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Survival at the Summits: Amphibian Responses to Thermal Extremes, Disease, and Rapid Climate Change in the High Tropical Andes

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

SURVIVAL AT THE SUMMITS: AMPHIBIAN RESPONSES TO THERMAL EXTREMES, DISEASE, AND RAPID CLIMATE CHANGE IN THE HIGH TROPICAL ANDES

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

BIOLOGY

by

Kelsey E. Reider

2018

To: Dean Michael R. Heithaus
College of Arts, Sciences and Education

This dissertation, written by Kelsey E. Reider, and entitled Survival at the Summits: Amphibian Responses to Thermal Extremes, Disease, and Rapid Climate Change in the High Tropical Andes, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Steven F. Oberbauer

Maureen A. Donnelly, Major Professor

Date of Defense: September 27, 2018

The dissertation of Kelsey E. Reider is approved.

Dean Michael R. Heithaus
College of Arts, Sciences and Education

Andrés G. Gil
Vice President for Research and Economic Development
and Dean of the University Graduate School

Florida International University, 2018

DEDICATION

To my parents, Ed and Carolyn Reider.

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ABSTRACT OF THE DISSERTATION

SURVIVAL AT THE SUMMITS: AMPHIBIAN RESPONSES TO THERMAL EXTREMES, DISEASE, AND RAPID CLIMATE CHANGE IN THE HIGH TROPICAL ANDES

by

Kelsey E. Reider

Florida International University, 2018

Miami, Florida

Professor Maureen A. Donnelly, Major Professor

Understanding biological responses to climate change is a primary concern in conservation biology. Of the ecosystems being rapidly impacted by climate change, those in the high-elevation tropics are among the most poorly studied. The tropical Andean biosphere includes record elevations above 5000 meters, where extreme environmental conditions challenge many organisms. In the Cordillera Vilcanota of southern Peru, frogs including *Pleurodema marmoratum* and *Telmatobius marmoratus* have expanded their ranges to 5244–5400 m into habitats created by glacial recession, making them among the highest recorded amphibians on Earth. To understand how hydrologic alterations from loss of glacial meltwater and climatic fluctuations affect these amphibians, I conduct a 36-month field study of reproductive phenology and develop a method to distinguish glacial meltwater-fed ponds and precipitation-fed ponds utilizing natural variation in stable isotopes of water (^{18}O, ^{2}H, and d-excess). My results suggest that some ponds critical for breeding populations may have lost their connection to glacial runoff. Ongoing deglaciation may transform these ponds from permanent to ephemeral habitats, leading to the extirpation of the fully aquatic species, *T. marmoratus*. The 2015/2016 El Niño delayed the onset of the 2015 wet season and shortened the *P. marmoratum* breeding and tadpole development period in ephemeral ponds. I examine regional patterns of amphibian occupancy and prevalence of the deadly amphibian pathogen *Batrachochytrium dendrobaditis* in unexplored high-elevation zones that were until recent decades covered by permanent ice. Next, I examine adaptive strategies that allow these two frog species to persist in the harsh high-elevation environment. *Pleurodema marmoratum* withstands the daily freeze-thaw cycle by utilizing a wide thermal tolerance range (from below 0ºC to $CT_{max} > 32$ºC) and I report the first evidence of frost tolerance in a tropical frog. My research compares divergent strategies allowing two anuran species to persist through disease and variable, extreme conditions in high-mountain environments, providing a better understanding of responses to and consequences of climate change for some of the world's highest life forms.

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INTRODUCTION

Documenting the effects of global climate change and predicting what will happen to species as a result is one of the most pressing topics in ecology. Climate change in high elevation Andean ecosystems is already having potent effects on organisms and ecosystem function. Increasing temperatures, changing precipitation patterns, and hydrologic alterations are main concerns for species that live in high mountain environments, including humans.

High-elevations are particularly vulnerable to climate change because they are warming more rapidly than low elevations (Bradley et al. 2006, Pepin et al. 2015). Maximum and minimum air temperatures are increasing (+0.11°C / decade over the past 60 years), a trend that has intensified in the past 25 years (+0.34°C / decade; Vuille and Bradley 2000, Vuille et al. 2008, Marengo et al. 2011). Precipitation patterns from regional climate models show areas of increased and decreased precipitation for the region (Urrutia and Vuille 2009). Changes to patterns are less well understood and vary within different regions of the tropical Andes (Perry et al. 2014, 2017). However, general patterns include the shortening and intensification of wet seasons, while dry season drought lasts longer than they did previously (Kharin et al. 2007, O'Gorman 2012). Strong positive phases of ENSO have severe impacts on both temperature and the timing of onset and duration of the wet season.

One important impact of climate change that has the capacity to alter drastically many high elevation ecosystems is the rapid shrinking of glaciers, which creates new terrain for many species to colonize but results in the reduction of meltwater inflow to many aquatic habitats. More than 99% of all tropical glaciers are located in the Andes of

Peru, Ecuador, Venezuela, Colombia, Chile, and Bolivia and 70% of all tropical glaciers are located in Peru (Kaser 1999). Peru has the most thoroughly-studied glaciers in the tropical Andes (Vuille et al. 2008). Because temperature variation is low in the tropics, the Equilibrium Line Altitude (ELA; the altitude above which accumulation outpaces ablation) remains at a fairly constant altitude through the year. However, the ELAs of glaciers throughout the tropical Andes have been rising in elevation in recent decades, some by as much as 300 meters (reviewed by Vuille et al. 2008). Glaciers throughout the tropical Andes are retreating because of increased temperatures (see Marengo et al. 2011 for a review). Tropical glaciers are particularly sensitive to small temperature changes compared to temperate glaciers because glaciers in the tropics exist close to melting conditions year-round and accumulation can only occur during the wet season (Thompson et al. 2011).

Glaciers act as a vast store of water, which is slowly released during the months when there is no precipitation. Unlike temperate glaciers, where accumulation occurs in winter and ablation in summer, glaciers in the tropical Andes experience ablation and accumulation simultaneously throughout the year, and thus contribute to water runoff all year. Because precipitation is seasonal, the relative contribution of glacial meltwater is at its maximum during the dry season. As glaciers retreat, they initially increase in runoff as they lose mass (Mark and McKenzie 2007) until they reach a stage known as peak water, which is followed by a decrease in meltwater runoff (Huss et al. 2008). Glacially-fed waters, including streams, wetlands, ponds, and lakes, thus stand to be severely affected by glacier retreat and disappearance. The impacts of losing glacial meltwater go far beyond the highest mountain zones to adjacent mid-elevations and lowlands that, in the

Andes, tend to support large human populations and enormous biodiversity (Anderson et al. 2011, Buytaert et al. 2017, Huss et al. 2017).

Meltwater from glaciers increase the availability and size of wetlands surrounding glaciers, and they feed high mountain lakes and streams as they melt. As glaciers retreat and disappear, abrupt changes occur in surface hydrology upon which aquatic assemblages depend (Seimon et al. 2007, 2017; Jacobsen et al. 2012; Polk et al. 2017). High-Andean aquatic ecosystems are likely among the most vulnerable to climate change (Maldonado et al. 2011). In addition to hydrologic alterations associated with glacier recession, climate change causes increases in temperature that will affect metabolic rates, life cycles, community assembly, organic matter processing, and primary productivity of organisms (Jacobsen 2008). The elevational ranges of cold-adapted aquatic organisms could be restricted from below, while other species could expand their ranges upslope to seek thermal refugia in response to rising temperatures. Range shifts, both upward and downward, are one of the primary expected responses of wildlife to climate change (Chen et al. 2011). Upslope shifts may expose species to novel thermal regimes, and understanding the thermal tolerances of species at the expanding edge of their ranges are critical for understanding the limits of upslope range expansions. As organisms respond to climate change, they must migrate to different elevations or latitudes, adapt to the new conditions, or perish (Aitken et al. 2008).

