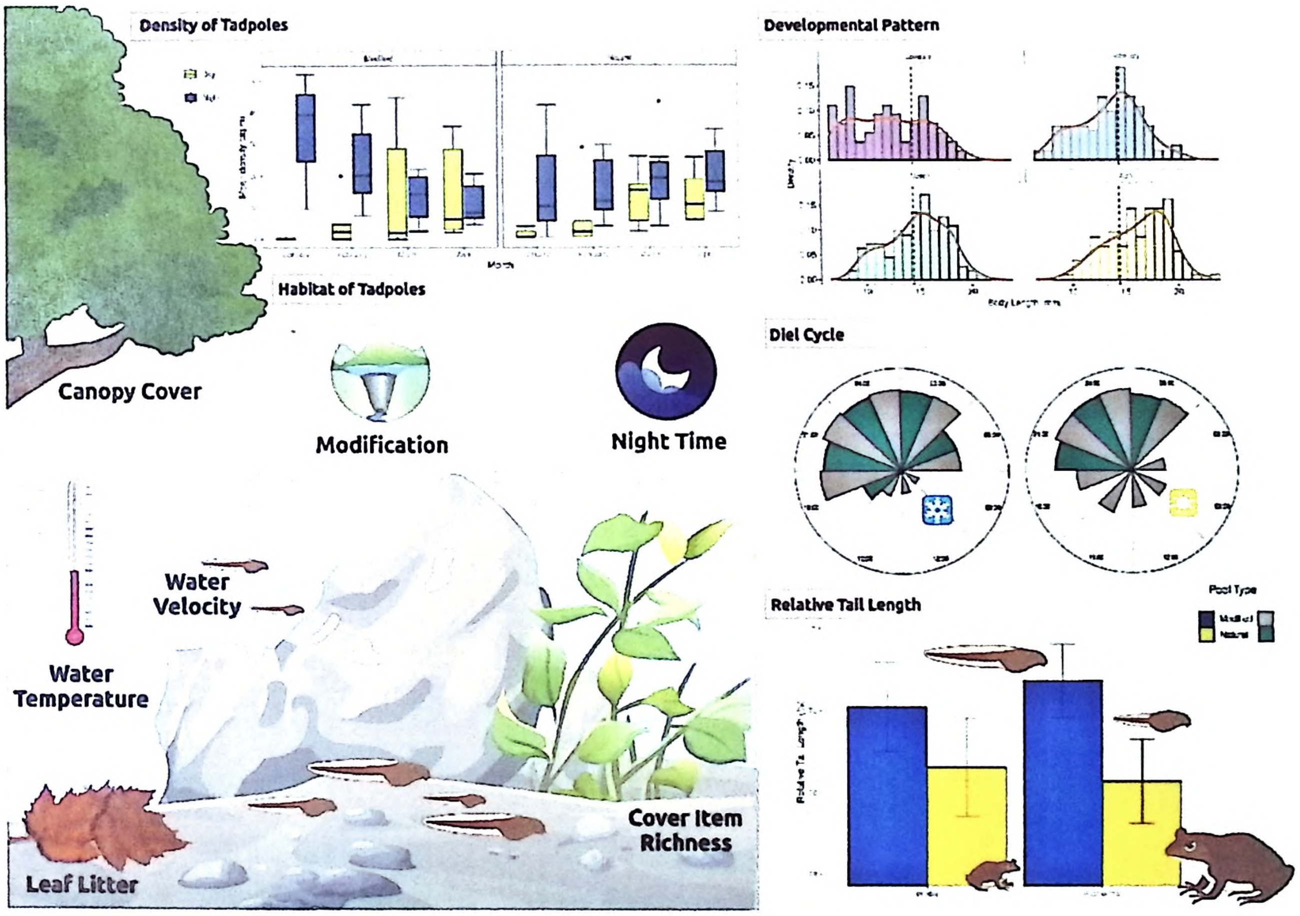
[1] Other abbreviations are mentioned along the text wherever necessary.

ABSTRACT

Overwintering is a unique phenomenon of delaying metamorphosis in amphibian larvae at low-temperature conditions prevailing at high elevation and latitude. This study is first of its kind in India to look at selected ecological aspects of overwintering tadpoles of *Nanorana* spp. in a Himalayan stream. I evaluated the temporal density of overwintering tadpoles in response to the temperature and disturbance gradient. Tadpole density was influenced by the time of the day, submerged leaf litter depth, canopy cover, cover item richness, and a cross-over effect of water temperature and canopy cover, as inferred from Generalized Linear Mixed Effect Modelling. Mean tadpole density at night time was higher in modified pools than natural pools during winter ($W=1355.5$, $p<0.05$), but there was no difference during daytime ($W=943.5$, $p=0.46$); the nocturnal pattern changed in the post-winter, where density was higher in natural pools ($W=2019.5$, $p<0.05$). Tadpole activity pattern varied significantly between pool types during post-winter, where modified pool population increased daytime activity and thus the activity overlap reduced from winter (90.8%) to post-winter (64.5%). Further, I discuss the influence of temperature fluctuations on tadpole density and behavioral thermoregulation. I report the differences in relative tail length of tadpoles in modified and natural pools between seasons, where the modified pool population are larger in relative tail length compared to natural population during post-winter ($t_{217}=3.6$, $p<0.05$, $d=0.488$). I propose two putative tadpole groups based on the morphometric measurements using PCA, and cluster analysis for further molecular identification. I present insights on the resource utilization pattern of overwintering tadpoles during winter and post-winter seasons using habitat availability and use analyses. Besides the understanding of poorly known larval ecology, the study provides some insights into the habitat simulation modelling with respect to habitat suitability criteria in the light of future altered stream flow.

Keywords: amphibian larva, metamorphosis, Western Himalaya, microhabitat use, temperature, adaptation, cold, development, life-history strategy, plasticity, stream, check dam, behavior

GRAPHICAL ABSTRACT



Graphics showing the temporal variation in mean tadpole densities across months and day-night sessions; Body size class variation across months as a proxy of the developmental pattern of tadpoles; diel cycle of tadpole counts between seasons and pool types; Larger relative tail length of modified pool populations (reduced body length); and the predictor variables that best explained the tadpole densities.

BACKGROUND

1 INTRODUCTION

Among vertebrates, amphibians have the most diverse life history strategies (Duellman, 1989; M.S. Johnson et al., 2017; Alford & Harris, 1988; Smith-Gill & Berven, 1979). Diversity of such strategies reach maximum in tropics, and lineages in the temperate region are perhaps further specialized with enhanced cold tolerance, change in breeding phenology and behavioral mechanisms (Vo & Gridi-Papp, 2017) and delayed metamorphosis (Brown, 1990).

In the low-temperature conditions of high elevation and latitude where average temperatures are lower and the growing season shorter than at lower elevation and latitude, amphibians delay their metamorphosis, and this is called overwintering (Ultsch et al., 1999). This can be also simply defined as spending the winter season in the larval stage (Fellers et al., 2001). Several anurans have been found to overwinter as larvae in temperate regions of the world (e.g., Brown, 1990; Fominykh & Liapkov, 2011; Lai et al., 2002; Logares & Úbeda, 2006; Lu et al., 2016; Pentek et al., 2019). The factors behind the plasticity in such life-history events however, are poorly known (Bradford, 1984; Walsh et al., 2008, 2016). Some factors influencing overwintering are seasonal changes, elevation, latitude, temperature and resource availability (Berven et al., 1979; Macedo, 2019; Pintar, 2000).

Previous studies have been focused on the relationship of overwintering with elevational gradients (Ultsch et al., 1999), developmental stage (Walsh et al., 2016), duration (Emerson, 1988) and temperature range (Collins, 1979; Lai et al., 2002) of overwintering events, and body size variation among overwintering tadpoles (Wilbur & Collins, 1973). In some populations, overwintering has been found to have critical role, where larger size at metamorphosis leads to greater adult fitness, survival and fecundity (Ficetola & De Bernardi, 2006; Walston & Mullin, 2007), whereas not highly adaptive in others (Fellers et al., 2001). This local adaptation has been investigated comparing low and high-altitude populations of *Rana temporaria* and found that temperature parameters act as strong selective pressure of such phenotypic variations despite high gene-flow (Muir et al., 2014).

In addition to the amphibian population, presence of overwintering tadpoles affects the aquatic community by influencing interspecific competition (Walston & Mullin, 2007), changes in predation patterns (Brodie & Formanowicz, 1983), and algal community structure (Collins, 1979; Dickman, 1968). As several hypotheses points at various factors behind the phenomenon, and affects the community structure, increases susceptibility towards global warming (Garner et al., 2010) and *Batrachochytrium dendrobatidis* (Narayan et al., 2014), overwintering provides us a good framework to study these aspects in light of climate change and disease ecology (Fig. 1).

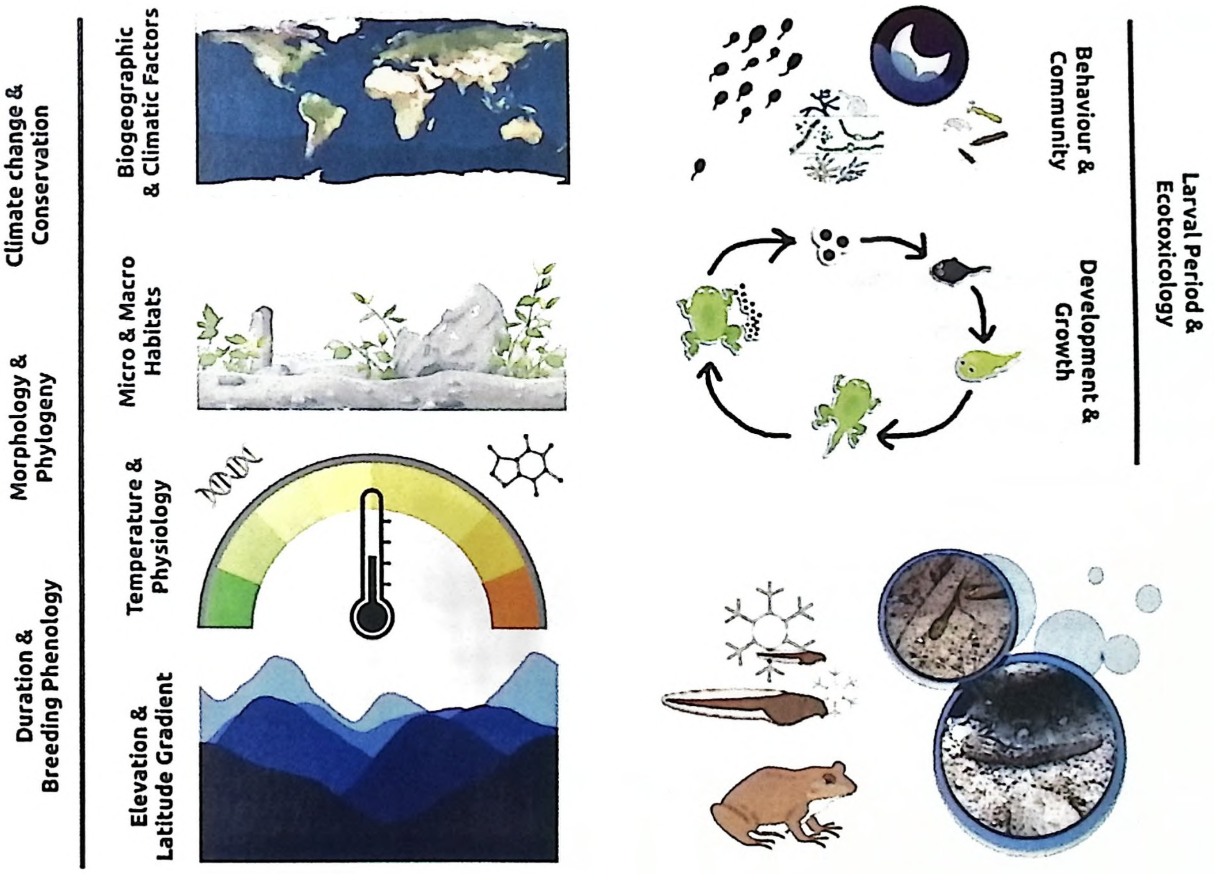


Fig. 1 Illustration showing various factors influencing overwintering tadpoles. Inset photographs are of overwintering tadpoles of *Nanorana sp.*, Western Himalaya, India. Design by V. Jithin

Several field and lab-based studies have been done to understand the phenomena (Aguirre-Becerra et al., 2018; Berven, 1982), even though the major questions are still unanswered.

The intra-population variation in overwintering tadpoles (Brown, 1990), degree of influence of various intrinsic and extrinsic factors, phylogenetic, geographic and taxonomic relationships are some of these questions (Jithin & Das, 2020).

2 LITERATURE REVIEW

2.a Global Scenario

While interpreting the published studies in amphibian larval overwintering, special attention needs to be taken due to the geopolitical bias in the research (Jithin & Das, 2020; Logares & Úbeda, 2004). Even though most of the studies are from North American region, the phenomenon occurs in other regions as well including 11.54% from the Asian region (Jthin & Das, 2020) (Fig. 2).

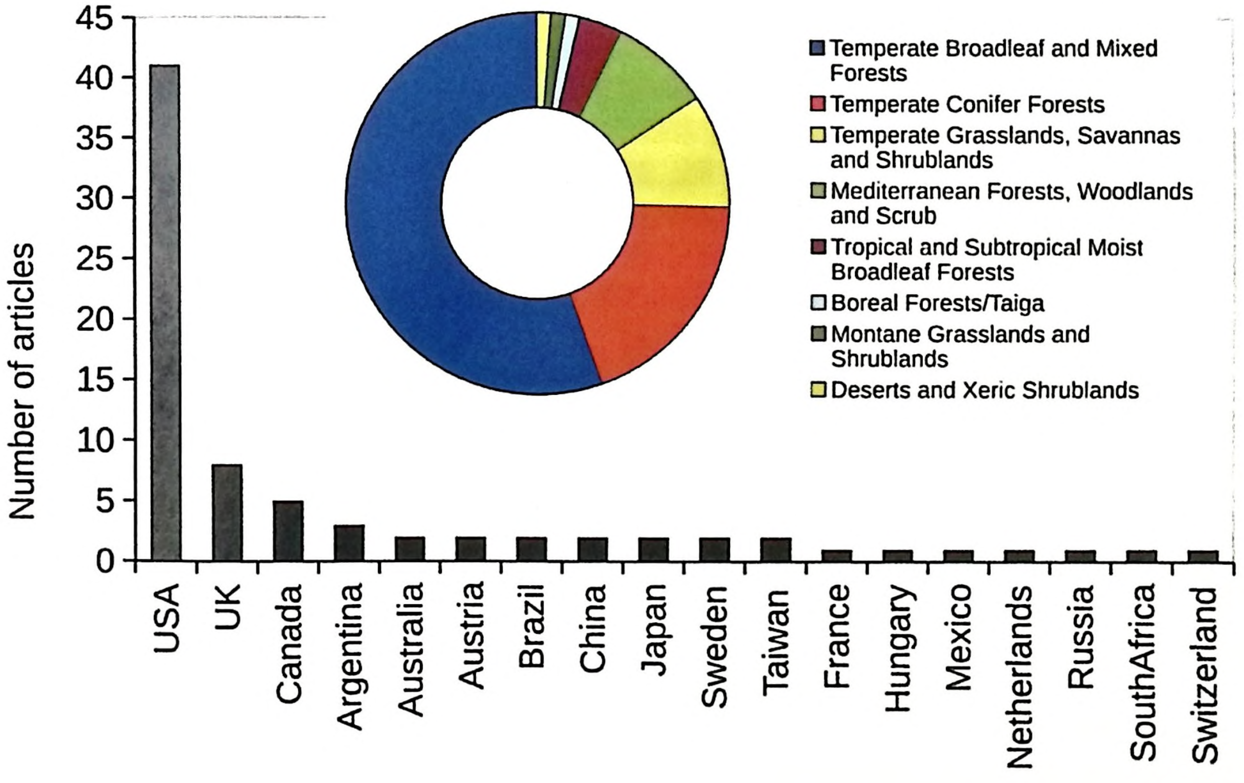


Fig. 2 Total number of articles published between 1958 and 2019 on overwintering anuran larvae (*N*=66) in relation to the country of affiliation of the authors (*N*=78). Inset chart shows the number of observations (*N*=83) and their terrestrial biomes. Modified from Jithin & Das (2020)

Various aspects of overwintering have been documented including seasonal changes, elevation, latitude, temperature and resource availability (Macedo, 2019; Pintar, 2000; Smith-Gill & Berven, 1979). Relationship with elevational gradients (Ultsch et al., 1999), developmental stage (Walsh et al., 2016), duration (Emerson, 1988), temperature range of overwintering events (Collins, 1979; Lai et al., 2002), and body size variation among overwintering tadpoles (Wilbur & Collins, 1973), development of different age classes and morphological variations with overwintering have been reported (Brown, 1990).

Ecophysiology and reproductive and developmental biology are the other major aspects of previous studies (Fig. 3) despite the increased understanding of relationship of overwintering with disease ecology, ecotoxicology for example, in cases where overwintering tadpoles act as disease reservoirs (Narayan et al., 2014).

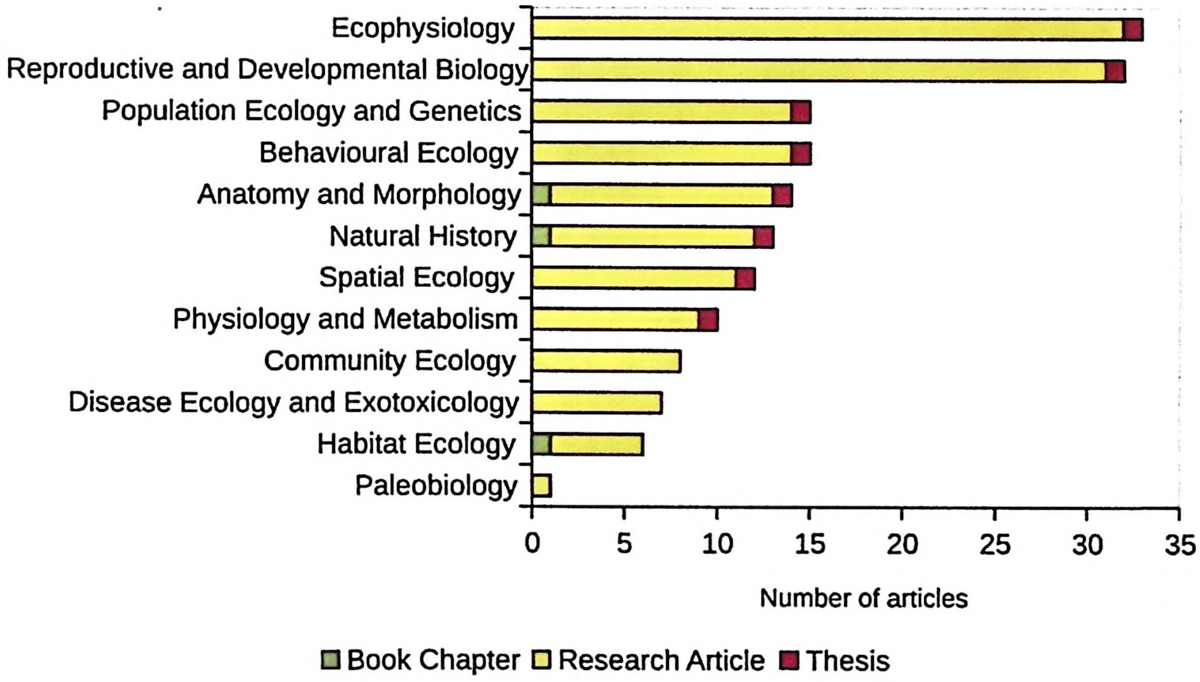


Fig. 3 Articles published on overwintering anuran larvae (*N*=66) according to the aspect of studies and type of publication. Category 'Research article' also includes natural history notes, and correspondence articles. Modified from Jithin & Das (2020)

Collins & Lewis (1979) reported that 13 species under the genus *Rana* and *Ascaphus truei* are known to overwinter in the continental U. S. According to Hsu et al. (2012), 40 species and 17 genera known to have overwintering tadpoles, but they did not provide any further details. In a global review, Jithin & Das (2020) found that at least 23 species of anurans are reported to have overwintering tadpoles (Fig. 4).

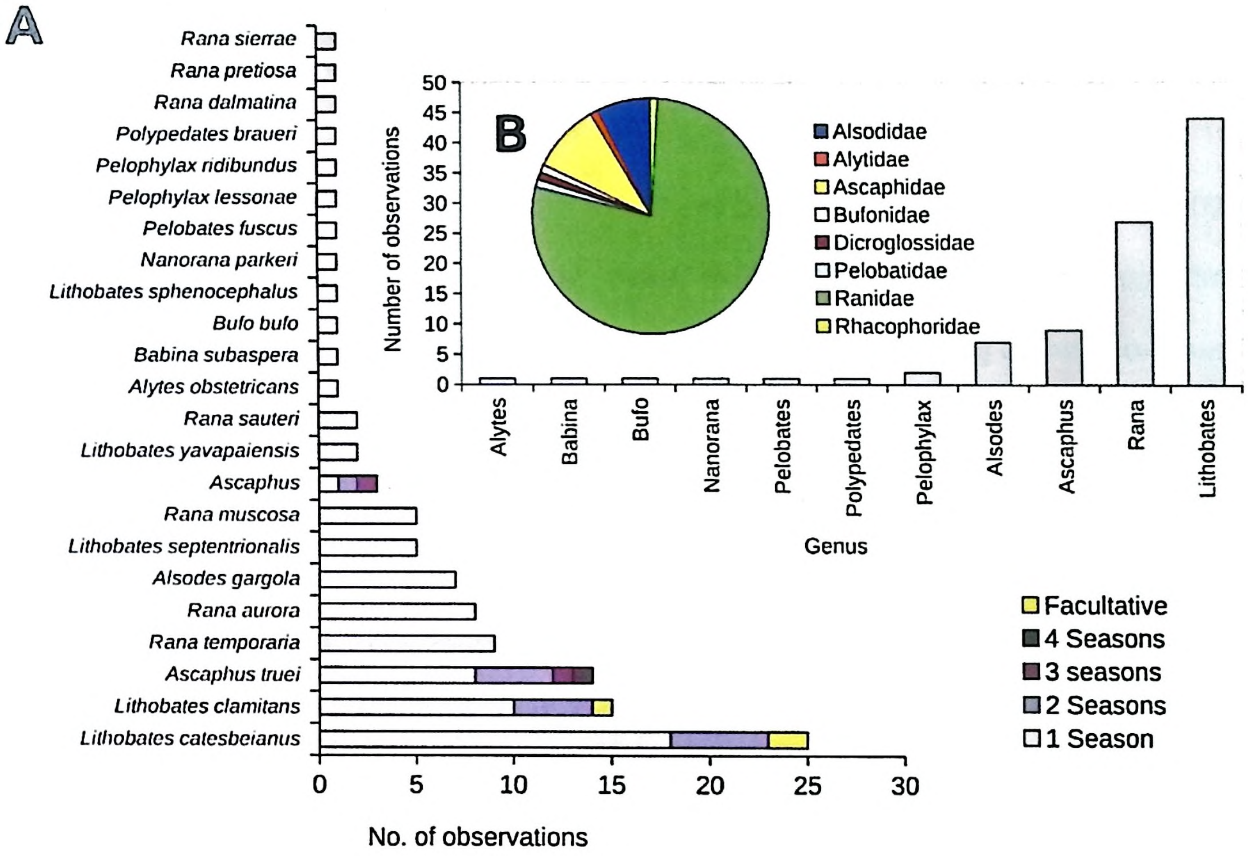


Fig. 4 Charts showing (A) number of observations for different overwintering durations; (B) taxonomic composition of the observations (*N*=95). Modified from Jithin & Das (2020)

Ranidae is one of the families mostly reported to be having overwintering tadpoles, but this can be an artefact of the bias in studies and (or) extensive studies done on these family in general which increased detection of overwintering in their tadpoles (Berven et al., 1979; Collins & Lewis, 1979; Hsu et al., 2012). Same reasons can be given for the high number of reports on the genera *Rana* and *Lithobates* (Frost, 2020; Hillis & Wilcox, 2005; Yuan et al., 2016).

Phylogenetically, overwintering trait is present from the primitive anuran family Ascaphidae to the advanced Ranidae (Jithin & Das, 2020). The study called for a detailed analysis of an extensive database of overwintering tadpoles in the light of changes in the ancient climate; 'equable climate problem' (Huber & Caballero, 2011) of warm extratropical annual mean and winter temperatures above freezing point during the early Eocene; high latitude warming concurrent with the Antarctic ice sheet expansion (Billups & Schrag, 2003); Eocene-Oligocene climate transition (Liu et al., 2009); and heterogeneity in global vegetation and rapid global cooling (Pound & Salzmann, 2017).

Observations of overwintering tadpole from India have been reported by Jithin & Das (2020) and the study emphasized the need of more explorations and documentation from Neotropics, Oriental and Sino-Japanese regions for a clear understanding of the geo-climatic affinities of overwintering tadpoles (Fig. 5). The study also confirmed the latitudinal gradient effect, but not the elevational effect, which warrants further investigations on altitude accounting for distribution ranges of amphibians. The study also pointed out the need of revision of the concept of 'early developmental stage overwintering' as an adaptive strategy considering the idea of the vulnerability of ice-damage to hind limbs (Lai et al., 2002; Walsh et al., 2008, 2016). The study also suggested some potential variables to be tested in further explorations. This include commonness or rarity of the species in the reported regions, ecomorphological characters of the tadpoles, physiological and morphological adaptations, length of growing seasons, temperature range and associated breeding season variations, and species interactions are some of the variables (Boone et al., 2004; Collins & Lewis, 1979; Hsu et al., 2012; Lai et al., 2002; Wallace & Diller, 1998; Walston & Mullin, 2007).

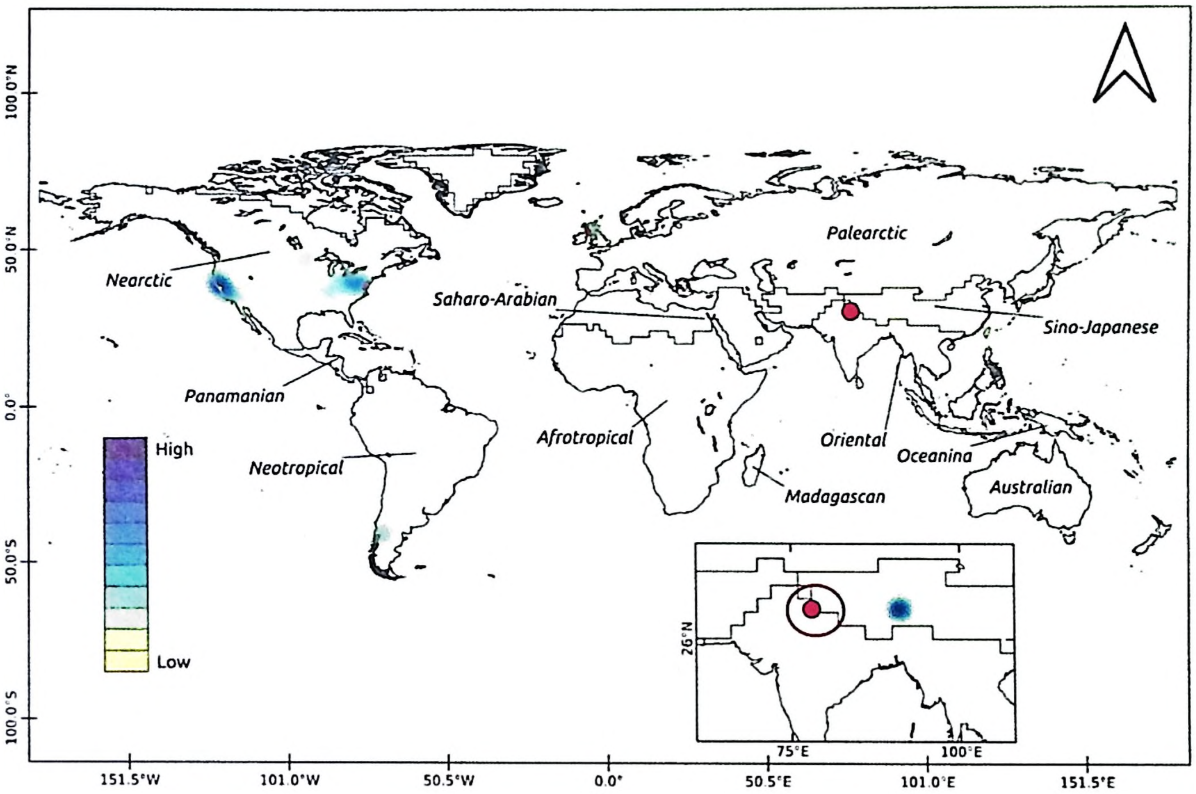


Fig. 5 A Heatmap showing the recorded observations of overwintering tadpoles (*N*=84) and their respective biogeographic realms from Jithin & Das (2020). The red dot indicates field-based data from India, and the location of the present study. Note the inset graphics to distinguish this observation from the Taiwan subtropical evergreen forest. From Jithin & Das (2020)

Aspects of physiological, behavioral and developmental biology of overwintering tadpoles remain poorly studied. Questions pertaining to adult and larval overwintering differences; advantages relating to the high tolerance of submergence hypoxia, energy expenditure of tadpoles during harsh winters (Bradford, 1983; Snodgrass et al., 2005; Tattersall & Ultsch, 2008), and endocrinological changes during overwintering are some of the important ones.

Bimodal distribution in the developmental stage pattern in overwintering tadpoles have been reported (Pintar, 2000; Walsh et al., 2008) showing the plasticity in development. Lai et al., (2002) suggested that the reduction in development would be accompanied by a reallocation of investment into growth. Studies from the field confirm the bimodal pattern which separates overwintering tadpoles from the non-overwintering ones suggesting the early developmental arrest (Walsh et al., 2008). This is contested by lab experiments where overwintering occurred at a relatively late stage (ca. stage 38) when limbs are well developed and some individuals arrested development onset of the metamorphic climax (stage 41) (Walsh et al., 2016).

Food resource availability has a significant influence on time to metamorphosis and thus overwintering. This has been shown by laboratory experiments in which low temperatures and limited food retarded both growth and development, resulting in overwintering; also showing the facultative nature of the strategy (Hsu et al., 2012). Lengths of the larval period have been found to be sensitive to changes in food level in lab experiments (Berven & Chadra, 1988). The diet influences the rate of growth and development, which essentially affects the timing of metamorphosis (Denver, 2019). Larval diet studies have shown the variability in the relative amounts of protein, carbohydrate and lipid in the natural diets which are essential for metamorphosis through influence on thyroid hormone function (Kupferberg, 1997). In this review by Kupferberg, he also suggests the seasonal and spatial variation in resources of varying nutritional content could have a large effect on size and timing of metamorphosis.

The temporal and spatial distribution of tadpoles are influenced by temperature fluctuation and light intensity variation (Beiswenger, 1977; Brattstrom, 1962; de Andrade et al., 2014; Leão-Pires et al., 2017; Whiteman & Buschhaus, 2003; Wollmuth et al., 1987). Reduced feeding behavior at night has been reported (Beiswenger, 1977; Warkentin, 1992) and low water temperature in the night is attributed to this observation. Microhabitat choices of tadpoles have been studied across seasons considering its relationship with predation risk, food availability and temperature. Altered patterns of microhabitat use by tadpoles may indicate the existence of spatial and temporal selective pressures which are still not well understood (Eterovick et al., 2010).

A more systematic, long-term monitoring framework for overwintering species will be helpful in understanding variations in breeding phenology, upslope shifts, and disease ecology in the light of climate change (Freeman et al., 2018; Garner et al., 2011).

2.b Indian Scenario

To the best of my knowledge, there are no studies from the country on overwintering tadpoles. The gap in study and description of larval forms or developmental modes compared to their adult forms of amphibians in the country have been reported (Sircar, 2010). Lack of tadpole behavioral ecology studies have also been highlighted considering the species richness, endemism, climatic variability and habitat heterogeneity in the country (Saidapur, 2001). Jithin & Das (2021) reported two overwintering tadpole populations of *Nanorana* sp. in Uttarakhand; Mussoorie Wildlife Sanctuary (Binog Mountain Quail WLS) and Dhanaulti. There is another population at Devalsari, Aglar valley of Tehri Garhwal district (30° 32' 37.13" N, 78° 11' 5.56" E, 1200 - 3048 m. asl.; *personal observation*).

3 IMPORTANCE OF THIS STUDY

1. Larval overwintering is a survival strategy of the Himalayan stream breeding frogs that remains poorly understood.
2. Insights into the overwintering aspect will help us understand the crucial influence of temperature on tadpoles in the stream environment.
3. The complex microhabitat interacting with temperature fluctuations provides us the opportunity to determine the potential use of these tadpoles as model organisms for further studies.
4. Since one of the expected focal species (*Nanorana minica*) is listed in the vulnerable category of the IUCN Red List, conservation plans need to be in accordance with their larval overwintering habitats and seasons taking the modification of stream sections into consideration.

4 FOCAL SPECIES AND SYSTEM

4.a Focal Species

A. Nanorana minica (Dubois, 1975)

Small Paa Frog or Nepal Paa Frog is a dicroglossid frog distributed in Nepal, Uttarakhand and Himachal Pradesh at elevations of 1000 to over 2400 m elevation (Bhattarai et al., 2020; Frost, 2021; Ohler et al., 2004). It is listed as a vulnerable species in the IUCN Redlist since its area of occupancy is less than 2,000 km^2, distribution is severely fragmented, and due to habitat degradation (Ohler et al., 2004). *N. minica* is found in montane subtropical forests and streams and breeding occur in streams. Larval ecology is poorly known and several larval descriptions of varying levels of completeness have been reported in Das & Dutta (2007). Reproductive ecology of the species is also not well understood. Major threat the species is facing are loss of habitat through forest clearance and waterway management (Ohler et al., 2004). Limb deformity in the species was reported by Banerjee et al. (2020) from Mussoorie WLS. A compilation of available larval description is given in the annexure. Three new batrachotrematid trematode species were discovered from *N. minica* (Rizvi et al., 2012). Benerjee et al. (2020) reported *N. minica* tadpoles are mostly seen scraping algae on the vertical walls of the modified pools and they are generally found around leaf litter in run and riffle sections of the stream. *N. minica* has an Evolutionarily Distinct and Globally Endangered (EDGE) score of 3.95 (EDGE, 2020; Isaac et al., 2007).

B. Nanorana vicina (Stoliczka, 1872)

Himalaya Paa Frog or Murree Frog is a dicroglossid frog distributed in the Himalayan front of India, north-central Pakistan from Kashmir, Himachal Pradesh to Punjab, and Uttarakhand. It is a high-altitude species (2000-3000 m. asl.) associated with montane streams, springs, fountains and other running water within open forest and grassland habitats (Frost, 2021; Ohler & Dutta, 2004). Banerjee et al. (2020) communicated *N. vicina* tadpoles feeding on Himalayan Toad (*Duttaphrynus himalayanus*) adult carrion. Gill et al., (2020b) described the external morphology, morphometry and oral disc (Gill et al., 2020a) of the tadpoles of *N.*

vicina from Pakistan. Tail end of the tadpoles are rounded (Dutta, 1999; Gill et al., 2020b). Gill et al. (2020b) also reported the difference in the Labial Tooth Row Formulae (LTRF) between lower and advanced development stages. Khan (2015) reported the eggs are found in patches of 10-30 clutch size, attached on rocks. He also documented the habitat as side pools along torrents and lentic-lotic areas. Sircar (2010) found the approx. 700 eggs per clutch attached on rock surface and crevices. The same study also reports the presence of mixed size classes in the same pool and speculates the breeding season in post-winter when snow starts to melt. Interspecific amplexus between *N. vicina* and *Duttaphrynus himalayanus* (Bufonidae) have also been recorded in the month of March (Jithin et al., 2021). Dutta (1999) reported that the species is quite active in night time and breeds during June and July when the tadpoles are found in the streams, from a population at the Great Himalayan National Park, Himachal Pradesh. The EDGE score of *N. vicina* is 2.56 (EDGE, 2020; Isaac et al., 2007).

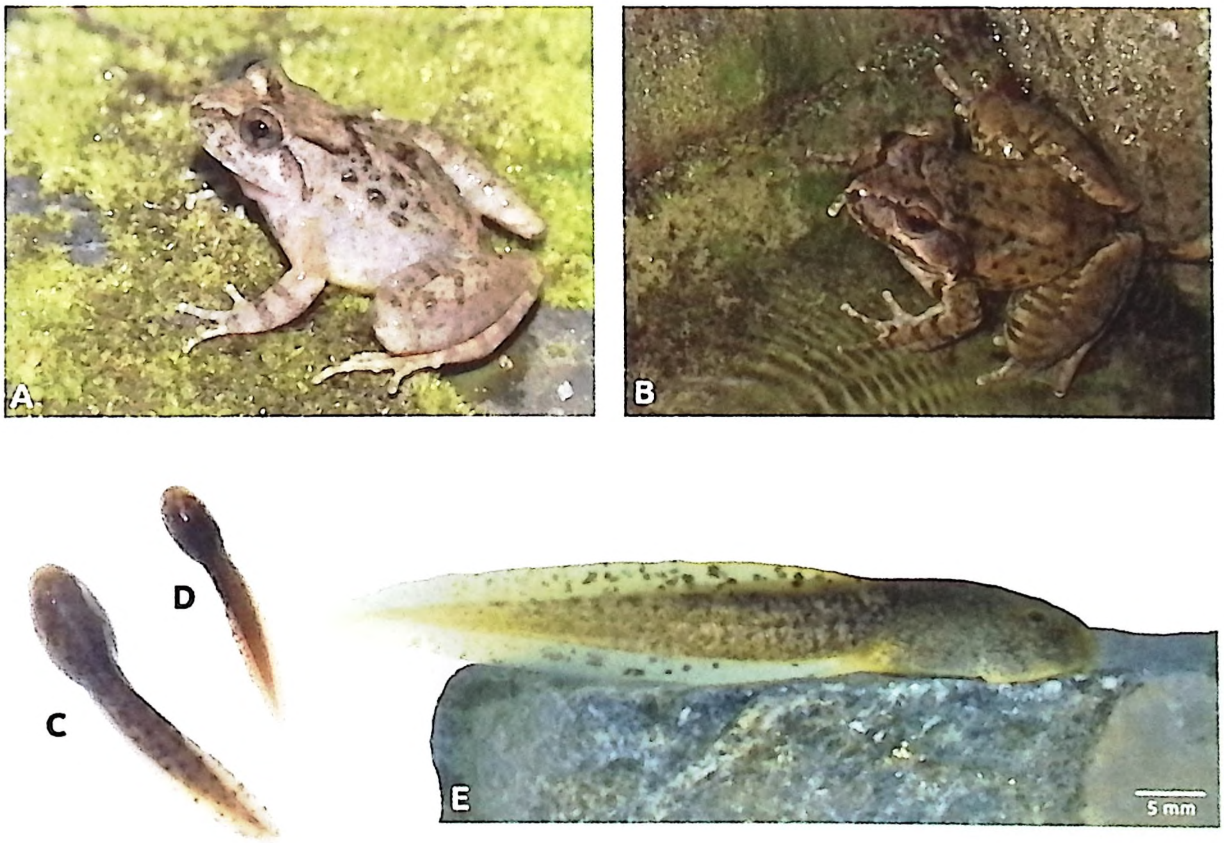


Fig. 6 Photographs of adult (A) *Nanorana minica* (B) *Nanorana vicina;* Dorsal view of tadpoles with (C) round-ended tail tip and (D) point-end tail tip; and (E) lateral view of *Nanorana* sp. tadpole. Photographs of adult frogs by Abhijit Das, and tadpoles by V. Jithin. Except E, all images are not to scale.

Saeed et al. (2021) studied the response of *N. vicina* tadpoles to different temperatures through metamorphosis, body size, deformities, fitness and survival. They found faster metamorphosis, reduction in body size, frequent developmental complications or deformities (edema and tail kinks), lower fitness and higher mortality at elevated temperatures using a combination of lab and field-based studies.

Natural History of the *Nanorana spp.* Tadpoles

Tadpoles of *Nanorana* spp. were found to be feeding on algae present on the walls of modified pools, bedrocks, boulders, cobbles, leaf litter surface, submerged log in pool; humus and carcass of Stone loach (*Schistura rupecula)* (*personal observation*). They are generally more active in night during winter, but the emergence pattern and activity varies with pool modification (Fig. 7) and both day and night during post-winter period. During winter, mostly they are not very active, and found resting on the pool substratum during both night and daytime. During day time, they are mostly found hiding under leaf litter, or inside rocky crevices. When disturbed with torch light in the night, most individuals quickly moved away from the point of light incidence, but some remains. I did not observe any predation events during the study period and tadpoles were generally co-occurring with water striders, odonate and damselfly larvae (*personal observations*).

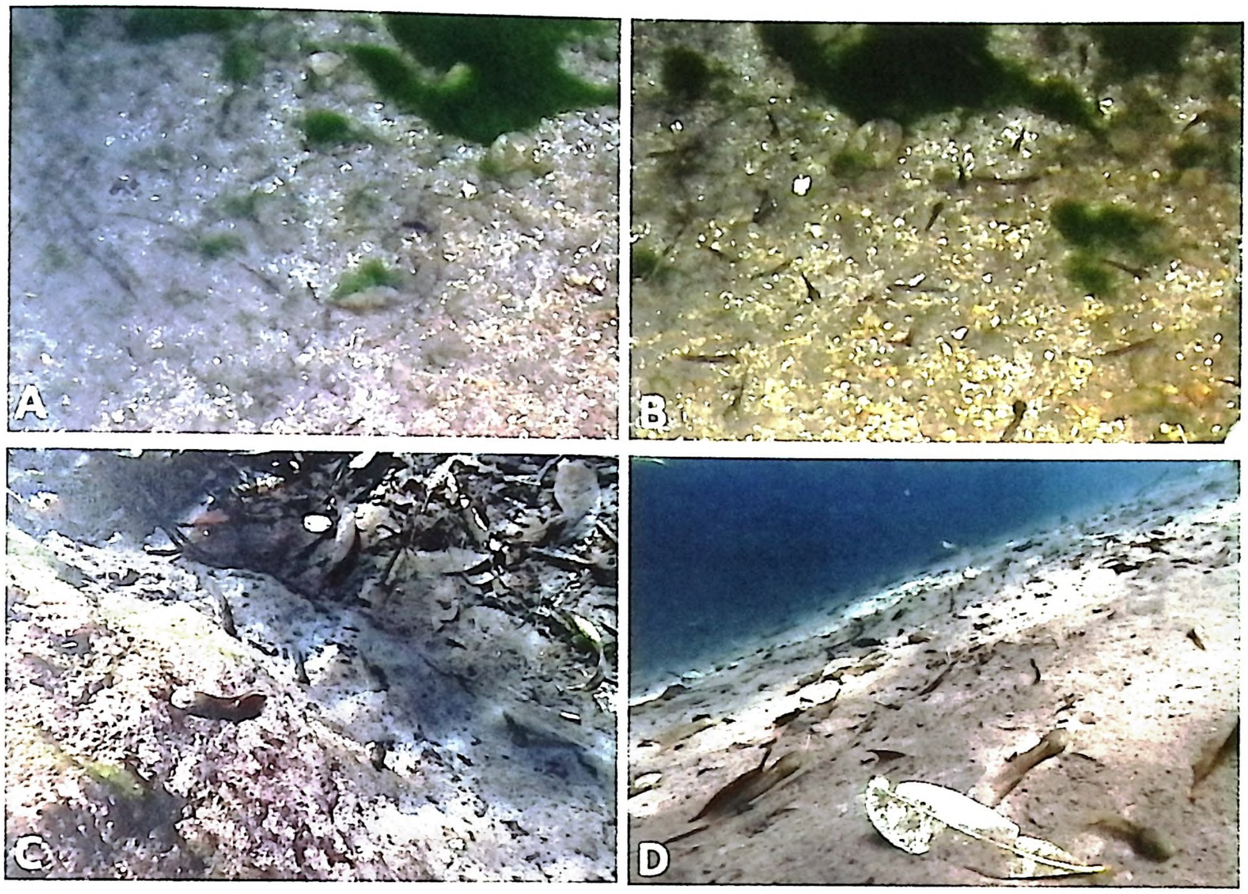


Fig. 7 Repeated photography showing the absence of tadpoles in (A) daytime and (B) presence in night during winter; Underwater photographs showing activity of tadpoles in the daytime in (C) natural pools, (D) modified pools during transition between winter to post-winter. Photographs by V. Jithin.

4.b Focal System

The streams in the study area are source of drinking water for the Mussoorie area. For water extraction and recharge purposes, check dams are built at varying intervals of the stream and a pump house of the Garhwal Jal Sansthan is situated in the compartment eight of the sanctuary. Mussoorie area depends on such montane streams and springs for its water supply. Mussoorie, being a highly tourist attraction place, faces huge water availability crisis (7.67 Minimal Liquid Discharge [MLD] supply against demand of 14.5 MLD) especially during the high tourist influx during summer (Mishra, 2016; Mishra & Upadhyay, 2018). In the study area, regular renovation of check dams and cleaning of silt happens (Fig. 8)

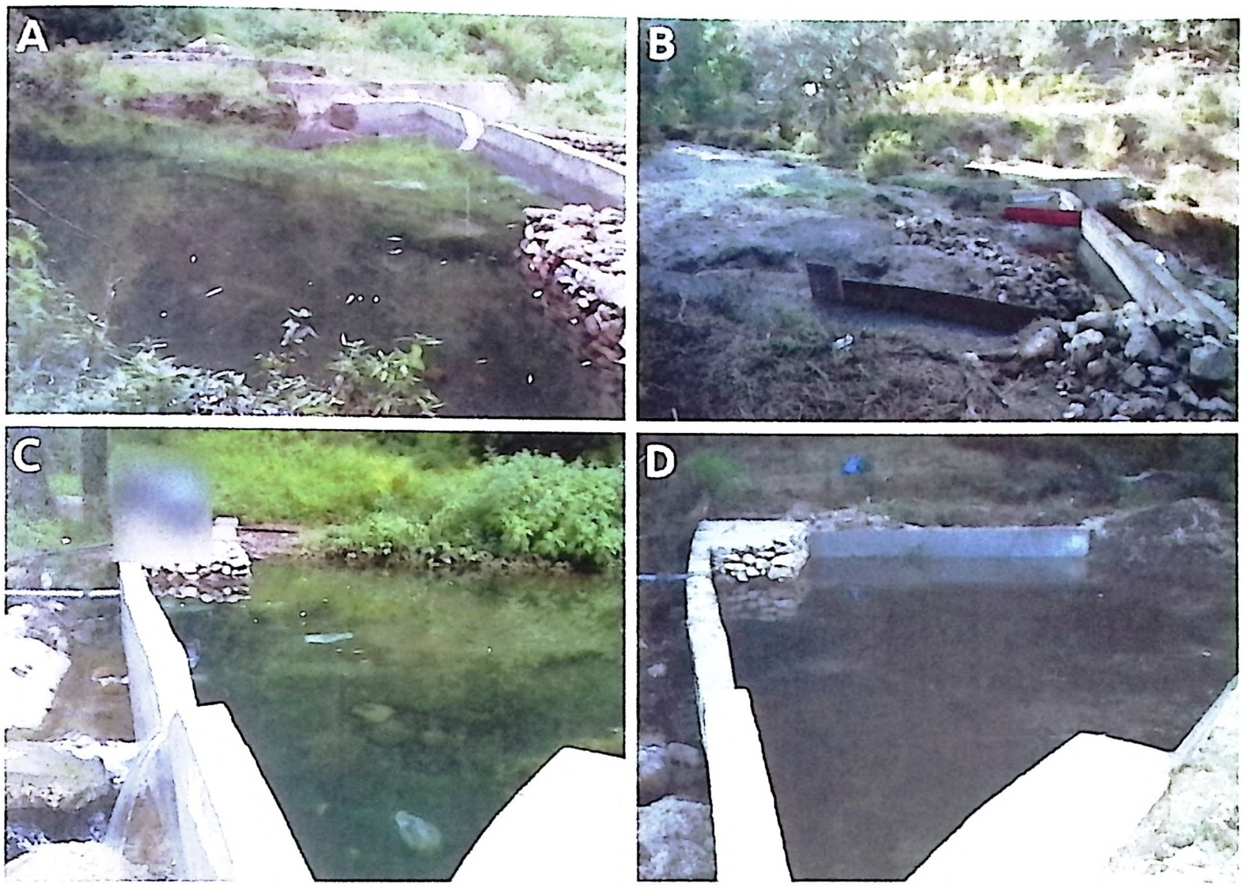


Fig. 8 Images showing renovation of modified pool in the focal stream during the study period. A) Pool during 2015 with full water and (B) during 2021 with dried streambed and renovation in progress. (C) Pool in 2015 showing the outflow of water (D) after renovation in 2021. Photographs by T. S. Rawat (A & C), V. Jithin (B &D)

The modified pools are very well used by the tadpoles, and this might be because of the highly nutrient rich humus substrate or the temperature gradient it provides. The algae that grow on the walls of check dam and logs inside the dammed portions are good feeding areas for tadpoles during post-winter (Fig. 9).

Fig. 9 Underwater photographs showing tadpoles feeding on the algal growth on (A) logs inside the check dams and (B) check dam walls

During maintenance works and renovation of these check dams, silt is removed, bottom is cleaned, pebbles and small boulders from other natural pools are deposited in the bed to facilitate the water filtration. This water directly goes to the pump for distribution via long pipes from each check dams through underground. During one such maintenance event during March 2021, the author quantified mortality of overwintering tadpoles. Thirty-two tadpoles found dead and 61 tadpoles left in the silt out of approx. 150 tadpoles in an area of 2.5 m^2. During the process, water is generally diverted from the natural flow way. The process takes at least two days to finish and by this time, tadpoles, fishes and crabs are either died or predated by birds (Fig. 10).

Fig. 10 Tadpole mortality during check dam maintenance and renovation; the pool portion (A) prior to renovation when the water flow stopped and diverted and (B) after renovation.

5 AIM

The study was aimed to investigate the morphological diversity and habitat use of overwintering tadpoles of *Nanorana* spp. and their response to the temperature and disturbance gradient in the pool sections of the stream.

6 OBJECTIVES

1. Assessing ecological correlates of density of overwintering tadpoles across natural and modified pools.
2. Understanding the morphological diversity of overwintering tadpoles.
3. Evaluating the variation in habitat availability and use by overwintering tadpoles.

7 HYPOTHESES

A. Multiple anuran larval species at early developmental stages (ca. Gosner 32-35) are overwintering in the study areas.

1. Preliminary morphological observations and distributional records from the study areas, suggests that tadpoles of sympatric species *N. minica* and *N. vicina* undergo overwintering (Banerjee et al., 2020; Jithin & Das, 2020).
2. It is predicted that overwintering in tadpoles should be occurring at an early developmental stage (Gosner 32-35) due to the vulnerability of ice-damage to hind limbs (Lai et al., 2002).
3. Field studies confirm the bimodal pattern which separates overwintering tadpoles from the non-overwintering ones suggesting the early developmental arrest (Walsh et al., 2008), which is contested by lab experiments where overwintering occurred at a relatively late stage (ca. stage 38) when limbs are well developed and some individuals arrested development onset of the metamorphic climax (stage 41) (Walsh et al., 2016).

B. Temporal and spatial microhabitat usage of overwintering tadpoles varies with the temperature gradient in natural and modified pools.

1. Temporal and spatial distribution of tadpoles are influenced by temperature fluctuation and light intensity variation (Beiswenger, 1977; Brattstrom, 1962; de Andrade et al., 2014; Leão-Pires et al., 2017; Whiteman & Buschhaus, 2003; Wollmuth et al., 1987).
2. Altered patterns of microhabitat use by tadpoles may indicate the existence of spatial and temporal selective pressures which are still not well understood (Eterovick et al., 2010).

3. Food resource availability has a significant influence on time to metamorphosis and thus overwintering. This has been shown by laboratory experiments in which low temperatures and limited food retarded both growth and development, resulting in overwintering; also showing the facultative nature of the strategy (Hsu et al., 2012).
4. Seasonal and spatial variation in resources of varying nutritional content could have a large effect on size and timing of metamorphosis (Kupferberg, 1997).

8 RESEARCH QUESTIONS

Objective I

A. Do density and behavior of tadpoles vary with season and modification of pools?

B. What are the best predictors of the temporal and spatial distribution patterns of the overwintering tadpoles?

Objective II

A. Which are the amphibian species overwintering in the study area? *

B. Are tadpoles of relatively lower developmental stages only overwintering?

Objective III

A. Whether the microhabitat use of overwintering tadpoles varies between natural and modified pools with the temperature gradient?

B. Whether diet composition and diet breadth of overwintering tadpoles varies across seasons? *

C. Is there a difference in microhabitat selection by overwintering tadpoles based on their size? **

*Even though samples have been collected, they were not processed due to logistical constraints imposed by COVID-19 lockdown. **Question not addressed.

MATERIALS & METHODS

1 STUDY AREA

The focal study stream, Dhobhighat (Ringali Gad) is flowing through the Mussoorie Wildlife Sanctuary (28° 60' - 31°28' N, 70°49' - 80°60' E) (formerly Binog Mountain Quail Wildlife Sanctuary) situated in Mussoorie hills, spreading over an area of 1081.97 hectares with an elevation range between 1500 and 2330 m. asl (Fig. 11). The area occurs in the outermost ridges of Himalaya in the west-east direction (Kumar et al., 2012; Mohan & Sondhi, 2015). The Binog hill lies to the north of the outer ridge, separated by the Ringali River. Banj oak forests, Chir Pine forests and Grasslands/ blanks are the major forest types in the sanctuary and classified as Himalayan moist temperate forest (Champion & Seth, 1968; Kumar et al., 2012).

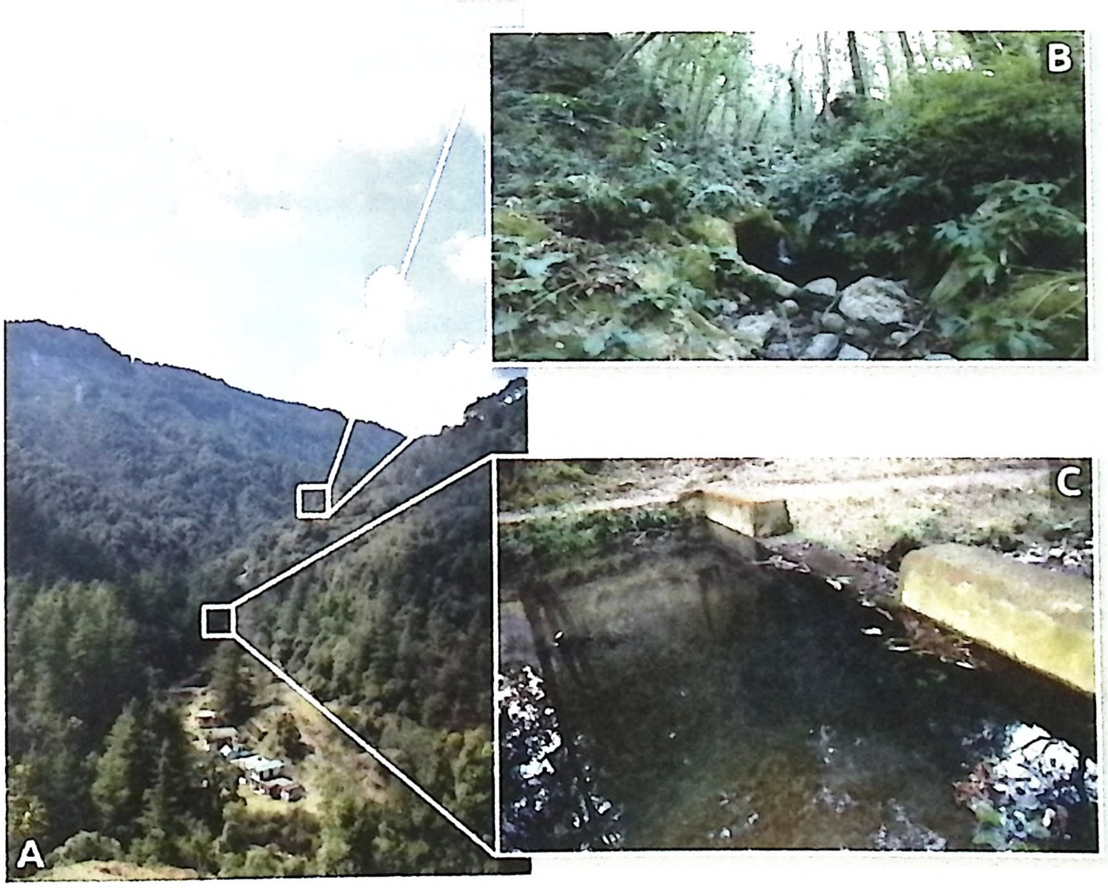


Fig. 11 Dhobhighat stream flowing through the (A) Mussoorie Wildlife Sanctuary; showing unmodified upstream; and (B) modified downstream sections. Photographs by V. Jithin

Some portions of the stream are dammed, creating large pool habitats in the outside sanctuary area. The check dams were initially built during 1960s for extraction of water for the Mussoorie area. Research on movement ecology of *Nanorana vicina* in the study area by Wildlife Institute of India team made initial observation of overwintering tadpoles in the stream. Later, a preliminary survey conducted as part of this thesis work during December 2019 confirmed the presence of overwintering tadpoles in the streams passing through the area. The area was chosen considering the presence of the overwintering tadpoles, accessibility and disturbance gradient in the stream.

The sanctuary comprises of eight forest blocks namely Bhadraj, Binog, Murrays Spring, Kandigaon, Municipal Garden, Bhilaru and Koti Kimoin. The study was conducted in the Dhobhighat stream portion flowing through the south of Binog block (338.74 Ha), a main drinking water source for the wild animals and source for the Mussoorie area. Mussoorie, the well-known tourist place forms the north-west boundary of the sanctuary. The sanctuary forms the catchment of Aglar river, a tributary of the Yamuna River. Dhobhighat is also the origin of the famous tourist destination Kempty falls in Mussoorie (Management Plan, 2010).

1.a Geology, Terrain and Climate

The youngest and tectonically active middle Himalayan eco-system is ecologically diverse, unique and fragile, which underlines the importance of the ecological understanding of this area (Dewan, 1990; Joshi & Joshi, 2011; Munsi et al., 2010). The underlying rocks generally have a dip of ~30"-40" towards the north-east and belong to the 'Purana' group. Limestones in various forms is the principal rock. In folds and favorable aspects, deep and moist soil can be seen. The hilly terrain has all the aspects represented, though northern aspect is most common. The Binog hill, an isolated hill in the north side of the outer ridge of Himalaya is separated by the Dhobhighat, a perennial water source (Management Plan, 2010).

The area is in the upper Gangetic plains moist deciduous forests terrestrial ecoregion (Olson et al., 2001) and temperate, dry winter, hot summer (Cwa) climate class (Kottek et al., 2006). The climate has well marked seasons. Winter (December-February) is characterized by snowfalls during January-February. A rapid transition from winter to summer happens through March and April, but occasional snowfalls may happen up to March. May and June are comparably warm with a maximum temperature up to 35°C. But this varies according to the elevation, and higher elevations are comparably cooler. During May end and June, thunder storms occur, but with a slighter rainfall. In northern aspects this ranges from 1800mm to 3000mm, but snow lies for a longer period compared to the more exposed southern slopes. According to the Management Plan (2010) of the sanctuary, the minimum temperature recorded during 2000–2010 indicates a range of 2.9°C–9°C, and maximum range 26.8°C-32.9°C; but generally, 0°C-5°C in winter and 20°C-30°C during summer. The monsoon sets in by July and it may last till September to October. During this time, moist atmosphere, less sunshine due to heavy clouds and mists can be observed. The autumn lasts from middle of September to December with clear atmosphere, and then slowly transform to winter with fall in temperature. During May and June, wind storms with hail storms are quite common in this area

1.b Flora

A total of 300 species of angiosperms, 31 species of Pteridophytes and 4 species of Gymnosperms are present in the sanctuary (Kumar et al., 2012). Dominant families among angiosperms were Asteraceae, Papilionaceae, Lamiaceae, Rosaceae, Poaceae, Ranunculaceae, Lauraceae, Polygonaceae, Urticaceae, and Rubiaceae. Among Gymnosperms, Coniferae and Cupressaceae families are present. Family Polypodiaceae was dominant among pteridophytes (Kumar et al., 2012). Some major canopy cover providing tree species includes *Quercus oblongata, Toona ciliata, Rhododendron arboreum, Cornus macrophylla* and *Pinus roxburghii.* Major shrubs and ferns include *Cirsium wallichii, Deutzia staminea, Hypericum oblongifolium, Araiostegia pseudocystopteris, Cyrtomium* *macrophyllum, Thelypteris erubescens, Lepisorus nudus, Polystichum discretum* and *Polystichum obliquum*. The Ringal Gad stream got its name from the Ringal bamboo (*Drepanostachyum falcatum*). Some major riparian vegetation elements include *Daphniphyllum himalense*, *Mahonia napaulensis, Sarcococca pruniformis, Daphne papyracea, Pteris cretica,* and *Dryopteris wallichiana.*

1.c Fauna

The sanctuary is designated as an Important Bird Area (IBA) (Mohan & Sondhi, 2015; Rahmani & Islam, 2004). Some common birds in the study area are Black-headed Jay (*Garrulus lanceolatus*), Great Barbet (*Psilopogon virens*), and White-throated Laughingthrush (*Lanthocincla albogularis*). The critically endangered Himalayan Quail (*Ophrysia superciliosa*) was last sighted in this area and is feared extinct. Kalij Pheasant (*Lophura leucomelanos*), Himalayan Griffon (*Gyps himalayensis*), White-capped Redstart (*Phoenicurus leucocephalus*), Spotted Forktail (*Enicurus maculatus*), Gray Treepie (*Dendrocitta formosae*), Blue Whistling-Thrush (*Myophonus caeruleus*), Blue-fronted Redstart (*Phoenicurus frontalis*), Plum-headed Parakeet (*Psittacula cyanocephala*), Slaty-headed Parakeet (*Psittacula himalayana*), Plumbeous Redstart (*Phoenicurus fuliginosus*), Himalayan Bluetail (*Tarsiger rufilatus*), Long-tailed Broadbill (*Psarisomus dalhousiae*), Eurasian Jay (*Garrulus glandarius*), Greater Yellownape (*Chrysophlegma flavinucha*), Lesser Yellownape (*Picus chlorolophus*), Collared Owlet (*Glaucidium brodiei*), Mountain Scops-Owl (*Otus spilocephalus*), Brown Fish-Owl (*Ketupa zeylonensis*), and Red-billed Blue-Magpie (*Urocissa erythroryncha*) are some of the bird species frequently encountered near the stream area.

The herpetofaunal community of the area include amphibians *Nanorana minica* (Small Paa Frog or Nepal Paa Frog), *Nanorana vicina* (Murree Hill Frog or Himalayan Paa Frog), *Duttaphrynus himalayanus* (Himalayan Toad), *Amolops formosus* (Beautiful Stream Frog), *Amolops jaunsari* (Jaunsar Stream Frog); and reptiles *Boiga multifasciata* (Many Banded

Tree Snake), *Herpetoreas platyceps* (Himalayan Keelback), *Gloydius himalayanus* (Himalayan Pitviper), *Lycodon mackinnoni* (Mackinnon's Wolf Snake), *Oligodon arnensis* (Common kukri snake), *Ptyas mucosa* (Oriental Ratsnake), *Sibynophis collaris* (Common Many-tooth Snake), *Asymblepharus himalayanus* (Himalaya Ground Skink), *Eurylepis* cf. *taeniolata* (Ribbon sided skink), *Japalura kumaonensis* (Kumaon Mountain Lizard or Kumaon forest agama), and *Laudakia tuberculata* (Tuberculated Agama or Kashmir Rock Agama) (Nawani et al., 2021).

Himalayan Goral (*Naemorhedus goral*) is the flagship species of the area. Other mammals include Leopard (*Panthera pardus*), Nepal Gray Langur (*Semnopithecus schistaceus*), Barking Deer (*Muntiacus muntjak*), Indian Crested Porcupine (*Hystrix indica*), and Asian Black Bear (*Ursus thibetanus*) (Management Plan, 2010).

Butterflies include Common map (*Cyrestis thyodamas*), Six-bar Swordtail (*Graphium eurous*), Walnut Blue (*Chaetoprocta odata*), Garhwal Ace (*Thoressa aina*), Mussoorie Bush Bob (*Pedesta masuriensis*), Pale Green Sailer (*Neptis zaida*), Himalayan Blackvein (*Aporia leucodice*), Spotless Grass Yellow (*Eurema laeta*), Indian Oakblue (*Arhopala atrax*), Tailed Punch (*Dodona eugenes*), Common Forester (*Lethe insana*), Himalayan Fivering (*Ypthima nikaea*), Peacock Pansy (*Junonia almana*), Blue Admiral (*Kaniska canace*), Himalayan Spotted Flat (*Celaenorrhinus munda*), Common Bluebottle (*Graphium sarpedon*), Large Cabbage White (*Pieris brassicae*), Indian Tortoiseshell (*Aglais caschmirensis*) (Boruah et al., 2020; Singh & Sondhi, 2016).

The major fish species reported in this region are *Schizothorax richardsonii* (Snow trout), and *Schistura sp.* (Stone loach) as mentioned in the Management Plan (2010).

1.d Management

Even though there is no human settlement inside the protected area, there are villages and private estates present outside the sanctuary, within 5km radius of boundary and the steam bank. Main source of incomes includes agriculture and livestock rearing. Livestock grazing is very common feature even inside the sanctuary. People are dependent on the natural forest resources. A pump house of the Garhwal Jal Sansthan is situated in the forest block Binog in compartment eight and the Jwala Devi temple in compartment four. Active soil and water conservation works recommended in the Management Plan (2010-2020) and are being carried out by the forest department. The portions of the stream outside the PA are heavily modified since it is flowing through private resorts, agricultural fields, and human habitations. Threats the area facing include denudation of forest, mining, watershed development, water extraction and stream modifications, fire incidents, unscientific land-use practice, and developmental activities.

2 SAMPLING METHODS

2.a Distribution and Microhabitat of Tadpoles

Portion of the Dhobhighat stream was selected as the intensive study area along the sanctuary. Four natural and four modified pool habitats were randomly chosen from a 0.5km stretch of this second order stream (Fig. 12). Concrete walls are present with wider broad-crested weirs (Davis, 1938) in the modified pools at both ends (M2) or in one end (Fig. 13). From the selected pool sections, six were delineated for intensive sampling. This included three natural pools (N1-N3, Fig. 14) and three modified pools (M1-M3, Fig. 15). Modified pools in the study area are majorly check-dams created for water extraction, recharge and soil erosion prevention. Details of all the intensive study sites are given in Table 1. The remaining two pools (B1 and B2) were used for behavioral sampling of tadpoles. Details of these pools are given in the respective section.

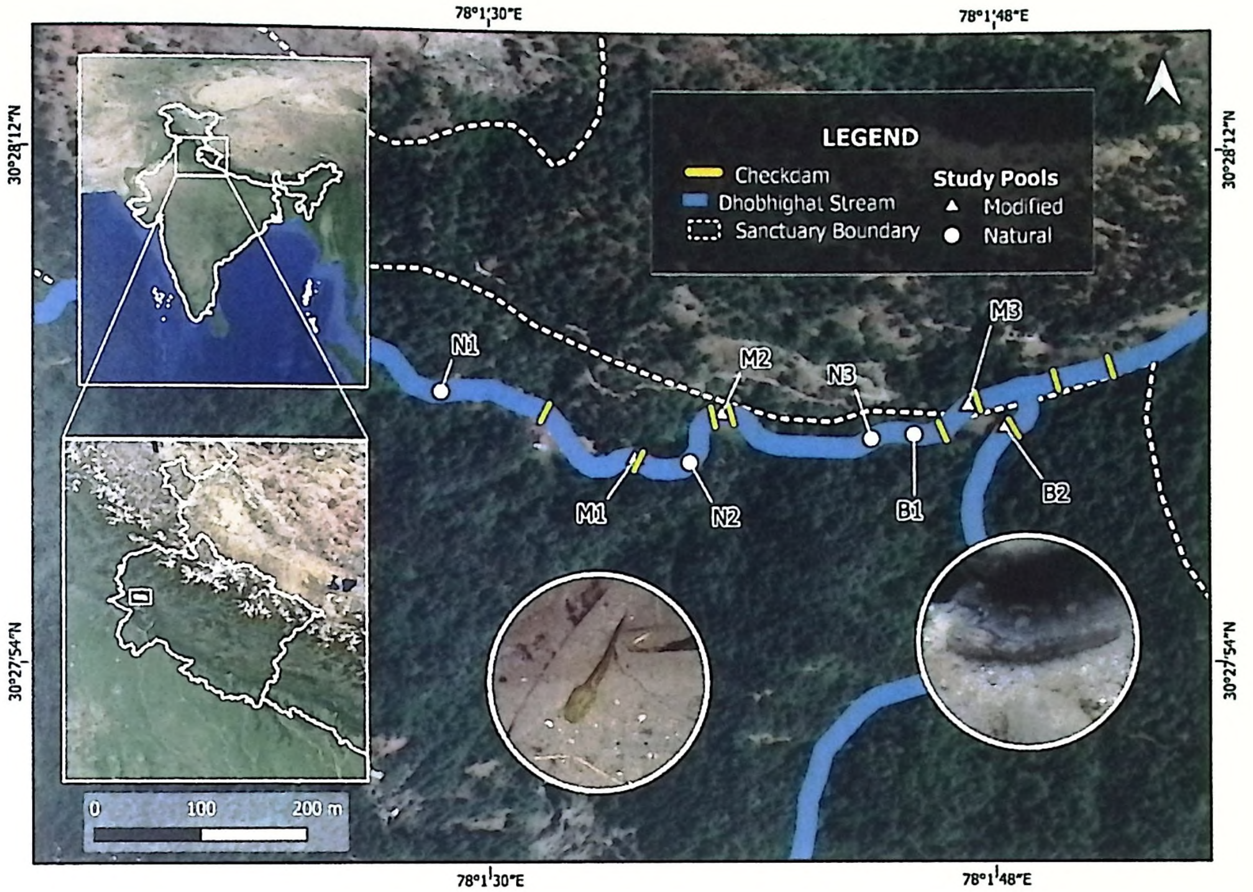


Fig. 12 Map showing the study area with study pools and check dam locations. Inset pictures shows overwintering tadpoles in the streambed. Photographs by V. Jithin

Table 1 Details of the intensive study sites (pool sections of the stream); CS- Cross Sections

Name	Pool Type	Slope (°)	GPS (N)	GPS (E)	Altitude (m. asl.)	Flow Direction	Actual Length (m)	No. of CS*
N1	Natural	5	30.46751	78.02444	1680	89 E	7.5	4
N2	Natural	1	30.46719	78.02699	1666	30 NE	14	7
N3	Natural	5	30.46734	78.02879	1642	130 E	9.5	5
M1	Modified	0	30.46698	78.02636	1648	60 NE	23.5	12
M2	Modified	0.5	30.4674	78.02738	1682	180 SE	25.8	13
M3	Modified	0.5	30.46781	78.02983	1648	90 E	27	14
B1	Natural	0	30.46724	78.02922	1645	50 NE	6.5	6
B2	Modified	0	30.46731	78.03011	1641	4 N	2.3	3

At each pool, detailed habitat inventory was carried out using multiple transects placed across 2m intervals along the stream. Depth (cm), velocity (m/s), wetted width (m) and substrate composition (bedrock, boulder, small boulder, cobble, gravel, sand, humus, leaf litter, algae, and other substrates) were recorded at every 1m interval (Grant et al., 1990; J. A. Johnson et al., 2020) in these transects.

Fig. 13 Check dam structure of Pool M1 showing the concrete wall with wider broad-crested weirs

Habitat-forming structures or cover items (bedrock undercut, boulder undercut, logwood, root undercut, submerged and overhanging vegetation) were quantified visually. Canopy cover was measured at three different points at each pool using a Spherical Densiometer (James C. Doster, Forest Densiometers, FL, Model A). To get accurate results, at each of these points, measurements were taken for four cardinal directions and averaged. For comparison across the pools, the average canopy cover value of all three points was used.

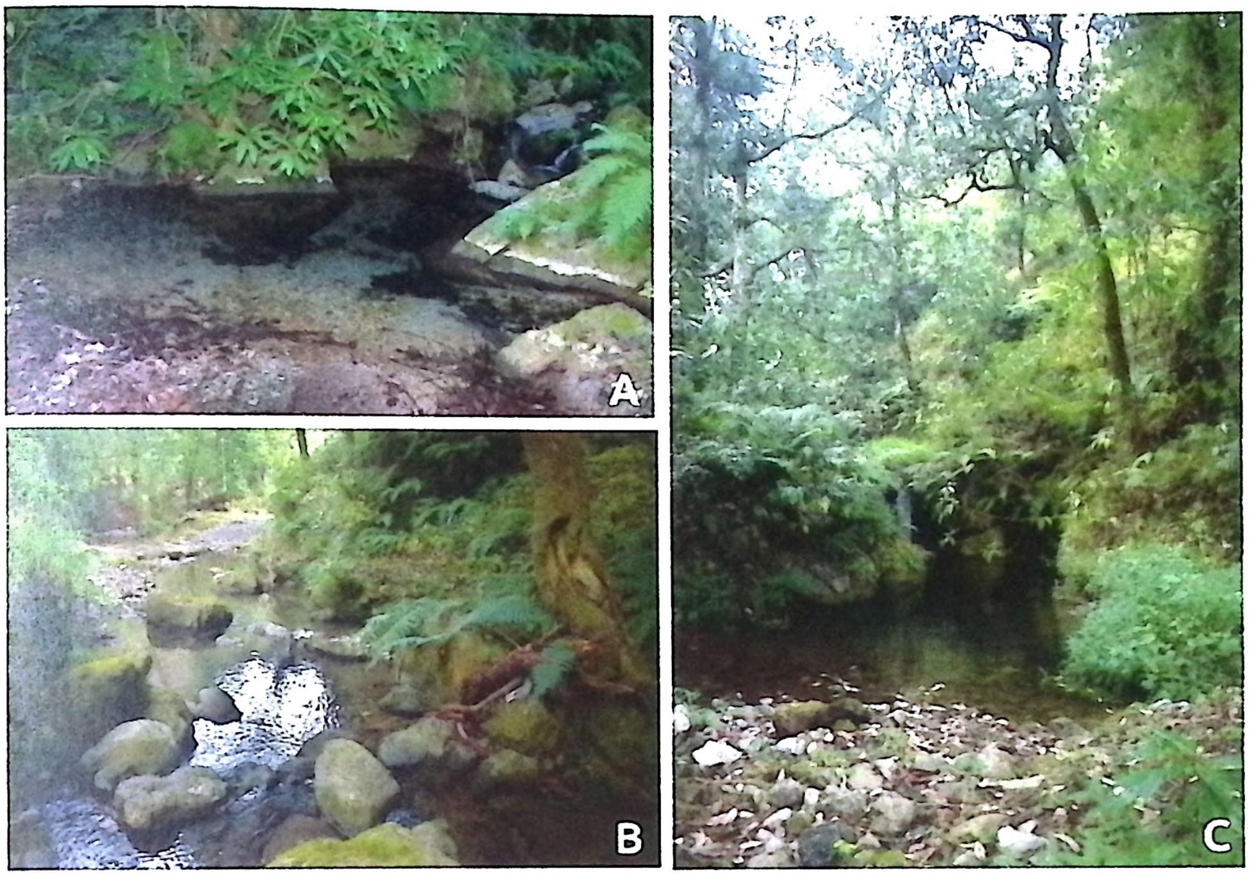


Fig. 14 Natural pools surveyed during the study showing the habitat. (A-N1, B-N2, C-N3)

All the habitat inventories were done post-density and behavioral sampling in the pools to avoid potential disturbance to the tadpole distribution. Except for M1 and M2, all pools were sampled by one observer wearing a wader (Magreel Chest Waders, USA) walking through the stream and recording the flow, depth and substrate at each point. Depth was recorded using a steel tape attached to a plastic pipe, and flow was measured using a digital flowmeter (MFP126-S Advanced Stream Flow Meter, GeoPacks). Substrate type was recorded at each of these points. In M1 and M2, where depth and humus accumulation does not allow the observer to walk through, an inflatable boat (Intex Explorer 200) was used for sampling. A Portable Depth Finder (DepthTrax(R) 1H - HawkEye(C), (DT1H), Norcross Marine Products, Inc.) was used to measure deeper areas (>80cm). Surface area of pools were calculated as length x mean width (m^2); and volume as length x mean width x mean depth (m^3). Habitat variables, their attributes and score category values applied in the study (adopted from Mercado-Silva et al. 2012) are given in Table 2.

Table 2 Habitat variables for pools in the study area. (Modified from Mercado-Silva et al., 2012)

Variable	**Score**	**Attributes and variable units**
Depth	1	Maximum depth <0.5 m
	2	Maximum depth 0.5 - 1 m
	3	Maximum depth > 1 m
Water Velocity	1	Maximum velocity <0.05 m/s
	2	Maximum velocity 0.05-0.5 m/s
	3	Maximum velocity > 0.5 m/s
Substrate Diversity	1	Soft sediments and leaf litter covering ≥ 75% of the pool.
	2	Rocky substrates covering ≥ 75% of the pool
	3	A mixture of 7 or more substrate types.
	4	A mixture of 6 or fewer substrate types.
Cover Availability	1	No. of cover items per volume 0-1
	2	No. of cover items per volume 2-5
	3	No. of cover items per volume >5

b Water Quality Analyses

Three water samples from each of the pools collected during day and night. Water temperature, pH, Total Dissolved Solids (TDS), Electrical Conductivity (EC) were measured using a portable pH meter (Aquasole Digital Pen Type Meter, AM-P-PH, 0-14 Range, 2% Accuracy, 0.1 Resolution) and a portable EC/TDS meter (Aquasole Digital Pen Type Meter, AM-P-EC, 0-9990 ppm, 0-9990 us/cm Range; 0.5 NaCl Factor, 2% Accuracy, 1ppm Resolution) with in-built temperature sensor.

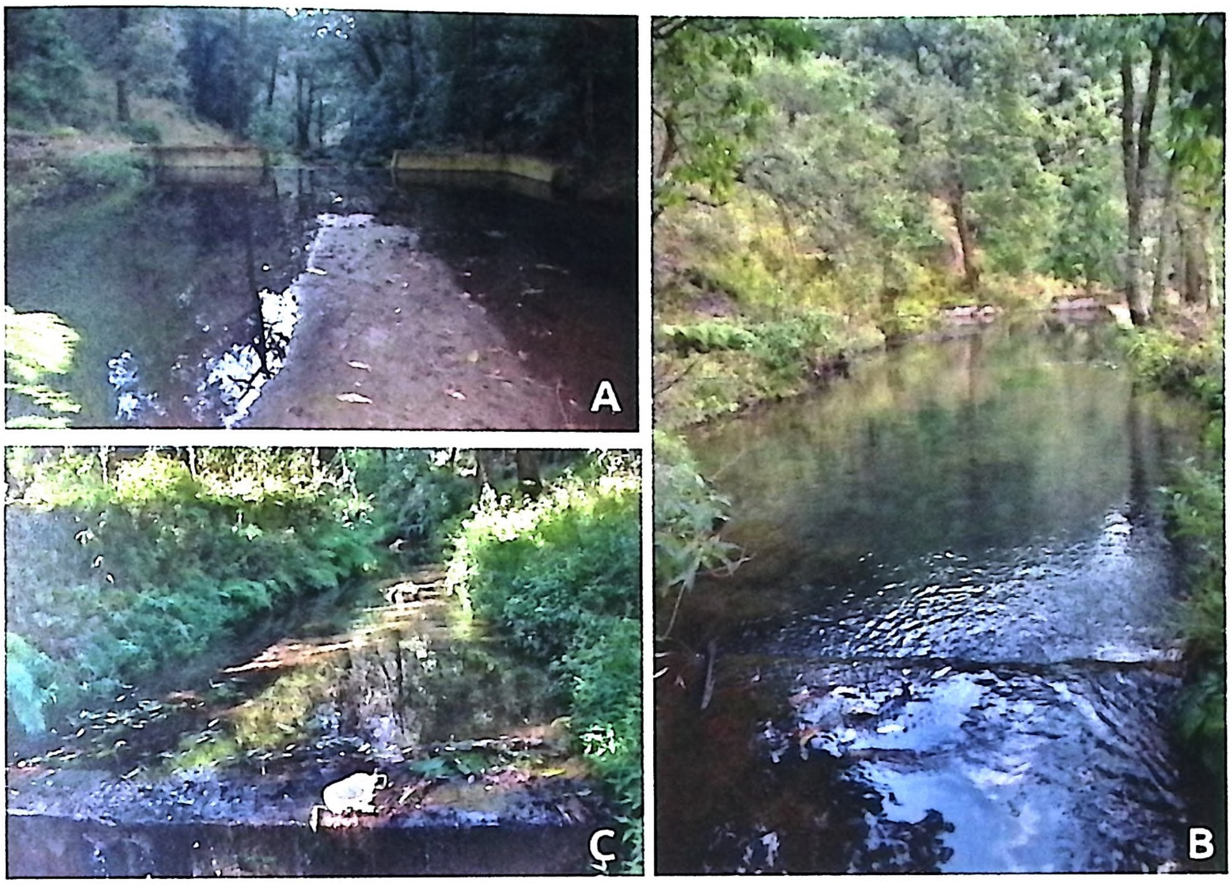


Fig. 15 Modified pools surveyed during the study showing the habitat. (A-M1, B-M2, C-M3)

For measuring Dissolved Oxygen (DO), three replicates of surface water samples (5 cm depth) were carefully collected in narrow-mouth BOD bottles (35 ml). To this, 0.5 ml of Manganous Sulfate ($MnSO_4$) solution, and 0.5 ml alkali-iodide (KI+KOH) solution were added and mixed thoroughly. These bottles were then carried to the field station for estimating dissolved oxygen in the sample using the modified iodometric (Winkler) method (Baird et al., 2017). The stored samples were protected from strong sunlight, and titration was carried out as soon as possible. For titration, a few drops of H_2SO_4 added to the fixed sample and allowed to dissolve the precipitate thoroughly. 5 ml of dissolved solution was pipetted and titration was carried out with standard Sodium Thiosulfate solution in the presence of starch as an indicator. Titration was continued till getting concordant values.

The following formula was used to calculate the dissolved oxygen level:

$$DO(mg/L) = \frac{V_1 N \times 8 \times 1000}{V_4(V_2 - V_3/V_2)}$$

Where, V_1 = volume of titrant (ml); N = normality of titrant (0.025); V_2 = volume of sampling bottle (mL); V_3 = volume of manganous sulphate + alkali iodide solutions added (ml); and V_4 = volume of fraction used for titration (mL). The equivalent weight of oxygen = 8.

c Tadpole Density Survey

Tadpole densities during the day and night at each pool were calculated visually using a $0.5m^2$ quadrat sampling at random locations. Nighttime observations were done with a headtorch (Trek 500, 25-200 lumens, Forclaz, Decathlon) (for a discussion on usage of artificial light for assessing nocturnal tadpole density, see Heath, 1975 and Leão-Pires et al., 2017). In pools M1 and M2, where large area and terrain do not allow the observer to sample the complete area, an inflatable boat was used for sampling (Fig. 16). Based on our observation that the tadpoles are not disturbed to a large extent by the boat movement, but by the light; the boat was moved gently without flashing the light towards the water. At each sampling location, the boat was gently stopped and all lights were turned off for 60 seconds. Sampling was done after this, by quickly turning on the light and counting the tadpoles. To avoid errors due to tadpole movement, the quadrats were placed with a minimum distance of 2m. The density of tadpoles was calculated from this using the formula:

$$Density = \frac{Number\ of\ tadpoles}{Grid\ area}$$

During the tadpole density estimation, the following microhabitat features were recorded: mean depth (cm), substrate and cover types present, water and air temperatures (°C), relative humidity (%), velocity (m/s), leaf litter and humus depth (cm), presence of floating/ submerged algae, fish, and invertebrates (surface or benthic invertebrates). Water surface radiant temperature was measured using Extech RH401 digital psychrometer with built-in IR thermometer (FLIR Systems, U.S.). Water temperature at 5cm below water surface was also measured occasionally using mercury thermometer and an automatic sensor in AM-P-EC digital pen type EC/TDS meter (Aquasole Digital, Rakiro Biotech Systems, India) to compare the difference in water temperature measured by different instruments.

Fig. 16. Tadpole density survey and habitat measurements in the field; (A) grid-based visual counting of tadpoles, (B) Night survey using inflatable boat in modified pools, (C) water depth measurement in transect point.

d Morphometry

To see the change in morphological features, morphological data collected from the live specimens in the field was used (*N*=388). The observer randomly caught at least ten individuals (five each during day and night) from each pool using a hand net and carefully placed them on a white plate with a scale (in mm) and photographed using a Nikon D5300

DSLR camera (Nikon Corporation, Tokyo, Japan). To calibrate these images, initial samples (*N*=75) were measured using vernier calipers (Mitutoyo Digimatic Caliper 500-151-30). Substrate type, depth (cm), body weight (g), surface water Temperature (∘C), air temperature (∘C) and relative humidity (%) were also recorded. The photographs were later matched to unique individual IDs based on the metadata using EXIFTool (v.11.88). After manual verification, the images were used to measure Body Length (BL), Tail Length (TL), Total Length (TL), Max. Tail Height (MTH) with ImageJ (v.1.53e) (Schneider et al., 2012). This post-processed data was tested for accuracy using the field-based data. This data was used for the temporal size-class distribution analysis.

e Behavioral Sampling

Two pools (B1 & B2) apart from the regular sampling pools were monitored weekly for focal pool observations. These pools were inventoried and used for scan sampling of tadpoles (Table 1). Scans were done during eight-time windows (06:00-07:00; 09:00-10:00; 12:00-13:00; 15:00-16:00; 18:00-19:00; 21:00-22:00; 24:00-01:00; 03:00-04:00) in a day. The observer reached the pools in the first 30 minutes of the time window and scanned the pool for a maximum of 10 minutes. All visually detectable tadpoles were counted without disturbing the tadpoles, by gently walking along the bank of the pool. During the night, a headtorch (Trek 500, Forclaz, 25-200 lumens, Decathlon) was used to detect the tadpoles. Red Light was used while approaching the pool prior to sampling to avoid light disturbance. Generally, both the pool bottoms were characterized by sand, leaf litter and humus. Natural pool (B1) had bedrocks, boulders, cobbles and gravels with sparse aquatic vegetation on rocks. The modified (B2) pool was devoid of these, and in one side it had concrete wall (Fig. 17).

Fig. 17 Behavior study pools; (A) natural pool-B1, (B) modified pool-B2

Tadpole counts in each session were converted to the percentage of the maximum number of tadpoles recorded that day from the pool. After each sampling, water temperature (psychrometer), air temperature, and relative humidity were recorded using an IR thermometer (Extech RH401 Digital Psychrometer with Built-in IR thermometer, FLIR Systems, U.S.) at a fixed point in the pool. Each tadpole was classified into a pre-defined activity state (Table 3) based on (Marian et al., 1980; Stynoski & Noble, 2012). Moon illumination data were acquired using '*lunar*' (Lazaridis, 2014) package in R (R Core Team, 2021).

Table 3 Ethogram used in the scan sampling modified from Stynoski & Virginia (2012) and Marian et al. (1980).

Activity	**Description**
Stationary	Tadpoles are stationary (not moving) at the streambed.
Feeding	Tadpoles scraping on detritus in stream sediment, leaf litter, rocks and concrete wall with slow movements and leaving scrape marks.
Moving	Tadpoles are moving with flexible tail movements (swimming).
Surfacing	Tadpole moving up and breaking the surface with the snout.

f Morphometric Analysis

Tadpole specimens (*N*=49) collected during December 2019 and October 2020 and preserved in 10% formalin. Staging after Gosner (1960) and morphological measurements were done using Olympus SZX10 Microscope and Digital Calipers (Mitutoyo Digimatic Caliper 500-151-30, 0-150mm range, ±0.02mm accuracy). Measurements involving the nares, eyes, and spiracle as landmarks were taken from the centers of the structures, and body terminus is the point where the axis of the tail myotomes contacts the body wall (Altig, 2007). For morphological measurements, I followed a modified version of the proposed standard tadpole body measurements of Haas & Das (2011) developed from Altig & McDiarmid (1999) (Fig. 18; Table 4). Selected measurements from Haas & Das (2011) were taken. In addition to this, vent tube length was also measured (Fabrezi et al., 2009).