The Cordillera Vilcanota, a principal mountain range in the central Andes of Peru, is the focus of my dissertation. The Cordillera Vilcanota is the second-most heavily glaciated range in the tropics. Pioneering research on the Quelccaya Ice Cap in the eastern Vilcanota yielded the first and longest annually resolved ice core paleoclimate records in the tropics, leading to a paradigm shift in our understanding of climate variability in the Neotropics (Thompson 1980; Thompson et al. 1984, 1986, 2006). Since that early groundbreaking work, the Vilcanota has become the epicenter of decades of research efforts focused on understanding the glaciological, ecological, and social consequences of climate change (Seimon et al. 2005, 2007, 2017; Halloy et al. 2006; Nemergut et al. 2007; Buffen et al. 2009; Salzmann et al. 2013; Hanshaw and Bookhagen 2014; Buytaert et al. 2017; Perry et al. 2014, 2017; Darcy et al. 2018; and many others).

In the Cordillera Vilcanota, maximum temperatures are increasing at rates comparable to an isothermal elevation increase of 7-10 m/year (+0.07ºC/year, Seimon et al. 2017). Ice lost since 1985 represents 30% of glacial cover by area (i.e., from 440 km^2 in 1962 to 297 km^2 in 2006) and 45% of ice volume (Salzmann et al. 2013). Glacially-fed lakes are increasing in area suggesting that increasing glacial meltwater is temporarily enlarging their boundaries. In contrast, lakes formerly downstream from glaciers that have already disappeared are shrinking, presumably because of continued glacial retreat (Hanshaw and Bookhagen 2014). A new ice-free pass bisects the core glaciated areas of the Vilcanota, and first became ice-free around 1980 (Seimon et al. 2007). The ice-free area is currently widening at 18.4 m/yr^{-1}, a rate that has increased in the last decade (Seimon et al. 2007, 2017). The new pass acts as a corridor, permitting access, possibly for the first time in thousands of years, from the northern Amazonian slope to the high-Andean plain on the southern side of the range to wildlife and to humans, including tourists and those seeking mineral deposits in the newly exposed rocks.

Ongoing glacier recession has facilitated the expansion of the biosphere to global elevation records in the Cordillera Vilcanota. Amphibians, along with the pathogenic chytrid fungus (*Batrachochytrium dendobaditis, Bd*), are now found from 5240–5400 m in recently deglaciated terrain (Seimon et al. 2007). Three species of frogs are known to occur at or above 5000 m in the Vilcanota: *Rhinella spinulosa* has been reported from 290–5244 m, *Telmatobius marmoratus* occurs from 1800–5244 m, and *Pleurodema marmoratum* has been recorded from 3200–5400 m (Seimon et al. 2007, Correa et al. 2010, IUCN Red List 2017). At such elevations in the tropical Andes, atmospheric pressure is just 50% of that at sea level, surface temperatures oscillate from winter to summer conditions on a daily basis, and animals and plants must develop behavioral and physiological adaptations to the extreme conditions. In my dissertation, I investigate amphibian responses to rapid deglaciation and adaptations to the extreme environment at the upper limits of the tropical Andean biosphere.

In Chapter 1, I establish the usefulness of the stable isotopes of water ($\delta^{18}O$ and $\delta^{2}H$) in understanding glacial meltwater contributions to surface waters in the high Andes. Despite the ubiquitous use of water stable isotopes in ice cores as indicators of past climates, measurements of stable isotopes in lakes and ponds in tropical high mountain environments remain scarce. I provide some of the first records of stable isotope composition of ponds, precipitation, glacial meltwater, snowpack, and shallow groundwater from the southern Andes of Peru, and provides a needed baseline for monitoring hydrological processes in the rapidly warming high Andes.

In Chapter 2, I examine the effects of climate change and deglaciation on two spatial scales. At a regional scale, I report on patterns of anuran occupancy in ponds and wetlands in the recently deglaciated zone of several previously unexplored valleys. At a local scale, I studied breeding phenology of two anurans with contrasting life histories in the recently deglaciated zone between 5240-5400 m. I employed the stable isotope framework developed in Chapter 1 to infer information about water sources to amphibian breeding habitat. Chapter 2 also examines the impact of a strong El Niño on amphibian habitat availability and reproduction.

In Chapter 3, I explore adaptations of *Pleurodema marmoratum*, a global elevation record holding amphibian in the Cordillera Vilcanota, to the extreme environmental conditions above 5000 m. I establish the thermal tolerance range of *P. marmoratum* and test the species for frost tolerance. I compare the thermal environment experienced by *P. marmoratum* to that of *T. marmoratus*, because although these anurans live in the same biome and at the same elevations, they live in very different microhabitats characterized by contrasting thermal regimes.

In Chapter 4, I investigate post-epizootic host-pathogen dynamics in *P. marmoratum* and *T. marmoratus* at the edge of the biosphere and the upper limits of known *Bd* pathogenicity. I study the seasonal and ontogenetic patterns of *Bd* dynamics intensively at a local scale over two hydrologic years, and survey *Bd* prevalence in amphibian populations inhabiting new ice-free locations throughout the Vilcanota.

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Chapter I. Water stable isotope framework for monitoring surface waters in the rapidly deglaciating high Andes

ABSTRACT

Stable isotopes of water ($\delta^{18}O$ and $\delta^{2}H$) are useful tools for understanding past climates, as well as for investigating hydrologic and climatic variability in the present. Since the first tropical ice cores were collected from the Quelccaya Ice Cap in the Cordillera Vilcanota, southern Peru, a great deal of information has been generated on the isotopic composition of precipitation and ice in the central Andes. However, measurements of stable isotopes in surface waters in tropical high mountain environments remain scarce. We examined the stable isotope composition of ponds, precipitation and glacial meltwater at high elevations in the Peruvian Andes to examine how $\delta^{18}O$ and $\delta^{2}H$ stable isotope values and d-excess vary in space and through time. We found a $\delta^{18}O$ – $\delta^{2}H$ relationship in precipitation that supports previous findings from the Central Andes. We document ^{18}O enrichment of 5.6‰ in snowpack in the dry season compared to the wet season. We found that precipitation from the 2015-2016 El Niño was enriched in heavy isotopes by 4.7‰ for $\delta^{18}O$ and 36.1‰ for $\delta^{2}H$. Surface ponds were strongly affected by evaporative enrichment in the dry season. Some semi-permanent ponds remain depleted in ^{18}O in the dry season, indicating an incoming source of ^{18}O-depleted water. Since the primary source of isotopically depleted water is glacial meltwater, these ponds will likely reduce in size or disappear as glacial retreat continues, thereby threatening the

sustainability of the biological communities that depend on them. Our study provides some of the first records of stable isotope composition of ponds, precipitation, glacial meltwater, snowpack, and shallow groundwater from the southern Andes of Peru, and provides a needed baseline for monitoring hydrological processes in the rapidly warming high Andes.