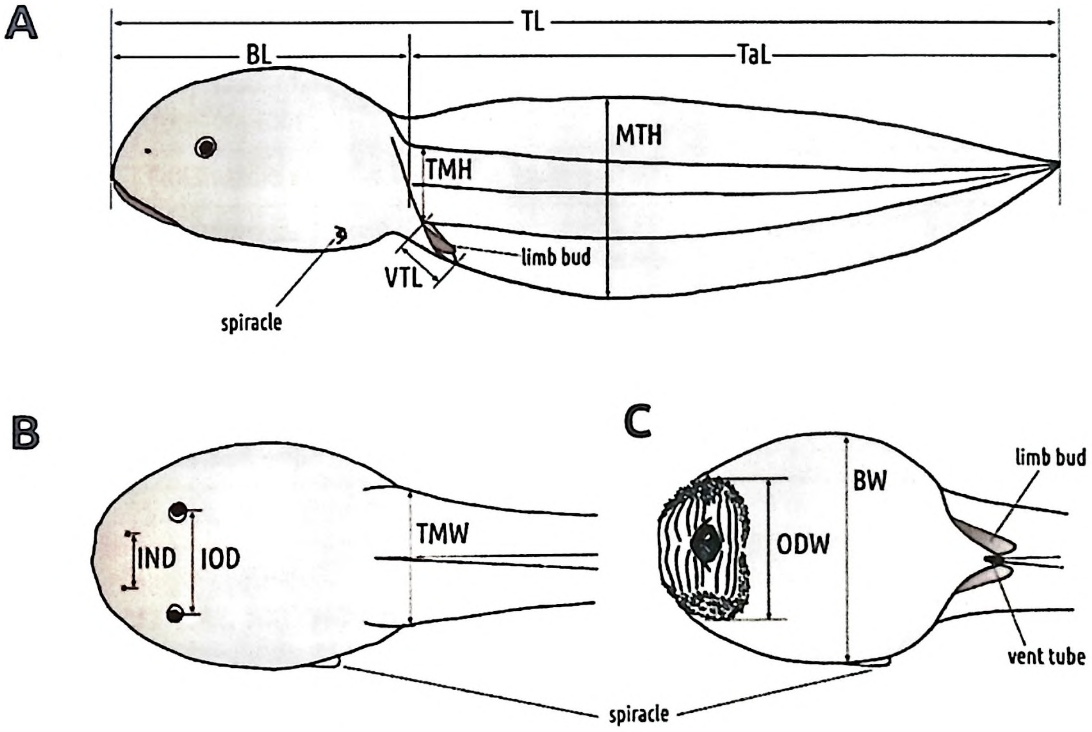


Fig. 18 Illustration showing the standard tadpole body measurements taken. Original illustration by K.M. Adila Farisa

Labial Tooth Row Formulae (LTRF) were generated as mentioned in Altig (2007) where the number of tooth rows per labium is shown in a fractional format in which the numerator depicts the number of upper tooth rows and denominator indicates the lower tooth rows. The tooth rows are numbered serially from the upper disc margin toward the mouth on the upper (anterior-A) labium and from the mouth toward the lower margin on the lower labium (posterior-P). Natural gaps among rows are noted in parentheses. Relative tail length (TaL%) as percentage of total length was calculated (see Merilä et al. 2004 for a discussion).

Table 4 Table showing the morphometric variables, their codes and definitions

Sl. No.	Code	Measurement
1	BL	Body length from snout to the point where the axis of the tail myotomes meets the body wall
2	TaL	Tail length (=TL-BL)
3	TL	Total length
4	TMH	Tail muscle height at the body-tail junction, where ventral line of musculature meets trunk contour
5	TMW	Tail muscle width at the same level as TMH
6	MTH	Maximum tail height
7	IND	Internarial distance
8	IOD	Interorbital distance
9	BW	Maximum body width
10	ODW	Oral disc width
11	VTL	Vent tube length

3 ANALYTICAL METHODS

All datasets in which atmospheric temperature (ºC), water temperature (ºC), and relative humidity (%) values included were combined to get the overall weather pattern. Based on the temperature pattern, the study period was divided into winter (January-February) and post-winter (March-April) seasons for comparisons in further analyses (Fig. 47, Table 9). The diurnal patterns of water temperature and relative humidity (RH) patterns across months are given in (Fig. 48-49). To assess difference in temperature between pools, Kruskal-Wallis test was used and Dunn test for pairwise comparisons with effect size calculation η^2. Wilcoxon rank sum test with continuity correction were used to see the difference in mean values with effect size *r* when data was non normally distributed. Paired t-tests with Cohen's *d* values were used when data was normally distributed to compare difference in mean values. To asses variation in mean tadpole density, one-way repeated measure ANOVA was used on log transformed data with η_p^2 as effect size. Pearson's product-moment correlations were utilized for assessing linear correlations between variables. Due to lack of sufficient data points during winter daytime to check the effect of diurnally and pool type on the mean tadpole density, pooled data was used for analysis. Effect sizes are reported in the results wherever necessary (Fritz et al., 2012; Tomczak & Tomczak, 2014).

To see how two independent variables in combination, affect the dependent variable, two-way mixed ANOVA were used with pair-wise post-hoc tests and effect size η_p^2. Adjusted p-values were used for interpretations. When outliers were present, two-way between-within subjects ANOVA on the trimmed means was performed using 'WRS2' package (Mair & Wilcox, 2020). The outliers were not removed since the observations were resulted from the high algal accumulation due to blockage of the dam opening during the study period.

Since different equipment was used to measure water temperature in different datasets considering logistical reasons and requirements, to understand the limitations in using temperature measurements from various instruments, a comparison of paired measurements datasets was carried out. Correlation and differences among the measurements were assessed. Since we found significant differences, usage of any of these sub-datasets in further detailed analyses (e.g., microhabitat modelling) required special attention. Considering the availability of point-data, IR (psychrometer) measurements were considered for further use. Values from the automatic sensor were used to calculate mean water temperature of pools in further analysis.

In the behavior dataset, at each time sampling session (8 sampling sessions a day), the total number of tadpoles detected was converted to the proportion of the total number of tadpoles detected that day. Hourly variation in mean proportional tadpole count between pool types and seasons was visualized as circular plots (rose diagrams) with segments in package 'season' (Barnett et al., 2020) and daily activity pattern of tadpoles using 'overlap' package (Ridout & Linkie, 2009) in R. Activity overlaps were estimated using Dhat4 estimator. Difference between proportions of tadpoles engaged in various activities across time of the day was assessed using equality of proportions tests with continuity correction. To assess the homogeneity of means of the circular distributions of tadpole counts between seasons and pool types, I used Watson-Williams test after assessing the von Mises distribution of data using Watson's test (Pewsey et al., 2013) available in the package 'circular' (Agostinelli & Lund, 2017).

Based on the Pearson correlation values, redundant variables were removed. Principal component analysis (PCA) was used to see the difference between individual pools and their common characters with respect to modification. Generalized Linear Mixed Models (GLMMs) were developed and tested on the data to identify a suitable model explaining the density of tadpoles explained by the habitat variables. Before finalizing the explanatory variables, a correlation matrix was created and multicollinear variables were removed. Since the response variable was grid-based, more appropriate variables (mean depth and velocity of grids) were chosen instead of depth and velocity indices of the pools. Since we did not observe any predatory events/ tadpole mortality during the course of this study, and no information available on the actual predators of the tadpoles, presence of invertebrates was removed from the list. GLMM was used considering the nature of the dataset containing repeated measurements from the same pools. The response variable was count data (tadpole density) and thus, zero inflation and over dispersion was assessed prior to modelling. Developing two separate models for natural and modified pools was not possible because of the reduced levels of the blocking variable, i.e., the pool number (less than five).

Thus, density of tadpoles was modelled using habitat variables and pool modification as a fixed effect, and pool identity as random effect.

The following explanatory variables were used to build the models:

1. Mean canopy cover % (Continuous)
2. Habitat-forming structure / Cover item richness (Categorical)
3. Mean depth (Continuous)
4. Humus depth (Continuous)
5. Leaf litter depth (Continuous)
6. Mean DO level (Continuous)
7. Water temperature (Continuous)
8. Presence of check dam/ Modification of pool (Presence-Absence)
9. Time of the day (Day/Night)
10. Season (Winter/Post-winter)
11. Substrate item richness (Categorical)
12. Mean water velocity (Continuous)

Tadpole density (count data) per grid was related to habitat variables using negative binomial logit-linked mixed-effect GLMMs (Zuur et al., 2009). An optimized random effect structure was chosen (1+Winter | Pool), indicating that the effect of the season will vary between pools; random intercepts for season and random slopes for pools influenced by the season. This term was added to account for the variability among pools influenced by the season. To account for zero inflation, an interaction of time of the day with the season was added as a zero-inflation formula in the model. The GLMMs were evaluated using the glmmTMB package (Brooks et al., 2017) in R.

Instead of considering all subset models (dredging) from a saturated model, a set of models were built based on the literature review on habitat ecology of tadpoles and hypotheses formulated in the field. All candidate models were diagnosed in a simulation-based approach using qq-plot to detect overall deviations from the expected distribution, tests for correct distribution, dispersion, zero-inflation and outliers, and residuals against predicted values plot for assessing heteroscedasticity using the package DHARMa (Hartig, 2021) in R. In addition to this, models with gigantic confidence intervals around parameter estimates were also removed (Bolker et al., 2009). The models with no misspecification problems were considered for ranking (Table 14). The ranking was done based on Akaike information criterion (AIC) (Burnham & Anderson 2002) using the MuMIn package (Bartoń, 2020). The models with ΔAIC < 2 were considered to have enough support. Since only the top model had an ΔAIC < 2, no model averaging was done. Only the top model was used for interpretation, and predictions were done only for significant variables ($p<0.05$). Positive or negative associations are indicated by the sign of regression coefficients. Nakagawa's R^2 (P. C. Johnson, 2014) was used to assess the model. Using the model, tadpole densities were predicted based on different variables across seasons, pool types and time of the day.

The morphology dataset was created from the pre-field work time (2019 December and 2020 October), since the collected samples during the field work was not processed due to logistical reasons at the time of COVID-19 lockdown. Putative species classification of the samples was carried out using Labial Tooth Row Formula (LTRF) from Sircar (2010) and Gill et al., (2020) based on a literature summary (Table 11). A note regarding this process is given in the annexure. Based on all the morphometric measurements, a hierarchical cluster analysis using Unweighted Pair Group Method with Arithmetic mean (UPGMA) was carried out. Based on the clusters obtained, putative species status was assigned and this classification was subjected to PCA to see the separation between the groups based on the morphometric variables.

Morphological measurements (mm) taken in the field (or post-processed from digital images) were used to see the size-class patterns, variation in percentage tail length and growth of tadpoles across time. Before analysis, we removed all observations with uncertainties (missing habitat variable data, damaged tadpoles etc.). After removing this data (15.8 %), we analyzed measurements for 363 individuals. Data from tadpole individuals whose Body Length (BL), Total Length (TL) and Maximum Tail Length (MTH) were measured using both (i) Vernier callipers in the field and (ii) ImageJ software (Ferreira & Rasband, 2012) were used to see the reliability of the data from image post-processing for further analysis.

To see the size class pattern and asses multimodality, I used density plots in combination with histograms and Hartigan's dip test statistic for unimodality (Hartigan & Hartigan, 1985) using package 'diptest' (Maechler, 2021). Since it was not possible to do the staging of tadpoles in the field due to logistical reasons, size-class of Body Length (BL) and Total Length (TL) were taken as substitutes for the development stage in this analysis (Brown, 1990).

The habitat availability and use data measured through cross-sections, visual assessment and grid sampling were categorized into classes. A classification of 12 categories each for depth (0, 0.01–0.1, 0.11–0.3, 0.31–0.6, 0.61–1.0, 1.01–1.2, 1.21–1.5, 1.51–1.8, 1.81–2.0, >2.0 m), and velocity (0, 0.01–0.1, 0.11–0.2, 0.21–0.3, 0.31–0.4, 0.41–0.5, 0.51–0.6, 0.61–0.9, 0.91–1.2, >1.2 m/s) was done (J.A. Johnson et al., 2017; J.A. Johnson et al., 2021). Cover items and substrate items were classified as earlier mentioned. Temporal replicates were pooled across seasons for further analysis.

Using the same data, I generated habitat suitability criteria (HSC) curves for overwintering tadpoles based on the habitat availability and use. For this, frequencies of observed available habitat and use by the tadpoles were generated at each class intervals of depth, velocity, cover item and substrate item. From the estimated relative frequencies of utilization and availability, preference of tadpoles for each class interval of the variables using the following formula (Bovee, 1982):

$$P_i = U_i / A_i$$

where P_i is the relative preference value, U_i is the use proportion, and A_i is the available proportion for particular class interval of the measured variable. The calculated preference values were normalized to the scale of 0-1 using the formula:

$$P_n = P_i / P_{max}$$

where P_n is the normalized preference index at each class interval of the variable and P_{max} is the maximum index of preference for the range of a variable P_i. Using P_n, HSCs were generated and visualized.

I used Ivlev's electivity index, E_i (Ivlev, 1961) to measure the use of a resource (p_i) compared to its availability (q_i). The index ranges from −1 to 1, where positive values indicate active selection, negative values for avoidance or inaccessibility and zero showing random selection.

$$E_i = \frac{p_i - q_i}{p_i + q_i}$$

All datasets were cleaned and analyzed using different packages available in R (v.v.4.1.0) (R Core Team, 2021). Other packages which are not mentioned above include 'lubridate' (Grolemund & Wickham, 2011), 'ggplot2' (Wickham, 2016), 'ggpubr' (Kassambara, 2020), 'ggridges' (Wilke, 2021), 'dplyr' (Wickham et al., 2021), 'rstatix' (Kassambara, 2021), 'dunn.test' (Dinno, 2017), 'GGally' (Schloerke et al., 2021), ' cluster' (Maechler et al., 2021), 'factoextra' (Kassambara & Mundt, 2020), 'tidyr' (Wickham, 2021), 'corrplot' (Wei & Simko, 2021), 'sjPlot' (Lüdecke, 2021), 'ggeffects' (Lüdecke, 2018), 'lme4' (Bates et al., 2015), and 'performance' (Lüdecke et al., 2021).

RESULTS

1 TEMPERATURE VARIATION IN STUDY POOLS

1.a Major Study Pools

Means of water temperatures across the major study pools varied significantly during both day and night sessions across the seasons. For daytime mean water temperature during winter (Kruskal-Wallis $X^2(5)=13.39$, p=0.02, $\eta^2=0.05$), pairwise comparison using Dunn's test revealed that the water temperature was significantly higher in pool M3 compared to pool N2 ($p_{adj}=0.01$). Other pools did not show significant differences in the post-hoc test. During post-winter, daytime water temperature significantly varied across pools ($X^2(5)=12.08$, p=0.03, $\eta^2=0.03$), but no pairwise differences were found in the post-hoc test.

In the case of nighttime during winter ($X^2(5)=19.64$, p=0.001, $\eta^2=0.08$), pairwise post-hoc tests showed that pool M3 had significant higher mean water temperature compared to pools M1 ($p_{adj}=0.01$), M2 ($p_{adj}=0.001$), N2 ($p_{adj}=0.03$), and N3 ($p_{adj}=0.02$). During post-winter ($X^2(5)=17.87$, p= 0.003, $\eta^2=0.05$), M3 showed higher mean water temperature in nighttime compared to M1 ($p_{adj}=0.004$), M2 ($p_{adj}=0.02$), N1 ($p_{adj}=0.03$), and N3 ($p_{adj}=0.02$) (Fig. 19).

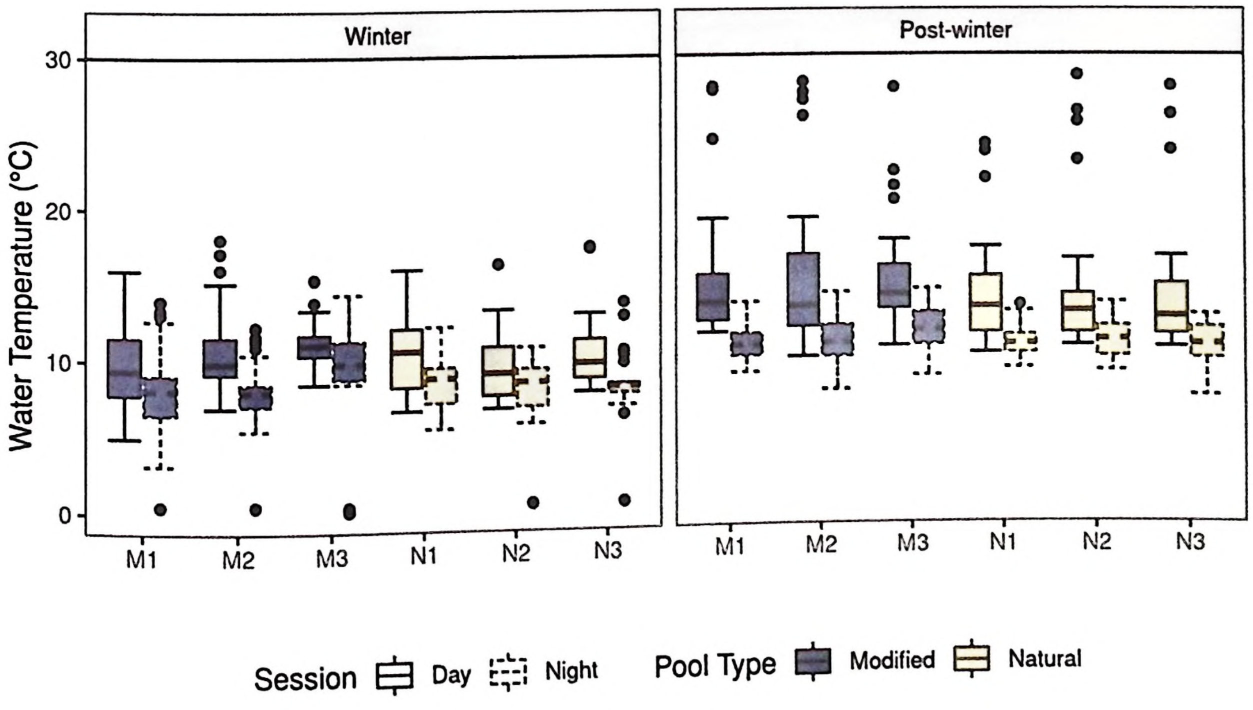


Fig. 19 Water temperature across study pools during the study period

When pools were combined based on their types (natural and modified), mean water temperatures during nighttime did not vary significantly across pool types in both winter (Wilcoxon rank sum test with continuity correction, W=4893.5, p=0.44, r=0.06) and post-winter (W=10567, p=0.16, r=0.08). Even though the daytime mean water temperature showed no significant difference across pool types during winter (W=4812.5, p=0.18, r=0.08), mean water temperature in modified pools was higher during daytime (mean±CI=14.7±0.63) than natural pools (mean±CI=13.9±0.73) during post-winter (W=10124, p=0.001, r=0.08) (Fig. 20).

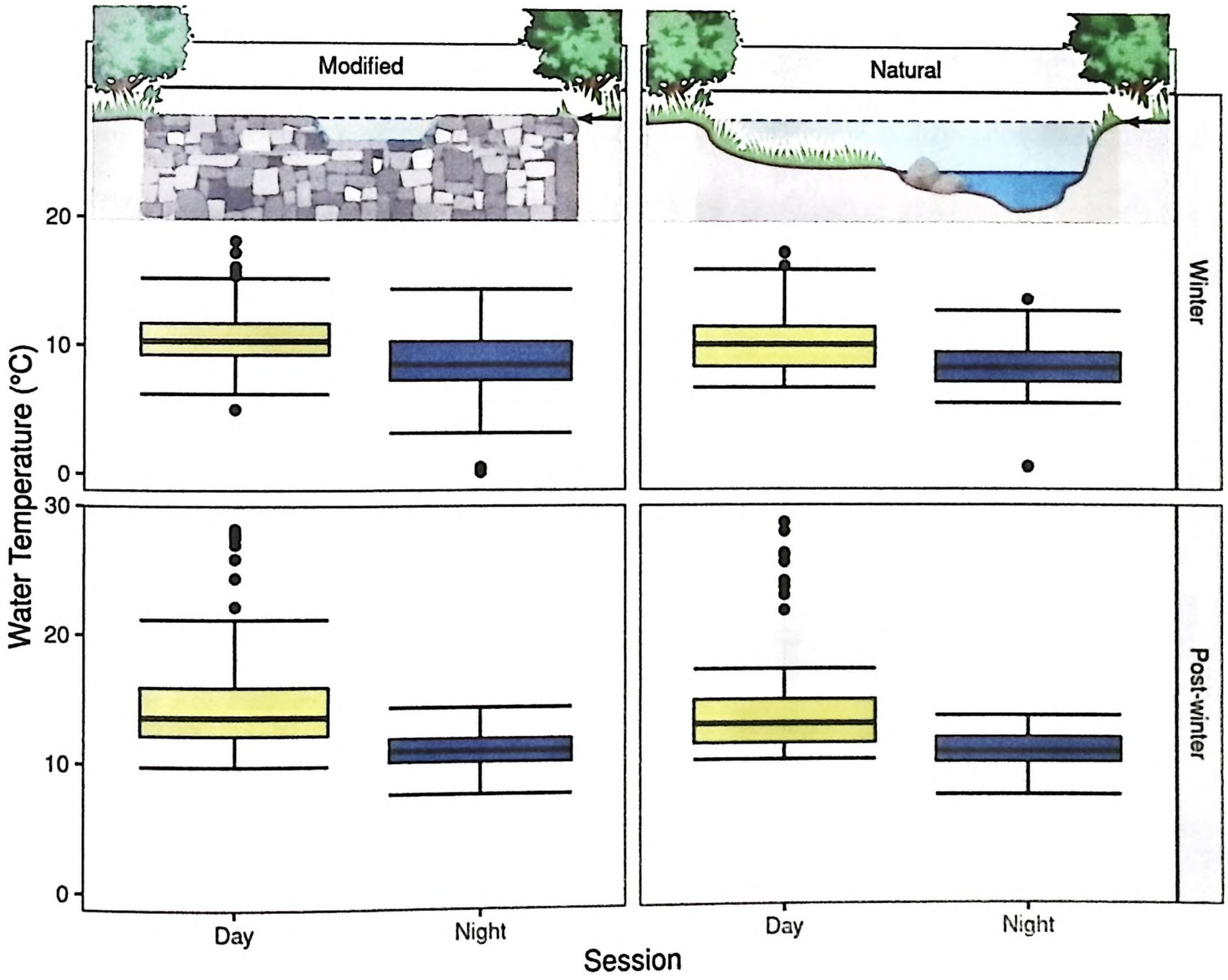


Fig. 20 Diurnal water temperature variation across major study pools

1.b Behavior Study Pools

During winter, there were significant differences in water temperatures in the nighttime (t(17)=-8.42, p<0.001, d=-1.98) and daytime (t(13)=-9.91, p<0.001, d=-2.65) in a paired t-tests between pool types. Even though air temperature did not significantly vary in daytime (t(13)=1.52, p=0.15, d=-0.41) during winter, a significant difference was found in nighttime (t(17)=3.12, p=0.006, d=0.74).

During post-winter, air temperature did not vary significantly in nighttime (t(19)=-0.99, p=0.33, d=-0.22) and daytime (t(21)=1.14, p=0.28, d=0.24), but the water temperature showed significant difference in nighttime (t(19)=-3.99, p<0.001, d=-0.89) contrasting to the insignificant difference in daytime (t(21)=-0.08, p= 0.94, d=-0.02) (Fig. 21).

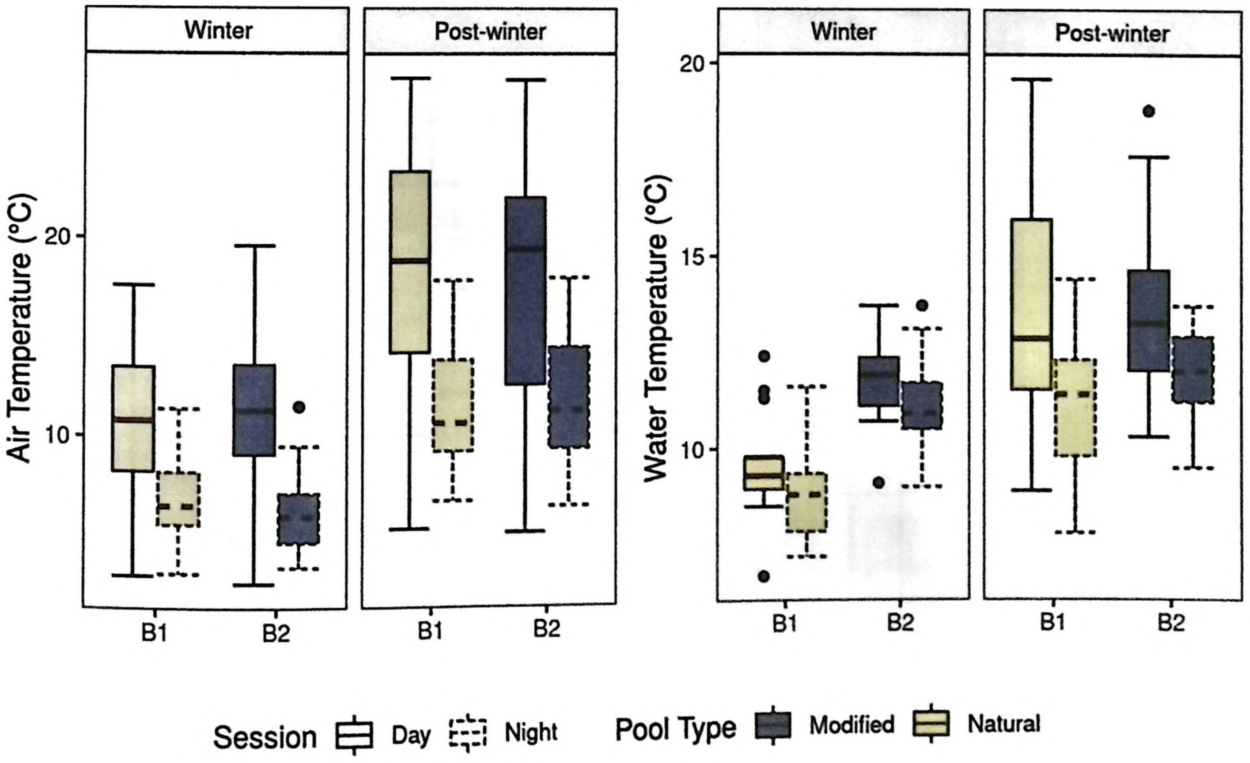


Fig. 21 Water and air temperature profiles of the behavior study pools

2 WATER QUALITY PARAMETERS

A summary of the water quality parameters is given in the Table 5.

Table 5 Summary of water quality parameters measured during the study period (2021 January – April), values in 95% confidence interval.

Parameter	Month	Mean	N	Min.	Max.
DO (mg/L)	January	8.22 ± 0.53	12	6.99	9.52
	February	9.04 ± 0.55	18	7.38	11.5
	March	7.89 ± 0.46	24	5.41	10.15
	April	6.87 ± 0.69	24	4.96	12.4
EC (µS/cm)	January	436.42 ± 9.48	12	419	470.67
	February	425.4 ± 3.37	24	412	442.67
	March	423.75 ± 6.82	30	342	453.5
	April	433.25 ± 8.97	24	352	463.67
pH	January	8.54 ± 0.13	12	8.2	8.8
	February	8.53 ± 0.05	24	8.3	8.77
	March	8.42 ± 0.07	30	7.95	8.87
	April	8.44 ± 0.12	24	8.07	9.23
TDS (ppm)	January	218.17 ± 4.76	12	209.33	235.33
	February	214.07 ± 3.04	24	206	242
	March	211.6 ± 3.4	30	171	226.5
	April	216.28 ± 4.48	24	175.67	231.67

Details regarding the instrument comparison are given in the annexure (Note 1).

2.a Dissolved Oxygen (DO)

Mean DO levels did not vary between day and night time but with a negligible effect size (paired t-test, t(23)=0.33, p=0.74, d=-0.07). Mean DO level significantly varied across months ($F_{1.75,17.53}$=14.68, p<0.001, η_p^2=0.6), but not between natural and modified pools ($F_{1,10}$=0.25, p=0.63, η_p^2=0.02), and there were no significant two-way interactions between pool types and months ($F_{1.75,17.53}$=1.2, p=0.32, η_p^2=0.11) (Fig. 22).

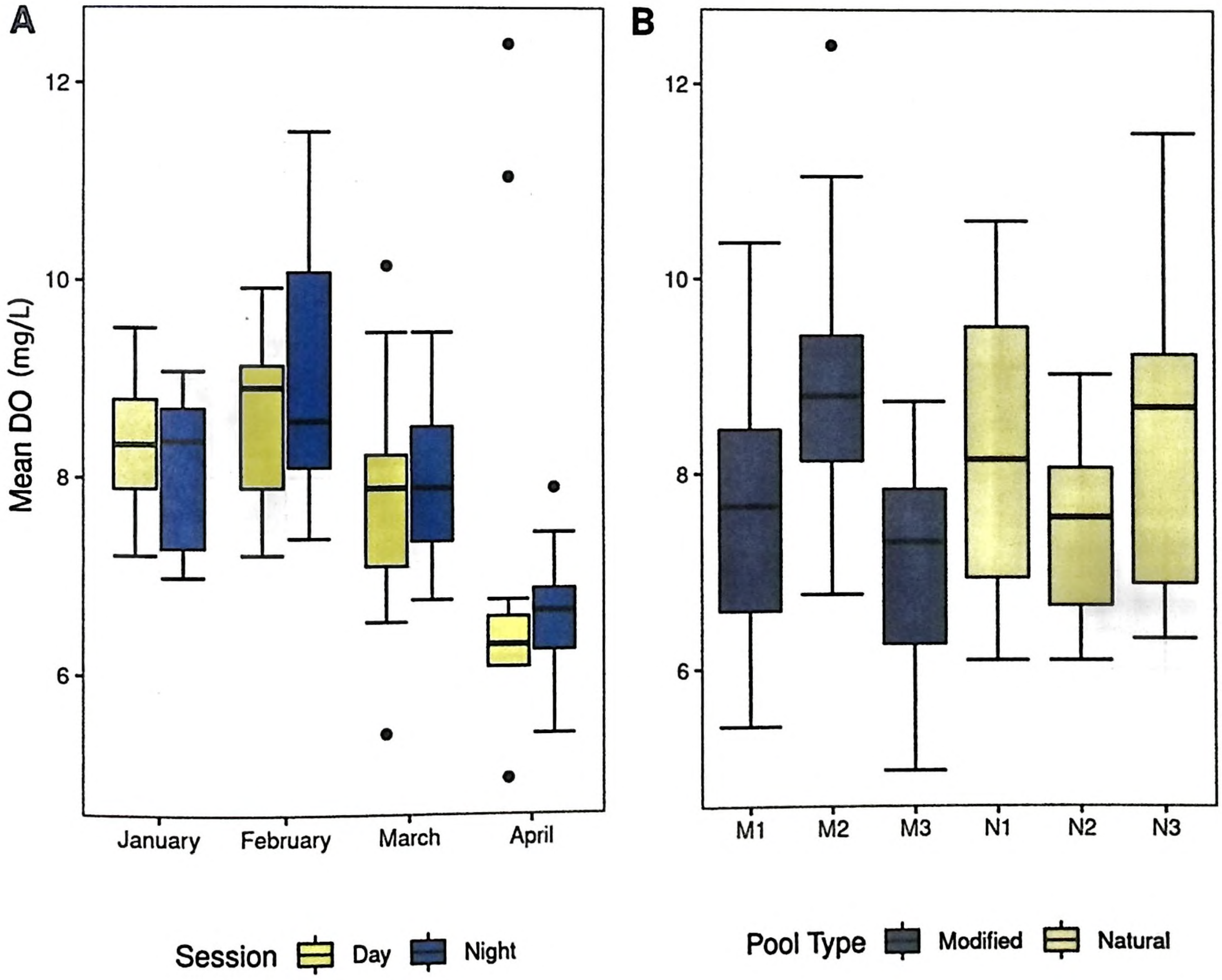


Fig. 22 Variation in Dissolved Oxygen levels (A) monthly diurnal pattern; (B) across pools

A pair-wise comparison showed that there was significant difference in mean DO level between February and March ($p_{adj}<0.001$), and all months with April (January:$p_{adj}=0.021$; February:$p_{adj}=0.003$; March:$p_{adj}=0.037$).

2.b pH

Mean pH levels did not vary between day and night time with a negligible effect size (paired t-test, t(23)=-0.17, p=0.87, *d*=-0.04). Mean pH levels varied significantly across months ($F_{3,3.5437}=10.62$, p=0.03), but not across pool types ($F_{1,4.1201}=6.64$, p=0.06), and did not have any significant two-way interaction between pool type and month ($F_{3,3.5437}=0.3$, p=0.82) (Fig. 23).

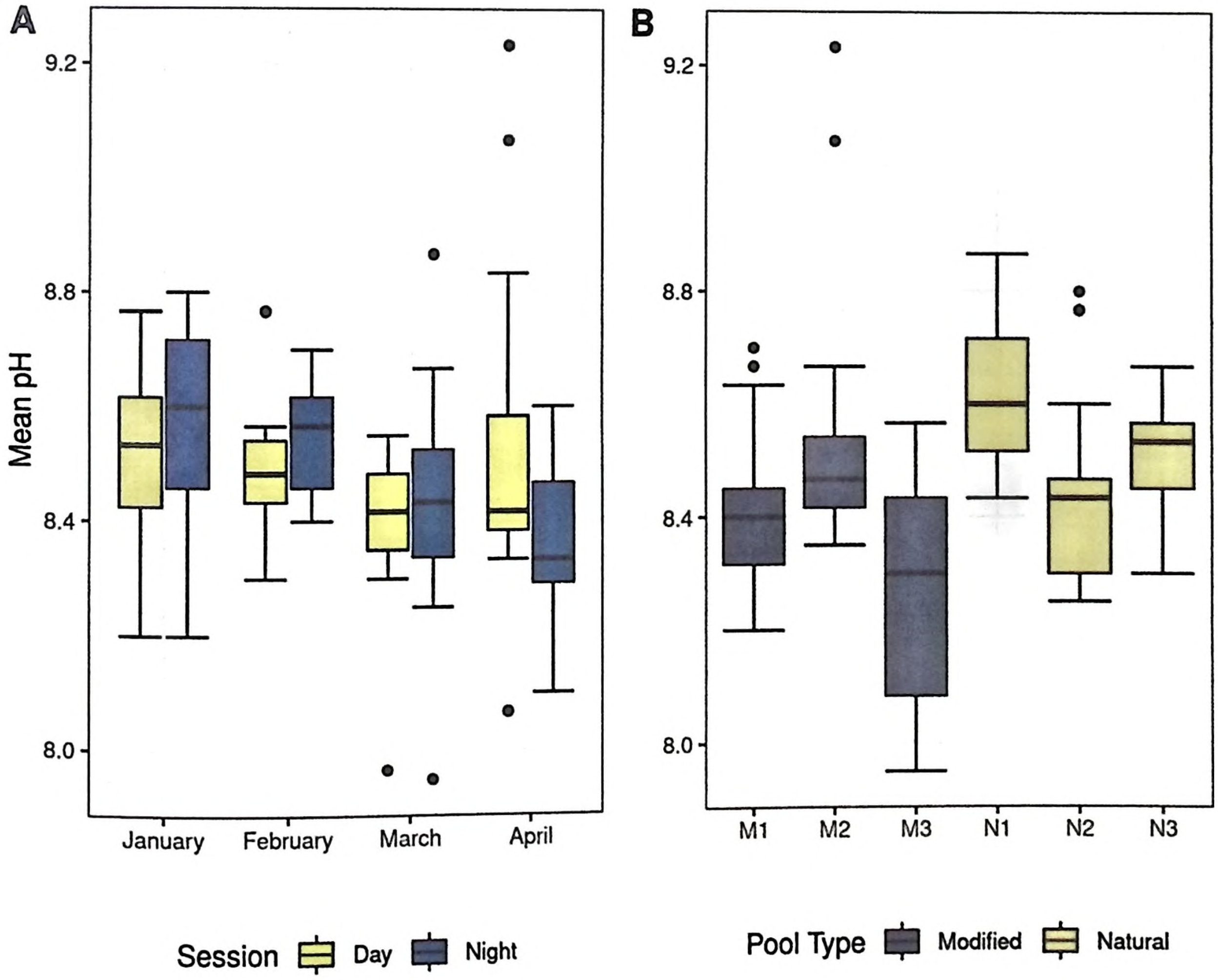


Fig. 23 Variation in pH levels (A) monthly diurnal pattern; (B) across pools

2.c Total Dissolved Solids (TDS)

Mean TDS levels were significantly higher during night than day time with a moderate effect size (paired t-test, t(23)=-2.88, p=0.009, *d*=-0.59). Mean TDS levels varied significantly across months ($F_{3,4.4033}$=11.6, p=0.02), but not across pool types ($F_{1,4.2641}$=1.05, p=0.36), and did not have any significant two-way interaction between pool type and month ($F_{3,4.4033}$=0.03, p=0.99). (Fig. 24).

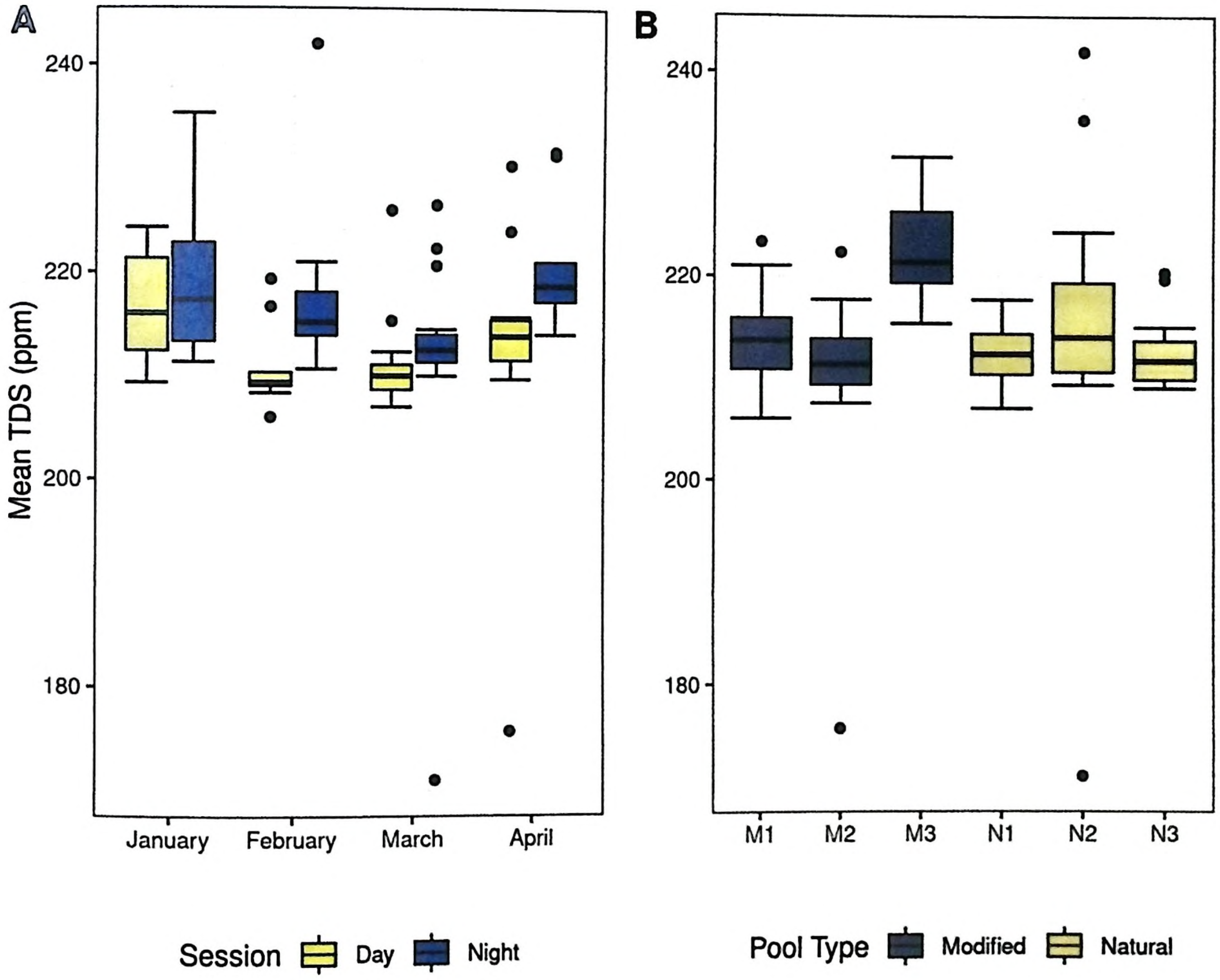


Fig. 24 Variation in TDS levels (A) monthly diurnal pattern; (B) across pools

2.d Electrical Conductivity (EC)

Mean EC levels were significantly higher during night than day time with a moderate effect size (paired t-test, t(23)=-2.5, p= 0.02, *d*=-0.51), a pattern similar to TDS as expected. Mean EC levels varied significantly across months ($F_{3,4.3891}$=11.8, p=0.02), but not across pool types ($F_{1,4.1024}$=1.22, p=0.33), and did not have any significant two-way interaction between pool type and month ($F_{3,4.3891}$=0.003, p=1). (Fig. 50).

TDS and DO were negatively correlated (r=-0.45, t(76) = -4.42, p<0.001); pH and DO were positively correlated (r=0.58, t(76)=6.13, p<0.001); and there was a strong positive correlation between EC and TDS (r=0.92, t(88)=21.93, p<0.001) (Fig. 51).

3 POOL HABITAT

Canopy cover (%) was significantly high for natural pools compared to modified pools (Wilcoxon signed rank test with continuity correction; V=35, p<0.001, r=0.8). A deviation from this pattern is pool M3.

Natural pools had a high proportion of boulder and bedrock under cuttings compared to other covers. In the case of modified pools, except log and root undercuts, other covers are in similar proportions. Humus is the major substrate of modified pools followed by leaf litter and gravel. In natural pools, major proportions of the substrate are occupied by gravels, bedrocks, and leaf litter. An increase in green algae in the post-winter period, and thus reduction in humus covered area was clear. Slight changes in water velocity were noted in natural pools as expected. Modified pools were almost devoid of any detectable flow. Compared to natural pools, modified pools have high proportions of deeper areas. Proportional charts showing variation of depth, velocity, substrate and cover items across individual pools, pool types and seasons are given in annexure (Fig. 53-56). A summary of the habitat variables collected during the study period are given in Table 6.

Table 6 Summary of habitat variables of pools averaged for modified and natural pools (*N*=42) during the study period (2021 January – April), values in 95% confidence interval.

Variable	Pool Type	Mean	Min.	Max.	SD
Mean Canopy Cover (%)	Modified	82.31 ± 3.11	67.5	100	9.98
	Natural	92.05 ± 0.97	86.74	96.53	3.11
Cover Index	Modified	2.43 ± 0.16	2	3	0.5
	Natural	3 ± 0	3	3	0
Depth Index	Modified	2.33 ± 0.26	1	3	0.85
	Natural	1.95 ± 0.23	1	3	0.73
Velocity Index	Modified	1.48 ± 0.23	1	3	0.74
	Natural	2 ± 0.1	1	3	0.31
Substrate Index	Modified	2.67 ± 0.26	1	4	0.85
	Natural	2.57 ± 0.23	2	4	0.74
Habitat Area (m^2)	Modified	158.9 ± 10.94	108	200.6	35.12
	Natural	36.78 ± 3.22	24.03	53.57	10.33
Habitat Volume (m^3)	Modified	75.44 ± 12.99	20.25	128.02	41.68
	Natural	10.93 ± 1.45	5.46	19.35	4.66
Mean Depth (m)	Modified	0.45 ± 0.07	0.18	0.77	0.23
	Natural	0.31 ± 0.04	0.12	0.54	0.14
Mean Velocity (m/s)	Modified	0 ± 0	0	0.02	0.01
	Natural	0.02 ± 0	0	0.05	0.01

PCA showed that mean dissolved oxygen level, and cover index were the important variables shaping the natural pools. At the same time, modified pool habitats are different from the natural habitats by the depth index, pool volume, and mean water temperature (Fig.

57a). Even though pool M3 was not matching with the modified pool broad characteristics (confirming the field experience of a check dam structure at the outflow, but mostly resembling a natural pool), I did not remove or reclassify the pool (Fig. 57b).

4 DEVELOPMENTAL PATTERN, MORPHOLOGY AND BEHAVIOUR OF THE TADPOLES

4.a Body Size Class Variation in Tadpoles

Shifts in distribution from lower to higher size classes was observed in the tadpole population across seasons, but no significant deviation from unimodality (January: Hartigan's *D*=0.05, *p*=0.39; February: *D*=0.02, *p*=0.96; March: *D*=0.03, *p*=0.79; April: *D*=0.03, *p*=0.83). Presence of tadpoles from wide range of size classes was observed across all months (e.g., TL in January: 19.6 – 65.6 mm) was also observed (Fig. 25, Table 7).