INTRODUCTION

The rapid melting of glaciers on a worldwide scale is one of the most significant impacts of the warming global climate (Medwedeff and Roe 2017). High elevations are particularly vulnerable because they are warming more rapidly than low elevations (Bradley et al. 2006, Pepin et al. 2015). The tropical Andes are subject to ongoing warming and the associated loss of freshwater reserves in glacial ice (Barnett et al. 2005, Bradley et al. 2006, Viviroli and Weingartner 2008, Viviroli et al. 2011). Glaciers in the tropical Andes are a critical water resource and contribute to water runoff all year. Alterations to water resources, driven by climate change, have been recognized as a serious threat to human populations and ecosystems in the Andes (Barry and Seimon 2000, Anderson et al. 2011, Bury et al. 2011). The relative contribution of glacial meltwater is outsized during the dry season and during drought because of the strong seasonality of precipitation delivery (Mark et al. 2005, Buytaert et al. 2017). As glaciers retreat, they initially increase in runoff as they lose mass until they reach their maximum contribution, followed by an abrupt decrease in meltwater inputs to downstream areas (Braun et al. 2000, Nolin et al. 2010, Baraer et al. 2012, Carey et al. 2014). Glacially fed waters, including streams, wetlands, ponds, and lakes, thus stand to be severely affected by glacier retreat and disappearance.

Air temperatures are increasing across the high-elevation zones of the tropical Andes (+0.11°C / decade over the past 60 years), a trend that has intensified in the past 25 years (+0.34°C / decade; Vuille and Bradley 2000, Vuille et al. 2008, Marengo et al. 2011). Tropical glaciers are highly sensitive to small temperature changes compared to temperate glaciers because glaciers in the tropics can experience melting conditions year-round. Within the tropics, there are distinctive patterns in the cycles of accumulation and ablation. Whereas the humid inner tropics (i.e., Ecuador, Colombia) remain wet year-round and experience simultaneous accumulation and ablation of glacial ice throughout the year, the outer tropics (i.e., Peru and Bolivia) have a pronounced annual dry season (Kaser 2001). Although ablation occurs year-round, accumulation occurs only during the wet season (Kaser and Osmaston 2002). The strong seasonality of accumulation in the outer tropics means that very small changes in air temperature and freezing level altitude can cause large-scale changes in glacial cover (Thompson et al. 2011).

Stable isotope ratios of water (δ^2H and $\delta^{18}O$) can reveal much about the history of different water sources because they fractionate systematically as a result of phase changes in the hydrological cycle (e.g., condensation, melting, and evaporation; Gat 1980, Kendall and Caldwell 1998). Evaporation is likely a critical process affecting high-elevation water balance and the interpretation of paleo-isotopic records derived from alpine lake sediment cores. The isotopic signal of evaporation in water stable isotopes (i.e., deuterium excess; hereafter "d-excess") is a result of differences in the fractionation rates for ^{18}O and ^{16}O in evaporating waters. In tropical climatology, considerable importance is placed on paleoclimate inferences drawn from stable isotope values in past precipitation preserved in and extracted from glaciers in the southern Peruvian Andes (e.g., Quelccaya Ice Cap in the Cordillera Vilcanota). The ~1800-year annually resolved records in ice cores from Quelccaya challenged the prevailing view that tropical climate has been historically stable and unchanging (Thompson et al. 1984) and are among the most important proxy records for climate in the late Holocene (Thompson et al. 2013). Initially, the ratios of ^{18}O to ^{16}O (the stable isotopes of oxygen) in the Quelccaya ice cores were interpreted as a proxy for temperature (Thompson et al. 1986). Although the ^{18}O ratio preserved in polar ice seems to serve as a reliable record of past temperatures, in tropical ice cores other precipitation processes (e.g., vapor history, cloud type, and post-depositional change) are the central determinants of stable isotope ratios (Vimeux et al. 2009, 2011; Samuels-Crow et al. 2014). Atmospheric processes that affect precipitation isotope ratios in the tropical Andes include intensity of El Niño Southern Oscillation (ENSO; Thompson et al. 1984, Vuille and Werner 2005), seasonal differences caused by the South American Summer Monsoon (SASM; Hurley et al. 2015), and air masses originating from different sources (i.e., the Amazon, the Pacific Ocean, and from high-latitude South America). Advances in the understanding of precipitation processes and stable isotope variation in the Vilcanota have improved interpretations of the Quelccaya paleoclimate records (Hurley et al. 2015, 2016).

Precipitation is the key freshwater input to mountain hydrological systems. Historically, tropical precipitation is assumed to be convective and occur during the afternoon. Recent research in the southern Andes suggests an important role of nocturnal stratiform precipitation (Perry et al. 2014), which likely has different isotopic composition compared to convective precipitation (Aggarwal et al. 2016). In addition, strong El Niño years receive higher-than-average precipitation throughout the hydrological year in the Cordillera Vilcanota whereas La Niña is associated with negative precipitation anomalies (Perry et al. 2014, 2017). The positive precipitation anomalies in the Vilcanota during El Niño years contrasts with the expected pattern throughout the Central Andes, where El Niño is associated with a dry phase and La Niña expected to be the wet phase (Vuille 1999). Although an increasing amount of information is available for stable isotope trends in precipitation from the central Andes, local variation in stable isotope ratios is important. For example, stable isotope research in the Bolivian Altiplano revealed significant unexplained local variation in $\delta^{18}O$ among meteorological stations (Fiorella et al. 2015).

Much of the information about the stable isotope compositions of modern Andean precipitation has been drawn from regional climatological inferences that may not adequately exemplify the patterns present in the Cordillera Vilcanota. Until recently, stable isotope studies from waters in the high Andes have concentrated almost entirely on northern Peru (focused on the Cordillera Blanca) and northern Bolivia (especially in the Cordillera Real). Stable isotope techniques in the tropical Andes have been utilized to characterize contributions of different sources of runoff (Mark and Seltzer 2003, Baraer et al. 2009) and document changes to the hydrological cycle caused by melting glaciers (Mark and McKenzie et al. 2007).

Lakes, ponds, and streams, in particular, play important roles in the hydrology and ecology of montane regions (Anderson et al. 2011). Lakes in glacierized regions are especially important sentinels of climate change because they integrate many important processes such as changes in glacial meltwater input, precipitation patterns, temperature, and evaporation, and are vital resources supporting biodiversity and human populations at high elevations (Shi et al. 2017). Measurements of $\delta^{18}O$, δD, and d-excess values of surface lake water are lacking from the high Andes, restricting our ability to understand potential changes to local and regional water resources under changing glacier cover and precipitation patterns. Stable isotope studies that consider the broad range of surface waters (e.g., lakes and ponds, glacial meltwater) are necessary to understand the hydrological cycle and determine future water resource limitations. The loss of glacial meltwater from continued deglaciation is considered a threat to water resources, alpine biodiversity and ecosystem services (Brown et al. 2007, Brown and Milner 2012, Jacobsen et al. 2012). Stable isotope data from the Cordillera Vilcanota remain scarce, despite the clear utility of stable isotope approaches and the urgent need for information about water resources in the rapidly changing Andean environment.

The Cordillera Vilcanota has become a natural laboratory for the study of rapid deglaciation, including studies of paleoclimate reconstructions, precipitation delivery, and glaciological and ecological responses to climate change (Thompson et al. 2006, Nemergut et al. 2007, Seimon et al. 2007, 2017; Perry et al. 2017, Darcy et al. 2018). We examined, for the first time, variability in the stable isotope composition of ponds, precipitation and other source waters occurring in a recently deglaciated ice-free pass in the proglacial zone at high elevation in the Cordillera Vilcanota. Our primary goal was to examine how $\delta^{18}O$ and $\delta^{2}H$ stable isotope and d-excess values vary among different water types and between the wet and dry seasons. Our study provides the first record of stable isotope composition of ponds, precipitation, glacial meltwater, snowpack, and shallow groundwater from the southern Peruvian Andes and provides a critical baseline for identifying changes in stable isotope composition of high-elevation lakes.