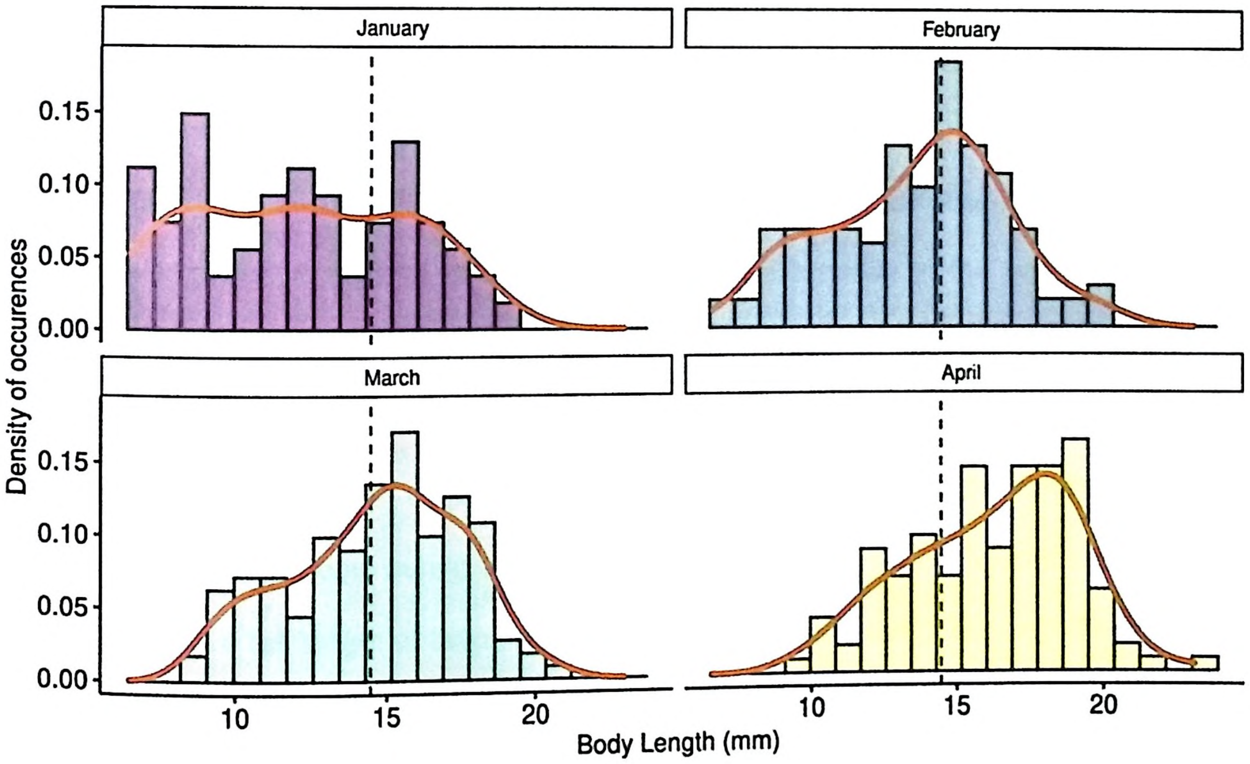


Fig. 25 Size-class distribution of tadpoles across months based on the Body Length (BL), dashed lines indicate the mean value of total length for all individuals

Total body length also showed the pattern (Fig. S11). Extreme variation in size-classes at different time points was confirmed.

Table 7 Summary of the Total Length (mm) class distribution across months.

Month	N	Total Length	Min.	Max.	SD
January	62	39.9 ± 3.2	19.6	65.6	12.6
February	118	45.3 ± 2.1	21.9	68.8	11.4
March	128	48.6 ± 1.7	27.3	69.1	10
April	121	54.4 ± 2	30.5	75.4	11.1

4.b Body Size and Relative Tail Length Variation

As expected, a general increasing trend in the body size was detected from winter to post-winter (Fig. 59-60). In both winter and post-winter seasons, mean total length of tadpoles seems to be lower in natural pools compared to modified pools. During winter, the mean total body length of the natural pool population was significantly lower than the modified pool population ($t(142)=2.4$, $p=0.02$, $d=0.4$). The same trend appears in the post-winter period ($t(217)=4.71$, $p<0.001$, $d=0.64$) (Fig. 58).

The relative tail lengths of tadpole populations were not significantly different between natural and modified pools during winter ($t(141)=1.82$, $p=0.07$, $d=0.31$). Contrasting to this, the mean relative tail length of tadpoles in the natural pool population was significantly lower than the modified pool population during post-winter ($t(217)=3.6$, $p<0.001$, $d=0.49$) (Fig. 26).

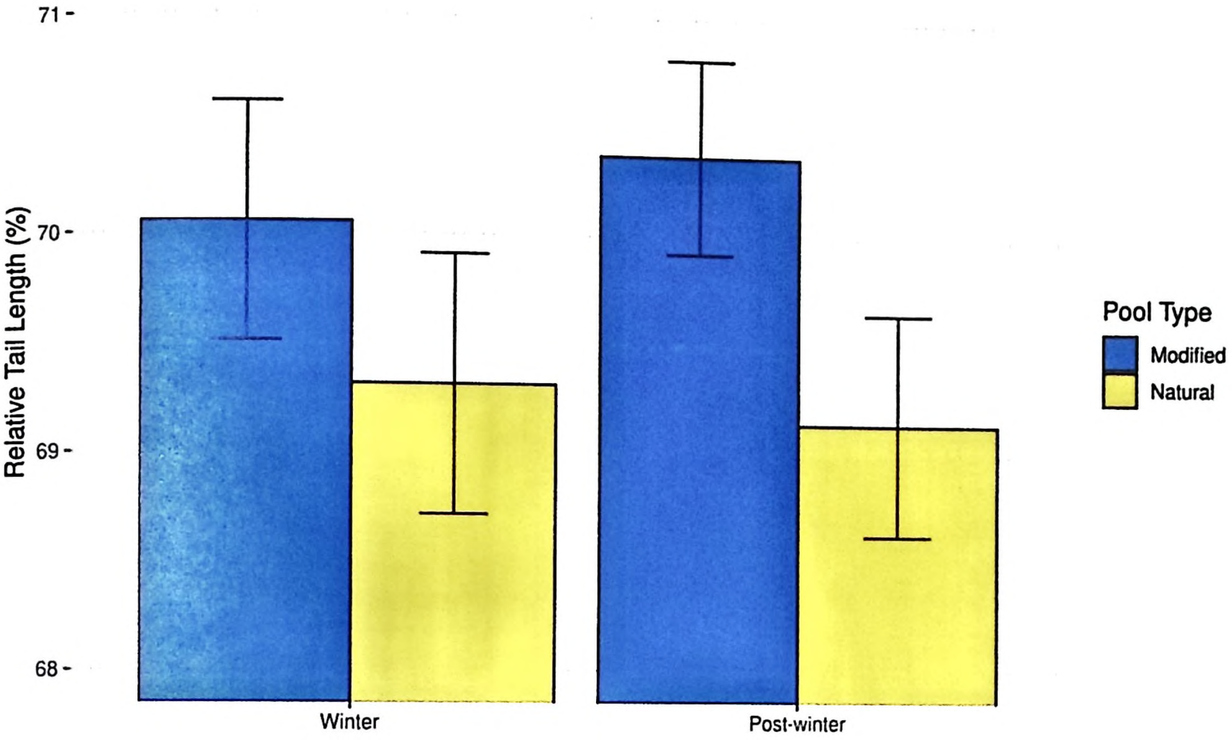


Fig. 26 Variation in relative tail length across seasons and pool types. Error bars indicating 95% confidence intervals.

4.c Designation of Putative Tadpole Groups

A summary of all morphometric measurements for three putative groups are given in the Table 13 in annexure. There was significant difference of body length across tadpole groups (Kruskal-Wallis $X^2(2)=30.46$, $p<0.001$, $\eta^2=0.62$). *Nanorana minica* group was the larger-bodied group followed by *Nanorana vicina* and the unidentified group (Fig. 27, Table 12, Note 2). All groups had significant pairwise difference between them (*N. minica* and *N. vicina:* $p_{adj}=0.002$, *N. minica* and Unidentified: $p_{adj}<0.001$, *N. vicina* and Unidentified: $p_{adj}=0.01$).

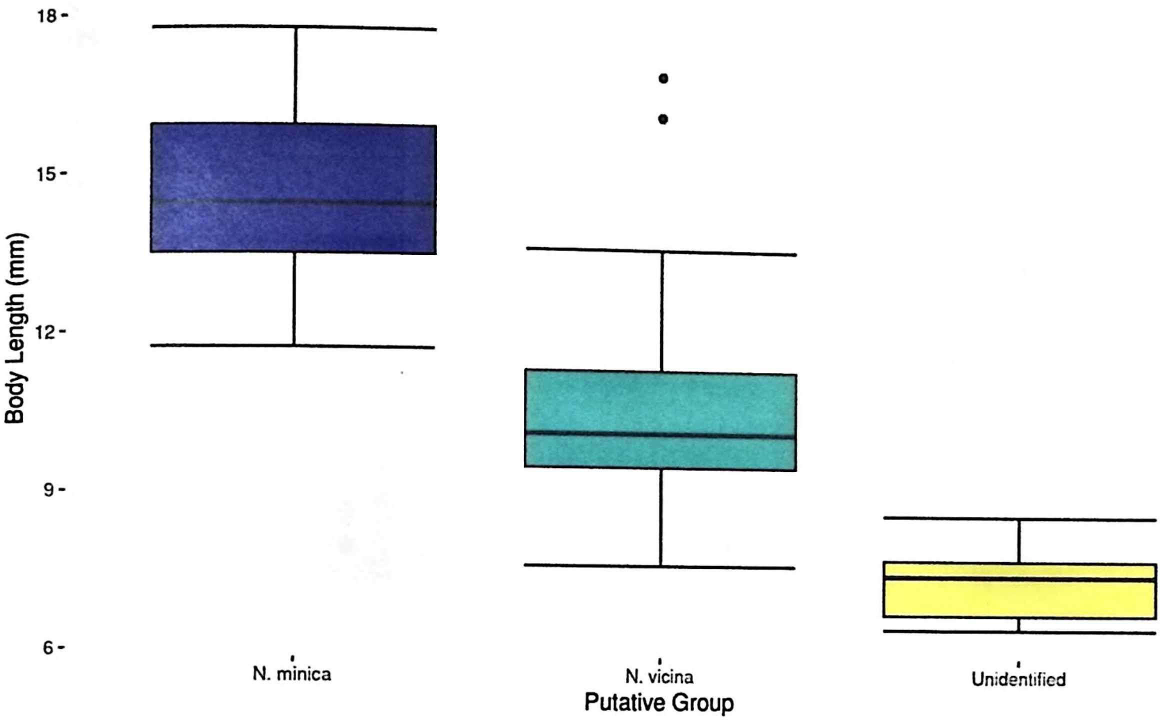


Fig. 27 Body Length (BL) variation across three putative tadpole groups

The hierarchical cluster analysis indicated the possibility of two major groups, (i) *N. vicina*+ unidentified, and (ii) *N. minica* with a few *N. vicina* individuals (Fig. 28).

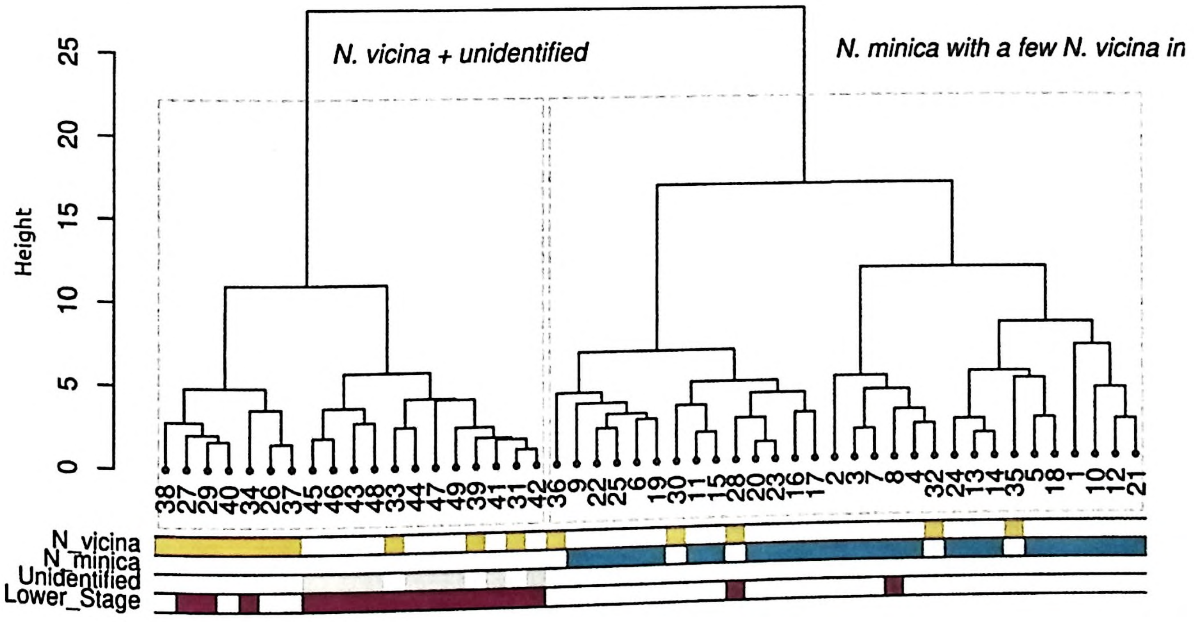


Fig. 28 Hierarchical cluster plot based on all morphological measurements. Node end indicates specimen ID and colour bars indicating pre-species identification based on tail shape, and whether stage is lower than 26 (Gosner, 1960).

PCA of the morphometric measurements for all three groups also showed similar results (Fig. 29). First and second principal component axis showed 86.6% and 7.8% of variance respectively.

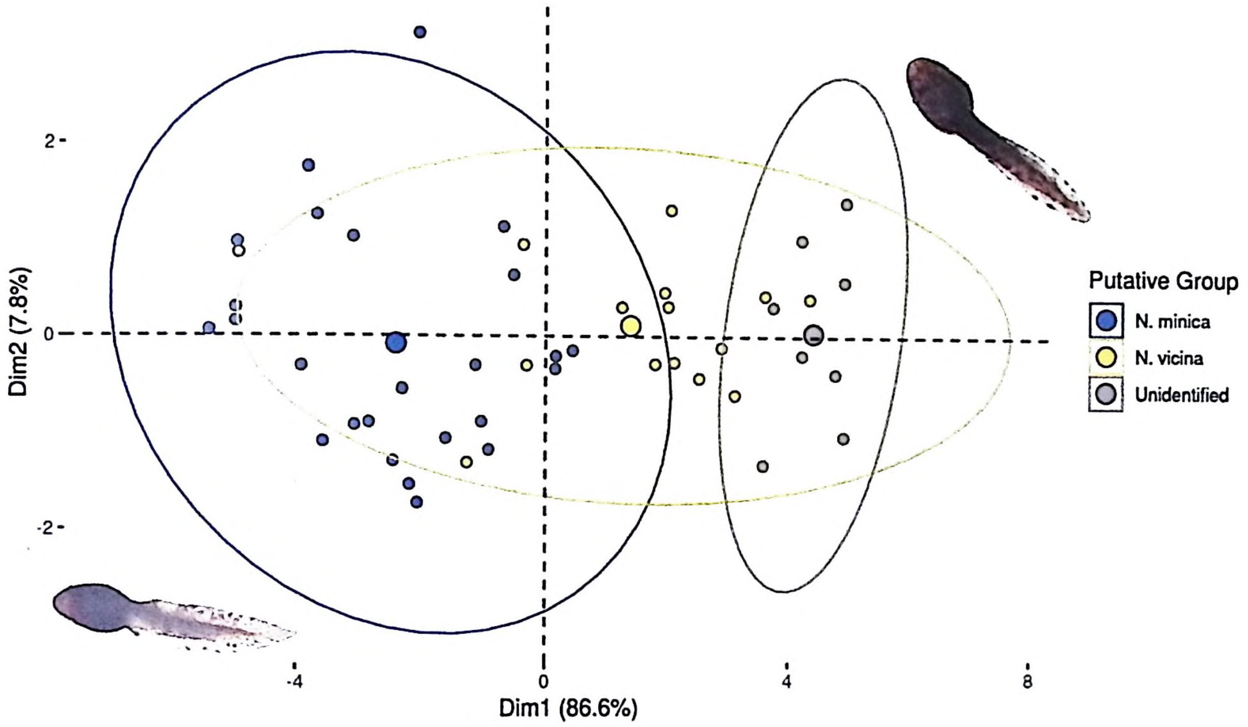


Fig. 29 2D Principal component analysis plot showing overlap between three putative species based on the morphometric measurements. Note that all points of group 'unidentified' are overlapping with *N. vicina* group. Illustration by K.M. Adila Farisa

Fig. 61 depicts the variance explained and contribution of variables to different axes. All variables except relative tail length and internarial distance contributed more than 7.5% variance each to the first principal component. Relative tail length contributed more than 80% contribution to the second axis, while other measurement remained below 10%. Individuals with ID 28, 30, 32 and 35 are clustered in the group ii, which can be due to mis-identification of tail-end shape, or lower stage. This might also have an influence on the overlap in the PCA.

4.d Behavior of Tadpoles

A Watson-Williams test of homogeneity of means on the activity pattern distributions showed significant difference between natural and modified pools in the post-winter (W=121.43, df=2, p<0.001), but not in winter (W=4.25, df=2, p=0.1196). The activity pattern overlap between natural and modified pool populations decreased from winter (90.8%) to post-winter (64.5%) (Fig. 30).

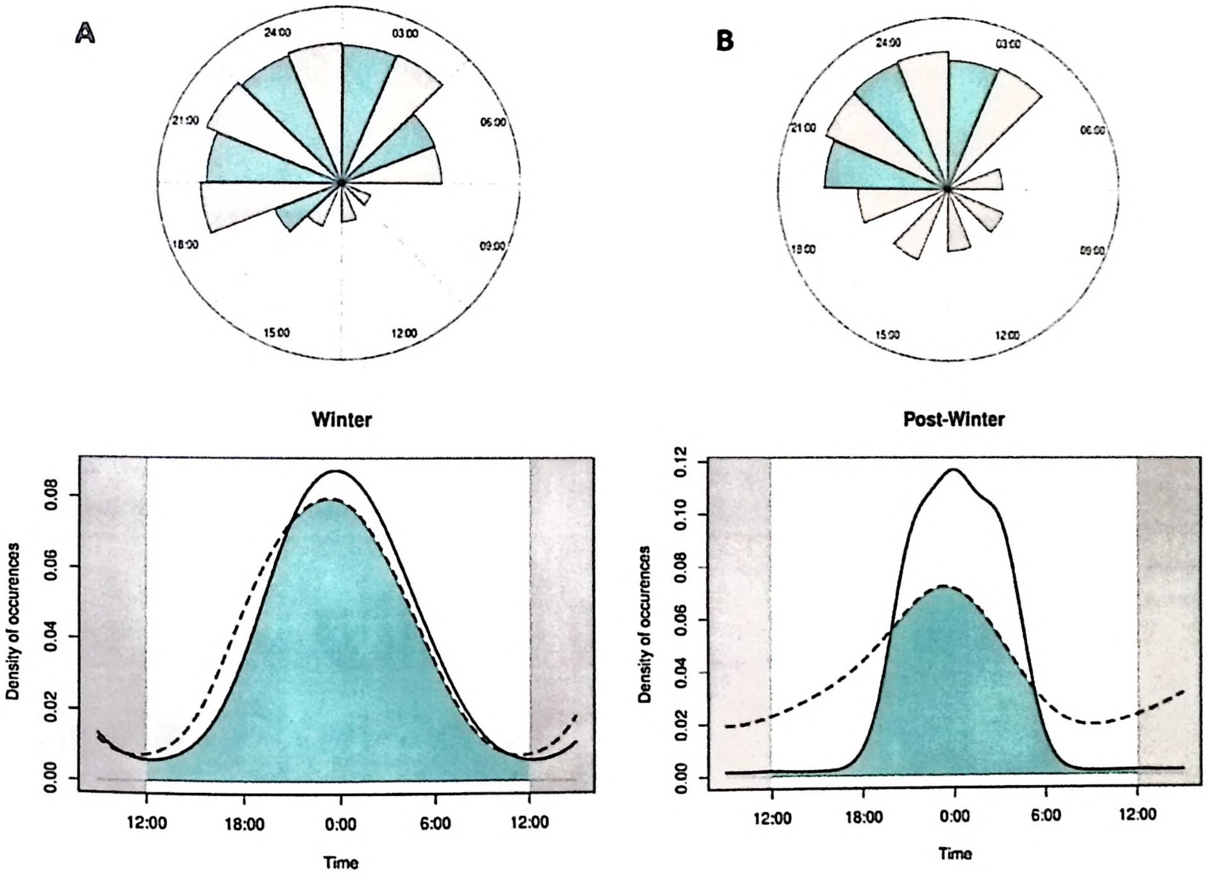


Fig. 30 Variation in mean proportional tadpole count in the natural (green) and modified (grey) pools across (A) winter and (B) post-winter seasons in relation to time (24:00=midnight, 12:00=noon IST). The plots below showing overlap between activity of modified (dashed line) and natural pool (continuous line) populations.

Natural pool population restricted their activity (coming out of cover/ hiding places) to the night time (21:00-03:00) in the post-winter period, whereas the modified pool population increased the proportion of tadpoles active in daytime (06:00-15:00). Circular plots showing the full transition across sampling sets are given in Fig. 62. While the natural pool population completely avoided daytime (09:00-15:00), a small proportion of the modified pool population was still active in the post-winter. During winter, small proportions of natural pool population was active during the transition between night and day time (06:00-09:00), and transition between day and night time (18:00-21:00). Proportions of tadpole counts were not linearly correlated to the moon illumination (r=0.06, t(168)=0.72, p=0.48) and water temperature did not have a strong correlation (r=-0.24, t(168)=-3.23, p=0.002).

A clear difference in the counts and activity pattern of tadpoles across months can be inferred from Fig. 31 depicting the proportion of tadpoles engaged in various activities (stationary, moving, feeding and surfacing).

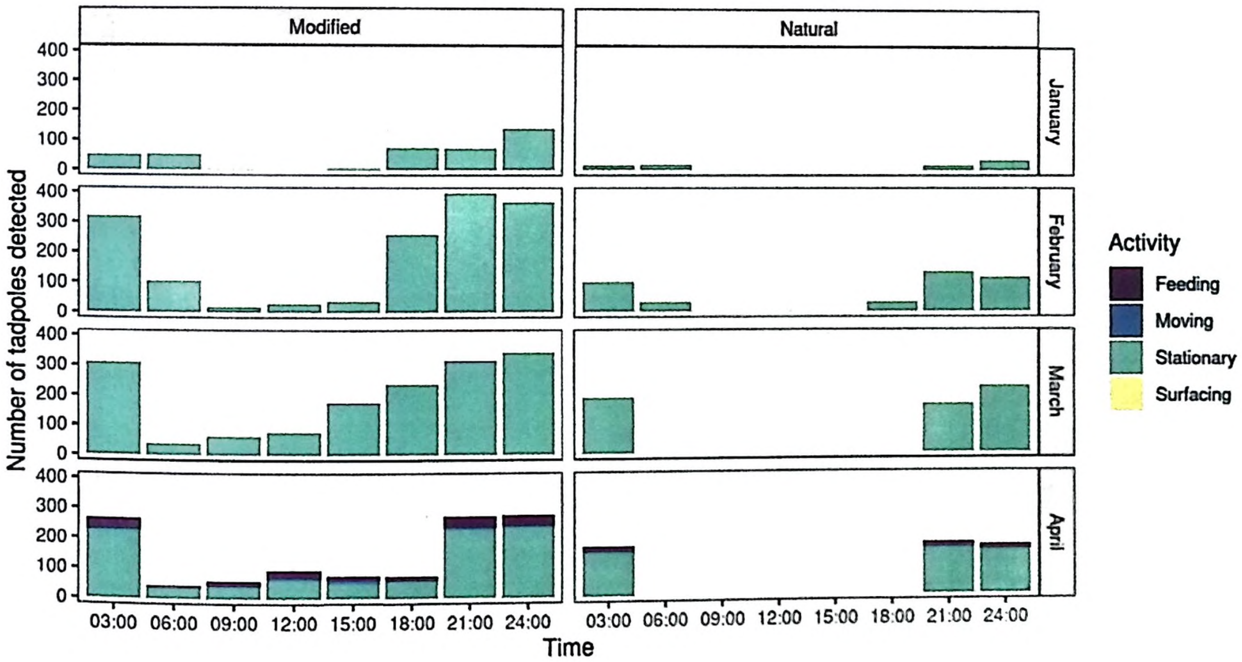


Fig. 31 Proportions of tadpoles in various behavior states in natural and modified pools with diurnal and monthly variation

There were no tadpoles engaged in moving, surfacing and feeding during winter. The proportion of these activities increased in the post-winter period. An equality of proportions test with continuity correction showed significant difference between proportions of stationary tadpoles ($X^2(1)=12.49$, $p<0.001$), feeding tadpoles ($X^2(1)=7.21, p=0.007$), and moving tadpoles ($X^2(1)=8.36, p=0.004$); but not in the case of surfacing tadpoles ($X^2(1)=0.13$, p=0.72) between night (*N*=4607) and day (*N*=1588) time. The hourly pattern of activity and count in modified and natural pools shows feeding and moving activities are not restricted to peak activity time (21:00-03:00), at least in the case of modified pool population.

5 DENSITY OF TADPOLES

5.a Temporal Variation

There were clear differences in the mean tadpole density patterns across the major study pools during both day and night sessions across the seasons (Fig. 32).

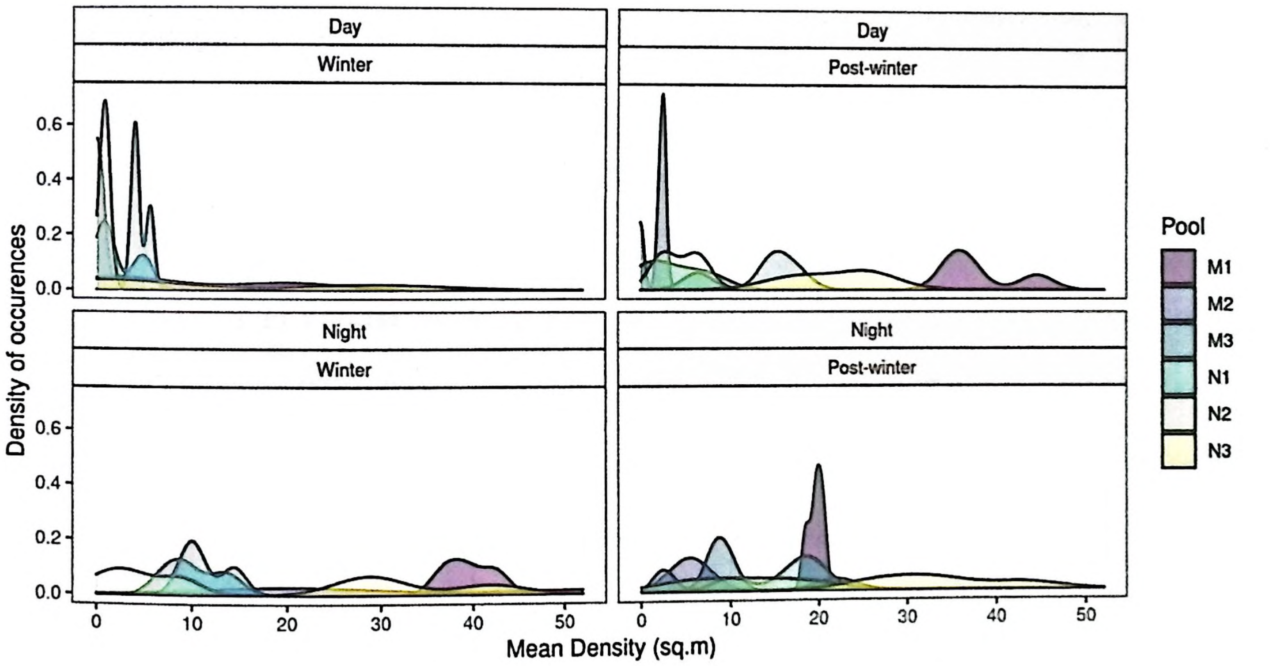


Fig. 32 Density plots showing the seasonal and diurnal pattern of mean tadpole densities in major study pools.

A clear diurnal variation in mean tadpole density was evident and the degree of this variation was different between winter and post-winter seasons (Fig. 33, Fig. 63-64). The mean tadpole density pooled across sessions did not vary significantly across months ($F_{1.42, 15.7}=3.52$, p=0.067, $\eta_p^2=0.243$).

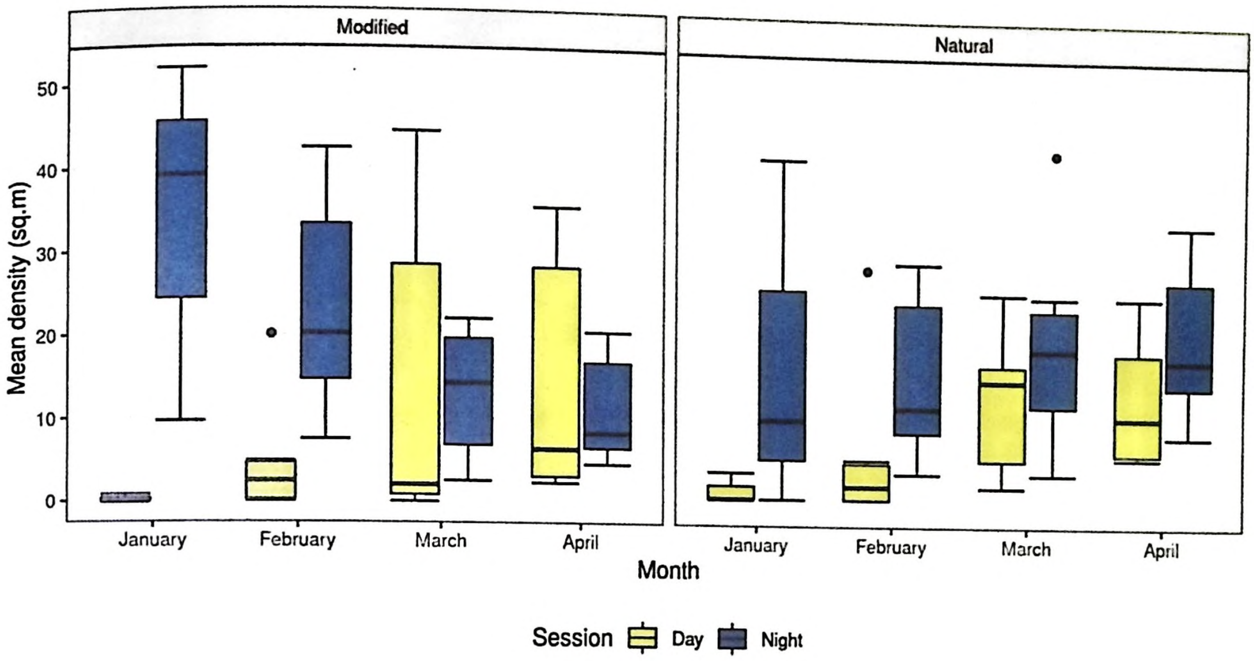


Fig. 33 Monthly and diurnal variation in mean tadpole density averaged across sampling sessions.

When the diurnal variation analyzed separately for two different pool types, night density in modified pools varied significantly across months (Kruskal-Wallis $X^2(3)=11.76$, $p=0.008$, $\eta^2=0.06$), but not in natural pools ($X^2(3)=4.43$, $p=0.22$, $\eta^2=0.62$). A pair-wise comparison showed no significant difference between any months in the night density of modified pools. Density during day time varied significantly in both natural ($X^2(3)=18.57$, $p<0.001$, $\eta^2=0.147$) and modified pools ($X^2(3)=19.7$, $p<0.001$, $\eta^2=0.13$). Pair-wise post-hoc tests showed significant difference between April and February ($p_{adj}=0.008$); April and January ($p_{adj}=0.002$); January and March ($p_{adj}=0.017$) in natural pools, and April and February ($p_{adj}=0.011$); and April and January ($p_{adj}<0.001$) in modified pools.

The pooled data using the mean densities in both seasons and sessions showed mean tadpole density at nighttime was significantly higher in the modified pools (mean±CI=25.9±7.01) than natural pools (mean±CI=15.8±5.67) (Wilcoxon rank sum test with continuity correction, W=1355.5, p=0.02, r=0.25) during winter. But there was no significant difference during daytime (W=943.5, p=0.46, r=0.08). The diurnal variation in density of tadpoles across months is given in Table 13 in the annexure.

During post-winter, nighttime mean density was significantly higher in natural pools (mean±CI=19.7±3.68) than modified pools (mean±CI=12.3±2.59) (W=2019.5, p=0.001, r=0.26). There was no significant difference in mean density of natural and modified pools in the daytime (W=2543, p=0.15, r=0.12).

5.b Temperature, Depth and Tadpole Density

Mean water temperature and mean tadpole density was positively correlated significantly in natural pools during daytime (r=0.45, p=0.041). A decreasing trend in modified pools during nighttime was observed, but the relationship was not statistically significant (r=-0.37, p=0.098) (Fig. 34).

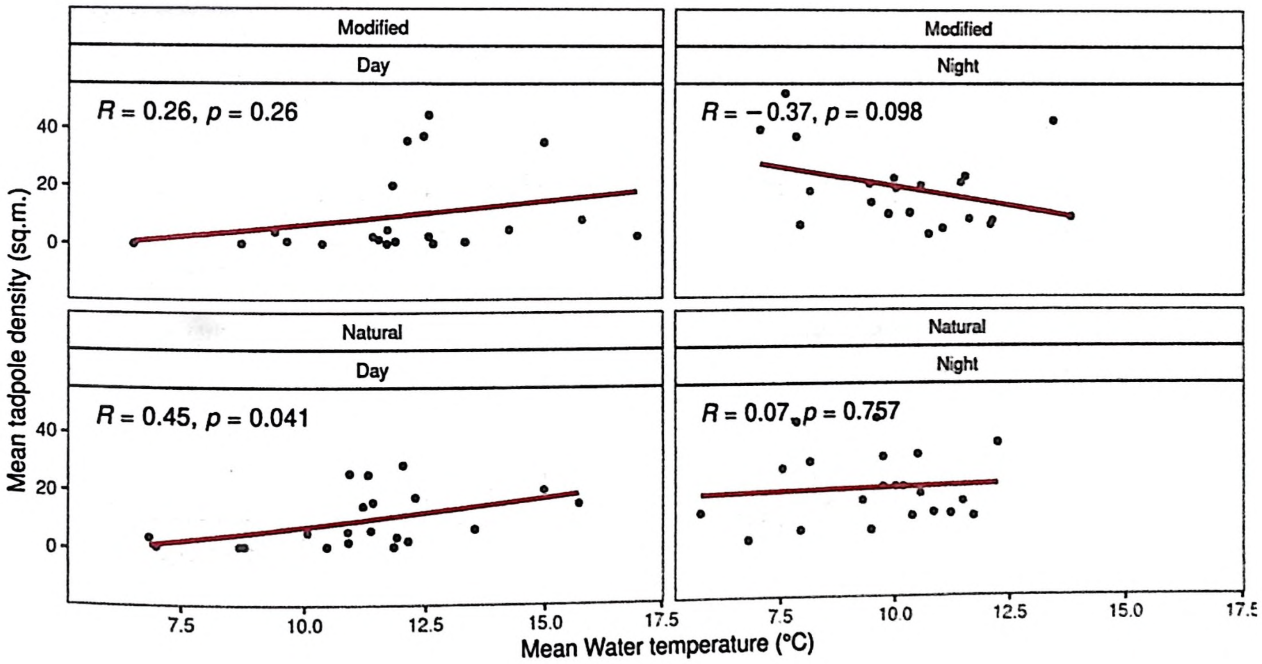


Fig. 34 Relationship between mean tadpole density and mean water temperature across pool types between day and night time

Mean water depth had a statistically significant positive influence on mean tadpole density in natural pool during both day and night time (day: r=0.69, p<0.001; night: r=0.63, p=0.002). Similar positive relationship was observed in modified pools during night time, but this was not statistically significant (Fig. 35).

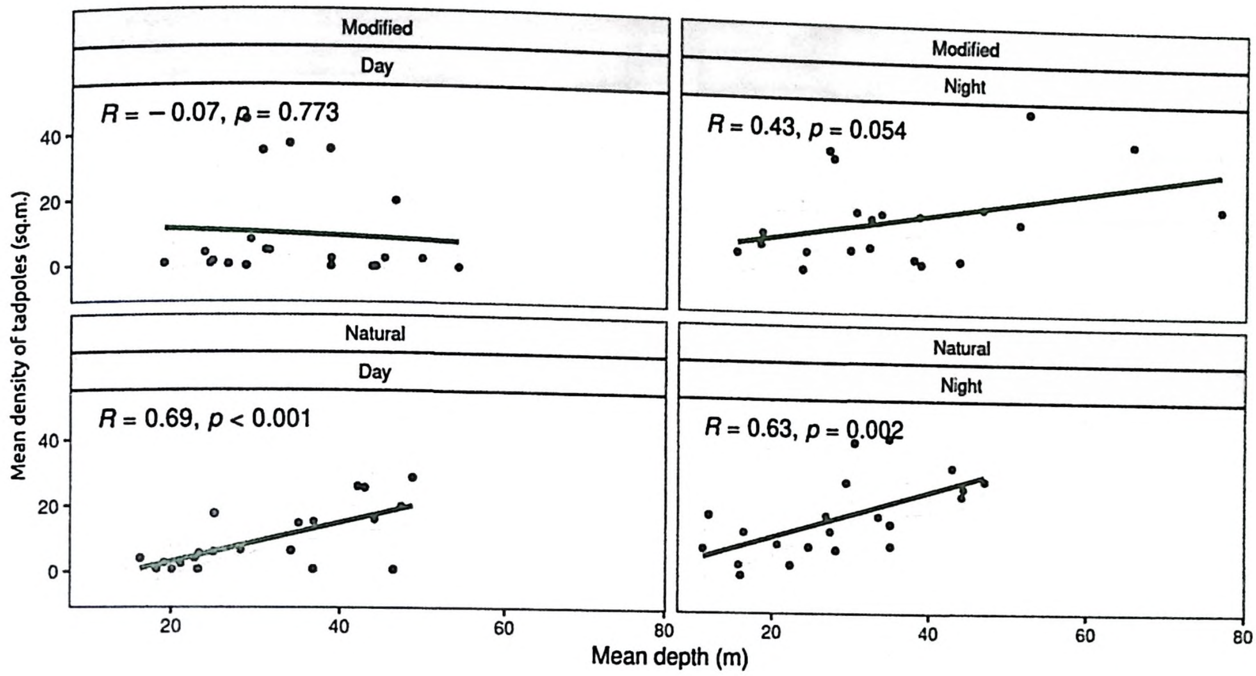


Fig. 35 Relationship between mean tadpole density and mean water depth across pool types between day and night time.

5.c Best Predictors of Tadpole Density

Strong multicollinearity (r>60%) was detected between EC and TDS, (r=91.16%), mean pool volume and depth index (r=67.34%), air and water temperature (r=78.87%), modification and pool volume (r=71.98%), and canopy cover percentage and pool volume (r=-84.27%) (Fig. 65). There were no outliers in the data, but large number of zeros were detected (37.4%, Fig.66). In the comparison of generalized linear mixed models, tadpole density was best predicted by the time of the day, interaction of mean canopy cover with water temperature (positive influence), richness of cover items, mean canopy cover percentage, water temperature, leaf litter depth, velocity and an interaction of day of the time with pool modification (negative influence) (Table 8, Fig. 36). Detailed summary of the best model is given in the annexure (Table 10).

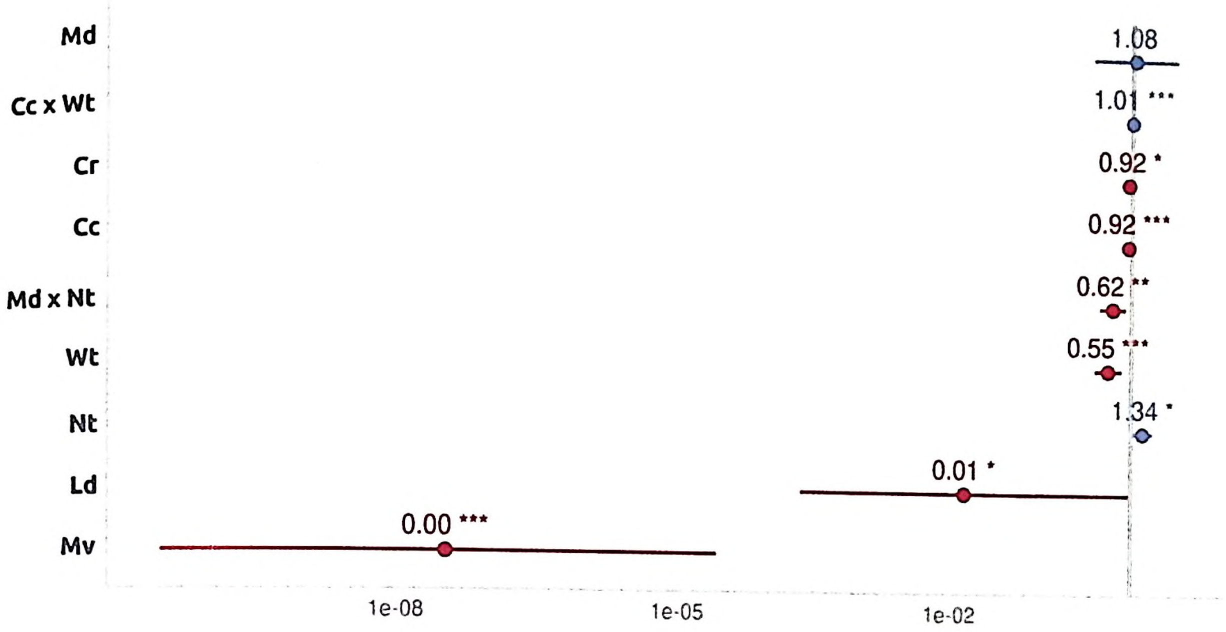


Fig. 36 Forest-plot depicting incidence rate ratios of various predictor variables in the top model. Red colour indicates negative influence and blue indicates positive influence. Significance codes: *p≤0.05; **p<0.01; ***p<0.001. *Abbreviations*: Water Temperature (Wt), Cover Item Richness (Cr), Mean Velocity (Mv), Leaf Litter Depth (Ld), Modification - Check Dam (Md), Mean Canopy Cover.

Table 8 Table showing all supported models (ΔAICc<10). All models include a random effect of (1 + Winter | Pool) and the zero-inflation formula. Bolded terms are significant predictor variables (p<0.05) in the model. *Abbreviations*: Water Temperature (Wt), Cover Item Richness (Cr), Mean Velocity (Mv), Leaf Litter Depth (Ld), Mean DO Level (Do), Modification - Check Dam (Md), Mean Canopy Cover Percentage (Cc), Night (Nt).

Model	**df**	**logLik**	**AICc**	**ΔAICc**	**Weight**
Ld + Mv + Cc x Wt + Md x Nt + Wt + Cr	18	-1450.24	2937.93	0	0.74
Ld + Cc x Wt + Md x Nt + Wt x Nt + **Cr** + Do + **Mv**	20	-1449.56	2940.89	2.96	0.17
Ld + **Mv** + **Cc x Wt** + Md + Nt + **Cr**	17	-1454.47	2944.23	6.3	0.03
Ld + **Mv** + **Cc** x **Wt** + Md + Wt x Nt + Cr	18	-1453.84	2945.13	7.2	0.02
Ld + **Mv** + Do + **Cc** x **Wt** + Md + Wt x Nt + **Cr**	19	-1453.38	2946.37	8.44	0.01
Ld + **Mv** + Do x Nt + **Cc x Wt** + Md + Wt x Nt + **Cr**	20	-1452.78	2947.34	9.4	0.01
Ld + **Mv** + **Do** x Nt + Cc + Md + Wt + Nt + **Cr**	18	-1455.04	2947.53	9.6	0.01

Predicted (estimated marginal means) tadpole density across various velocity and water temperature classes during day and night time in natural and modified pools are given in Fig. 37. Predicted tadpole densities for day and night times are given in Fig. 38. Predictions based on cover item, and leaf litter depths are given in the annexure (Fig. 68-69).