METHODS

Study Site

The Cordillera Vilcanota is located in the outer tropical Andes at approximately 13.85°S, 71.05°W, in the Peruvian departments of Cusco and Puno (Fig. 1). The Cordillera Vilcanota is the second most heavily glaciated range in Peru (ANA 2014) and is home to the largest tropical glacier on Earth, the Quelccaya ice cap (Albert et al. 2014). Glaciers in Peru are shrinking rapidly as atmospheric temperature and the freezing level altitude increase (Thompson et al. 2006, Vuille et al., 2008; Bradley et al. 2009, Rabatel et al., 2013; Salzmann et al., 2013, Albert et al. 2014, Hanshaw and Bookhagen 2014). In the Cordillera Vilcanota, the ice lost since 1985 represents 30% of glacial cover by area and 45% of ice volume (Salzmann et al. 2013). Glacially fed lakes are increasing in area, whereas lakes that have lost their connection to glacial meltwater are shrinking, presumably because of continued glacial retreat (Hanshaw and Bookhagen 2014). Our study focused on surface waters in a recently deglaciated ice-free pass in the Cordillera Vilcanota between the large, high-elevation glacially fed lakes Singrinacocha and Sibinacocha. The new ice-free pass bisects the core glaciated areas and first became ice-free around 1980 (Seimon et al. 2007). The ice-free area is currently widening at 18.4 m/yr^{-1}, a rate that has increased in the last decade (Seimon et al. 2007, 2017).

Above 5000 meters above sea level (masl) in the Vilcanota, precipitation peaks in the early afternoon and at night (Perry et al. 2014, 2017). Afternoon precipitation is primarily convective moisture from the Amazon, whereas moisture from the Amazon Basin and moisture originating from other sources, including the Pacific Ocean, contribute to nighttime stratiform precipitation (Perry et al. 2017). The wet season lasts from October to April, with a dry season from May to September (Perry et al. 2017). We classified samples collected during October to April as wet season samples, and samples collected from May to September as dry season samples. More than half of wet season precipitation occurs during December – February, and only 1-2% of annual precipitation falls during the core dry season from June – August (Perry et al. 2014). Above 5000 masl, precipitation falls primarily as snow or graupel, rime-covered snow (Perry et al. 2017).

Although there has been extensive paleoclimate research in the Cordillera Vilcanota, there are no long-term weather records. We determined precipitation patterns during the study period using data available from the National Weather Service of Peru (SENAMHI) meteorological stations located in the region (Appendix 1.1). We analyzed average monthly precipitation using data from the three stations with the most complete records from 2012–2017: Ccatcca (55 km NW at 3730 masl), Sicuani (56 km SSW, 3570 masl), and Macusani (77 km SE, 4350 m). We determined cumulative daily precipitation using data from four SENAMHI stations with 21-year records available: Ccatcca, Pomacanchi (60 km SW, 3200 masl), Santa Rosa (100 km SSE, 3990 masl), and Progreso (150 km SE, 3980 masl). The weather station closest to our study area is operated by Appalachian State University at Murmurani Alto (5050 masl, 5 km from and 190 vertical m below our precipitation stable isotope collecting point; Appendix 1.1). Annual precipitation at the Murmurani Alto weather station was 623 mm in 2012 –2013, 749 mm in 2013 –2014, and 732 mm in 2014 –2015 (Poremba et al. 2015). The average annual precipitation and cumulative precipitation is comparable between the SENAMHI weather station data used in the present study and the Murmurani Alto weather station. Despite the distance and difference in elevation we believe the SENAMHI stations are representative of precipitation patterns in the Cordillera Vilcanota region.

Field Sampling

Monthly mean, maximum, and minimum shaded surface temperatures were determined from the mean of two iButton thermochrons logging every 30 minutes from August 2013 to June 2016. There are a few gaps in the record. The iButtons were suspended 1 cm above the surface inside a rock cairn located at the study site (5240 masl).

We collected syntopic water samples over three days in December 2013 and February 2014 (wet season), and May and June 2014 (dry season) for analysis of stable isotopes of oxygen and hydrogen. We collected water samples from precipitation, glacial melt, and surface lakes and ponds located within the new ice-free corridor that bisects the Cordillera Vilcanota. Most ponds, 19 of 20, were located between 5240–5400 masl. We sampled one spring and one pond at a lower elevation (5000 masl). We collected additional samples from springs and snowpack. Glacier melt samples were from surface waters draining directly from the Osjollo Anante glacier. We collected snowpack samples from the top of easily accessible portions of the glacier. Snowpack samples were collected with a trowel from the full depth of accumulated snow (ranging from 10–80 cm). Shallow spring samples were collected where water emerged from the ground near ponds. We collected grab samples of water from semi-permanent and temporary ponds at varying distances from the glaciers. Water samples were collected in new or acid-washed high-density polyethylene 30 ml Nalgene bottles. Precipitation samples were collected opportunistically from the study site from July 2013 – June 2016 at 5235 masl. Precipitation samples were collected by setting out a flat collecting plate at 7 am, and then sampling from the full accumulation depth of the snow at 7am, prior to the initiation of melting. Snow depth for a subset of precipitation samples were measured on the plate and used to calculate amount-weighted isotope values.

Water samples were filtered through 0.45 μm syringe filters. We rinsed bottles and syringes with sample water prior to filling. All water sample bottles and vials were filled completely to minimize headspace. Sample lids were tightly sealed, and caps were wrapped with Parafilm (Bernis NA, Wisconsin, USA) and electrical tape to prevent evaporation. After collection, water samples were transported by foot, horse, truck, and airplane, and stored in a dark, cool location until they were analyzed.

Laboratory Analyses

All isotope results are reported in delta (δ) notation in per mil units (‰) calculated as:

$$\delta = \left(\frac{\mathrm{R_{sample}}}{\mathrm{R_{standard}}} - 1\right) \times 1000 \quad (\mathrm{Eq.\,1})$$

where R is the ratio of ^{2}H to ^{1}H atoms or ^{18}O to ^{16}O atoms in the sample and the standard is Vienna Standard Mean Ocean Water (VSMOW). We used precipitation samples (N = 25) with standardized depth measurements to calculate an amount-weighted precipitation value according to

$$\delta_{\mathrm{W}} = \frac{\sum_{i=1}^{n} P_i \delta_i}{\sum_{i=1}^{n} P_i} \quad (\mathrm{Eq.\,2})$$

where P_i and δ_i are daily precipitation depth (in mm) and the $\delta^{18}O$ or $\delta^{2}H$, respectively. Deuterium excess (d-excess or 'd') of each water sample was estimated using

$$d = \delta D - 8\delta^{18}O \quad (Eq. 3)$$

following Dansgaard (1964). In terrestrial environments, d-excess values <10 are presumed to have undergone some evaporation. For lakes and ponds, low d-excess values indicate an increasing role of evaporation in water balance (Brooks et al. 2014).

Water samples were analyzed for stable isotopes of oxygen and hydrogen on a Los Gatos Liquid Water Isotope Analyzer (LWIA) at Florida International University. A sequential series of two internal laboratory standards (LGR–2C and LGR–4C) and two international standards (GISP and SLAP2) were analyzed between each sample to assess the accuracy and precision of measurements. The agreement between measured and expected values of standards are available in Appendix 1.2.The average measurement of accuracy was 0.02‰ for $\delta^{18}O$ and 0.01‰ for $\delta^{2}H$, using the absolute value of the difference between the expected and observed values. The average measurement of precision was 0.29‰ for $\delta^{18}O$ and 1.32‰ for $\delta^{2}H$, using the average standard deviation from repeated measurements of each standard.