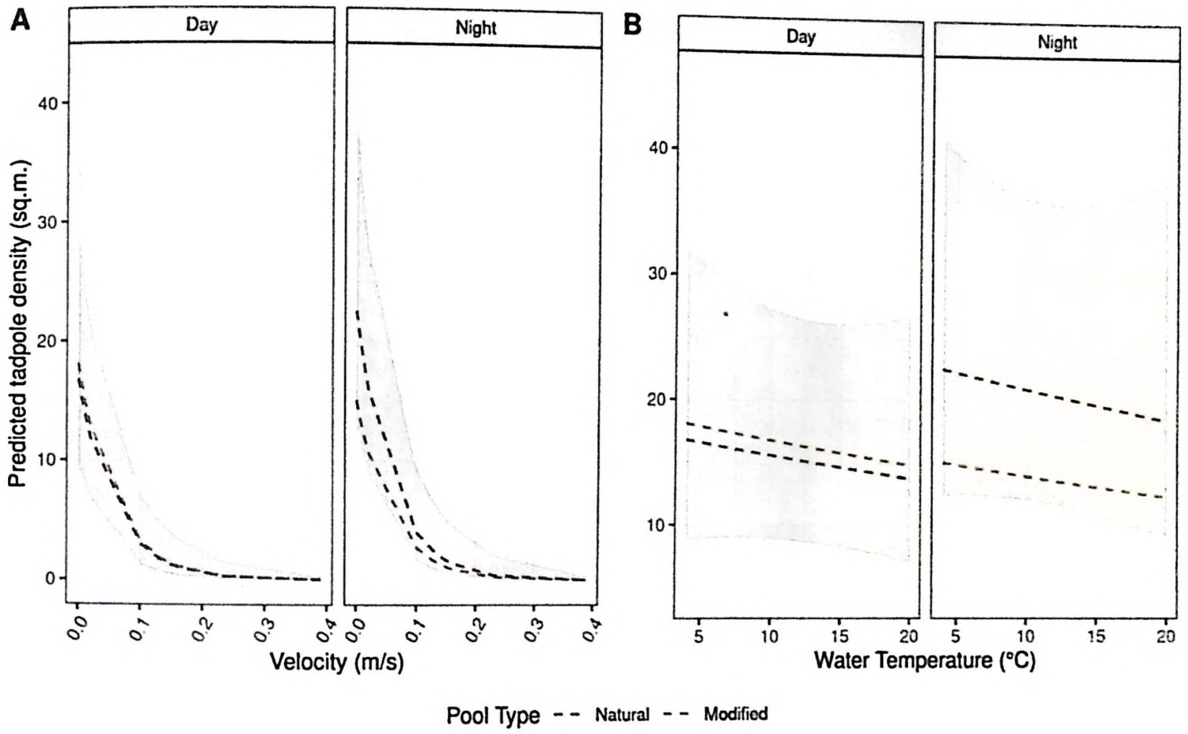


Fig. 37 Predicted tadpole density along (A) water velocity and (B) water temperature ranges in modified and natural pools during day and night time (95% CI).

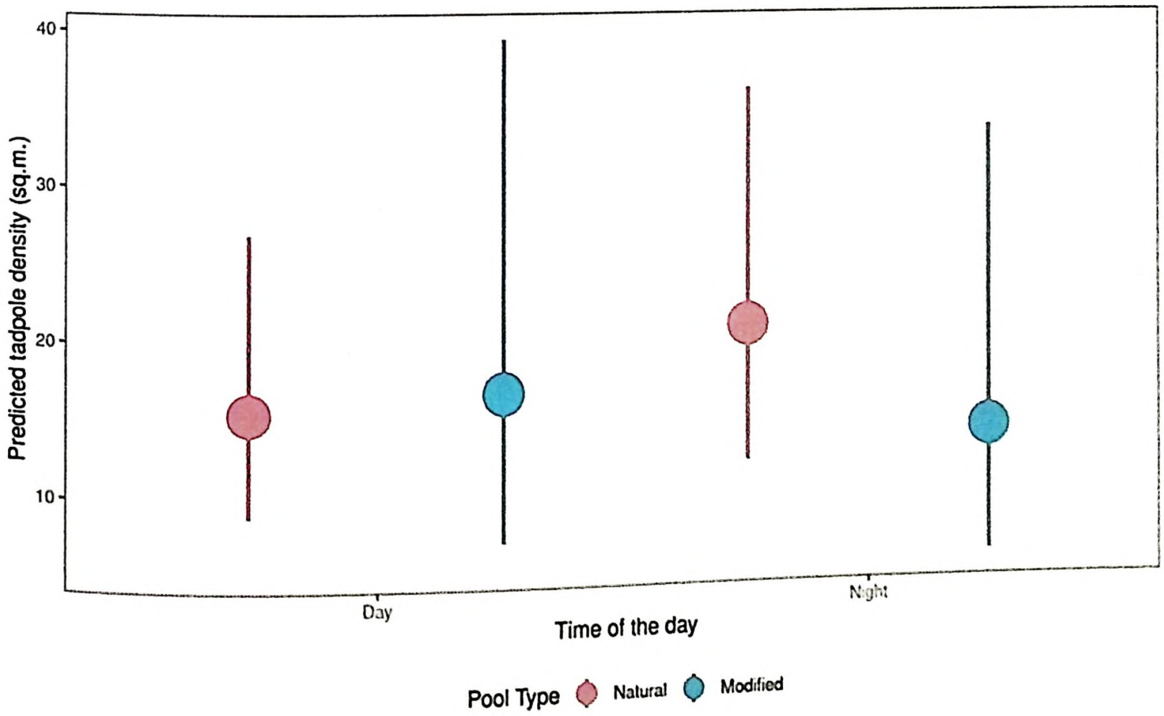


Fig. 38 Predicted tadpole density during night and daytime in modified and natural pools (95% CI).

6 HABITAT OF TADPOLES

6.a Habitat Availability, Use & Suitability Curves

Tadpoles were largely using moderate depth areas compared to shallow and deep areas irrespective of pool type and season. Tadpoles were mostly using a range of 0.01-0.2m depth (Fig. 39). Proportional use of such areas often exceeded the available proportion.

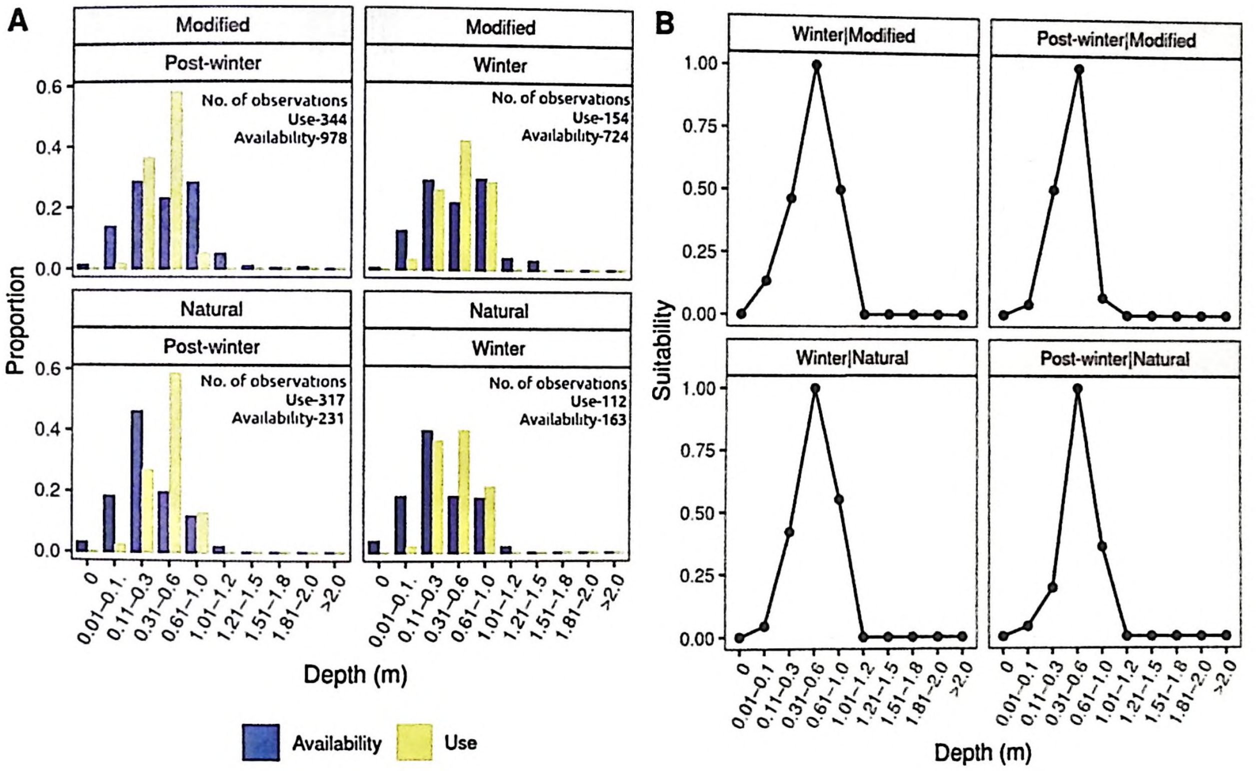


Fig. 39 (A) Relative frequency of different classes of depth available and use and (B) depth habitat suitability criteria (HSC) curves of the overwintering tadpoles.

During both winter and post-winter, tadpoles mostly used stagnant areas (0 m/s) of natural and modified pools (Fig. 40).

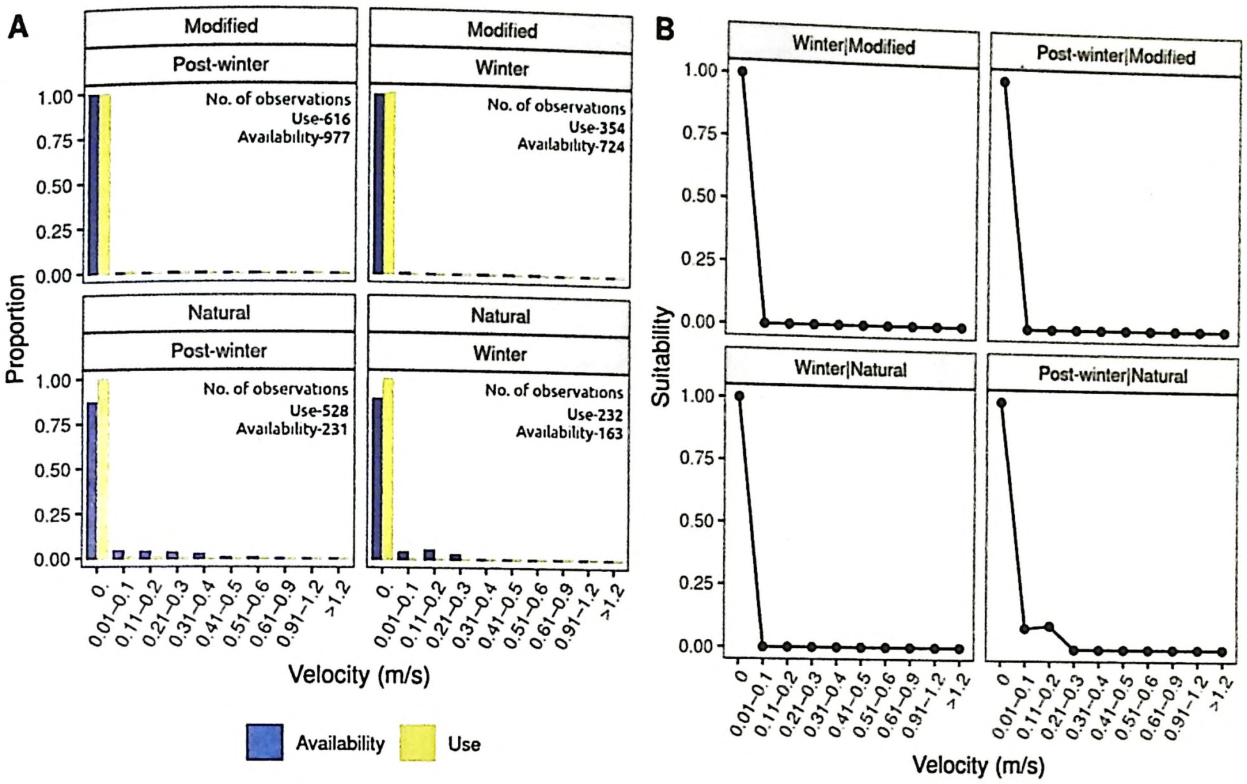


Fig. 40 (A) Relative frequency of different classes of velocity available and use and (B) velocity habitat suitability criteria (HSC) curves of the overwintering tadpoles.

Substrate use by tadpoles varied across pool types and seasons (Fig. 41). During winter, the modified pool population used leaf litter, algae and sand proportion greater than their availability. Algae, leaf litter, humus, and sand were the preferred items in natural pools in winter. During post-winter, modified pool population used sand greater than their availability. Generally, tadpoles used substrates according to their availability. Natural pool population showed preference towards sand, cobble and algae during post-winter. In general, the mostly used in modified pools are humus and leaf litter.

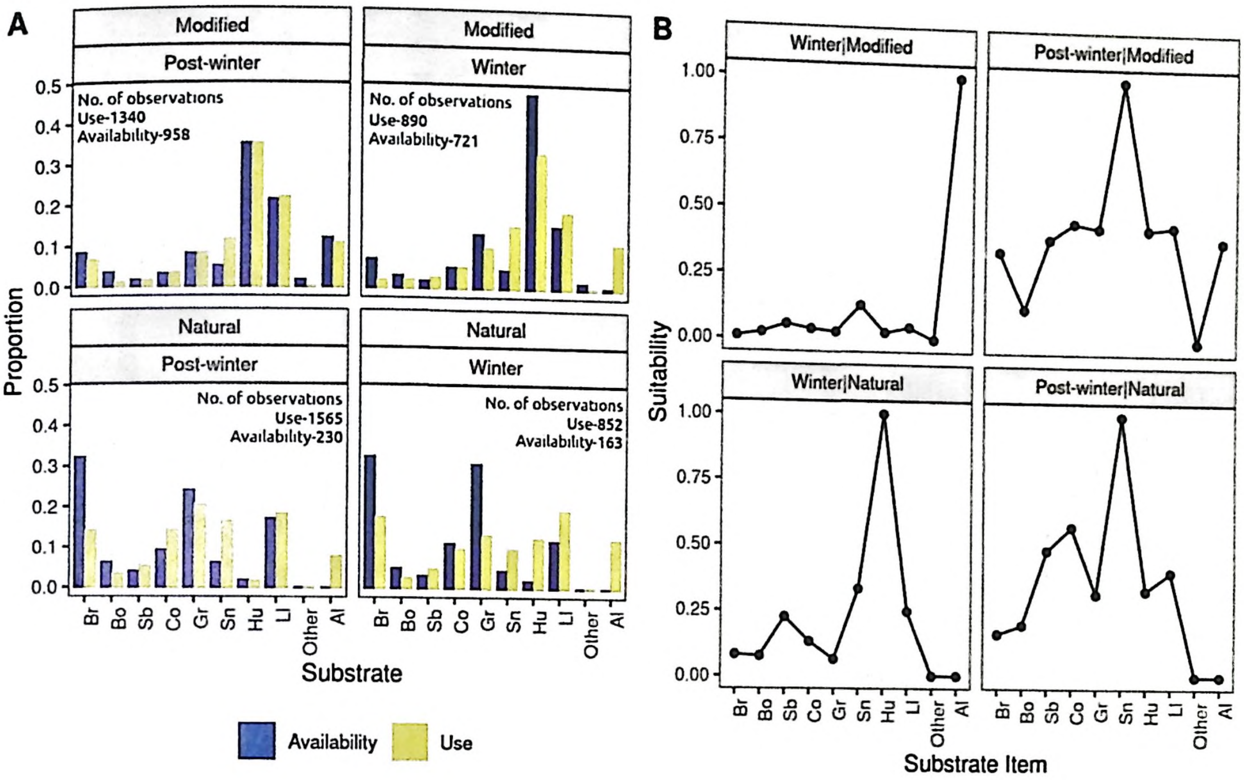


Fig. 41 (A) Relative frequency of different classes of substrate available and use and (B) substrate habitat suitability criteria (HSC) curves of the overwintering tadpoles.

Cover item use in natural pool by tadpoles remained the same in both winter and post-winter (Fig. 42). Tadpole used bedrock, submerged vegetation, and overhanging vegetation greater than their available proportions. In case of modified pools, high proportional use of submerged and overhanging vegetation was found during post-winter compared to winter. Proportional use of root and log undercuts was high during winter compared to post-winter in modified pools.

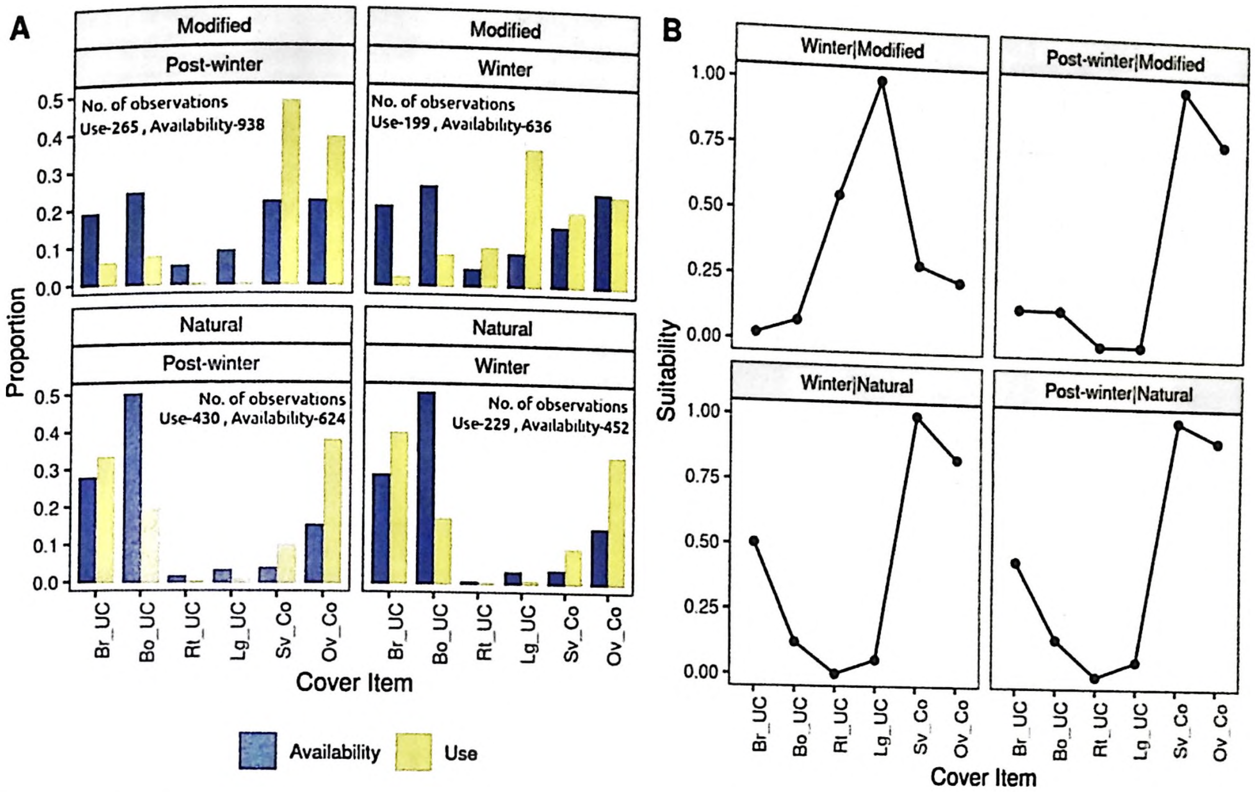


Fig. *42* (A) Relative frequency of different classes of cover items available and use and (B) cover habitat suitability criteria (HSC) curves of the overwintering tadpoles.

6.b Resource Electivity by Tadpoles

Overwintering tadpoles generally avoided both shallow and deeper areas of the pools across seasons and pool types (Fig. 43).

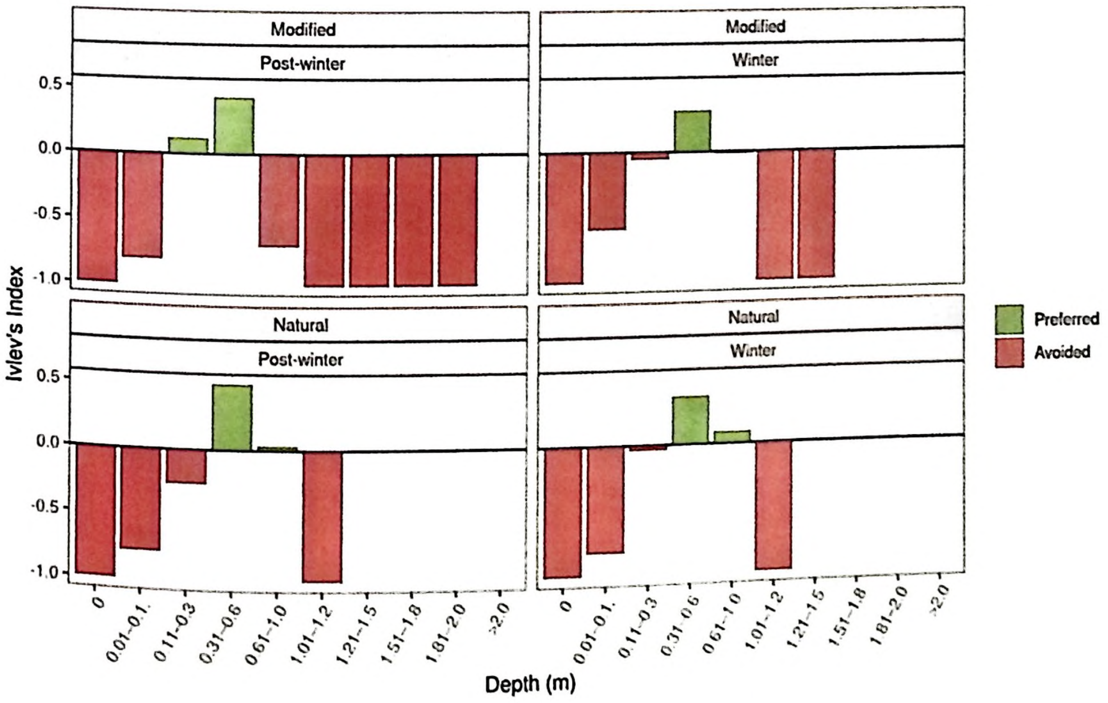


Fig. **43** Avoidance and preference of different classes of water depth by the overwintering tadpoles across seasons and pool types.

Tadpoles preferred low-velocity areas (0-0.05 m/s) in both modified and natural pools (Fig. 44) while avoiding the other areas.

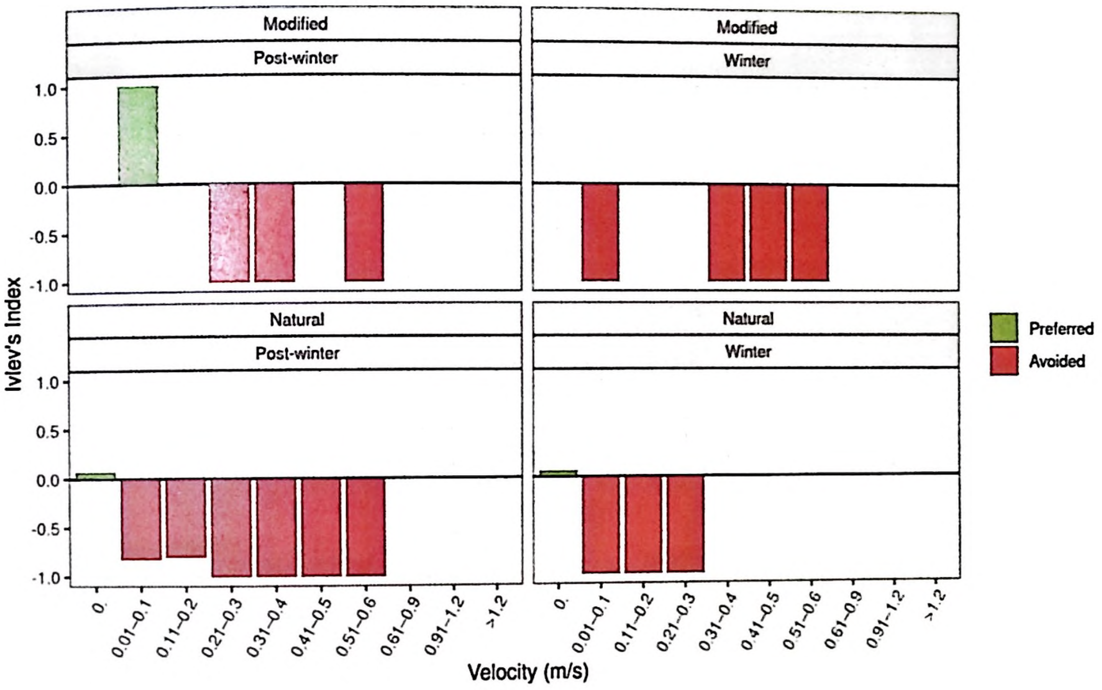


Fig. 44 Avoidance and preference of different classes of water velocity by the overwintering tadpoles across seasons and pool types

Tadpoles preferred algae, leaf litter, sand, and small boulder as substrate in modified pools during winter (Fig. 45). But in post-winter, highly preferred item was sand. Avoidance of other substrates (e.g., concrete wall) was clear in both seasons. In natural pools, preferred items in winter were algae, humus, sand, leaf litter, and small boulders. Gravel, cobbles, boulders and bedrocks were avoided in winter. In post-winter, cobble was preferred and humus was avoided as contrasting to winter.

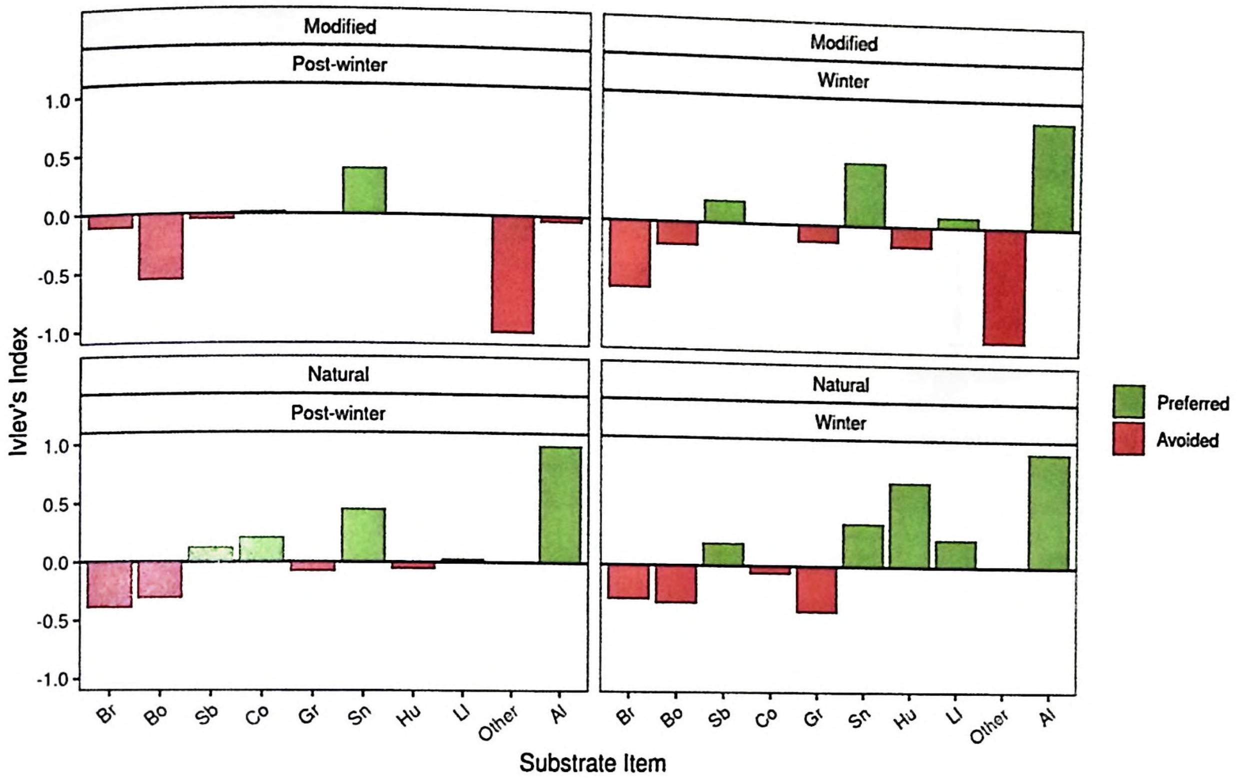


Fig. 45 Avoidance and preference of different types of substrate items by the overwintering tadpoles across seasons and pool types.

In natural pools, cover item preference and avoidance were consistent across seasons (Fig. 46). Preferred items were bedrock undercuts, overhanging and submerged vegetations. Boulder, log and root undercuts were avoided by the tadpoles in natural pools. While tadpoles in the modified pools preferred submerged vegetation, root, and log undercuts during winter, they avoided root and log undercuts during post-winter and preferred submerged and overhanging vegetation during post-winter. Bedrock and boulder undercuts were avoided by the modified pool population in both the seasons.

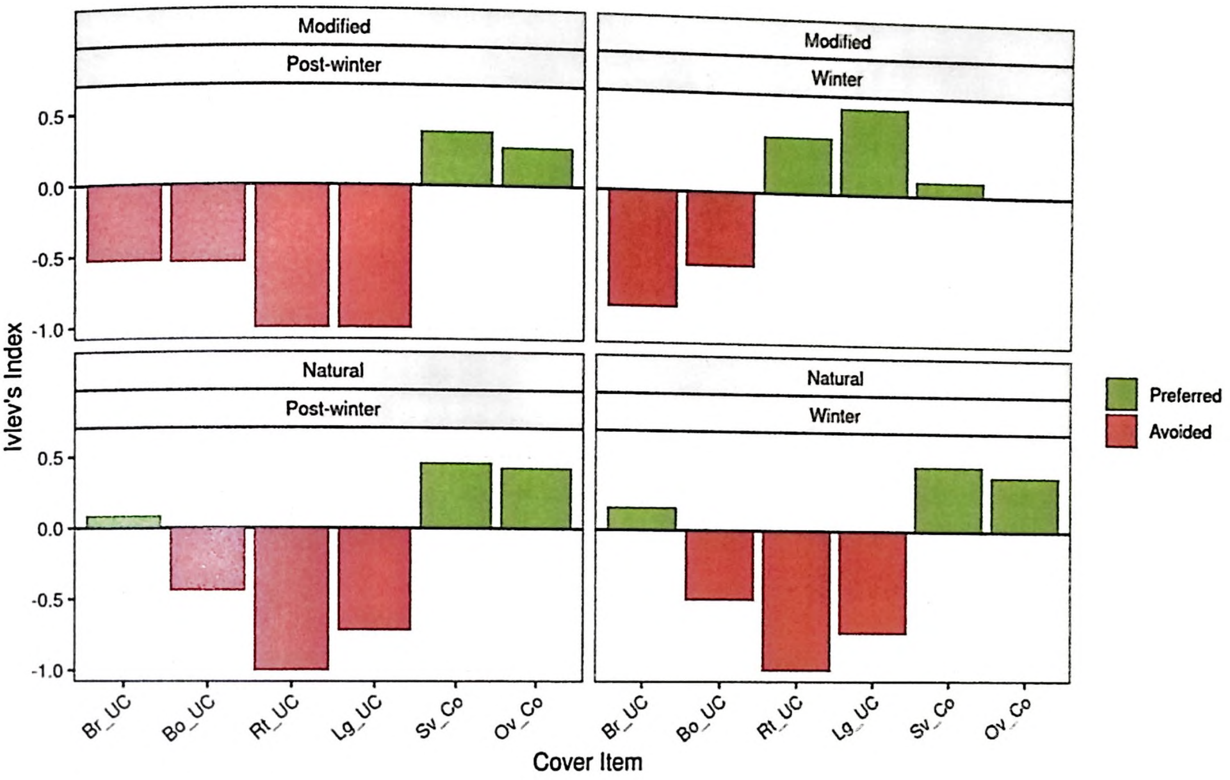


Fig. 46 Avoidance and preference of different types of cover items by the overwintering tadpoles across seasons and pool types.

DISCUSSION

Different aspects of evolution of adaptive intrinsic growth rates have been proposed among different taxa. This includes slow growth as an adaptive strategy to deal with nutrient stress, rapid growth requirement for reaching the minimum size quickly, limitation of growth in species that require early life maturation by the tradeoff between growth rate and development, and rapid growth as a compensation for slow growth due to environmental conditions (Arendt, 1997). In anurans, larval growth has been shown to be influenced by both temperature and food availability (Alvarez & Nicieza, 2002; Burraco et al., 2021; Hsu et al., 2012; Liess et al., 2013). Environmental conditions such as low temperature and short growing seasons in the high elevation areas forces amphibian populations to have long larval periods (Brown, 1990; Walsh et al., 2008) which is called as overwintering. Overwintering tadpoles are found to be exhibiting seasonal differences in their energetic physiology by changes in membrane composition, metabolic enzyme activities and locomotor performance (Gray et al., 2016).

Bimodal distribution of developmental stages with individuals that remain in relatively lower developmental stages throughout the seasons have been documented in several species including *Rana temporaria* (Walsh et al., 2008). Walsh et al., (2008) also suggested that the decision to overwinter as larvae or not seems to be made relatively early in the season. A later experimental study showed that tadpoles did not arrested their larval development early in the season contrary to the field observations (Walsh et al., 2016). Overwintering tadpoles have been reported from various parts of the globe, but regional biases are existing in the literature due to non-documentation of the phenomena or lack of explorations (Jithin & Das, 2020). Despite the amazing opportunity tadpoles provide to learn many complex biological phenomena and evolutionary aspects, studies on tadpoles are still in the developmental stage in India (Hebbar et al., 2019; Saidapur, 2001; Sircar, 2010).

The present study results showing high temporal variation in water temperature and tadpole emergence in diurnal, and seasonal scales underlines the importance of taking temperature fluctuations rather than mean temperature when studying changing climate and performance of organisms (Dillon et al., 2016; Ma et al., 2015). Energy fluxes across the stream zones are integrated by water temperature, and they are influenced by the physical characteristics and anthropogenic disturbances of the stream and atmospheric conditions, and the biology of the stream (Kurylyk et al., 2015; MacKenzie, 2008; Strauch et al., 2017; Tague et al., 2007).

Even though I did not find any difference in daytime water temperature between pool types in winter, the temperature was high in modified pools during post-winter. One possible reason behind the heating up of modified pool water during daytime is the reduced canopy cover or openness of such pools. This is supported by the fact that the temperature was not high in one modified pool (M3), which resembled a natural pool in terms of canopy cover and riparian vegetation. Canopy cover acts as a resource gradient for tadpoles through its effect on water temperature, dissolved oxygen, and abundance and composition of periphyton (Halverson et al., 2003; Schiesari, 2004; Skelly et al., 2002). The diel patterns of dissolved oxygen and water temperature are also influenced by canopy cover gradient (Broadmeadow et al., 2011; Werner & Glennemeier, 1999). The night time water temperature did not vary between pool types and the seasons and this may be one of the reasons why tadpoles are more active in the night time during both the seasons and pool types.

In the pools where behavioral observations were carried out, air temperature was not different in the daytime, but comparatively high temperature in natural pool during winter. This might be due to the heat trapped in the atmosphere for long duration due to high canopy cover in the modified pool. In post-winter, there was no difference between the pool air temperatures. In winter, both day and night time water temperature was high in modified pool compared to the natural pool. Modified pool showed high water temperature in the nigh time during post-winter. The increased water temperature in modified pool can be explained by two reasons. One is the effect of canopy cover and other being the high microbial respiration from the humus and leaf litter deposition (Gökbulak, 2002) in the substrate in these pools.

In the case of dissolved oxygen levels, it reduced from winter to post-winter without any diurnal or pool type variations. Total dissolved solids levels (or conductivity) varied across months, but not pool types. High levels of TDS during night, except in January, the peak winter time was observed. Diurnal and seasonal fluctuations in stream water flow, biogeochemical processes, water temperature, and the biological uptake by biofilm communities are some of the reasons behind these variations (Gribovszki et al., 2010; Loperfido et al., 2010; Nimick et al., 2011). Negative correlation between DO and TDS, and positive correlations of pH with DO and EC with TDS were detected. Correlation between EC and TDS in natural waters is very well known (Rusydi, 2018; Thirumalini & Joseph, 2009). Mean canopy cover, cover item index, substrate index, and velocity index were higher for natural pools as expected. In contrast to this, excluding M3, all modified pools were of high surface area, volume, and depth index. The variation in characters of modified pools have been assessed by earlier studies. It is shown that the dammed pools show lower velocity, finer substrate, higher water temperature, lower dissolved oxygen, and worse biological indices of water quality compared to the main stream channel (Kang & Kazama, 2012). Check dam construction often strongly modify the morphology of active stream channel by retaining high amount of sediment in the upstream and subsequently affect the downstream bank stability (Boix-Fayos et al., 2007; Fortugno et al., 2017; Korpak & Lenar-Matyas, 2019).

The study showed that body size class distribution varies from winter to post-winter period with a shift to larger size classes where extreme variation in size classes can be observed. This confirms our field observations of tadpoles of varying developmental stages during all the seasons. The presence of a wide range of size classes (19-80 mm) varying across time strengthens the idea of overwintering cohorts. Even though we have confirmed the presence of different age class cohorts in the study population based on the body length (and total length) frequencies, and observations of diverse stage classes at the same time, more detailed analysis of the age-size classes considering multi-season coverage, development duration tracking using individual tagging, body weight, species identity, and limb differentiation (Brown, 1990).

The variation in relative tail length in response to increasing level of environmental stress (low temperature and limited food availability) have been found in *Rana temporaria* tadpoles (Merilä et al., 2004) in a latitudinal gradient. Increasing relative tail length, or the change in body growth can be an indication of the thermal and food stress tadpoles facing in the modified pools in the post-winter. Even though my study design was not capable to tease out the effect of species identity, and the developmental stage on this aspect, the variation in the tail length gives a good framework for further investigation. Tadpole growth is found to be reduced due to Daily Thermal Fluctuations (DTFs), and species which cannot compensate the variation in temperature by adjusting growth and development will be the most affected by this (Kern et al., 2015).

I propose two different putative tadpole groups based on morphometric measurements and LTRF formulae, which can be further confirmed using molecular identification. But, considering the change in LTRF across developmental stages, as reported by other studies (e.g., Gill et al., 2020), especially in this case where both the species might have overlapping formulae across stages, it is not recommended to rely on the LTRF to confirm species identity. Even if both the species have stable and non-overlapping LTRF, without clearly defined morphological distinction, species identification in the field will be problematic considering the variability observed in tail end shape, a character assumed to be different between the potential groups. The 'unidentified group', I proposed prior to the analyses can be the lower stage tadpoles of *N. vicina* (Gill et al., 2020), as suggested by the cluster analysis. When comparing the group and stage-wise cluster plots, it can be seen that the *N. vicina* classified in the second cluster are most probably the advanced stages (above 30) except one (stage 27).

Seasonal difference in the diurnal emergence or activity pattern of tadpoles in natural and modified pools were found in the study. During post-winter, natural population restricted their major activity to night, but the modified pool population increased daytime activity while keeping the peak activity time in the night. In winter, natural population showed slight activity during the transition periods (06:00-09:00, 18:00-21:00) between day and night in contrast to the post-winter time. The overlap between activity times between natural and modified pool populations reduced from winter to post-winter and activities such as feeding, moving and surfacing increased in post-winter. Diel cycle of distribution and activity of tadpoles have been studied previously in *Bufo americanus* on the basis of temperature and light availability (Beiswenger, 1977). The study found that tadpoles were scattered all the parts of the pond, but cooler shallowest part during night. The activity (feeding, swimming) increased according to light intensity and tadpoles moved to shallow areas when the area begin to warm. The close correlation between diel cycle and light intensity, light-triggered increase in activity, inactivity during low-light conditions were reported. In our study, we did not categorize the tadpoles according to their body size and depth of pool area they occupied. This was due to the uniform depth of the modified pool compared to the depth gradient present in the natural pool.

The ability of tadpoles to identify and occupy warm microhabitats is considered as an adaptive strategy considering the positive relationship between rate of development and temperature (Beiswenger, 1978). Study on *Polypedates megacephalus* tadpoles reared in container habitats has shown that tadpoles use both behavioral thermoregulation and metabolic compensation for dealing cope up with the thermal fluctuations in the environment due to the thermal heterogeneity in the habitat (Wu et al., 2007). The same study also found that the tadpoles evolved metabolic compensation to maintain physiological homeostasis under different thermal regimes over the larval period in the man-made water bodies or during seasonal variations in temperature where they do not experience daily thermal gradient. Diel activity pattern during early and late winter have been studied in Atlantic salmon (*Salmo salar*) juveniles using radiotelemetry and reduced activity in daytime have been documented compared to night time (Hiscock et al., 2002). Transition from uniform diel cycle activity to nocturnal behavior during winter in salmonids have been documented thoroughly (for a discussion, see Hiscock et al., 2002) and this behavior has been attributed to the avoidance of diurnal endothermic predators, increased feeding efficiency in relation to the cost of energy intake (Gries et al., 1997).

I found clear diurnal variations across months in the mean tadpole density in the major study pools, but no variation when day and night densities pooled. Thus, the season and time of sampling would have an effect on the tadpole density estimates if these variations are not taken into account. The low counts of tadpoles during daytime can be due to hiding of tadpoles under suitable cover items. Hiding in refugia during daytime and becoming increasingly nocturnal and emergence for feeding in night during winter by salmonids have been documented before (Fraser et al., 1995). Both experimental and field study showed this 'switch' happens in other seasons as well if the temperature is low, and there is no underlying inherent annual rhythm or photoperiodic influence (Fraser et al., 1993, 1995). Behavior of taking shelter under leaf litter or humus during daytime and becoming exposed on the stream bottom during the night time is known for non-overwintering tadpoles such as *Leptodactylus pentadactylus* and this is attributed primarily to avoid leaf-scrapers, a predator (Silva & Giaretta, 2008). It is to be noted here that surfacing was a very rare activity in the study population during the study period. Behavioral thermoregulation of tadpoles by using suitable microhabitats may be limited by the unavailability of such areas, especially in modified habitats of low richness and abundance of cover items or thermal variability (Balogová & Gvoždík, 2015; Hutchison & Dupré, 1992).

The slightly increasing tadpole emergence or density during daytime in both pool types across months can be an indication of the beginning of metamorphosis, with high feeding activity on the periphytons growing on the walls of the modified pools and rocky substrates in the natural pools, and generally on the leaf litter. High algal growth observed in the modified pools during the post-winter, compared to the natural pools might be the reason behind the slow shift of tadpole density from night time to day time in the modified pools. The daytime density of tadpole in both natural and modified pools was almost similar in contrast to the night time pattern. In night, tadpole density was high in the modified pools compared to the natural pools across seasons. This can be an indication of high relative abundance of tadpoles in the modified pools as observed in the study area. Diurnal and annual shift in *Rana catesbeiana* tadpole distribution patterns in human made ponds have been studied by (Nie et al., 1999) Nie et al. (1999) and concluded that the shifts are most likely related to temperature patterns and not predator presence. Avoidance of large, overwintering tadpoles as prey by predators have been also documented previously (Brodie & Formanowicz, 1983) which needs to be tested in the Nanorana spp. tadpoles.

An assessment of the depth-temperature-density relationships across pools showed varying patterns. Water temperature was positively correlated to tadpole density in natural pools during daytime. But I did not find any relationship in other scenarios, *i.e.,* Nighttime in natural pool, and both day and night in modified pools. Positive relationship with depth and density was found in natural pools both during night and day time. Night time distribution of tadpoles in the deep areas can be explained by the warmth available in such areas (Beiswenger, 1978). The same pattern in the daytime might be an artefact of the availability of more food items in the deeper areas, or a general tendency of the tadpoles to remain in the low-light areas similar to fishes like *Danio rerio* (Currie et al., 2016; Serra et al., 1999). Experiments conducted on *Rana temporaria* have shown that critical life history decisions may not be strongly influenced by photoperiodic cues, with differences between populations, a pattern contrasting to several insect species (Laurila et al., 2001). They also suggested the possibility of influence of other environmental factors on photoperiodic cues based on the interactive effect of temperature and photoperiod on several traits.

Considering the potential interactive effect of several factors (i.e., light intensity, temperature, food availability, and predator avoidance), simplicity of the current study design and very few published studies on tadpoles in this regard, no conclusions are being drawn at this stage. There was no relationship between tadpole density and depth in modified pools, and this was expected due to the almost negligible depth gradient in modified pools except M2. Seasonal variation in these patterns needs to be studied and this might throw more light on the depth-temperature-density relationships of overwintering tadpoles.

The best predictors of tadpole densities were time of the day, interaction of mean canopy cover with water temperature (negative influence); richness of cover items, mean canopy cover percentage, water temperature, leaf litter depth, velocity and an interaction of day of the time with pool modification (positive influence). Overwintering tadpoles in the study pools preferred moderately deep areas in both pool types across seasons and avoided shallow and deep areas of the pools. The tadpoles used flow refuge and zero flow areas in both pool types across seasons. The modified pool population showed preference towards algae, leaf litter, sand, and small boulder as substrate in modified pools during winter, but this was changed to sand as highly preferred substrate in post-winter. Avoidance of other substrates (e.g., concrete wall) in the modified pools was clear in both the seasons.

In natural pools, tadpoles preferred algae, humus, sand, leaf litter, and small boulders in winter and avoided gravel, cobbles, boulders and bedrocks. In post-winter, cobble was preferred and humus was avoided. While tadpoles in the modified pools preferred submerged vegetation, root, and log undercuts during winter, they avoided root and log undercuts during post-winter and preferred submerged and overhanging vegetation during post-winter. Bedrock and boulder undercuts were avoided by the modified pool population in both the seasons. Contrastingly, cover item preference and avoidance were consistent across seasons in natural pools. Preferred items were bedrock undercuts, overhanging and submerged vegetations while avoided items included boulder, log and root undercuts. This seasonal patterns in substrate and cover item preference and avoidance points out the relative advantages and disadvantages of these items in varying environmental conditions in terms of refugia from predation, temperature, flow, light and availability of food resource.

Occurrence of *Nyctibatrachus major* tadpoles was found to be influenced by the presence of leaf litter, high relative humidity, and canopy cover over the stream in the Western Ghats

(Girish & Krishnamurthy, 2009). Occurrence of these tadpoles was negatively influenced by the reduced canopy cover and the authors suggested that the increased light level and elevated water and air temperature might be the reasons behind this. Influence of canopy cover on stream water temperature, especially daily fluctuations have been established by multiple studies (St-Hilaire et al., 2000; Studinski et al., 2012; Warren et al., 2013).

Similarly, density variation of *Ascaphus truei* tadpoles within stream have been found to be associated with the substrate size, embeddedness and water velocity (Hawkins et al., 1988). Influence of water velocity on habitat selection have been studied in tadpoles in rainforest stream (Richards, 2002) and adaptation to water flow by means of morphological and behavioral attributes are documented in species living in rapidly flowing and turbulent habitats, but not in others from a little or no water flow (Altig & Johnston, 1989). Even though *Nanorana spp.* in the study area are present in run and riffle sections of the stream in relatively less abundance, generally they are observed not swimming frequently, or moving slowly in the substrate or swimming away from point of disturbance. Oral disc of *N. vicina* and *N. minica* are characterized by suctorial, many small, closely spaced marginal papillae with LTRF formulae larger than 2/3 (Type 'D') (Altig & Johnston, 1989; Gill et al., 2020; Ray, 1999; Sircar, 2010; This study). *N. vicina* is reported to be adapted for the lotic habitat and tadpoles are often seen clinging on to the rocky surfaces in slow water flowing areas (Gill et al., 2020; Sircar, 2010).

It has been found that individuals of different sizes and developmental stages within species use habitat differently (Alford, 1986; Wollmuth & Crawshaw, 1988). Tadpoles use microhabitats electively and diverse microhabitat choice patterns can be found across different anuran species (de Melo et al., 2018; Eterovick & Barata, 2006; Hiragond & Saidapur, 2001; Sun et al., 2021). Even though seasonal variation in microhabitat usage patterns have been documented, the selective pressures behind this plasticity, which might be varying through space and time are not well understood (Eterovick et al., 2010). The substrate preference of tadpole might be based on the camouflaging potential or the thermal advantage (Eterovick et al., 2018). Habitat selection is regarded as a complex process in tadpoles which varies temporally and spatially based on multiple cues (Nie et al., 1999; O'Hara, 1981). However, it is important to note that experimental studies have shown the habitat preference of overwintering tadpoles of *Rana catesbiana* varies according to their age, and change in size might be an influential factor for this change (Smith, 1999).

Apart from fish and benthic invertebrates, Habitat Suitability Curves (HSCs) have been previously developed for tadpoles of *Rana boylii* and *Rana sierrae* in California for generating Habitat Suitability Index (HSI) for use in flow recommendations for hydropower relicensing (Bondi et al., 2013; Yarnell et al., 2012, 2019). The present study is the first one to develop HSCs for tadpoles in India, apart from the studies on fishes and Black Necked Crane (J.A. Johnson et al., 2017; J.A. Johnson et al., 2021; Nale et al., 2017). HSCs generated in this study can be used in Physical Habitat Simulation modelling (PHABSIM) which can predict the effects of altered or reduced flow on stream biology (Bovee et al., 1998; Jowett et al., 2008). Thus, this study can be useful in preparing conservation plans for Himayalan streams, where large scale hydrological alterations are taking place (Gergan, 2020; Grumbine & Pandit, 2013; Pandit & Grumbine, 2012).

Evidence for direct consequences of climate change on phenological shifts are growing (Cohen et al., 2018) and such phenological delays and advancement have been very well documented in amphibians (Arietta et al., 2020; Klaus & Lougheed, 2013). Larger size and prolonged larval period have been attributed to the phenotypic expressions due to the changes in environmental temperature (Berven et al., 1979). Experimental studies on some of the overwintering and non-overwintering tadpoles of temperate areas assessing the tolerance limits and locomotory sensitivity towards temperature fluctuations at local scales (Bonino et al., 2020) found that the tadpoles are adapted to low temperature and none of them were under thermal stress which might affect their survival in the present time or under a moderate climate change scenario. It is hypothesized that combined or individual effect of abiotic factors correlated with climatic variation, biotic factors, and changes in timing, duration, and number of annual breeding periods are responsible for overwintering larval frog populations (Collins, 1979).

Species such as the *Nanorana minica* with restricted distribution may be more susceptible to climate change and vulnerable to local extinctions (Perotti et al., 2018). Appropriate conservation actions are needed in accordance with the unique life history pattern of these amphibians, especially since the Himalayan region is predicted to be warming at higher rate than the global average (Christensen et al., 2007; Dimri et al., 2018). A recent global review has also suggested that tadpoles of narrowly distributed species which develop on lotic habitat or on land are most sensitive to modification in their habitat (Nowakowski et al., 2017). This study shows that montane stream-dwelling amphibians such as *Nanorana spp.* with overwintering tadpoles have high potential to act as good models for assessing influence of temperature fluctuation on larval period, breeding phenology, and survival probability as they are well adapted to seasonally dynamic environment and modified habitats (Wheeler et al., 2015).

CONCLUSIONS

The present study confirms overwintering tadpoles in the study area using body size class distribution and shows the notable temporal variation in water temperature, water quality parameters and tadpole densities in diurnal, and seasonal scales. Considering the clear difference in tadpole diurnal activity pattern, I recommend further sampling of these overwintering tadpoles to be carried out in fixed time intervals during both day and night time. To enumerate tadpoles in a pool, more active tadpole catching techniques such as minnow trapping can be employed. But considering the depth, substrate nature, difficulty in wading, disturbance to the tadpoles, and complexity of cover items in the study area, passive sampling employed in this study can be an alternative and give good indicator of tadpole density, assuming no tadpoles are hiding under refugia during both day and night.

The hypothesis of overwintering of only lower developmental stages does not seem to be true for the study population. I present the details of the tadpole behavior in the study population including hiding in refugia during daytime, becoming increasingly nocturnal and emerge in night during winter which is earlier reported in some fish species. A preliminary assessment of the depth-temperature-density relationships across pools showed varying patterns and I suggest the need of detailed seasonal analysis of this aspect. This confirms that modification of the stream habitat influences tadpole temporal distribution patterns and behavior.

I report the variation in the patterns of tadpole emergence or density and related variables between the natural and modified pools (check dams) and discuss potential effects of modification of the stream habitat on the daily thermal fluctuations, tadpole habitat selection, stress, and behavioral thermoregulation. I found preliminary evidence for variation in relative tail length, where modified pool population had higher relative tail length than natural pool population suggesting increasing level of environmental stress in such areas. Through analyzing the activity patterns, I report the seasonal and diurnal difference in tadpole behavior between natural and modified pool populations and potential causes behind this.

Using morphometric measurements and LTRF formulae, I propose two different putative tadpole groups which can be further confirmed using molecular identification, and discuss potential issues with field identification of tadpole species.

The overwintering tadpole density was best predicted by time of the day, interaction of mean canopy cover with water temperature (negative influence); richness of cover items, mean canopy cover percentage, water temperature, leaf litter depth, velocity and an interaction of day of the time with pool modification (positive influence). I discuss the preference and avoidance of different habitat variables between natural and modified pools across seasons and developed Habitat Suitability Curves (HSCs) for the tadpoles.

Overwintering tadpoles in the study pools preferred moderately deep areas in both pool types across seasons and avoided shallow and deep areas of the pools. The tadpoles used relatively no flow areas in both pool types across seasons. Substrate and cover item preference varied across pool types and seasons, and I discuss the potential reasons behind these patterns.

The HSCs developed in this study can be used for understanding the effects of altered or reduced flow on the tadpoles and the stream biology in general. This will be useful in stream restoration activities and developing conservation plan in light of the modifications which can happen in future due to high demand of water in the area.

The overwintering tadpoles can act as good models for assessing the effect of climate change in the Himalayan region. Long-term research on the species breeding biology and larval response towards temperature fluctuations can contribute to the increasing knowledge on the influence of global warming on amphibians.

This study strengthens the assumption that there are factors beyond temperature and food availability which are behind overwintering in addition to several other contributions to the larval ecology of the focal species, and natural history. This preliminary study will also act as a starting point for such long-term monitoring and research for the benefit of conservation of the local species and similar amphibians elsewhere in the country.

Conservation Implications

1. From the present findings, I found that the depth suitability of overwintering tadpoles is 30-60cm. Thus, the pool habitats should be maintained with this depth range during the lean season (winter).
2. Since Himalayan streams are subjected to large scale hydrological alterations, this might have great impact on the existence of the *Nanorana* spp.
3. The habitat suitability curves can be used in any hydrological model for the estimation of ecological flow required for the *Nanorana* spp. in the Himalayan region.

Limitations of the Present Study and Recommendations

1. Incomplete sampling of winter season, and no data from pre-winter do not allow us to conclude and explain many patterns observed, and thus a whole-year study needs to be done.
2. Species identities are not resolved and breeding biology of the focal species are not yet understood due to the cryptic life of the adults. Thus, research on the breeding biology need to be strengthened and molecular identification of tadpoles need to be carried out.
3. To tease out effect of multiple variables and their interactions, controlled and mesocosm studies need to be done on habitat selection of tadpoles, influence of temperature, canopy cover on tadpole density etc.
4. Samples collected during the study (tadpole specimens, leaf litter, periphytons, and sediments) were not processed and analyzed due to the limitations paused by COVID-19. This need to be followed up to throw light on the feeding ecology,

physiology, morphology, and neuroendocrinology of the tadpoles.

5. To establish the actual overwintering duration, individual tagging and monitoring of the tadpoles is essential.
6. Ex-situ experiments on the thermal preference of overwintering tadpoles need to be carried out and variation in body temperature in response to the ambient temperature need to be assessed.
7. Based on the effect sizes and design issues reported in the present study, appropriate sample sizes and study designs need to be planned for further explorations.
8. Research question 3(C) was not addressed due to difficulty in classification of tadpoles based on their size in the field.
9. Research question 2(A) [confirmation of species identity], and 3(B) [diet analyses] were not addressed during the fieldwork due to logistical difficulties during COVID-19 lockdown. This should be carried out and further information on this aspect will be crucial in understanding the species-specific difference in ecology and diet pattern.

REFERENCES

Agostinelli, C., & Lund, U. (2017). *Circular* (0.4-93) [R Package]. https://r-forge.r-project.org/projects/circular/

Aguirre-Becerra, H., García-Trejo, J. F., Peña-Herrejón, G. A., Muniz-Afonso, A., Feregrino-Pérez, A. A., & Soto-Zarazúa, G. M. (2018). Effect of Two Levels of Temperature and Natural and Artificial Photoperiod on Growth and Metamorphosis of Bullfrog Tadpoles in an Intensive Rearing System. *North American Journal of Aquaculture*, *80*(4), 388–396. https://doi.org/10.1002/naaq.10045

Alford, R. A. (1986). Habitat Use and Positional Behavior of Anuran Larvae in a Northern Florida Temporary Pond. *Copeia*, *1986*(2), 408–423. JSTOR. https://doi.org/10.2307/1444999

Alford, R. A., & Harris, R. N. (1988). Effects of larval growth history on anuran metamorphosis. *The American Naturalist*, *131*(1), 91–106.

Altig, R. (2007). Comments on the descriptions and evaluations of tadpole mouthpart anomalies. *Herpetological Conservation and Biology*, *2*(1), 1–4.

Altig, R., & Johnston, G. F. (1989). Guilds of anuran larvae: Relationships among developmental modes, morphologies, and habitats. *Herpetological Monographs*, 81–109.

Altig, R., & McDiarmid, R. W. (1999). Body plan. In *Tadpoles: The biology of anuran larvae* (pp. 24–51). University of Chicago Press.

Alvarez, D., & Nicieza, A. (2002). Effects of temperature and food quality on anuran larval growth and metamorphosis. *Functional Ecology*, *16*(5), 640–648.

Arendt, J. D. (1997). Adaptive Intrinsic Growth Rates: An Integration Across Taxa. *The Quarterly Review of Biology*, *72*(2), 149–177. JSTOR. https://www.jstor.org/stable/3036336

Arietta, A. A., Freidenburg, L. K., Urban, M. C., Rodrigues, S. B., Rubinstein, A., & Skelly, D. K. (2020). Phenological delay despite warming in wood frog Rana sylvatica reproductive timing: A 20-year study. *Ecography*, *43*(12), 1791–1800.

Baird, R. B., Eaton, A. D., Rice, E. W., Bridgewater, L., & others. (2017). *Standard methods for the examination of water and wastewater* (Vol. 23). American Public Health Association Washington, DC.

Balogová, M., & Gvoždík, L. (2015). Can newts cope with the heat? Disparate thermoregulatory strategies of two sympatric species in water. *PLoS One*, *10*(5), e0128155.

Banerjee, K., Baruah, B., & Das, A. (2020). First Record of Carrion Feeding by Tadpoles of Duttaphrynus himalayanus and Nanorana vicina from Western Himalaya, India. *Tropical Natural History*, *20*(3), 268–271.

Barnett, A. G., Baker, P. J., & Dobson, A. J. (2020). *season: Analysing Seasonal Data R Functions* (0.3.12) [R Package].

Bartoń, K. (2020). *MuMIn: Multi-Model Inference* (1.43.17) [R Package]. https://CRAN.R-project.org/package=MuMIn

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Beiswenger, R. E. (1977). Diel patterns of aggregative behavior in tadpoles of Bufo americanus, in relation to light and temperature. *Ecology*, *58*(1), 98–108.

Beiswenger, R. E. (1978). Responses of Bufo tadpoles (Amphibia, Anura, Bufonidae) to laboratory gradients of temperature. *Journal of Herpetology*, 499–504.

Berven, K. A. (1982). The Genetic Basis of Altitudinal Variation in the Wood Frog Rana sylvatica. II. An Experimental Analysis of Larval Development. *Oecologia, 52*(3), 360–369. JSTOR. https://www.jstor.org/stable/4216627

Berven, K. A., & Chadra, B. G. (1988). The relationship among egg size, density and food level on larval development in the wood frog (Rana sylvatica). *Oecologia*, *75*(1), 67–72.

Berven, K. A., Gill, D. E., & Smith-Gill, S. J. (1979). Countergradient Selection in the Green Frog, Rana clamitans. *Evolution*, *33*(2), 609–623. JSTOR. https://doi.org/10.2307/2407784

Bhattarai, S., Gurung, A., Lamichhane, B., Regmi, R., Dhungana, M., Kumpakha, B., & Subedi, N. (2020). Amphibians and Reptiles of Chure Range, Nepal. *President Chure Terai-Madhesh Conservation Development Board and National Trust for Nature Conservation, Khumaltar, Lalitpur, Nepal*, *60*.

Billups, K., & Schrag, D. P. (2003). Application of benthic foraminiferal Mg/Ca ratios to questions of Cenozoic climate change. *Earth and Planetary Science Letters*, *209*(1–2), 181–195.

Boix-Fayos, C., Barberá, G., López-Bermúdez, F., & Castillo, V. (2007). Effects of check dams, reforestation and land-use changes on river channel morphology: Case study of the Rogativa catchment (Murcia, Spain). *Geomorphology*, *91*(1–2), 103–123.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J.-S. S. (2009). Generalized linear mixed models: A practical guide for ecology and evolution. *Trends in Ecology & Evolution*, *24*(3), 127–135.

Bondi, C. A., Yarnell, S. M., Lind, A. J., & Lind, A. (2013). Transferability of habitat suitability criteria for a stream breeding frog (Rana boylii) in the Sierra Nevada, California. *Herpetological Conservation and Biology*, *8*(1), 88–103.

Bonino, M. F., Cruz, F. B., & Perotti, M. G. (2020). Does temperature at local scale explain thermal biology patterns of temperate tadpoles? *Journal of Thermal Biology*, *94*, 102744.

Boone, M. D., Little, E. E., & Semlitsch, R. D. (2004). Overwintered Bullfrog Tadpoles Negatively Affect Salamanders and Anurans in Native Amphibian Communities. *Copeia*, *2004*(3), 683–690. JSTOR. https://www.jstor.org/stable/1448490

Boruah, B., Kumar, A., & Das, A. (2020). Cirsium wallichii DC.(Asteraceae): A key nectar source of butterflies. *Journal of Threatened Taxa*, *12*(14), 17049–17056.

Bovee, K. D. (1982). *A guide to stream habitat analysis using the instream flow incremental methodology* (Vol. 1). Western Energy and Land Use Team, Office of Biological Services, Fish and Wildlife Service, U.S. Department of the Interior, Washington, D. C.

Bovee, K. D., Lamb, B. L., Bartholow, J. M., Stalnaker, C. B., & Taylor, J. (1998). *Stream habitat analysis using the instream flow incremental methodology*. Geological Survey Reston Va Biologicalresources Div.

Bradford, D. F. (1983). Winterkill, Oxygen Relations, and Energy Metabolism of a Submerged Dormant Amphibian, Rana Muscosa. *Ecology*, *64*(5), 1171–1183. JSTOR. https://doi.org/10.2307/1937827

Bradford, D. F. (1984). Water and Osmotic Balance in Overwintering Tadpoles and Frogs, Rana muscosa. *Physiological Zoology*, *57*(4), 474–480. JSTOR. https://www.jstor.org/stable/30163349

Brattstrom, B. H. (1962). Thermal control of aggregation behavior in tadpoles. *Herpetologica*, *18*(1), 38–46.

Broadmeadow, S., Jones, J., Langford, TEL, Shaw, P., & Nisbet, T. (2011). The influence of riparian shade on lowland stream water temperatures in southern England and their viability for brown trout. *River Research and Applications*, *27*(2), 226–237.

Brodie, E. D., & Formanowicz, D. R. (1983). Prey Size Preference of Predators: Differential Vulnerability of Larval Anurans. *Herpetologica*, *39*(1), 67–75. JSTOR. https://www.jstor.org/stable/3892477

Brooks, M. E., Kristensen, K., Van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Machler, M., & Bolker, B. M. (2017). GlmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, *9*(2), 378–400.

Brown, H. A. (1990). Morphological variation and age-class determination in overwintering tadpoles of the tailed frog, Ascaphus truei. *Journal of Zoology*, *220*(2), 171–184. https://doi.org/10.1111/j.1469-7998.1990.tb04301.x

Burraco, P., Laurila, A., & Orizaola, G. (2021). Limits to compensatory responses to altered phenology in amphibian larvae. *Oikos*, *130*(2), 231–239.

Champion, H. G., & Seth, S. K. (1968). *A revised survey of the forest types of India*. Manager of publications, Govt. of India.

Christensen, J. H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, I., Jones, R., Kolli, R. K., Kwon, W.-T., Laprise, R., Magaña Rueda, V., Mearns, L., Menéndez, C. G., Räisänen, J., Rinke, A., Sarr, A., & Whetton, P. (2007). Regional Climate Projections. In *In Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.)*. Cambridge University Press.

Cohen, J. M., Lajeunesse, M. J., & Rohr, J. R. (2018). A global synthesis of animal phenological responses to climate change. *Nature Climate Change*, *8*(3), 224–228.

Collins, J. P. (1979). Intrapopulation Variation in the Body Size at Metamorphosis and Timing of Metamorphosis in the Bullfrog, Rana Catesbeiana. *Ecology*, *60*(4), 738–749. JSTOR. https://doi.org/10.2307/1936611

Collins, J. P., & Lewis, M. A. (1979). Overwintering Tadpoles and Breeding Season Variation in the Rana pipiens Complex in Arizona. *The Southwestern Naturalist*, *24*(2), 371–373. JSTOR. https://doi.org/10.2307/3670934

Currie, S. P., Doherty, G. H., & Sillar, K. T. (2016). Deep-brain photoreception links luminance detection to motor output in Xenopus frog tadpoles. *Proceedings of the National Academy of Sciences*, *113*(21), 6053–6058.

Das, I., & Dutta, S. K. (2007). Sources of larval identities for amphibians of India. *Hamadryad*, *31*(2), 330.

Davis, H. (1938). Instructions for conducting stream and lake surveys. *U. S. Bureau of FisheriesFishery Circular*, *26*, 55.

de Andrade, E. V. E., Palhas, I. J. L., & de Moura, G. J. B. (2014). Diurnal habitat segregation by tadpoles in two temporary ponds in an Atlantic Rainforest remnant, Northeastern Brazil. *Hydrobiologia*, *723*(1), 181–194.

de Melo, L. S. O., Garey, M. V., & de Cerqueira Rossa-Feres, D. (2018). Looking for a place: How are tadpoles distributed within tropical ponds and streams? *Herpetology Notes*, *11*, 379–386.

Denver, R. J. (2019). Tadpole Behavior and Metamorphosis. In *Encyclopedia of Animal Behavior* (Second, Vol. 2, pp. 514–518). Elsevier. https://doi.org/10.1016/B978-0-12-809633-8.01074-8

Dewan, D. (1990). Ecological Havoc in Himalayas. *Himalayas, Environmental Problems*, 1.

Dickman, M. (1968). The effect of grazing by tadpoles on the structure of a periphyton community. *Ecology*, *49*(6), 1188–1190.

Dillon, M. E., Woods, H. A., Wang, G., Fey, S. B., Vasseur, D. A., Telemeco, R. S., Marshall, K., & Pincebourde, S. (2016). Life in the frequency domain: The biological impacts of changes in climate variability at multiple time scales. *Integrative and Comparative Biology*, *56*(1), 14–30.

Dimri, A., Kumar, D., Choudhary, A., & Maharana, P. (2018). Future changes over the Himalayas: Maximum and minimum temperature. *Global and Planetary Change*, *162*, 212–234.

Dinno, A. (2017). *dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums* (1.3.5) [R Package]. https://CRAN.R-project.org/package=dunn.test

Duellman, W. E., 1989. Alternative life-history styles in anuran amphibians: evolutionary and ecological implications, in: Bruton, M.N. (Ed.), Alternative Life-History Styles of Animals, Perspectives in Vertebrate Science. Springer Netherlands, Dordrecht, pp. 101–126.

Dutta, S. K. (1999). Assessment of Herpetofauna: Diversity, Distribution, Ecological Requirements and Responses to Human Activities in GHNP and WLSs. *Bhubaneswar, Orissa*, 1–16.

EDGE. (2020). *EDGE Amphibians| EDGE of Existence*. http://www.edgeofexistence.org/amphibians/

Emerson, S. B. (1988). The giant tadpole of Pseudis paradoxa. *Biological Journal of the Linnean Society*, *34*(2), 93–104.

Eterovick, P. C., & Barata, I. M. (2006). Distribution of tadpoles within and among Brazilian streams: The influence of predators, habitat size and heterogeneity. *Herpetologica*, *62*(4), 365–377.

Eterovick, P. C., Lazarotti, I., Franco, B. P., & Dias, C. J. (2010). Seasonal variation of tadpole spatial niches in permanent streams: The roles of predation risk and microhabitat availability. *Austral Ecology*, *35*(8), 879–887.

Eterovick, P. C., Mendes, I. S., Kloh, J. S., Pinheiro, L. T., Václav, A. B. H. P., Santos, T., & Gontijo, A. S. B. (2018). Tadpoles respond to background colour under threat. *Scientific Reports*, *8*(1), 1–8.

Fabrezi, M., Quinzio, S. I., & Goldberg, J. (2009). Giant tadpole and delayed metamorphosis of Pseudis platensis Gallardo, 1961 (Anura, Hylidae). *Journal of Herpetology*, *43*(2), 228–243.

Fellers, G. M., Launer, A. E., Rathbun, G., Bobzien, S., Alvarez, J., Sterner, D., Seymour, R. B., & Westphal, M. (2001). Overwintering tadpoles in the California red-legged frog (Rana aurora draytonii). *Herpetological Review*, *32*(3), 156–157.

Ferreira, T., & Rasband, W. (2012). ImageJ user guide. *ImageJ/Fiji*, *1*, 155–161.

Ficetola, G. F., & De Bernardi, F. (2006). Trade-off between larval development rate and post-metamorphic traits in the frog Rana latastei. *Evolutionary Ecology*, *20*(2), 143–158.

Fominykh, A., & Liapkov, S. (2011). The formation of new characteristics in life cycle of the marsh frog (Rana ridibunda) in thermal pond conditions. *Zhurnal Obshchei Biologii*, *Query date: 2020-04-19 10:10:18*, Article Query date: 2020-04-19 10:10:18. https://www.ncbi.nlm.nih.gov/pubmed/22292280

Fortugno, D., Boix-Fayos, C., Bombino, G., Denisi, P., Quinonero Rubio, J. M., Tamburino, V., & Zema, D. A. (2017). Adjustments in channel morphology due to land-use changes and check dam installation in mountain torrents of Calabria (southern Italy). *Earth Surface Processes and Landforms*, *42*(14), 2469–2483.

Fraser, N. H., Metcalfe, N. B., Heggenes, J., & Thorpe, J. E. (1995). Low summer temperatures cause juvenile Atlantic salmon to become nocturnal. *Canadian Journal of Zoology*, *73*(3), 446–451.

Fraser, N. H., Metcalfe, N. B., & Thorpe, J. E. (1993). Temperature-dependent switch between diurnal and nocturnal foraging in salmon. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *252*(1334), 135–139.

Freeman, B. G., Scholer, M. N., Ruiz-Gutierrez, V., & Fitzpatrick, J. W. (2018). Climate change causes upslope shifts and mountaintop extirpations in a tropical bird community. *Proceedings of the National Academy of Sciences*, *115*(47), 11982–11987.

Fritz, C. O., Morris, P. E., & Richler, J. J. (2012). Effect size estimates: Current use, calculations, and interpretation. *Journal of Experimental Psychology: General*, *141*(1), 2.

Frost, D. R. (2020). Amphibian Species of the World: An Online Reference. Version 6.1 [Data set]. In *American Museum of Natural History*. https://doi.org/doi.org/10.5531/db.vz.0001

Frost, D. R. (2021). *Amphibian Species of the World: An Online Reference. Version 6.1*. American Museum of Natural History, New York, USA.

Garner, T., Rowcliffe, J., & Fisher, M. (2011). Climate change, chytridiomycosis or condition: An experimental test of amphibian survival. *Global Change Biology*, *Query date: 2020-04-19 13:02:19*, Article Query date: 2020-04-19 13:02:19. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2486.2010.02272.x

Garner, T., Rowcliffe, M., & Fisher, M. (2010). *Climate, chytridiomycosis or condition: An experimental test of amphibian survival*. https://hal.archives-ouvertes.fr/hal-00599520

Gergan, M. D. (2020). Disastrous hydropower, uneven regional development, and decolonization in India's Eastern Himalayan borderlands. *Political Geography*, *80*, 102175.

Gill, S., Rais, M., Saeed, M., Ahmed, W., & Akram, A. (2020a). A note on ontogeny and oral disc of tadpoles of Murree Hills Frog (Nanorana vicina,[Stoliczka 1872]). *Herpetology Notes*, *13*, 1003–1007.

Gill, S., Rais, M., Saeed, M., Ahmed, W., & Akram, A. (2020b). The tadpoles of Murree Hills Frog Nanorana vicina (Anura: Dicroglossidae). *Zootaxa*, *4759*(3), 440–442. https://doi.org/10.11646/zootaxa.4759.3.11

Girish, K. G., & Krishnamurthy, S. V. B. (2009). Distribution of tadpoles of large wrinkled frog Nyctibatrachus major in central Western Ghats: Influence of habitat variables. *Distribution of Tadpoles of Large Wrinkled Frog Nyctibatrachus Major in Central Western Ghats: Influence of Habitat Variables*, 153–160.

Gökbulak, F. (2002). Effect of American bison (Bison bison L.) on the recovery and germinability of seeds of range forage species. *Grass and Forage Science*, *57*(4), 395–400.

Grant, G. E., Swanson, F. J., & Wolman, M. G. (1990). Pattern and origin of stepped-bed morphology in high-gradient streams, Western Cascades, Oregon. *Geological Society of America Bulletin*, *102*(3), 340–352.

Gray, K. T., Escobar, A. M., Schaeffer, P. J., Mineo, P. M., & Berner, N. J. (2016). Thermal acclimatization in overwintering tadpoles of the green frog, Lithobates clamitans (Latreille, 1801). *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *325*(5), 285–293.

Gribovszki, Z., Szilágyi, J., & Kalicz, P. (2010). Diurnal fluctuations in shallow groundwater levels and streamflow rates and their interpretation–A review. *Journal of Hydrology*, *385*(1–4), 371–383.

Gries, G., Whalen, K. G., Juanes, F., & Parrish, D. L. (1997). Nocturnal activity of juvenile Atlantic salmon (Salmo salar) in late summer: Evidence of diel activity partitioning. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*(6), 1408–1413.

Grolemund, G., & Wickham, H. (2011). Dates and Times Made Easy with lubridate. *Journal of Statistical Software*, *40*(3), 1–25. https://www.jstatsoft.org/v40/i03/

Grumbine, R. E., & Pandit, M. K. (2013). Threats from India's Himalaya dams. *Science*, *339*(6115), 36–37.

Haas, A., & Das, I. (2011). Describing East Malaysian tadpole diversity: Status and recommendations for standards and procedures associated with larval amphibian description and documentation. *TROPICAL VERTEBRATES IN A CHANGING WORLD*.

Halverson, M., Skelly, D., Kiesecker, J., & Freidenburg, L. (2003). Forest mediated light regime linked to amphibian distribution and performance. *Oecologia*, *134*(3), 360–364.

Hartig, F. (2021). *DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models* (0.4.1) [R Package]. https://CRAN.R-project.org/package=DHARMa

Hartigan, J. A., & Hartigan, P. M. (1985). The dip test of unimodality. *The Annals of Statistics*, 70–84.

Hawkins, C. P., Gottschalk, L. J., & Brown, S. S. (1988). Densities and habitat of tailed frog tadpoles in small streams near Mt. St. Helens following the 1980 eruption. *Journal of the North American Benthological Society*, *7*(3), 246–252.

Heath, A. G. (1975). Behavioral Thermoregulation in High Altitude Tiger Salamanders, Ambystoma tigrinum. *Herpetologica*, *31*(1), 84–93. JSTOR. https://www.jstor.org/stable/3891993

Hebbar, P., Ravikanth, G., & Aravind, N. (2019). A review on the conservation genetic studies of Indian amphibians and their implications on developing strategies for conservation. *Journal of Genetics*, *98*(5), 1–8.

Hillis, D. M., & Wilcox, T. P. (2005). Phylogeny of the New World true frogs (Rana). *Molecular Phylogenetics and Evolution*, *34*(2), 299–314.

Hiragond, N. C., & Saidapur, S. K. (2001). Microhabitat choice of tadpoles of seven anuran species. *Current Herpetology*, *20*(1), 51–60.

Hiscock, M., Scruton, D., Brown, J., & Pennell, C. (2002). Diel activity pattern of juvenile Atlantic salmon (Salmo salar) in early and late winter. *Hydrobiologia*, *483*(1), 161–165.

Hsu, J.-L., Kam, Y.-C., & Fellers, G. M. (2012). Overwintering Tadpoles and Loss of Fitness Correlates In Polypedates braueri Tadpoles That Use Artificial Pools In A Lowland Agroecosystem. *Herpetologica*, *68*(2), 184–194. https://doi.org/10.1655/HERPETOLOGICA-D-11-00042.1

Huber, M., & Caballero, R. (2011). The early Eocene equable climate problem revisited. *Climate of the Past*.

Hutchison, V., & Dupré, R. (1992). Thermoregulation. In *Feder M.E., Burggren W.W. (Eds.), Environmental physiology of amphibians*. University of Chicago Press.

Isaac, N. J., Turvey, S. T., Collen, B., Waterman, C., & Baillie, J. E. (2007). Mammals on the EDGE: conservation priorities based on threat and phylogeny. *PloS One*, *2*(3), e296.

Ivlev, V. (1961). Experimental ecology of the feeding of fishes.[Translated by D. Scott] Yale Univ. *Press, New Haven*.

Jithin, V., & Das, A. (2020). *Defying metamorphosis: Overwintering in larval anurans—A research synthesis* [Term Paper (Unpublished)]. Wildlife Institute of India, Dehradun.

Jithin, V., Kumar, S., & Das, A. (2021). First record of interspecific amplexus between a Himalayan Toad, *Duttaphrynus himalayanus* (Bufonidae), and a Himalayan Paa Frog, *Nanorana vicina* (Dicroglossidae), from the Western Himalaya, India. *Reptiles & Amphibians*, *28*(2), 270–273. https://doi.org/10.17161/randa.v28i2.15579

Johnson, J. A., Sharma, A., Rajput, V., Dubey, V. K., & Sivakumar, K. (2020). Taxonomic and guild structure of fish assemblages in the streams of Western Himalaya, India. *Community Ecology*, 1–19.

Johnson, J. A., Watham, T., Gopi, G. V., & Sivakumar, K. (2021). Development of habitat suitability criteria for conservation of the black-necked crane in Nyamjang Chu River in eastern Himalaya, India in connection with a proposed hydropower dam. *River Research and Applications*, *37*(3), 321–329.

Johnson, J.A., Sivakumar, K., & Rosenfeld, J. (2017). Ecological flow requirement for fishes of Godavari River: Flow estimation using the PHABSIM method. *Current Science*, 2187–2193.

Johnson, M.S., Aubee, C., Salice, C.J., Leigh, K.B., Liu, E., Pott, U., Pillard, D., 2017. A review of ecological risk assessment methods for amphibians: Comparative assessment of testing methodologies and available data. Integrated Environmental Assessment and Management 13, 601–613. https://doi.org/10.1002/ieam.1881

Johnson, P. C. (2014). Extension of Nakagawa & Schielzeth's R2GLMM to random slopes models. *Methods in Ecology and Evolution*, *5*(9), 944–946.

Joshi, A. K., & Joshi, P. (2011). A rapid inventory of indicators of climate change in the middle Himalaya. *Current Science*, *100*(6), 831–833.

Jowett, I. G., Hayes, J. W., & Duncan, M. J. (2008). *A guide to instream habitat survey methods and analysis*. NIWA Wellington.

Kang, J. H., & Kazama, S. (2012). An ecological assessment of a dammed pool formed by a slit check dam. *International Journal of River Basin Management*, *10*(4), 331–340.

Kassambara, A. (2020). *ggpubr: "ggplot2" Based Publication Ready Plots*. https://CRAN.R-project.org/package=ggpubr

Kassambara, A. (2021). *rstatix: Pipe-Friendly Framework for Basic Statistical Tests* (0.7.0.) [R Package]. https://CRAN.R-project.org/package=rstatix

Kassambara, A., & Mundt, F. (2020). *factoextra: Extract and Visualize the Results of Multivariate Data Analyses* (1.0.7.) [R Package]. https://CRAN.R-project.org/package=factoextra

Kern, P., Cramp, R. L., & Franklin, C. E. (2015). Physiological responses of ectotherms to daily temperature variation. *Journal of Experimental Biology*, *218*(19), 3068–3076.

Khan, M. S. (2015). *Guild structure of Indus Valley amphibian tadpoles*.

Klaus, S. P., & Lougheed, S. C. (2013). Changes in breeding phenology of eastern Ontario frogs over four decades. *Ecology and Evolution*, *3*(4), 835–845. https://doi.org/10.1002/ece3.501

Korpak, J., & Lenar-Matyas, A. (2019). Stream channel changes as a result of sudden sediment release due to check dam lowering (Polish Carpathians). *Environmental Earth Sciences*, *78*(14), 1–13.

Kottek, M., Grieser, J., Beck, C., Rudolf, B., & Rubel, F. (2006). World map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift*, *15*(3), 259–263.

Kumar, A., Mitra, M., Singh, G., & Rawat, G. (2012). An inventory of the flora of Binog wildlife sanctuary, Mussoorie, Garhwal Himalaya. *Indian Journal of Fundamental and Applied Life Sciences*, *2*(1), 281–299.

Kupferberg, S. J. (1997). Bullfrog (Rana Catesbeiana) Invasion of a California River: The Role of Larval Competition. *Ecology*, *78*(6), 1736–1751. JSTOR. https://doi.org/10.2307/2266097

Kurylyk, B. L., MacQuarrie, K. T., Caissie, D., & McKenzie, J. M. (2015). Shallow groundwater thermal sensitivity to climate change and land cover disturbances: Derivation of analytical expressions and implications for stream temperature modeling. *Hydrology and Earth System Sciences*, *19*(5), 2469–2489.

Lai, S.-J., Kam, Y.-C., Hsu, F.-H., & Lin, Y.-S. (2002). Elevational effects on the growth and development of tadpoles of Sauter's frog Rana sauteri Boulenger in Taiwan. *Acta Zoologica Taiwanica 13 (1)*, 11–20.

Laurila, A., Pakkasmaa, S., & Merilä, J. (2001). Influence of Seasonal Time Constraints on Growth and Development of Common Frog Tadpoles: A Photoperiod Experiment. *Oikos*, *95*(3), 451–460. JSTOR. https://www.jstor.org/stable/3547501

Lazaridis, E. (2014). *lunar: Lunar Phase & Distance, Seasons and Other Environmental Factors* (0.1-04) [R Package]. http://statistics.lazaridis.eu

Leão-Pires, T. A., Giaretta, A., & Sawaya, R. J. (2017). The diurnal aggregation behavior in Phasmahyla cochranae tadpoles (Anura: Phyllomedusidae). *Basic and Applied Herpetology*, *31*, 117–123.

Liess, A., Rowe, O., Guo, J., Thomsson, G., & Lind, M. I. (2013). Hot tadpoles from cold environments need more nutrients–life history and stoichiometry reflects latitudinal adaptation. *Journal of Animal Ecology*, *82*(6), 1316–1325.

Liu, Z., Pagani, M., Zinniker, D., DeConto, R., Huber, M., Brinkhuis, H., Shah, S. R., Leckie, R. M., & Pearson, A. (2009). Global cooling during the Eocene-Oligocene climate transition. *Science*, *323*(5918), 1187–1190.

Logares, R., & Úbeda, C. (2004). *Alsodes gargola (Rana del Catedral). Overwintering Tadpoles*. https://lup.lub.lu.se/record/628790

Logares, R., & Úbeda, C. (2006). First insights into the overwintering biology of Alsodes gargola frogs and tadpoles inhabiting harsh Andean-Patagonian alpine environments. *Amphibia-Reptilia*, *27*(2), 263–267.

Loperfido, J., Just, C. L., Papanicolaou, A. N., & Schnoor, J. L. (2010). In situ sensing to understand diel turbidity cycles, suspended solids, and nutrient transport in Clear Creek, Iowa. *Water Resources Research*, *46*(6).

Lu, X., Ma, X., Fan, L., Hu, Y., Lang, Z., Li, Z., & ... (2016). Reproductive ecology of a Tibetan frog Nanorana parkeri (Anura: Ranidae). *Journal of Natural History* https://www.tandfonline.com/doi/abs/10.1080/00222933.2016.1205155

Lüdecke, D. (2018). ggeffects: Tidy Data Frames of Marginal Effects from Regression Models. *Journal of Open Source Software*, *3*(26), 772. https://doi.org/10.21105/joss.00772

Lüdecke, D. (2021). *sjPlot: Data Visualization for Statistics in Social Science* (2.8.8) [R Package]. https://CRAN.R-project.org/package=sjPlot

Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., & Makowski, D. (2021). performance: An R Package for Assessment, Comparison and Testing of Statistical Models. *Journal of Open Source Software*, *6*(60), 3139. https://doi.org/10.21105/joss.03139

Ma, G., Hoffmann, A. A., & Ma, C.-S. (2015). Daily temperature extremes play an important role in predicting thermal effects. *The Journal of Experimental Biology*, *218*(14), 2289–2296.

Macedo, A. (2019). *Life history of the coastal tailed frog (Ascaphus truei) across an elevational gradient in Northern California* [Master's thesis, Humboldt State University]. https://digitalcommons.humboldt.edu/etd/330/

MacKenzie, R. A. (2008). Impacts of riparian forest removal on Palauan streams. *Biotropica*, *40*(6), 666–675.

Maechler, M. (2021). *Diptest: Hartigan's Dip Test Statistic for Unimodality—Corrected* (0.76-0) [R Package]. https://CRAN.R-project.org/package=diptest

Maechler, M., Rousseeuw, P., Struyf, A., Hubert, M., & Hornik, K. (2021). *cluster: Cluster Analysis Basics and Extensions* (2.1.2.) [R Package]. https://CRAN.R-project.org/package=cluster

Mair, P., & Wilcox, R. (2020). Robust Statistical Methods in R Using the WRS2 Package. *Behavior Research Methods*, *52*, 464–488.

Management Plan. (2010). *Management Plan of Mussoorie Wild Life Sanctuary for the period (2010-11 to 2019-20)*. Mussoorie Forest Division, Yamuna Circle, Forest Department, Uttarakhand.

Marian, M. P., Sampath, K., Nirmala, A., & Pandian, T. (1980). Behavioural response of Rana cyanophylictis tadpole exposed to changes in dissolved oxygen concentration. *Physiology & Behavior*, *25*(1), 35–38.

Mercado-Silva, N., Lyons, J., Díaz-Pardo, E., Navarrete, S., & Gutiérrez-Hernández, A. (2012). Environmental factors associated with fish assemblage patterns in a high gradient river of the Gulf of Mexico slope. *Revista Mexicana de Biodiversidad*, *83*(1), 117–128.

Merilä, J., Laurila, A., Laugen, A. T., & Räsänen, K. (2004). Heads or tails? Variation in tadpole body proportions in response to temperature and food stress. *Evolutionary Ecology Research*, *6*(5), 727-738.

Mishra, P. R. (2016, March 26). Water worries back to haunt Mussoorie | Dehradun News—Times of India. *The Times of India*. https://timesofindia.indiatimes.com/city/dehradun/water-worries-back-to-haunt-mussoorie/articleshow/51565218.cms

Mishra, P. R., & Upadhyay, V. (2018, May 31). Mussoorie, Nainital not far from Shimla-like crisis, say residents, experts. *The Times of India*. https://timesofindia.indiatimes.com/city/dehradun/mussoorie-nainital-not-far-from-shimla-like-crisis-say-residents-experts/articleshow/64405990.cms

Mohan, D., & Sondhi, S. (2015). An updated checklist of the birds of Uttarakhand. *Uttarakhand Forest Department, Dehradun*.

Muir, A. P., Biek, R., Thomas, R., & Mable, B. K. (2014). Local adaptation with high gene flow: Temperature parameters drive adaptation to altitude in the common frog (Rana temporaria). *Molecular Ecology*, *23*(3), 561–574. https://doi.org/10.1111/mec.12624

Munsi, M., Malaviya, S., Oinam, G., & Joshi, P. (2010). A landscape approach for quantifying land-use and land-cover change (1976–2006) in middle Himalaya. *Regional Environmental Change*, *10*(2), 145–155.

Nale, J., Gosain, A., & Khosa, R. (2017). Environmental flow assessment of River Ganga–importance of habitat analysis as a means to understand hydrodynamic imperatives for a sustainable Ganga biodiversity. *Current Science*, *112*(11), 2187.

Narayan, E. J., Graham, C., McCallum, H., & Hero, J.-M. (2014). Over-wintering tadpoles of Mixophyes fasciolatus act as reservoir host for Batrachochytrium dendrobatidis. *PloS One*, *9*(3), Article 3.

Nawani, S., Deepak, V., Gautam, K. B., Gupta, S. K., Boruah, B., & Das, A. (2021). Systematic status of the rare Himalayan wolf snake Lycodon mackinnoni Wall, 1906 (Serpentes: Colubridae). *Zootaxa*, *4966*(3), 305–320.

Nie, M., Crim, J. D., & Ultsch, G. R. (1999). Dissolved Oxygen, Temperature, and Habitat Selection by Bullfrog (Rana catesbeiana) Tadpoles. *Copeia*, *1999*(1), 153–162. JSTOR. https://doi.org/10.2307/1447396

Nimick, D. A., Gammons, C. H., & Parker, S. R. (2011). Diel biogeochemical processes and their effect on the aqueous chemistry of streams: A review. *Chemical Geology*, *283*(1–2), 3–17.