Statistical Analyses

All statistical analyses were conducted in R version 3.2.1 (R Core Team 2015). We compared $\delta^{18}O$ and $\delta^{2}H$ for each type of water (i.e., precipitation, ponds, snowpack, glacier meltwater, springs) using one-way ANOVA. We compared wet and dry season $\delta^{18}O$ and $\delta^{2}H$ means for each water type, $\delta^{18}O$ and $\delta^{2}H$ means for permanent versus ephemeral ponds, and pond d-excess in the wet and dry season using Welch's t-tests, which account for unequal sample size and variance. We classified strong positive ENSO periods using the Ocean Niño Index (ONI; NOAA ERSST.v5). We compared precipitation samples collected during strong El Niño (i.e., during months with sea surface temperature anomalies > 1.0 in the Niño 3.4 region) and weak or El Niño (i.e., ONI < 1) using Welch's t. We fit linear least-squares regressions using lm() to determine the relationship between δ^{18}O and δ^{2}H in precipitation, to fit local evaporation lines, and to examine the effect of lapse rate on samples collected from ponds and springs.

RESULTS

Annual shaded surface temperature was 1.49° ± 3.8°C (mean ± SD) for the nearly three years of temperature data collected (Fig. 1.2a). Diurnal temperature variation was much greater than was annual temperature variation. Average monthly precipitation was highest in DJF and was lowest in JJA (Fig. 1.2a). Cumulative average precipitation totals were 724 mm in the 2013-2014 hydrologic year, 797 mm in 2014-2015, and 740 mm in 2015-2016. Cumulative precipitation during the three hydrologic years of this study was above the 1996-2017 long-term average of 675 mm (Fig 1.2b).

All precipitation samples collected were in the form of snow or graupel, or a mixture of both. The δ^{18}O and δ^{2}H values of all the precipitation samples plot near or slightly above the Global Meteoric Water Line: $\delta^{2}\mathrm{H} = 8\delta^{18}\mathrm{O} + 10$ (Eq. 4; GMWL; Craig 1961). Linear regression of the precipitation values resulted in a Local Meteoric Water Line (LMWL; Fig. 1.3) defined by the equation:

$$\delta^{2}\mathrm{H} = (8.26 \pm 0.14)\delta^{18}\mathrm{O} + (18.94 \pm 2.42) \quad \mathrm{Adj.}\, R^{2} = 0.99, N = 35 \ (\mathrm{Eq.}\ 5)$$

The δ^{18}O values of all precipitation samples ranged from -26.99‰ to -8.81‰. The δ^{2}H values of all precipitation samples ranged from -208.18‰ to -56.41‰. The amount-weighted precipitation values (± SE) were $\delta^{18}O = -15.89 \pm 0.84‰$ and $\delta^{2}H = -112.65 \pm 7.05‰$.

There were no significant differences in stable isotope signatures for precipitation, ponds, snowpack, glacier meltwater, and springs for $\delta^{18}O$ ($F_{4,143} = 1.42$, $p = 0.23$; Fig. 1.4a) or $\delta^{2}H$ ($F_{4,143} = 0.43$, $p = 0.79$; Table 1.1, Fig. 1.4b). Stable isotope signatures from ponds were significantly more depleted in ^{18}O and ^{2}H in the wet season compared to the dry season ($p < 0.05$; Table 1.2). There were no statistically significant seasonal differences for precipitation, glacial meltwater, springs, or snowpack (Table 1.2). Precipitation during the strong 2015/2016 El Niño was significantly enriched compared to samples collected during weak or neutral ENSO conditions for $\delta^{18}O$ ($t = 2.22$, $df = 29.15$, $p = 0.034$) and marginally non-significant for $\delta^{2}H$ ($t = 2.02$, $df = 29.85$, $p = 0.052$; Fig. 1.5).

Local Evaporation Lines (LELs) for dry season and wet season pond samples are plotted along the LMWL in Fig. 1.6. The average values of weighted precipitation, glacial melt water, shallow groundwater, and snowpack are plotted along the LMWL with the LELs for reference (Fig. 1.6). The LEL for dry season ponds (LEL_{dry}) was $\delta^{2}\mathrm{H} = (4.21 \pm 0.32)\delta^{18}\mathrm{O} - (61.3 \pm 4.50)\ \ R^2 = 0.89, N = 24\ (\mathrm{Eq.}\ 6)$. The LEL_{wet} was defined by: $\delta^{2}\mathrm{H} = (7.26 \pm 0.63)\delta^{18}\mathrm{O} - (3.99 \pm 10.85)\ \ R^2 = 0.81, N = 32\ (\mathrm{Eq.}\ 7)$ Assuming a linear relationship continues beyond the available data, the LEL_{dry} would intersect with the LMWL at (-19.81, -144.63). The intersection point of LEL_{wet} and the LMWL would be (-23.21, -172.67).

Ephemeral and permanent ponds did not differ in δ^{18}O (t = -0.42, df = 47.49, p = 0.68) or δ^{2}H (t = 0.25, df = 51.62, p = 0.80). We found no evidence for the elevation effect for these ponds in the wet season using the elevations of each individual sampling location for δ^{18}O (R^2 = -0.02, F = 0.02, df = 1, 54, p = 0.9) or δ^{2}H (Adj. R^2 = -0.015, F = 0.16, df = 1, 54, p = 0.7). We did not find a significant relationship between average isotopic values of springs in the wet season and average sub-watershed elevation (δ^{18}O R^2 = 0.0095; δ^{2}H R^2 = 0.00003).

Pond d-excess was significantly more negative in the dry season compared to the wet season (t = -5.35, df = 26.84, p = 0.00001), but d-excess values did not differ between the wet and dry season for precipitation, springs, snowpack, or glacial meltwater (Table 1.2, Fig. 1.7a). Nearly all sampled ponds had d-excess values similar to precipitation, glacial meltwater, and springs in the wet season. Many ponds had more negative d-excess values in the dry season (-9.29‰ ± 15.68) compared to the wet season (8.53‰ ± 5.21). However, a subset of ponds maintained high d-excess values in the dry season (Fig. 1.7b). The subset of ponds with high d-excess values in the dry season remained relatively depleted in ^{18}O (Fig. 1.7b) and grouped closer to dry season glacier and spring water than to the average for ponds in the plot of d-excess–δ^{18}O (Fig. 1.7a).

DISCUSSION

This study provides the first comprehensive record of stable isotope composition of ponds, precipitation, glacial meltwater, snowpack, and shallow groundwater from the southern Andes of Peru. The precipitation samples collected at 5230 masl are defined by a local meteoric water line (slope: 8.26, intercept: +18.94) that falls slightly above the

GMWL. The slope > 8 might reflect the temperature of condensation for solid precipitation (Clark and Fritz 2013). The slope is not as steep as it would be if predicted by condensation temperature alone, possibly because of additional fractionation (i.e., evaporation or sublimation) after condensation. Precipitation in the Vilcanota above 5050 masl is predominantly snow and graupel (Perry et al. 2017). The LMWL-Vilcanota is very similar to other tropical Andean MWLs that recorded precipitation or precipitation-derived samples including the Cordillera Blanca in northern Peru (slope: 8.27, intercept: + 16.30; Mark and Seltzer 2003), and the Cordillera Real in northern Bolivia (slope: 8.2, intercept: +14.5; Guido et al. 2016). The Vilcanota MWL is also similar to the results reported for subtropical Andean precipitation in Chile (slope: 8.29, intercept: +11.75; Hoke et al. 2013). Broad interpretations using LMWLs likely disregard important differences in source moisture history and climate characteristics. However, it is interesting that LMWLs for high Andean precipitation are so similar across 23° of latitude and may point to the usefulness of a regional Andean MWL for high elevations.

The average $\delta^{18}O$ value of precipitation was 4.7‰ higher during the 2015/2016 ENSO positive period compared to the ENSO neutral period. Enrichment during ENSO supports previous findings that more positive precipitation $\delta^{18}O$ values are associated with ENSO events in the central Andes (Vuille and Werner 2005). The enrichment in $\delta^{18}O$ is somewhat contradictory if the mechanism is related to the amount effect, because isotopically enriched ^{18}O values during ENSO are expected to coincide with below-average precipitation. However, in contrast to most of the central Andes, the Cordillera Vilcanota receives average or higher-than-average precipitation during ENSO periods, including the 2015/2016 ENSO (Perry et al. 2017). The significant isotopic enrichment during the ENSO period occurred despite local positive precipitation anomalies for the same period, suggesting that regional climatological patterns have a strong influence on ENSO-associated $\delta^{18}O$ values. If local precipitation processes such as the amount effect determined the isotopic composition in precipitation, we would expect to find depleted isotope values rather than enrichment. Our independent confirmation of ENSO-associated enrichment in precipitation stable isotope values is particularly valuable with respect to the interpretation of paleoclimate records that rely on $\delta^{18}O$ preserved in ice cores and alpine lake sediments to reconstruct past climate, including El Niño events. The Quelccaya Ice Cap, located at the eastern edge of the Cordillera Vilcanota, has been a key source of paleoclimate ice records used to reconstruct climate variability and past ENSO events (Thompson et al. 1986, 2006).

Kinetic fractionation by sublimation from the surface snow layer combined with very low precipitation during the dry season is thought to result in ^{18}O enrichment on the nearby Quelccaya Ice Cap (Hurley et al. 2015). Hurley et al. (2015) found post-depositional enrichment of $\delta^{18}O$ from about –20‰ in December-January (wet season) to –15‰ in May-June (dry season). Despite relatively low sampling effort of snowpack from the central Vilcanota, we detected a similar amount of dry-season enrichment in snowpack stable isotopes for the same months. In addition to having implications for the interpretation of stable isotope records preserved in ice cores, this result underscores the importance of collecting snow samples prior to alteration. The strong post-depositional effects on snow isotopic values may be high at elevations above 5000 masl.

Precipitation or shallow groundwater from an elevational gradient can typically be used to examine the elevational lapse rate for isotope values (Gonfiantini et al. 2001, Mark and McKenzie 2007). We did not detect a relationship between groundwater isotope values and elevation, although we focused on a subset of ponds that were geographically isolated (i.e., no surface inflow) and therefore likely to be fed mostly by precipitation or shallow groundwater. Precipitation samples collected along elevational gradients are needed to establish the isotopic lapse rate in the Vilcanota, but we do not expect the lapse rate to differ substantially from other studies that have calculated lapse rates in the Andes of Peru. Lapse rates have been measured as -7‰ per km from shallow groundwater in the Cordillera Negra, a non-glacierized range in northern Peru (Mark and McKenzie 2007), and -2.1‰ per km in the glacierized Cordillera Blanca of northern Peru (Burns 2013). Our study area was confined to a narrow elevational range, from 5230 – 5395 masl (with two outlying sites at 4970 masl). The lack of a strong effect of elevation in our isotope data suggests an important role of processes other than elevational controls on the isotope values of precipitation (e.g., glacial runoff and evaporation).

The wet-season local evaporation line (LEL_{wet}) had a lower intercept than the LMWL, indicating a role of evaporation in the stable isotope concentrations of some ponds. The LEL_{dry} had a much lower slope and intercept than the LEL_{wet} or LMWL. The $\delta^2H – \delta^{18}O$ relationship of ponds indicates that evaporation processes are extremely important in the dry season. We also infer from the distinct d-excess in different ponds that evaporation from pond surfaces was an important factor in determining stable isotope composition. Strong evaporative enrichment indicates that most surface ponds in our study area are closed-basin reservoirs filled primarily by seasonal precipitation, which matches observations that the majority of ponds in our study have no surface outlets. The strong evaporation signal appears to be independent of pond permanence, because we observed a strong evaporation signal for some ponds that remained full for the duration of the present study (July 2013 – June 2016). Only a small number of semi-permanent ponds have isotopic compositions that indicate sustained inflow of ^{18}O-depleted water in the dry season.

The intersection of the local evaporation line and LMWL approximates the average isotopic composition of water inflow to surface lakes (Gibson et al. 2005). The estimated isotopic composition was much more negative than snow, glacial meltwater, or springs sampled during our study. We sampled meltwater primarily at surface flow locations along the Osjollo Anante glacier, which is a large glacier that has undergone rapid retreat since the 1980s leading to the formation of the ponds studied here (Seimon et al. 2007; Albert et al. 2014, but note that Albert refers to the glacier as Japujapu). The most depleted water we sampled was shallow groundwater collected at a spring near a permanent pond. We may not have sampled the full isotopic composition of meltwater inflow that enters ponds through groundwater. Additional evidence suggests our average meltwater value was more positive than the full range of meltwater values: many of our pond water samples were more depleted than both our sampled precipitation or meltwater values. The remaining pond-sample isotopic values were much more positive than sampled precipitation or meltwater, probably because of evaporative enrichment. Additional groundwater sampling may better capture the stable isotope values of glacial meltwater. Other methods allow separation of isotopic composition into source components, e.g., end-member mixing analyses. We were unable to use end-member mixing analyses to estimate the proportion of meltwater contribution from glaciers or precipitation because the potential end members were too isotopically similar.

Seasonal differences in pond $\delta^{18}O$ and d-excess likely are the result of evaporation because there were no statistically significant seasonal differences in $\delta^{18}O$ and d-excess in precipitation, meltwater, springs, or snowpack. The ponds and lakes sampled in our study likely did not receive substantial meteoric waters that had already undergone evaporative isotopic enrichment before entering the catchment. An alternative possibility is that the ponds that appear to be highly sensitive to evaporation by these metrics (which we posit have limited connectivity to glacial meltwater inputs) could have greater evaporation signals than other lakes as a result of their surface area-to-volume ratios irrespective of glacial meltwater inflow (Brooks et al. 2014). However, evidence already exists that glacial lakes in the Cordillera Vilcanota are shrinking in response to losing their connection to glacial meltwater inputs (Hanshaw and Bookhagen 2013).

In the high-elevation environment, the principal dry season input is likely glacial melt or the previous wet season snow accumulation from the top of the glacier, since just 1-2% of annual precipitation falls during the dry season (Perry et al. 2017). Whether the runoff from the glacier is comprised mostly of old ice or old snow (i.e., snowpack), the runoff exists only because of the presence of glacial ice. Away from the glaciers, wet season precipitation melts within hours of falling, or at most within days during the colder months of May–August, because of the high daytime temperatures and solar radiation (personal observation). Our results show that the dry season ponds that likely receive glacial input plot along the LMWL in the depleted $\delta^{18}O$ – $\delta^{2}H$ and $\delta^{18}O$ – d-excess space even during the dry season. If these depleted dry season ponds were cut off from meltwater inputs, they would plot along the oblique LEL_{dry} that is constrained by water samples from ponds that are sensitive to evaporation. In addition, the same ponds have the highest d-excess and the smallest observed difference between their d-excess values in the wet season compared to the dry season, indicating that they are not highly sensitive to evaporation. These data support our interpretation that a small subset of ponds in our study area receive glacial meltwater in the dry season, while others are dependent on strongly seasonal precipitation. Our study demonstrates the strong role of evaporation in tropical high-elevation Andean lakes and provides a strong foundation for future research using the evaporation to inflow ratios to determine water balance for Andean regions undergoing rapid hydrologic alterations under climate change.