Nowakowski, A. J., Thompson, M. E., Donnelly, M. A., & Todd, B. D. (2017). Amphibian sensitivity to habitat modification is associated with population trends and species traits. *Global Ecology and Biogeography*, *26*(6), 700–712.

O'Hara, R. (1981). *Habitat selection behavior in three species of anuran larvae: Environmental cues, ontogeny, and adaptive significance*. ir.library.oregonstate.edu. https://ir.library.oregonstate.edu/dspace/handle/1957/9935

Ohler, A., & Dutta, S. (2004). IUCN Red List of Threatened Species: Nanorana vicina. *IUCN Red List of Threatened Species*. https://www.iucnredlist.org/en

Ohler, A., Dutta, S., & Shrestha, T. K. (2004). IUCN Red List of Threatened Species: Nanorana minica. *IUCN Red List of Threatened Species*. https://www.iucnredlist.org/en

Olson, D. M., Dinerstein, E., Wikramanayake, E. D., Burgess, N. D., Powell, G. V., Underwood, E. C., D'amico, J. A., Itoua, I., Strand, H. E., & Morrison, J. C. (2001). Terrestrial Ecoregions of the World: A New Map of Life on EarthA new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. *BioScience*, *51*(11), 933–938.

Pandit, M. K., & Grumbine, R. E. (2012). Potential effects of ongoing and proposed hydropower development on terrestrial biological diversity in the Indian Himalaya. *Conservation Biology*, *26*(6), 1061–1071.

Pentek, A., Sarospataki, M., & Zsuga, K. (2019). Larval overwintering of the Pelophylax esculentus complex in a sodic bomb crater pond near Apaj, Hungary. *North-Western Journal of Zoology*, *15*(1), 109.

Perotti, M. G., Bonino, M. F., Ferraro, D., & Cruz, F. B. (2018). How sensitive are temperate tadpoles to climate change? The use of thermal physiology and niche model tools to assess vulnerability. *Zoology*, *127*, 95–105. https://doi.org/10.1016/j.zool.2018.01.002

Pewsey, A., Neuhäuser, M., & Ruxton, G. D. (2013). *Circular statistics in R*. Oxford University Press.

Pintar, M. (2000). On overwintering larvae of the Common frog Rana temporaria Linnaeus 1758 (Anura: Ranidae). *Herpetozoa*, *13*, 181–186.

Pound, M. J., & Salzmann, U. (2017). Heterogeneity in global vegetation and terrestrial climate change during the late Eocene to early Oligocene transition. *Scientific Reports*, *7*, 43386.

R Core Team. (2021). *R: A Language and Environment for Statistical Computing* (4.1.0) [Ubuntu]. R Foundation for Statistical Computing. https://www.R-project.org/

Rahmani, A. R., & Islam, M. (2004). *Important bird areas in India*. IBCN, Bombay Natural History Society.

Raj, P. (2015). *Systematics of select amphibian species using their larval morphology and ontogeny* [Ph.D thesis]. Department of Wildlife Science, Saurashtra University. http://hdl.handle.net/10603/128885

Ray, P. (1999). *Systematic studies on the amphibian fauna of the District Dehradun, Uttar Pradesh, India* (Memoir 18(3); p. 102). Zoological Survey of India.

Richards, S. J. (2002). Influence of flow regime on habitat selection by tadpoles in an Australian rainforest stream. *Journal of Zoology*, *257*(2), 273–279.

Ridout, M., & Linkie, M. (2009). Estimating overlap of daily activity patterns from camera trap data. *Journal of Agricultural, Biological, and Environmental Statistics*, *14*(3), 322–337.

Rizvi, A. N., Bursey, C. R., & Bhutia, P. T. (2012). Three new species of Digenea (Batrachotrematidae) in Nanorana minica (Anura, Dicroglossidae) from Uttarakhand, India. *Acta Parasitologica*, *57*(2), 154–159.

Rusydi, A. F. (2018). Correlation between conductivity and total dissolved solid in various type of water: A review. *IOP Conference Series: Earth and Environmental Science*, *118*(1), 012019.

Saeed, M., Rais, M., Gray, R. J., Ahmed, W., Akram, A., Gill, S., & Fareed, G. (2021). Rise in temperature causes decreased fitness and higher extinction risks in endemic frogs at high altitude forested wetlands in northern Pakistan. *Journal of Thermal Biology*, *95*, 102809.

Saidapur, S. (2001). Behavioral ecology of anuran tadpoles: The Indian scenario. *Proceedings-Indian National Science Academy Part B*, *67*(6), 311–322.

Schiesari, L. (2004). *Performance tradeoffs across resource gradients in anuran larvae*. search.proquest.com. http://search.proquest.com/openview/37dc89185cfd806cd75e2b10618e47ce/1?pq-origsite=gscholar&cbl=18750&diss=y

Schloerke, B., Cook, D., Larmarange, J., Briatte, F., Marbach, M., Thoen, E., Elberg, A., & Crowley, J. (2021). *GGally: Extension to "ggplot2"* (2.1.1.) [R Package]. https://CRAN.R-project.org/package=GGally

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671–675. https://doi.org/10.1038/nmeth.2089

Serra, E., Medalha, C., & Mattioli, R. (1999). Natural preference of zebrafish (Danio rerio) for a dark environment. *Brazilian Journal of Medical and Biological Research*, *32*, 1551–1553.

Silva, W., & Giaretta, A. (2008). Further notes on the natural history of the South American pepper frog, Leptodactylus labyrinthicus (Spix, 1824)(Anura, Leptodactylidae). *Brazilian Journal of Biology*, *68*, 403–407.

Singh, A. P., & Sondhi, S. (2016). Butterflies of Garhwal, Uttarakhand, western Himalaya, India. *Journal of Threatened Taxa*, *8*(4), 8666–8697.

Sircar, G. (2010). *Reproductive mode of Himalayan Paa frog-Nanorana vicina* [Master's thesis]. School of Environmental Studies, University of Delhi.

Skelly, D., Freidenburg, L., & Kiesecker, J. (2002). Forest canopy and the performance of larval amphibians. *Ecology*, *83*(4), 983–992.

Smith, G. R. (1999). Microhabitat preferences of bullfrog tadpoles (Rana catesbeiana) of different ages. *Transactions of the Nebraska Academy of Sciences*, *25*, 73–76.

Smith-Gill, S. J., & Berven, K. A. (1979). Predicting Amphibian Metamorphosis. *The American Naturalist*, *113*(4), 563–585. JSTOR. https://www.jstor.org/stable/2460275

Snodgrass, J. W., Hopkins, W. A., Jackson, B. P., Baionno, J. A., & Broughton, J. (2005). Influence of larval period on responses of overwintering green frog (Rana clamitans) larvae exposed to contaminated sediments. *Environmental Toxicology and Chemistry*, *24*(6), 1508–1514. https://doi.org/10.1897/04-339R1.1

St-Hilaire, A., Morin, G., El-Jabi, N., & Caissie, D. (2000). Water temperature modelling in a small forested stream: Implication of forest canopy and soil temperature. *Canadian Journal of Civil Engineering*, *27*(6), 1095–1108.

Strauch, A. M., MacKenzie, R. A., & Tingley III, R. W. (2017). Base flow-driven shifts in tropical stream temperature regimes across a mean annual rainfall gradient. *Hydrological Processes*, *31*(9), 1678–1689.

Studinski, J. M., Hartman, K. J., Niles, J. M., & Keyser, P. (2012). The effects of riparian forest disturbance on stream temperature, sedimentation, and morphology. *Hydrobiologia*, *686*(1), 107–117.

Stynoski, J. L., & Noble, V. R. (2012). To beg or to freeze: Multimodal sensory integration directs behavior in a tadpole. *Behavioral Ecology and Sociobiology*, *66*(2), 191–199.

Sun, Z., Zhao, C., Zhu, W., Zhu, W., Feng, J., Su, S., & Zhao, T. (2021). Microhabitat features determine the tadpole diversity in mountainous streams. *Ecological Indicators*, *126*, 107647.

Tague, C., Farrell, M., Grant, G., Lewis, S., & Rey, S. (2007). Hydrogeologic controls on summer stream temperatures in the McKenzie River basin, Oregon. *Hydrological Processes: An International Journal*, *21*(24), 3288–3300.

Tattersall, G. J., & Ultsch, G. R. (2008). Physiological Ecology of Aquatic Overwintering in Ranid Frogs. *Biological Reviews*, *83*(2), 119–140. https://doi.org/10.1111/j.1469-185X.2008.00035.x

Thirumalini, S., & Joseph, K. (2009). Correlation between electrical conductivity and total dissolved solids in natural waters. *Malaysian Journal of Science*, *28*(1), 55–61.

Tomczak, M., & Tomczak, E. (2014). The need to report effect size estimates revisited. An overview of some recommended measures of effect size. *Trends in Sport Sciences*, *1*(21), 19–25.

Ultsch, G. R., Bradford, D. F., & Freda, J. (1999). Physiology: Coping with the Environment. In *Tadpoles: The biology of anuran larvae* (pp. 189–214). University of Chicago Press.

Vo, P., & Gridi-Papp, M. (2017). Low temperature tolerance, cold hardening and acclimation in tadpoles of the neotropical túngara frog (Engystomops pustulosus). *Journal of Thermal Biology*, *66*, 49–55. https://doi.org/10.1016/j.jtherbio.2017.03.012

Wallace, R. L., & Diller, L. V. (1998). Length of the Larval Cycle of Ascaphus truei in Coastal Streams of the Redwood Region, Northern California. *Journal of Herpetology*, *32*(3), 404–409. JSTOR. https://doi.org/10.2307/1565455

Walsh, P. T., Downie, J. R., & Monaghan, P. (2008). Larval over-wintering: Plasticity in the timing of life-history events in the common frog. Journal of Zoology, 276(4), 394-401. https://doi.org/10.1111/j.1469-7998.2008.00503.x

Walsh, P. T., Downie, J. R., & Monaghan, P. (2016). Factors affecting the overwintering of tadpoles in a temperate amphibian. *Journal of Zoology*, *298*(3), 183–190. https://doi.org/10.1111/jzo.12296

Walston, L. J., & Mullin, S. J. (2007). Responses of a Pond-Breeding Amphibian Community to the Experimental Removal of Predatory Fish. *The American Midland Naturalist*, *157*(1), 63–73. JSTOR. https://www.jstor.org/stable/4500595

Warkentin, K. (1992). Effects of temperature and illumination on feeding rates of green frog tadpoles (Rana clamitans). *Copeia*, 725–730.

Warren, D. R., Keeton, W. S., Bechtold, H. A., & Rosi-Marshall, E. J. (2013). Comparing streambed light availability and canopy cover in streams with old-growth versus early-mature riparian forests in western Oregon. *Aquatic Sciences*, *75*(4), 547–558.

Wei, T., & Simko, V. (2021). *R package "corrplot": Visualization of a Correlation Matrix* (0.88) [R Package]. https://github.com/taiyun/corrplot

Werner, E. E., & Glennemeier, K. S. (1999). Influence of forest canopy cover on the breeding pond distributions of several amphibian species. *Copeia*, 1–12.

Wheeler, C. A., Bettaso, J. B., Ashton, D. T., & Welsh Jr., H. H. (2015). Effects of Water Temperature on Breeding Phenology, Growth, and Metamorphosis of Foothill Yellow-Legged Frogs (Rana boylii): A Case Study of the Regulated Mainstem and Unregulated Tributaries of California's Trinity River. *River Research and Applications*, *31*(10), 1276–1286. https://doi.org/10.1002/rra.2820

Whiteman, H. H., & Buschhaus, N. L. (2003). Behavioral thermoregulation in field populations of amphibian larvae. In *Exploring Animal Behavior in Laboratory and Field* (pp. 79–84). Elsevier.

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org

Wickham, H. (2021). *tidyr: Tidy Messy Data* (1.1.3) [R Package]. https://CRAN.R-project.org/package=tidyr

Wickham, H., François, R., Henry, L., & Müller, K. (2021). *dplyr: A Grammar of Data Manipulation* (1.0.6) [R Package]. https://CRAN.R-project.org/package=dplyr

Wilbur, H. M., & Collins, J. P. (1973). Ecological Aspects of Amphibian Metamorphosis. *Science*, *182*(4119), 1305–1314. JSTOR. https://www.jstor.org/stable/1737516

Wilke, C. O. (2021). *ggridges: Ridgeline Plots in "ggplot2"* (0.5.3) [R Package]. https://CRAN.R-project.org/package=ggridges

Wollmuth, L. P., & Crawshaw, L. I. (1988). The effect of development and season on temperature selection in bullfrog tadpoles. *Physiological Zoology*, *61*(5), 461–469.

Wollmuth, L. P., Crawshaw, L. I., Forbes, R. B., & Grahn, D. A. (1987). Temperature selection during development in a montane anuran species, Rana cascadae. *Physiological Zoology*, *60*(4), 472–480.

Wu, H.-J., Yen, C.-F., & Kam, Y.-C. (2007). Metabolic compensation and behavioral thermoregulation of subtropical rhacophorid (Polypedates megacephalus) tadpoles in container habitats. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *146*(1), 101–106.

Yarnell, S. M., Lind, A. J., & Mount, J. (2012). Dynamic flow modelling of riverine amphibian habitat with application to regulated flow management. *River Research and Applications*, *28*(2), 177–191.

Yarnell, S. M., Peek, R. A., Keung, N., Todd, B. D., Lawler, S., & Brown, C. (2019). A lentic breeder in lotic waters: Sierra Nevada yellow-legged frog (Rana sierrae) habitat suitability in northern Sierra Nevada streams. *Copeia*, *107*(4), 676–693.

Yuan, Z.-Y., Zhou, W.-W., Chen, X., Poyarkov, N. A., Chen, H.-M., Jang-Liaw, N.-H., Chou, W.-H., Matzke, N. J., Iizuka, K., Min, M.-S., Kuzmin, S. L., Zhang, Y.-P., Cannatella, D. C., Hillis, D. M., & Che, J. (2016). Spatiotemporal Diversification of the True Frogs (Genus Rana): A Historical Framework for a Widely Studied Group of Model Organisms. *Systematic Biology*, *65*(5), 824–842.

Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. Springer Science & Business Media.

ANNEXURE

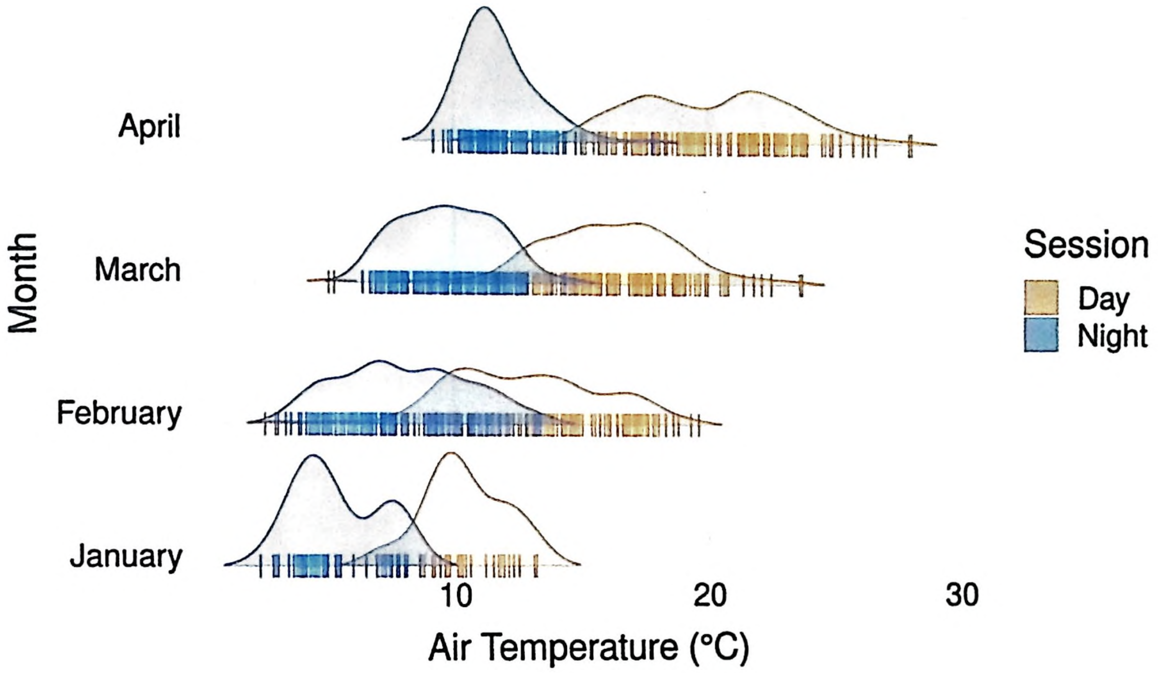


Fig. 47 Air temperature across the study period (2021 January - April)

Table 9 Weather data summary during the study period (2021 January - April)

	Air Temperature (^0C)				**Water Temperature (^0C)**				**Relative Humidity (%)**			
Month	**Mean**	**Max.**	**SD**	**Min.**	**Mean**	**Max.**	**SD**	**Min.**	**Mean**	**Max.**	**SD**	**Min.**
January	7.4	13.2	2.9	2.4	7.3	11.5	3.0	0.1	56.0	86.0	13.3	29.1
February	10.5	19.6	3.7	2.6	10.1	18.1	2.3	5.5	55.5	89.0	13.2	4.4
March	12.8	23.7	4.0	5.1	11.8	28.6	2.9	7.2	53.8	79.6	15.0	18.9
April	15.9	28.0	4.9	9.2	13.4	28.2	3.3	9.0	48.1	80.1	18.4	10.7

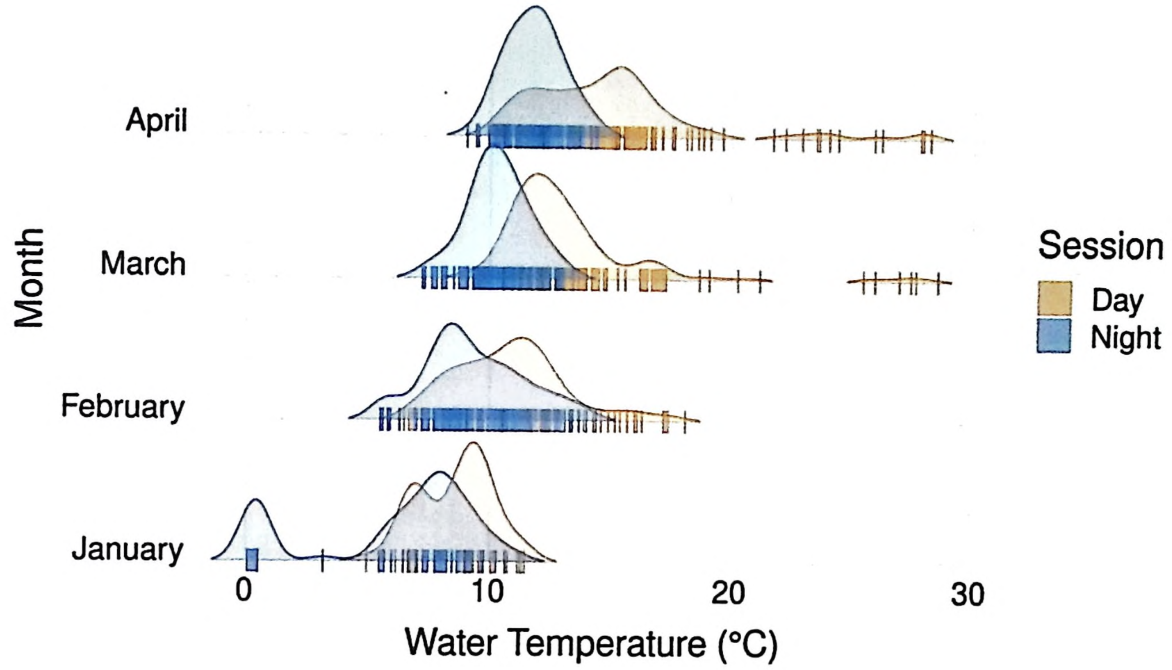


Fig. 48 Water temperature across the study period (2021 January - April)

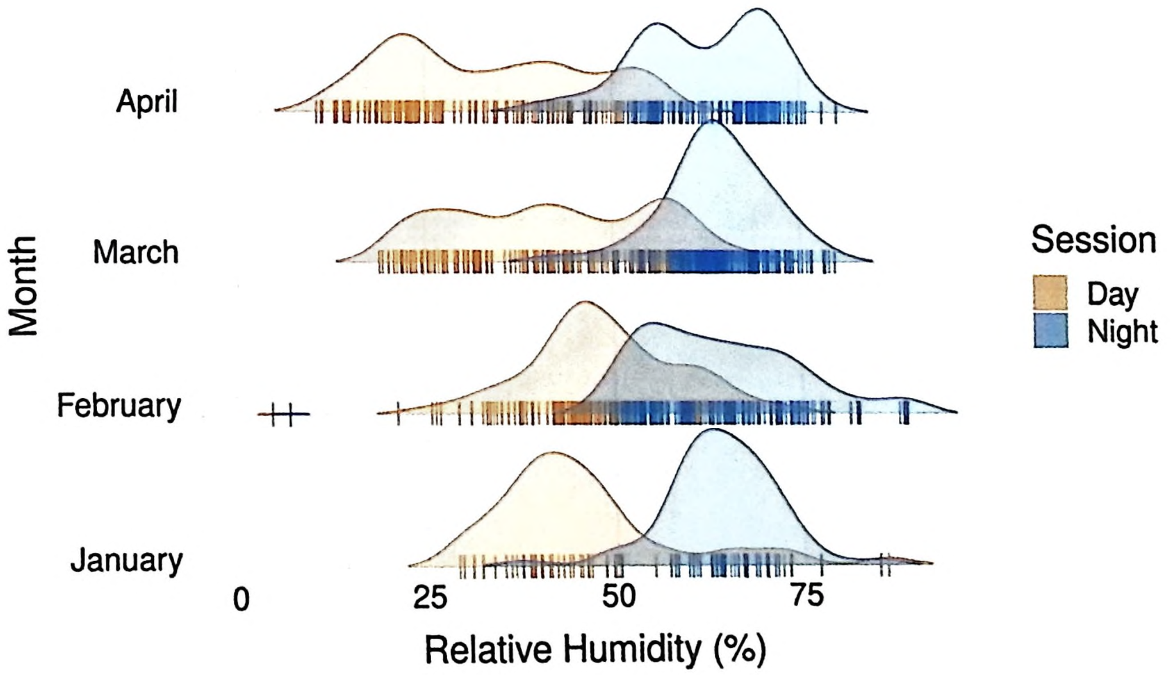


Fig. 49 Relative humidity across the study period (2021 January - April)

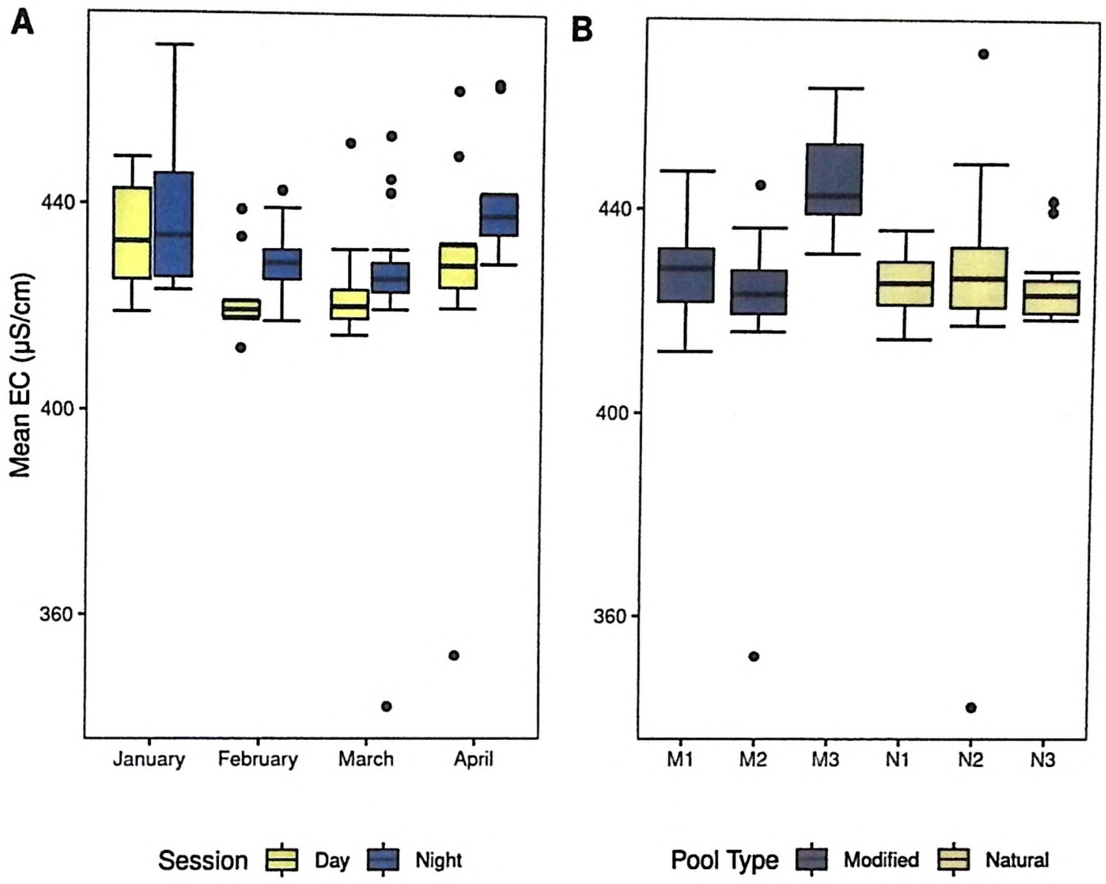


Fig. 50 Variation in EC levels (A) monthly diurnal pattern; (B) across pools

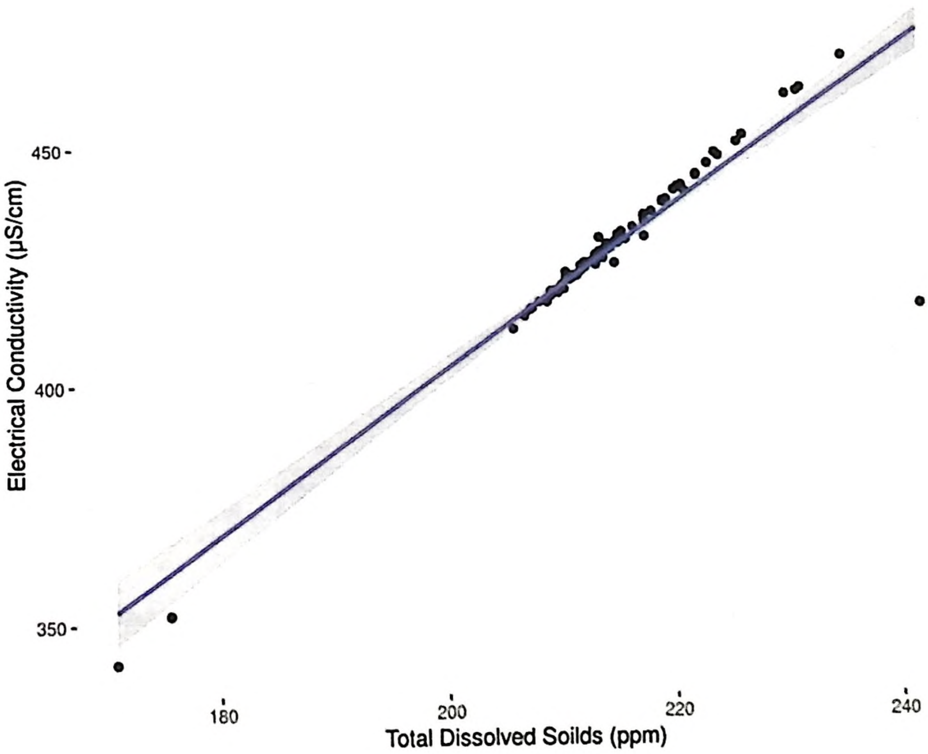


Fig. 51 Relationship between EC and TDS

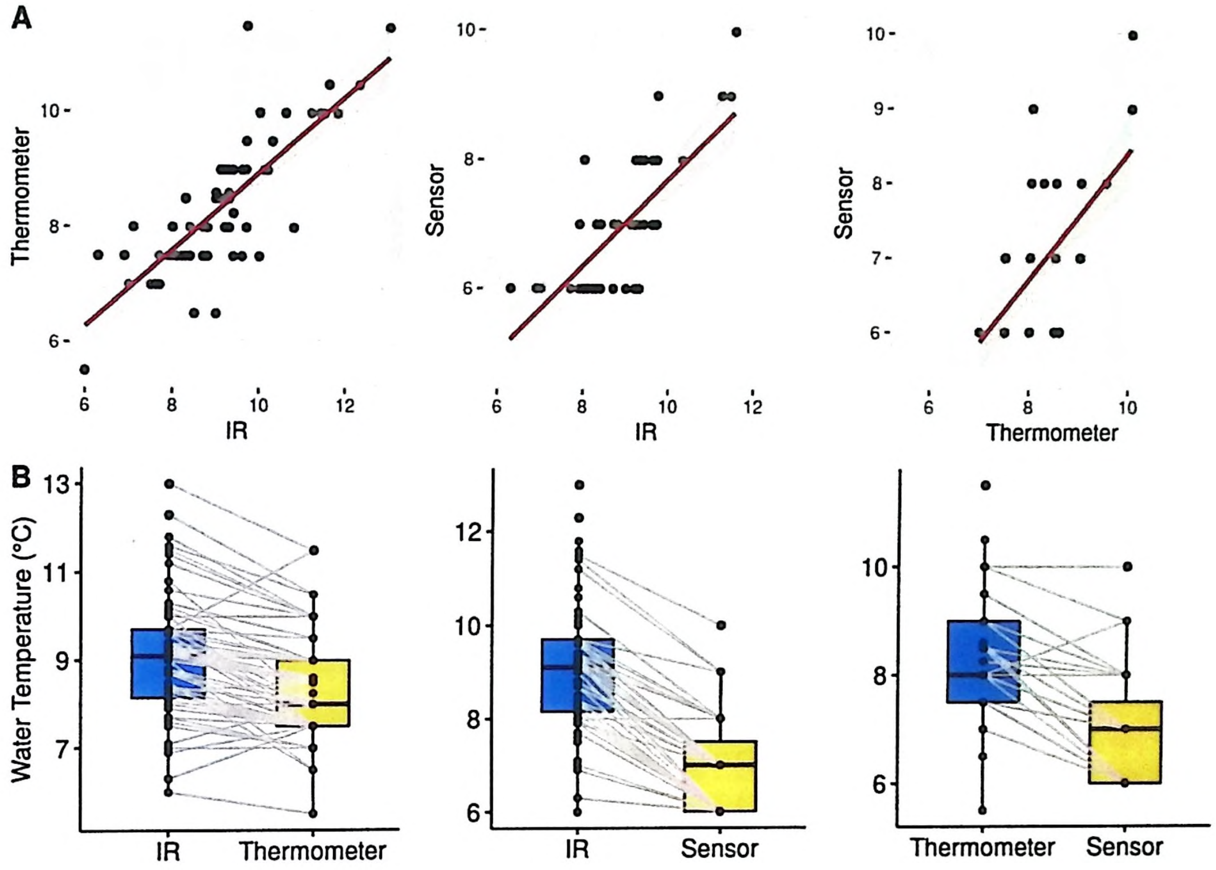


Fig. 52 Variations and relationships in water temperature measurements obtained with different approaches, indicated through (A) correlation plots and (B) paired boxplots.

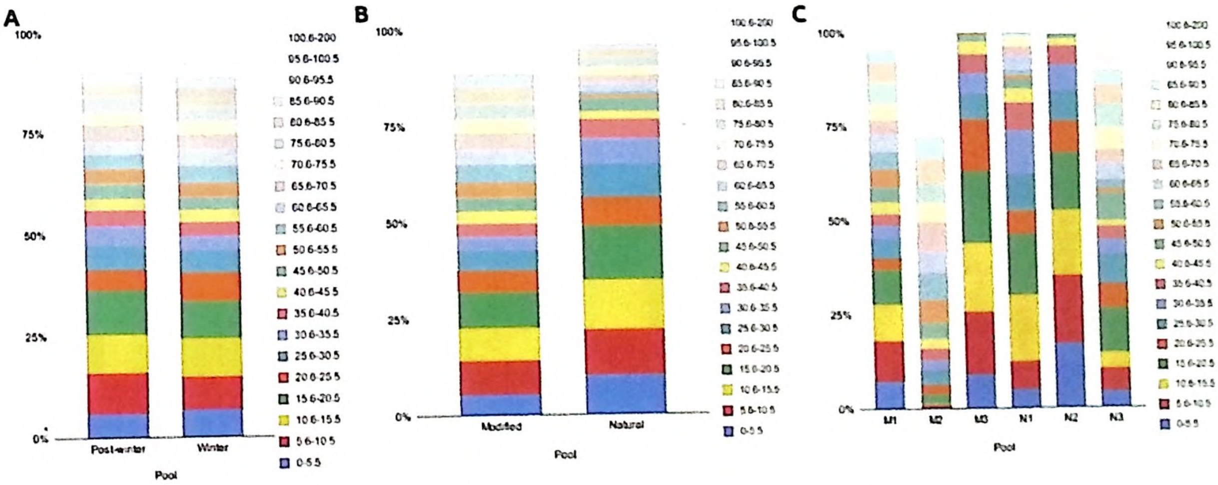


Fig. 53 Water depth profiles across (A) seasons (B) pool types and (C) individual pools averaged across months

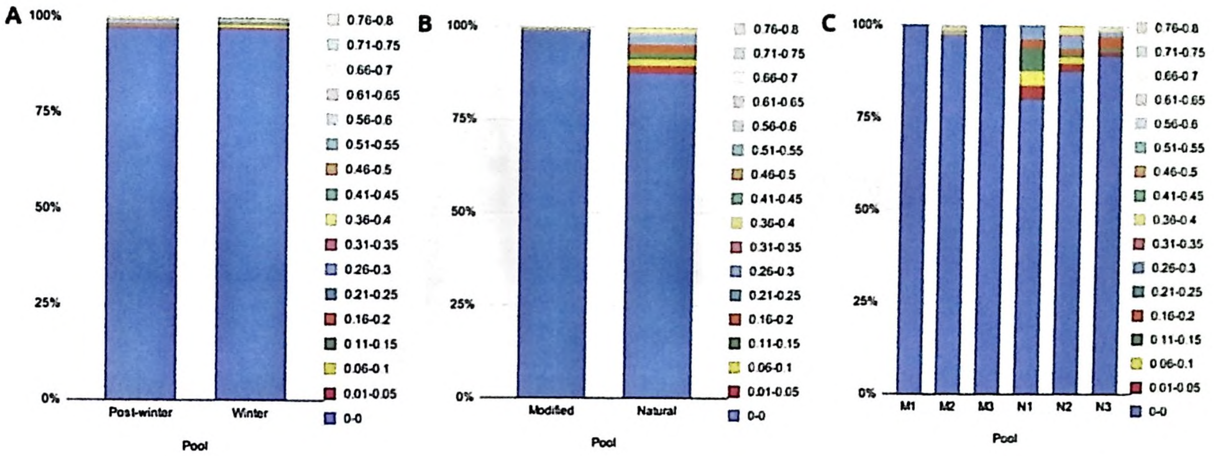


Fig. 54 Water velocity profiles across (A) seasons (B) pool types and (C) individual pools averaged across months

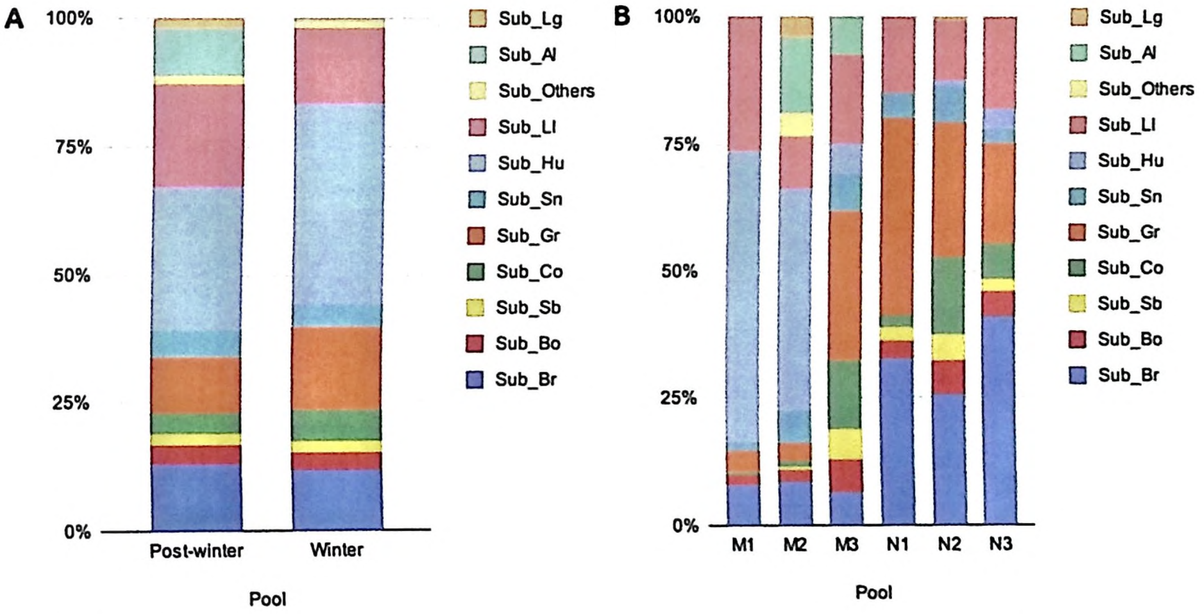


Fig. 55 Substrate profiles of pools across (A) seasons and (B) individual pools averaged across months

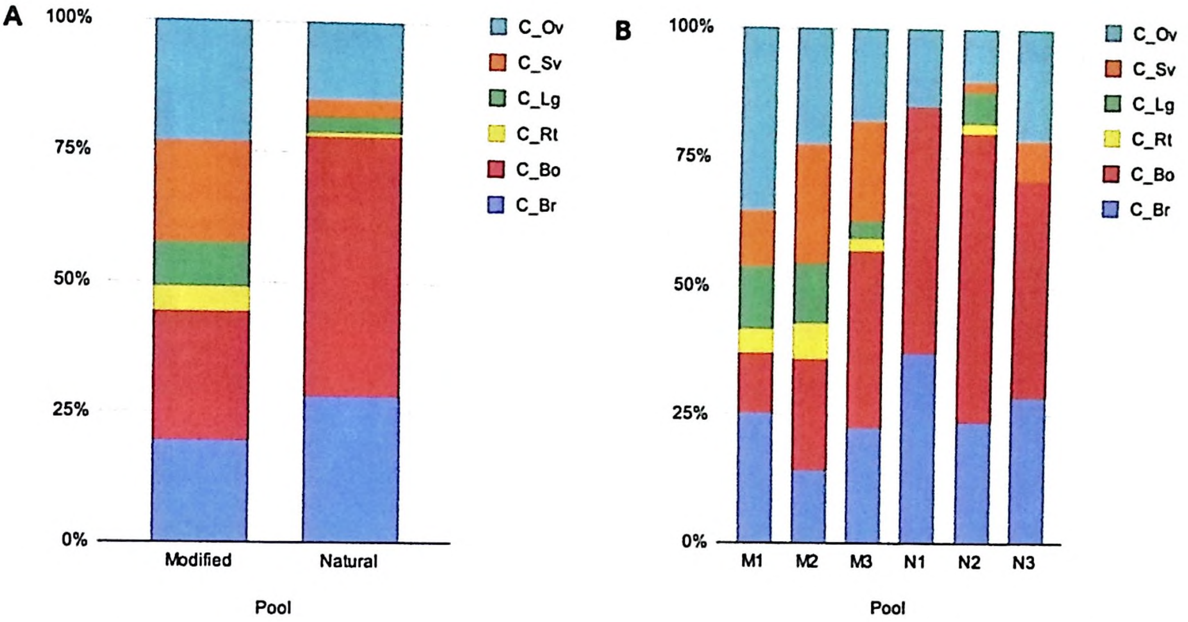


Fig. 56 Cover item profiles of pools across (A) pool types and (B) individual pools averaged across months

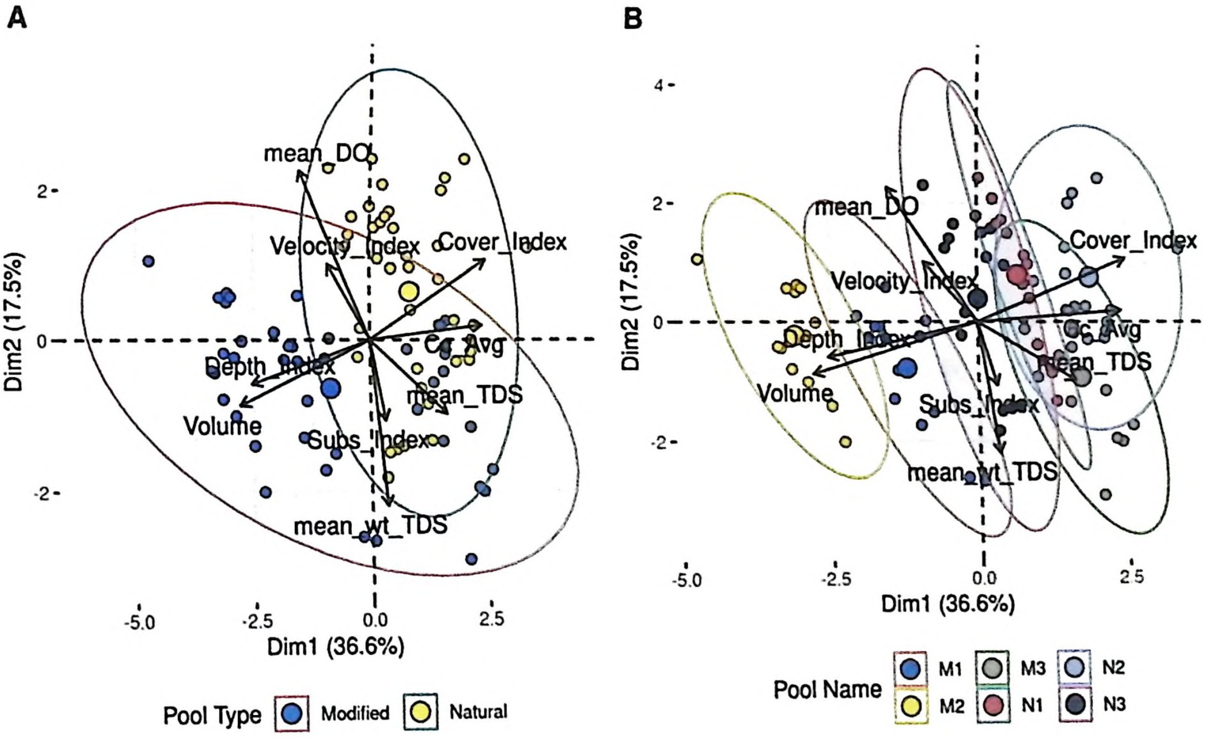


Fig. 57 2D PCA plot showing various habitat and environmental variables shaping natural and modified pool habitats based on (A) pool types, (B) individual pools

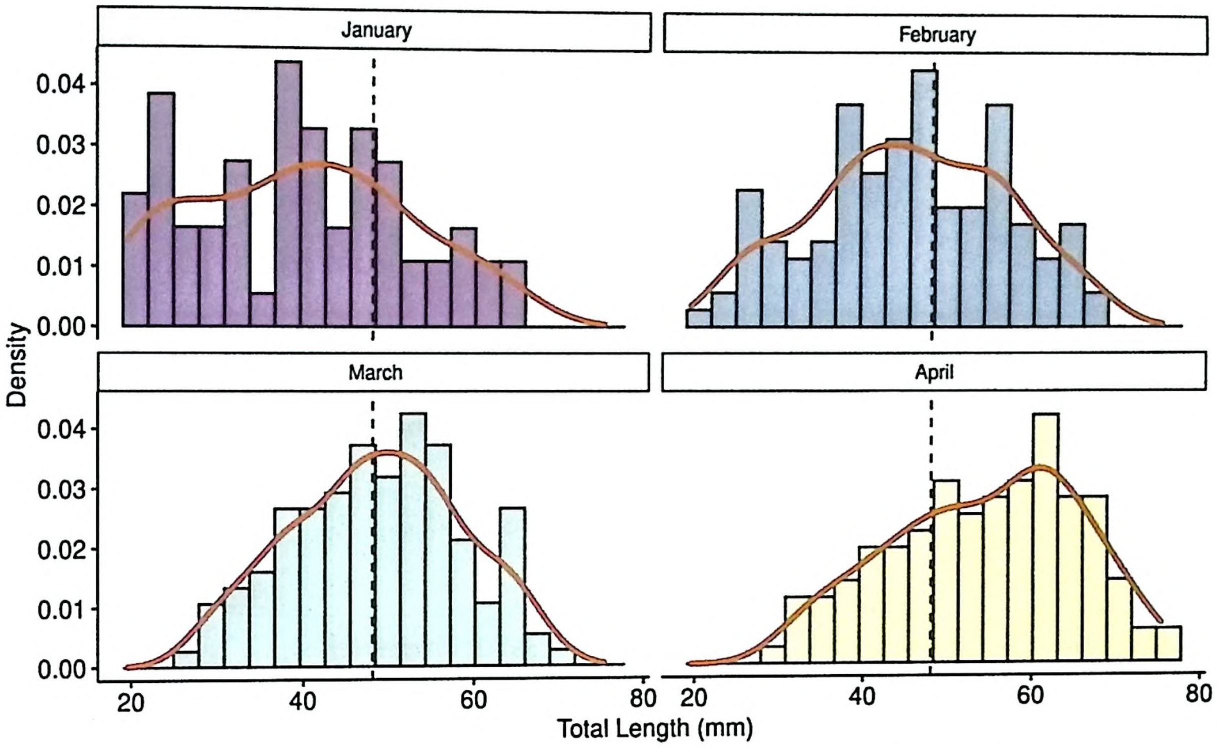


Fig. 58 Size-class distribution of tadpoles across months based on the Total Length (TL), dashed lines indicate the mean value of total length for all individuals.