Our study suggests that stable isotope techniques can augment inferences from changes observed in plant community composition in high elevation wetlands at some of the ponds and lakes we studied. Previous research has documented the death of perennial cushion plant peatlands and has attributed the plant cover losses to a changing water table as a result of the loss of glacial meltwater inflow (Seimon et al. 2017). Desiccation-tolerant tussock grasses are replacing the cushion plant wetlands (Seimon et al. 2017). Semi-permanent ponds that show strong evaporative enrichment in the dry season will likely experience additional drops in the dry-season water table as glacier retreat continues in the high Andes. Future studies should investigate the fate of biological communities that depend on these rapidly changing high-elevation wetlands. Long-term monitoring of pond stable isotope values may document a change in dry season stable isotope composition from depleted to enriched in continuously isotope depleted ponds, which could be used to document changing contributions of dry season inflow.

The effects of rapid deglaciation on biological communities goes far beyond the Vilcanota. First, water from the Cordillera Vilcanota contribute to two crucial river systems (the Vilcanota–Urubamba–Ucayali and Inambari–Madre de Dios) that provide water resources including hydroelectricity generation and irrigation to a large human population and ecosystem services from the high Andes to the lowland Amazon. Secondly, the effects of reduced water storage as a result of continued deglaciation is occurring throughout the glaciated regions of the world, and having a serious impact in the tropical Andes. Few ponds appear to receive hydrological inflow from glaciers during the dry season, meaning that surface waters in the highest elevation zones are likely to be reduced or disappear as glacial retreat continues and the communities that depend on them may be in danger if dependent on year-round water. Although our study focused on a small geographical area, our findings are broadly applicable to the high Andes and high mountain systems in general. Rapidly disappearing glaciers signify a worldwide loss of reliable stores of water that supply millions of people and rapidly changing montane ecosystems.

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TABLES

Table 1.1. Water stable isotope variation by season and source.

	N	Mean	SD	N	Mean	SD
		Dry Season			Wet Season	
Precip.	5			31		
δ^{18}O ‰		-13.71	6.44		-17.50	4.93
δ^{2}H ‰		-94.36	54.4		-125.50	40.79
d-excess		15.31	3.50		14.48	4.54
Glacial Melt.	14			18		
δ^{18}O ‰		-17.16	0.85		-17.35	1.16
δ^{2}H ‰		-124.74	7.46		-124.96	6.82
d-excess		13.20	3.85		13.85	3.65
Springs	6			4		
δ^{18}O ‰		-17.42	2.31		-19.10	0.59
δ^{2}H ‰		-127.60	13.52		-141.31	2.77
d-excess		11.72	6.01		11.48	3.18
Snowpack	8			5		
δ^{18}O ‰		-14.43	2.45		-20.04	4.77
δ^{2}H ‰		-106.98	19.77		-148.69	35.48
d-excess		8.44	1.14		11.62	3.64
Ponds	24			32		
δ^{18}O ‰		-13.71	3.85		-17.10	1.47
δ^{2}H ‰		-118.96	17.16		-128.28	11.86
d-excess		-9.29	15.68		8.53	5.21

Table 1.2. Results of t-tests comparing mean stable isotope composition of different water sources in the wet and dry seasons.

	t	*df*	*p*
Precip.			
$\delta^{18}O$ ‰	1.26	4.79	0.27
$\delta^{2}H$ ‰	1.23	4.75	0.28
d-excess	0.47	6.40	0.65
Glacial Melt			
$\delta^{18}O$ ‰	1.14	16.77	0.27
$\delta^{2}H$ ‰	0.98	15.23	0.34
d-excess	-0.49	29.25	0.62
Springs			
$\delta^{18}O$ ‰	1.70	5.94	0.14
$\delta^{2}H$ ‰	2.41	5.61	0.05
d-excess	0.08	7.79	0.93
Snowpack			
$\delta^{18}O$ ‰	2.44	5.34	0.05
$\delta^{2}H$ ‰	2.40	5.58	0.05
d-excess	-1.90	4.49	0.12
Ponds			
$\delta^{18}O$ ‰	4.10	28.08	<0.001
$\delta^{2}H$ ‰	2.28	38.72	0.04
d-excess	-5.35	26.8	<0.001

FIGURES

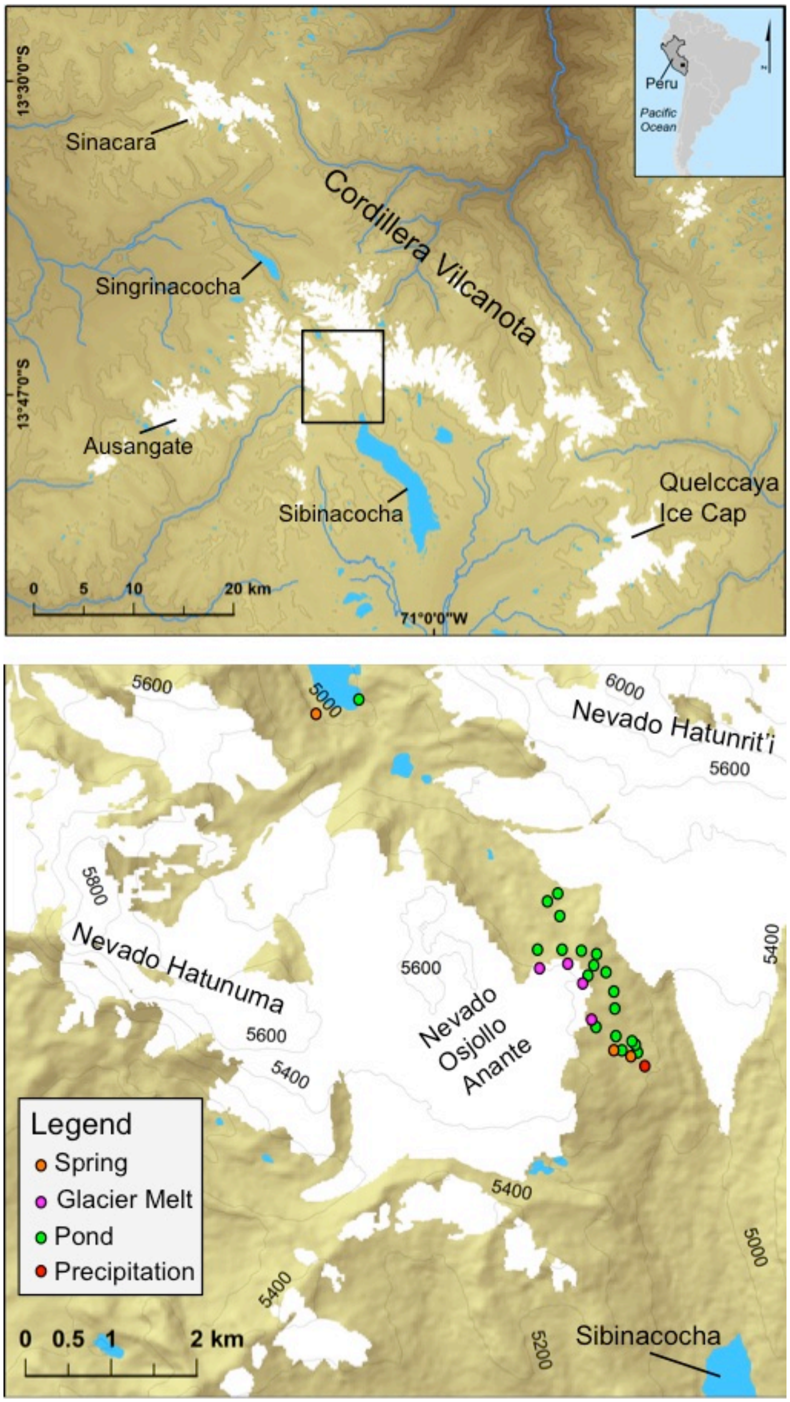


Figure 1.1. Map of the Cordillera Vilcanota study area. Top) Overview map with notable peaks and lakes. The new, ice-free corridor is visible between Sibinacocha and Singrinacocha. Inset shows location of study area within Peru. Bottom) Sampling locations in the recently deglaciated pass.