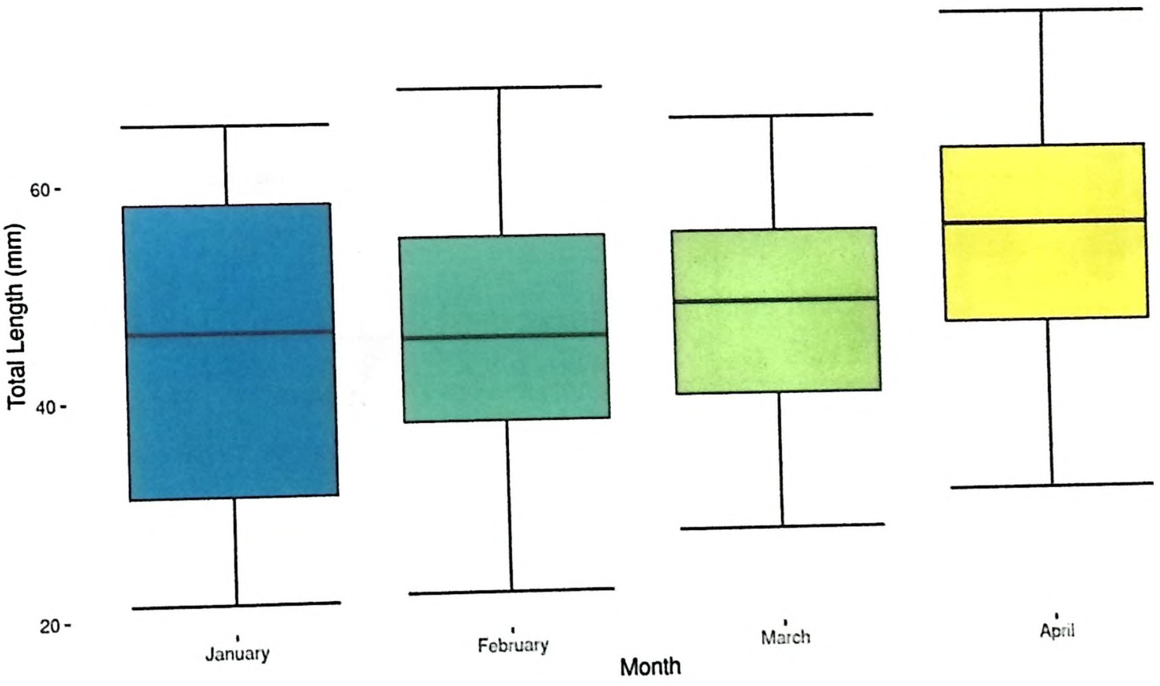


Fig. 59 Total Length variation across months

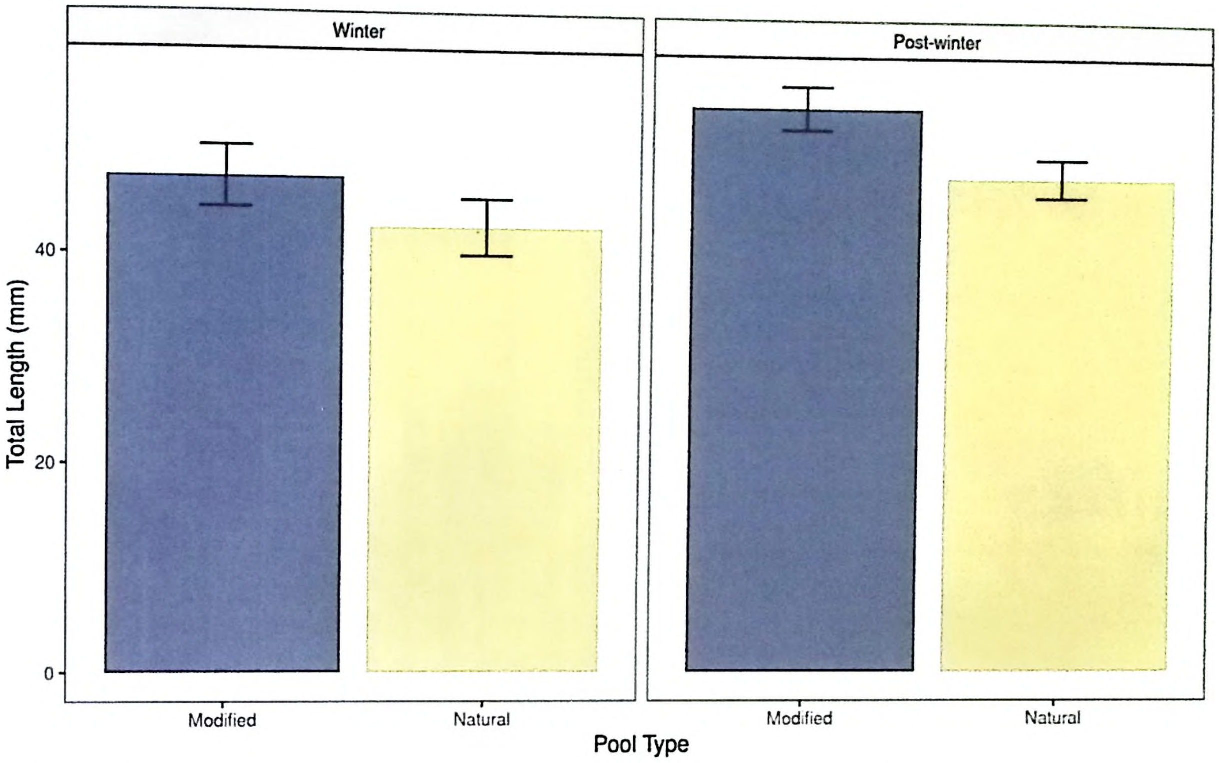


Fig. 60 Total Length variation across pool type and seasons. Error bars indicating 95% confidence intervals.

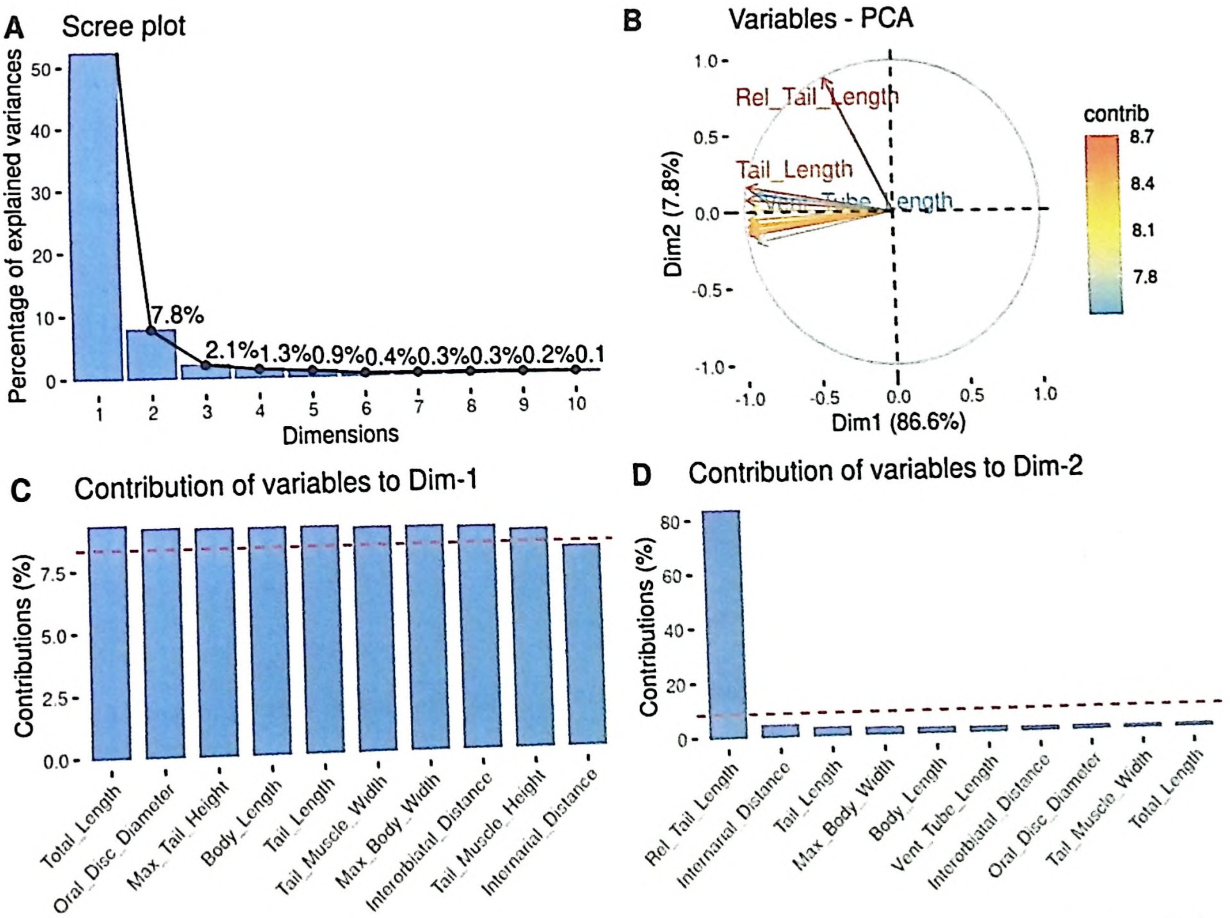


Fig. 61 PCA diagnosis plot explaining (A) percentage of explained variance by each axis generated, (B) overall contribution of variables to PCA, (C) percentage contribution of variable to axis 1 and (D) axis 2.

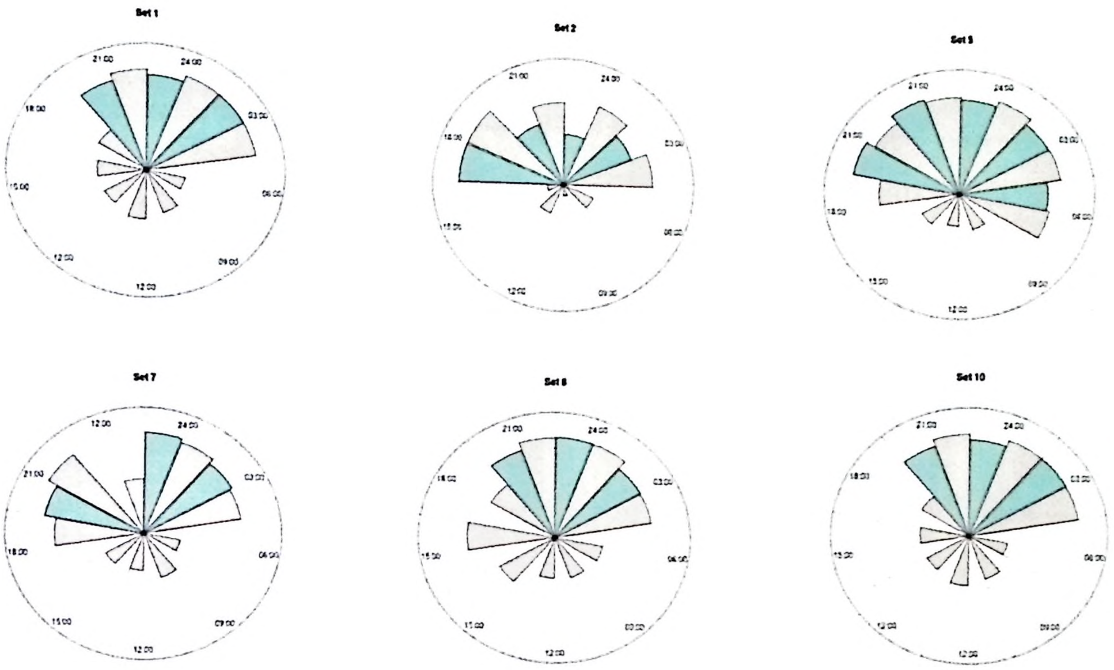


Fig. 62 Variation in mean proportional tadpole count in natural (green segments) and modified (grey segments) pools across sampling sets (sampling set number as subtitles; transition from January to April)

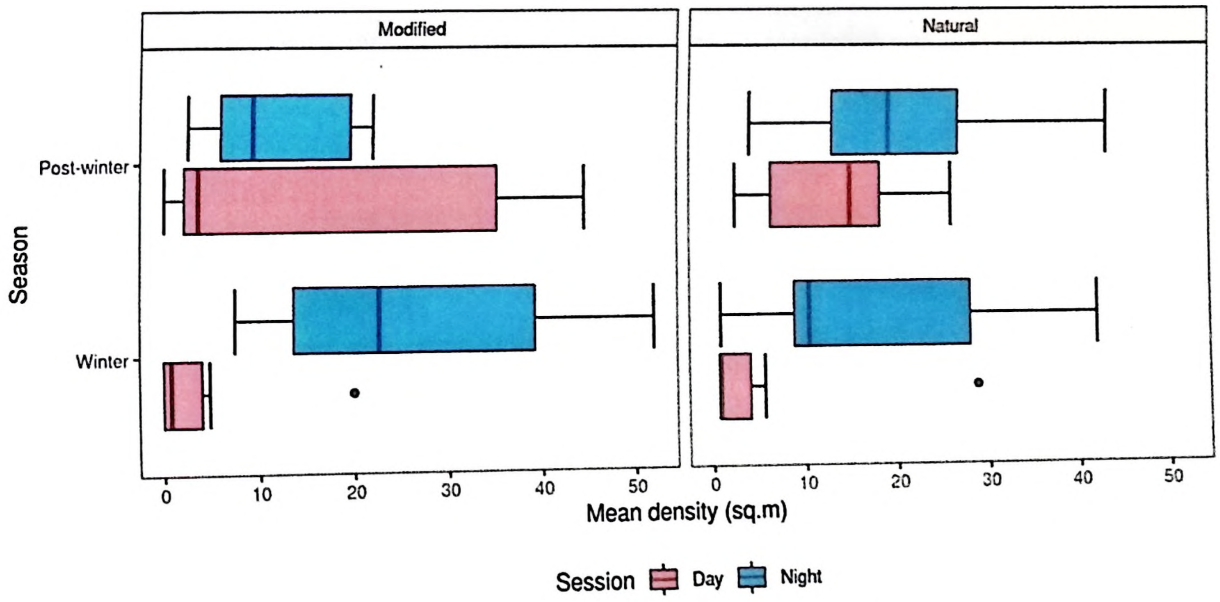


Fig. 63 Seasonal and diurnal variation in mean tadpole density of pool types averaged across sampling sessions

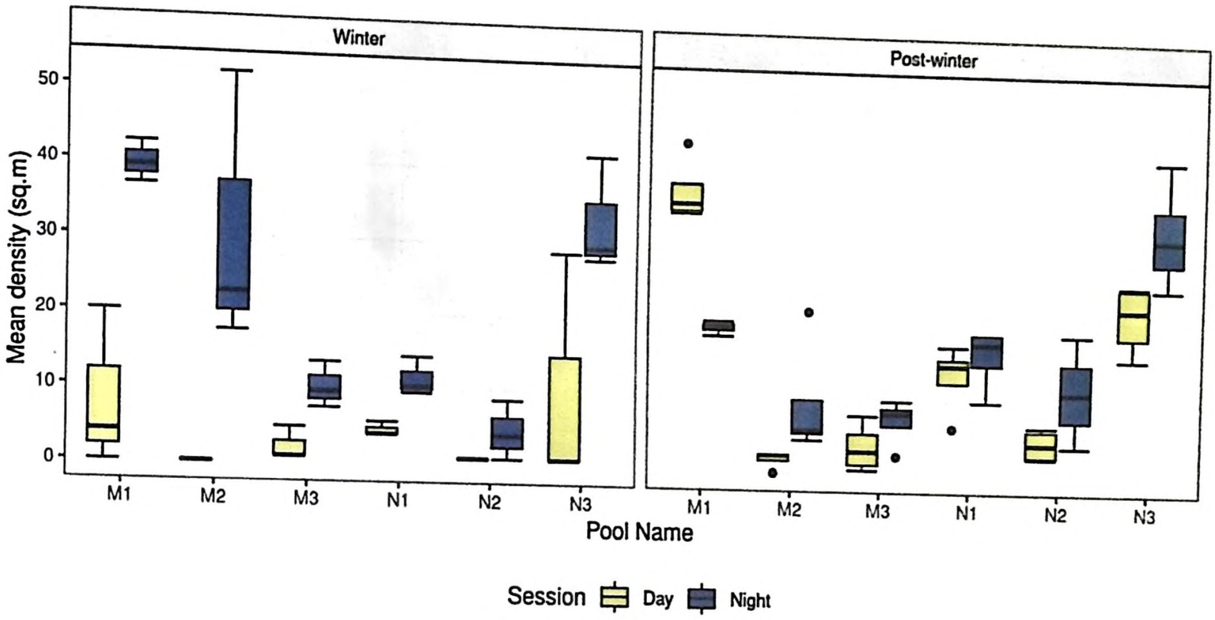


Fig. 64 Seasonal and diurnal variation in mean tadpole density of major pools averaged across sampling sessions

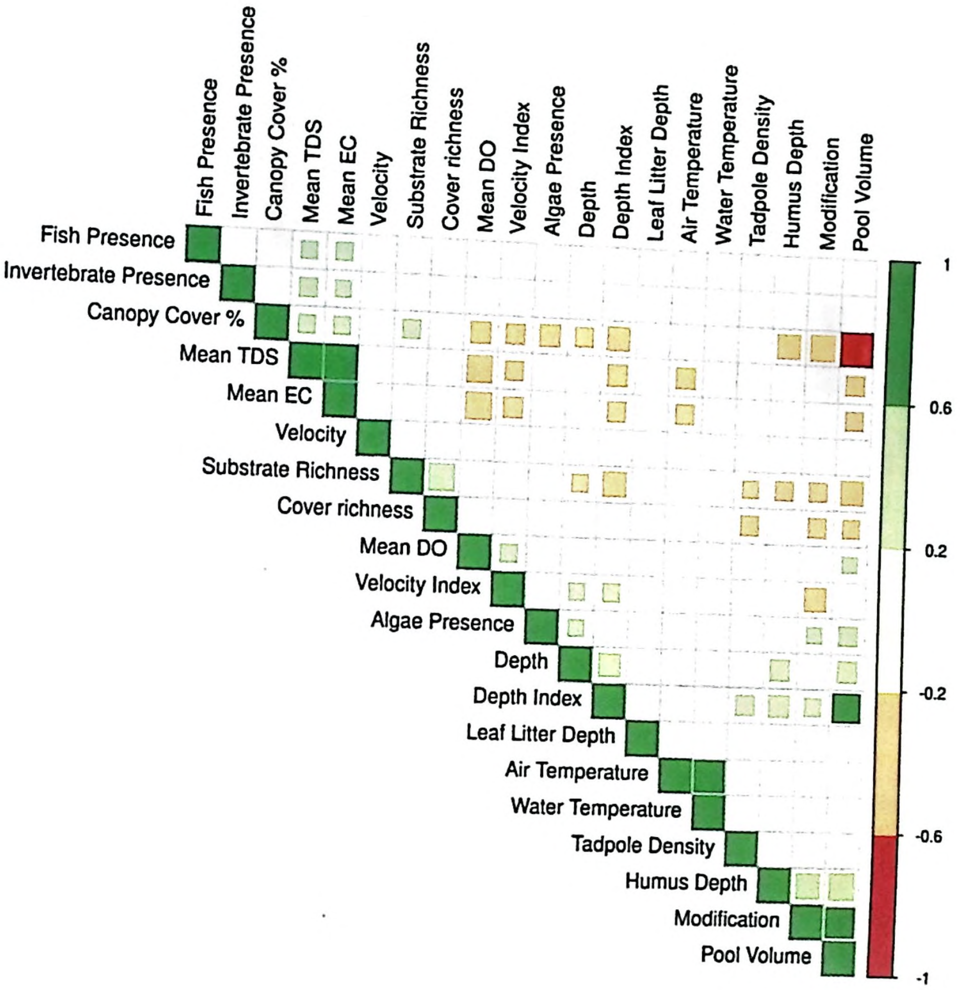


Fig. 65 Correlation matrix of all variables related to original tadpole density, assessed for multicollinearity.

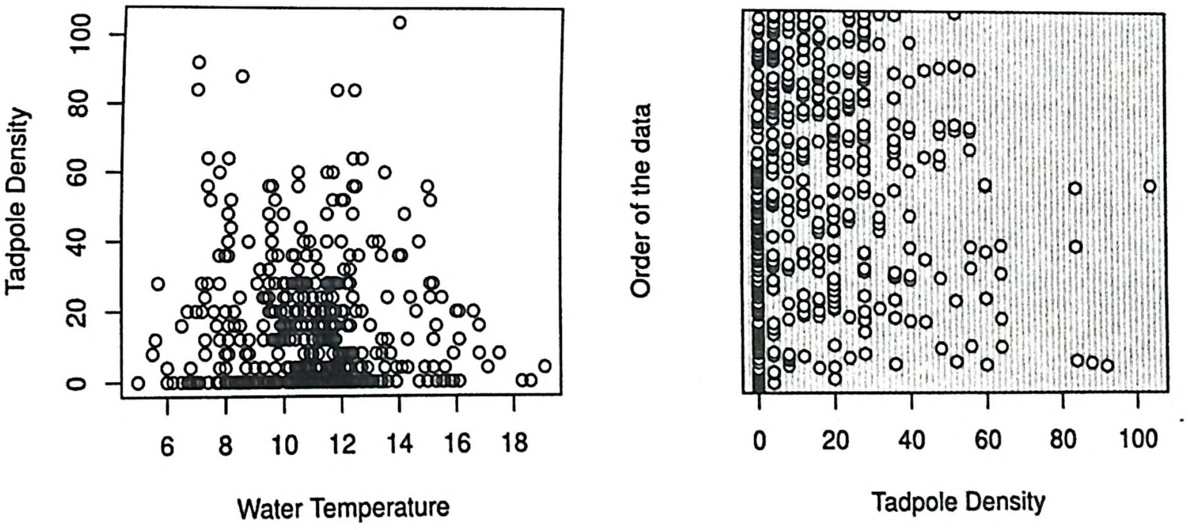


Fig. 66 Scatter plot and a Cleveland dot plot indicating the nature of tadpole density measurements, showing high number of zeros and absence of outliers.

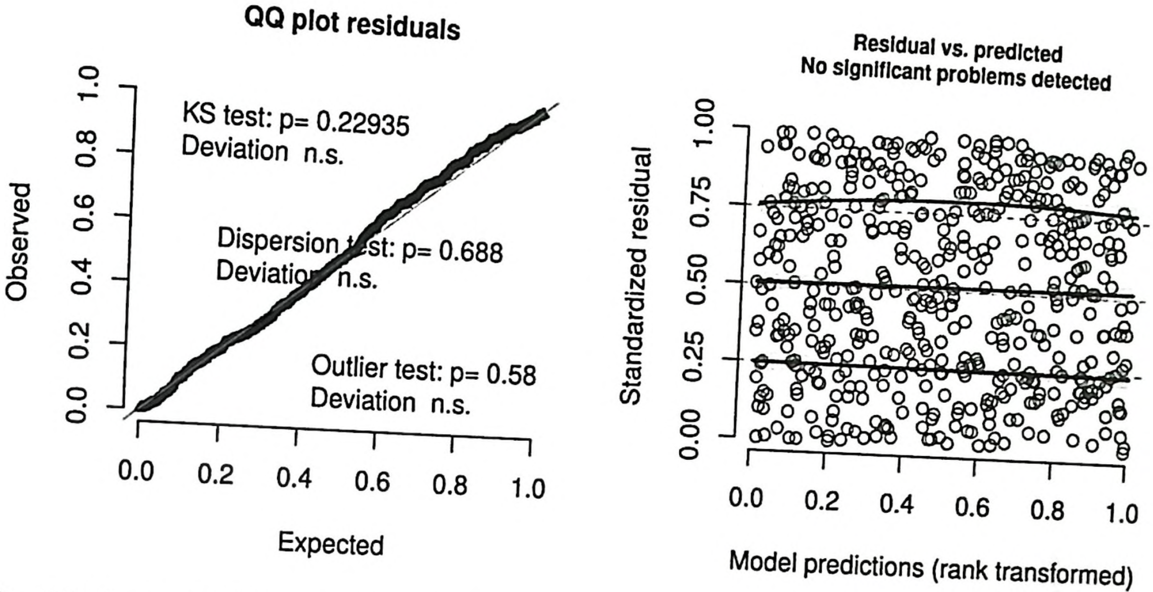


Fig. 67 Diagnostic plot for the selected model indicating compliance with the assumptions.

Table 10 Details of the selected model with incidence ratios of predictor variables, related confidence intervals and significance values. Bold values indicate <0.05 significance level. Significance codes: $*p \leq 0.05$; $**p < 0.01$; $***p < 0.001$. Marginal R^2 / Conditional R^2 = 0.227 / 0.386 (Nakagawa's R^2). $N_{observations}$= 492, N_{Pools}=6, Overdispersion parameter for nbinom2 family=3. *Abbreviations*: Water Temperature (Wt), Cover Item Richness (Cr), Mean Velocity (Mv), Leaf Litter Depth (Ld), Mean DO Level (Do), Modification - Check Dam (Md), Mean Canopy Cover Percentage (Cc), Night (Nt), Winter (Wr).

AIC	**BIC**	**logLik**	**deviance**	**df.resid**
2936.5	3012.1	-1450.2	2900.5	474

Random Effects (Conditional model):

Groups	**Name**	**Variance**	**SD**	**Correlation**
Pool	(Intercept)	0.2235	0.4728	
	Winter	0.1144	0.3382	-0.25

Fixed Effects (Conditional model):

	Estimate	SE	z value	Pr(>|z|)
(Intercept)	10.532827	2.249771	4.682	2.84e-06 ***
Ld	-4.229383	2.124087	-1.991	0.046464 *
Mv	-17.240882	3.425054	-5.034	4.81e-07 ***
Cc	-0.085098	0.025729	-3.307	0.000941 ***
Wt	-0.592370	0.169432	-3.496	0.000472 ***
Md	0.075149	0.541281	0.139	0.889580
Nt	0.296361	0.122043	2.428	0.015169 *
Cr	-0.078890	0.037418	-2.108	0.035002 *
Cc:Wt	0.006689	0.001955	3.421	0.000625 ***
Md:Nt	-0.479558	0.163816	-2.927	0.003418 **

Zero-inflation model:

	Estimate	SE	z value	Pr(>|z|)
(Intercept)	-0.4160	0.1686	-2.467	0.01361 *
Nt	-1.1743	0.2788	-4.212	2.53e-05 ***
Wr	1.5776	0.3019	5.226	1.73e-07 ***
Nt:Wr	-1.2698	0.4562	-2.783	0.00538 **

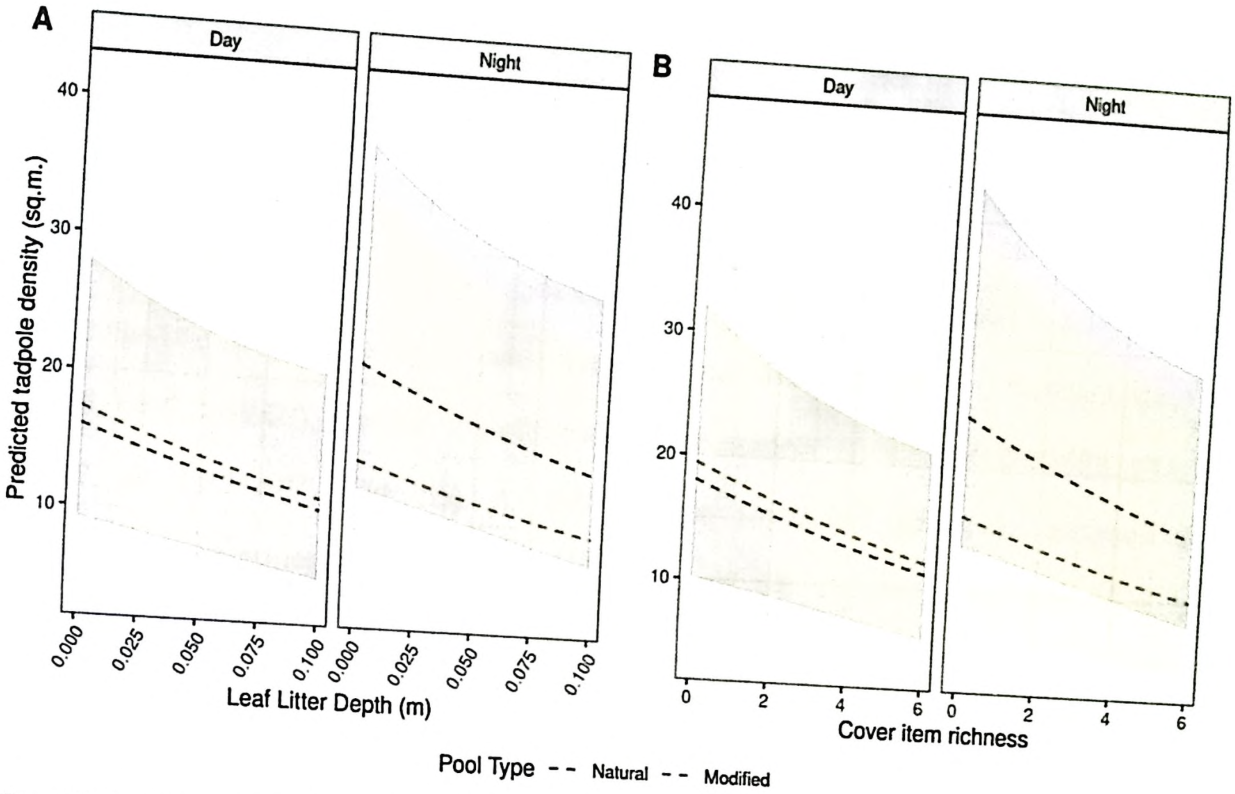


Fig. 68 Predicted tadpole density along (A) leaf litter depth and (B) cover item richness ranges in modified and natural pools during day and night time (95% CI).

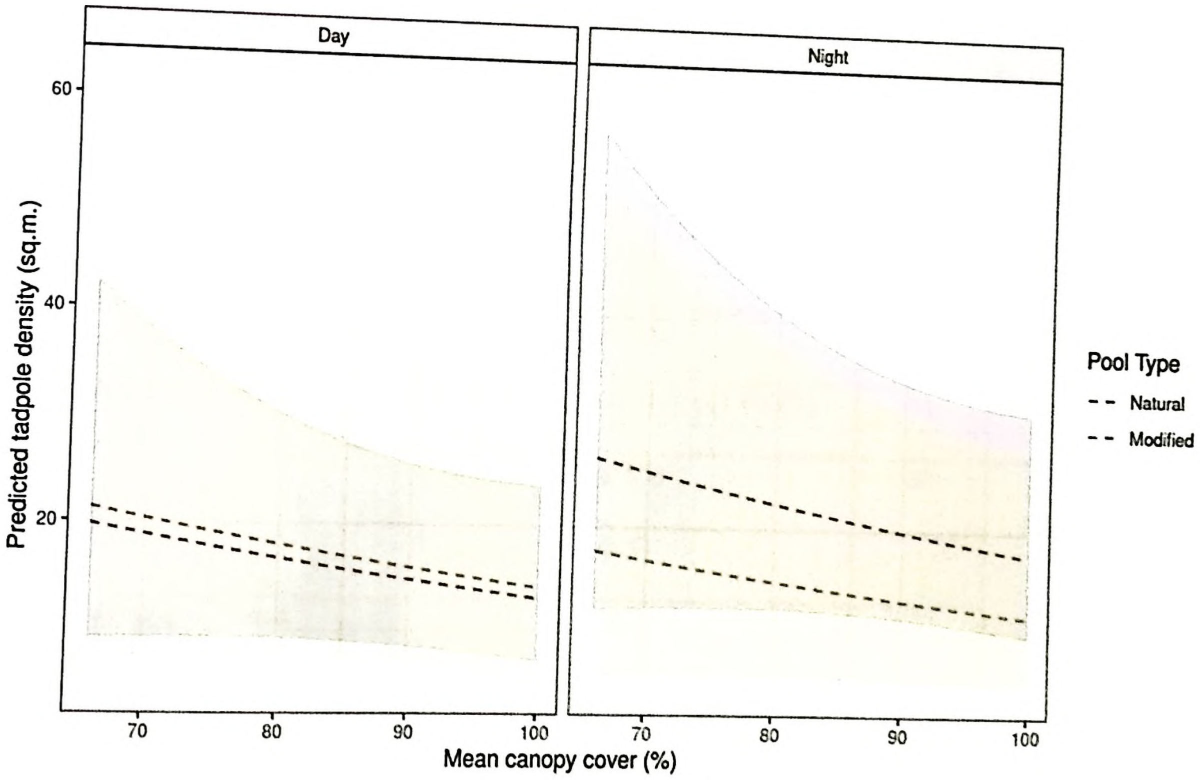


Fig. 69 Predicted tadpole density along the mean canopy cover percentage ranges in modified and natural pools during day and night time (95% CI).

NOTES

1. Comparison of Instruments: Even though measurements from all instruments were positively correlated to each other, degree of relatedness varied across, strongest relationship was between (A) Psychrometer and Thermometer (r=0.8030357, t(64)= 10.78, p= 5.028e-16), followed by (B) Psychrometer and automatic sensor (r=0.7423503, t(45) = 7.4325, p= 2.338e-09), and (C) Thermometer and automatic sensor (r=0.6817682, t(44) = 6.1817, p= 1.824e-07) (Fig. S5). All sets showed significant differences between the instrument measurements (A: V= 1804.5, p= 4.968e-08, ; B: V= 1081, p= 3.593e-09; C: V= 977.5, p= 1.431e-08).

2. Putative Species Designation Based on Literature Summary: Considering the literature summary (Table 14) and excluding Raj, 2015 (which is not matching with others), a preliminary classification of tadpoles was done for further analysis.

Table 11 Synthesis of LTRF from literature; staging in Gosner (1960)

Species Name	LTRF	Stage	Reference
Nanorana vicina	4(3-4)/3(1)	25	Gill et al., (2020 a & b)
	5(3-5)/3(1)	36	
	5(3-5)/3(1), 5(2-5)/3(1) or 6(3-6)/3(1)	41	
	5(3-5)/3(1)	35	Sircar (2010)
	6(4)/3(1)	-	Raj (2015)
Nanorana minica	5(4)/3(1)	-	
N.minica "Rana (Paa) tuberculata"	6-7(5)/3(1)	"Hind limb"	Ray, P. (1999)

I designated the three putative groups as (i) Nanorana vicina – 5(x)/3(1); (ii) Nanorana minica – 6(x)/3(1); (iii) Unidentified – 4(x)/3(1).

Table 12 Morphometric measurements of tadpoles in mm. For details, see the Methods section. Developmental stages are given in Gosner (1960), sample sizes are indicated in parentheses near the stage. Values are in 95% confidence intervals.

Putative Group	Stage	BL	TL	TaL %	TMH	TMW	MTH	IND	IOD	MBW	ODD	VTL
Nanorana minica	25 (1)	17.2	57.9	70.3	7	5.7	12.2	3.5	5.9	9.6	5.4	3.3
	27 (4)	14.3 ± 3.8	46.6 ± 14	69.2 ± 1.8	5.2 ± 2.5	4.2 ± 1.3	9.4 ± 3.1	3.2 ± 0.9	4.9 ± 1.4	8.5 ± 2.6	4.3 ± 1.4	2.7 ± 0.8
	28 (18)	14.4 ± 0.8	46.8 ± 3.3	69.1 ± 1.1	5.4 ± 0.6	4.3 ± 0.5	9.3 ± 0.6	3.3 ± 0.2	4.9 ± 0.2	8.4 ± 0.7	4.6 ± 0.3	2.7 ± 0.2
	29 (1)	14.9	51.8	71.3	5.2	4.8	9.7	2.8	5	8.8	5.2	3.4
	30 (1)	16	58.3	72.5	5.5	4	9.1	3.4	5.4	7.9	5.3	3.9
Nanorana vicina	25 (7)	9.3 ± 1.2	29.3 ± 4.1	68.4 ± 1.2	2.8 ± 0.6	2.1 ± 0.3	5.4 ± 0.6	2 ± 0.3	2.9 ± 0.6	5.1 ± 0.5	2.5 ± 0.3	1.6 ± 0.4
	26 (1)	9.6	30.1	68.1	2.6	2.3	6	2.6	3.6	5.7	3	1.8
	27 (2)	10.4 ± 3.8	33.3 ± 18.7	68.7 ± 6.2	3.1 ± 0	2.4 ± 5.6	6.1 ± 3.2	2.4 ± 1.9	3.4 ± 2.9	5.7 ± 3.6	2.9 ± 1.2	1.6 ± 1
	28 (3)	12.6 ± 7.6	43.2 ± 30.4	70.5 ± 3.1	4.8 ± 5.3	3.8 ± 4.2	7.8 ± 6.8	3.2 ± 1.9	4.5 ± 3.2	7.2 ± 5.6	3.9 ± 2.2	2.5 ± 3
	35 (1)	13.6	43.7	68.8	3.3	2.6	6.6	3.3	4.8	7	4.3	1.8
	36 (1)	16.9	50.9	66.7	4.4	4.5	7.3	2.9	4.2	7.8	4.3	1.9
Unidentified	25 (9)	7.4 ± 0.6	23 ± 1.6	67.7 ± 1.2	2.1 ± 0.4	1.6 ± 0.2	4.4 ± 0.4	1.5 ± 0.3	2.1 ± 0.3	4.2 ± 0.4	2.2 ± 0.2	1.2 ± 0.2

Table 13 Summary of diurnal tadpole density (sq. m.) across months. Values in 95% confidence interval. N indicates the number of grids

Month	Session	N	Mean ± CI	Min.	Max.	SD
January	Day	31	1 ± 1.4	0	20	3.7
	Night	29	25.1 ± 11.3	0	92	29.7
February	Day	59	6.3 ± 3.5	0	60	13.3
	Night	63	19.2 ± 4.5	0	64	17.7
March	Day	78	14 ± 5.1	0	104	22.8
	Night	78	15.7 ± 3.5	0	56	15.5
April	Day	77	14.3 ± 3.7	0	56	16.1
	Night	77	15 ± 2.7	0	52	12.1

Table 14 All models used for model selection with estimates (beta coefficients) for each term present in the model; blank cells indicate that term was not included in the model. All models include a random effect of (1 + Winter | Pool) and the zero-inflation formula. Abbreviations: Water Temperature (Wt), Cover Item Richness (Cr), Mean Velocity (Mv), Humus Depth (Hd), Leaf Litter Depth (Ld), Mean DO level (Do), Modification - Check Dam (Md), Mean Canopy Cover Percentage (Cc), Night (Nt), Winter (Wr), Substrate Richness (Sr), Mean Depth (Dp).

Model Name	(Int)	Cc	Cr	Ld	Do	Md	Nt	Sr	Mv	Wr	Wt	Cc:Nt	Ld:Mv	Do:Wr	Nt:Wt	Wr:Wt	Nt:Wr	Nt:Wr:Wt	Hd	Dp	Dp:Nt	Cr:Md	Do:Nt	Dp:Wt	Cc:Wt	Md:Nt	df	logLik	AICc	ΔAICc	weight
22	10.5	-0.1	-0.1	-4.2		0.1	0.3		-17.2		-0.6														0	-0.5	18	-1450.2	2937.9	0	0.7
23	10	-0.1	-0.1	-4.7	-0.1	0	0.4		-17.4		-0.5				0										0	-0.5	20	-1449.6	2940.9	3	0.2
20	9.7	-0.1	-0.1	-4.5		-0.2	0.1		-17.3		-0.5														0		17	-1454.5	2944.2	6.3	0
21	9.4	-0.1	-0.1	-4.5		-0.1	0.6		-17.3		-0.5				-0.1										0		18	-1453.8	2945.1	7.2	0
19	9	-0.1	-0.1	-4.9	0	-0.2	0.6		-17.5		-0.4				0										0		19	-1453.4	2946.4	8.4	0
18	9.1	-0.1	-0.1	-4.9	-0.1	-0.2	-0.1		-17.5		-0.4				0								0.1		0		20	-1452.8	2947.3	9.4	0
17	5.2	0	-0.1	-4.9	-0.1	-0.2	-0.6		-17.7		0												0.1				18	-1455	2947.5	9.6	0
14	3.7		-0.1	-4.8	-0.1	-0.1	0.6		-17.5	-0.5	0			0.1	-0.1												19	-1454.4	2948.4	10.4	0
16	4.3	0	-0.1	-4.8	-0.1	-0.2	1		-17.7		0	0															18	-1455.8	2949	11.1	0
10	5.3	0	-0.1	-5.1	-0.1	-0.2	0.3		-17.7	-2.7	0			0.1	0	0.2	1.8	-0.1		0.1							24	-1449.7	2950	12.1	0
15	4.6	0	-0.1	-4.8	-0.1	-0.2	0.6	0	-17.5		0				-0.1												19	-1455.3	2950.3	12.4	0
13	5.1	0	-0.1	-5.4	-0.1	0.1	0.9		-17.4	-1	0			0.2	-0.1					0.2							21	-1453.2	2950.4	12.5	0
12	5.7	0	-0.1	-5.6	-0.1		0.3		-17.7	-2.8	-0.1			0.1	0	0.2	2	-0.1		-1.4			0	0.1			25	-1449.1	2951	13	0
3	5	0	-0.1	-5	-0.1	-0.2	0.8	0	-17.7	-2.7	0	0		0.1	0	0.2	1.9	-0.1									25	-1449.6	2952	14	0
11	5.3	0	-0.1	-5.1	-0.1	-0.2	0.6		-17.7	-2.8	0			0.1	-0.1	0.2	1.8	-0.1		0.1			0				25	-1449.7	2952.1	14.2	0
2	4.6	0	-0.1	-5.1	-0.1	-0.1	1.6	0	-17.5	-1	0	0		0.1	-0.1	0.1											23	-1452.7	2953.7	15.8	0
1	4.6	0	-0.1	-5	-0.1	-0.1	1.6	0	-21.6	-1	0	0	1047.5	0.1	-0.1	0.1											24	-1451.6	2953.8	15.8	0
5	4.9	0	-0.1	-5.1	-0.1	-0.1	0.9		-17.7	-2.6	0	0		0.1	0	0.2	1.8	-0.1		0.3	-0.3						26	-1449.5	2954	16.1	0

4	4.9	0	-0.1	-5	-0.1	-0.2	0.8	0	-17.7	-2.7	0	0		0.1	0	0.2	1.9	-0.1	0.4								26	-1449.6	2954.2	16.2	0
9	5.1	0		-5.7	-0.1	-0.2	0.6	0	-17.8	-2.7	0			0.1	-0.1	0.2	1.5	-0.1		0.3	-0.4						25	-1451.3	2955.5	17.5	0
6	4.9	0		-5.7	-0.1	-0.1	0.9		-17.8	-2.7	0	0		0.1	-0.1	0.2	1.6	-0.1		0.3	-0.4						25	-1451.4	2955.7	17.7	0
7	4.9	0	-0.1	-5.1	-0.1	-0.1	0.9		-17.7	-2.6	0	0		0.1	0	0.2	1.8	-0.1		0.3	-0.3	0					27	-1449.4	2956.1	18.2	0
8	4.9	0		-5.8	-0.1	-0.1	0.9	0	-17.8	-2.7	0	0		0.1	-0.1	0.2	1.6	-0.1		0.3	-0.4						26	-1451.3	2957.6	19.7	0