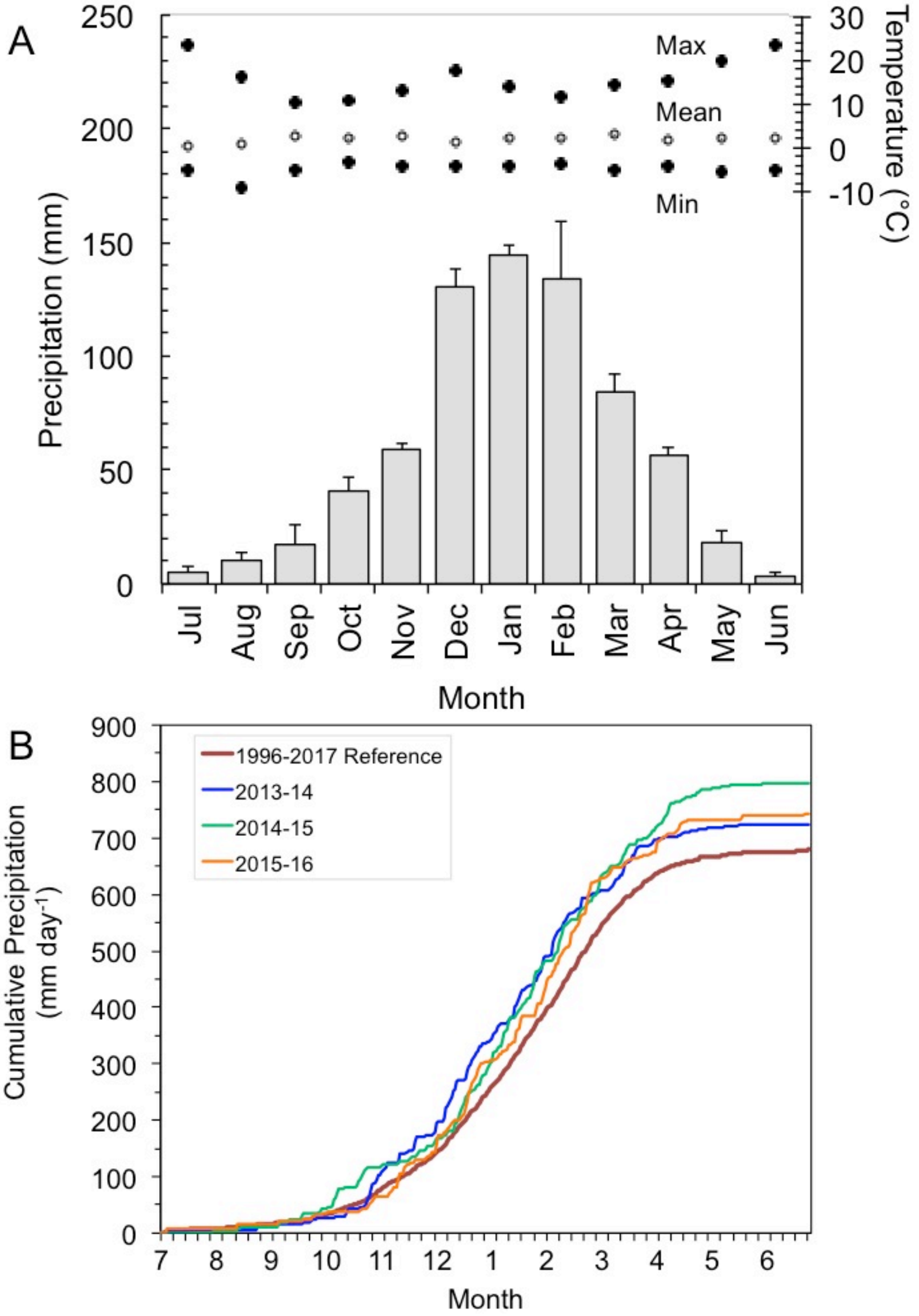


Figure 1.2. Climate (precipitation, temperature) patterns in the Cordillera Vilcanota. A) Average precipitation totals (mean ± SD) in the Cordillera Vilcanota. Data are from three SENAMHI weather stations (Sicuani, Ccatcca, and Macusani) for the period 2012-2017. Average air temperature (mean ± SD, open circles), average maximum, and average minimum air temperature (solid circles) from the study site at 5250 masl. B) Cumulative daily precipitation during the present study for four SENAMHI weather stations (Ccatcca, Pomacanchi, Santa Rosa, and Progreso) compared to 1996-2017 reference period.

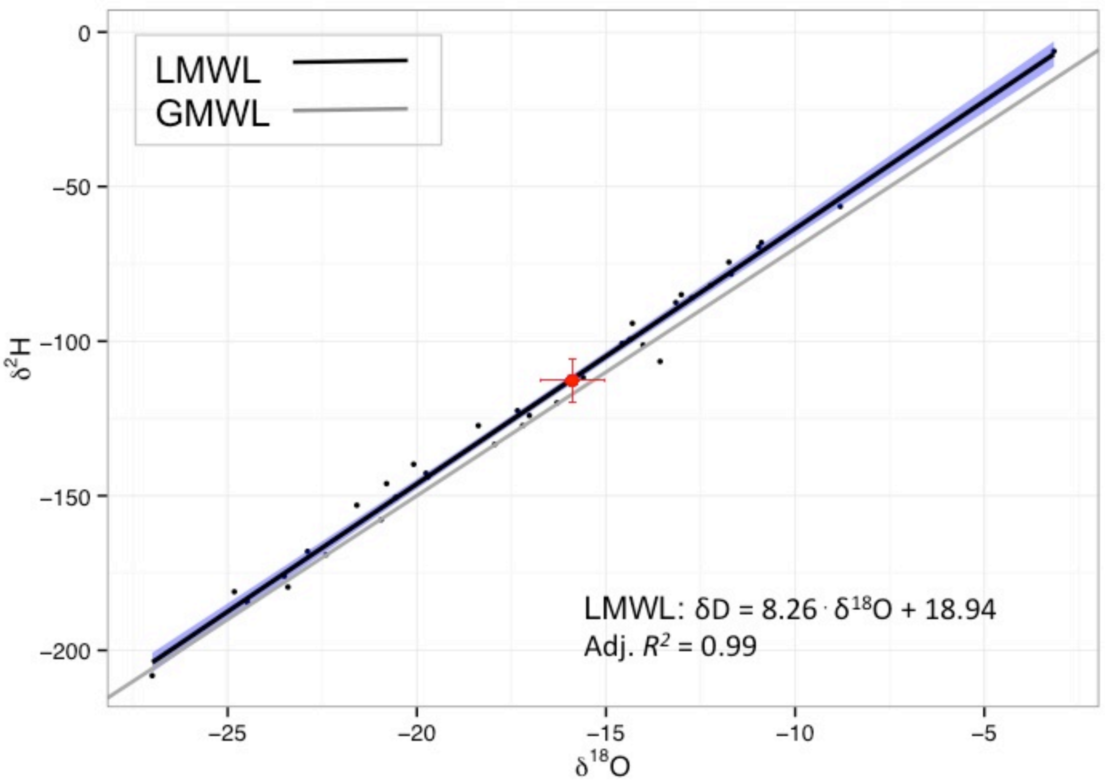


Figure 1.3. Local meteoric water line with 95% CI. The red point is the amount-weighted precipitation value (mean ± SE: δ^{18}O = -15.89 ± 0.84‰ and δ^{2}H = -112.65 ± 7.05‰) of the precipitation samples (black dots). The GMWL ($\delta^2\mathrm{H} = 8\delta^{18}\mathrm{O} + 10$) is shown for reference.

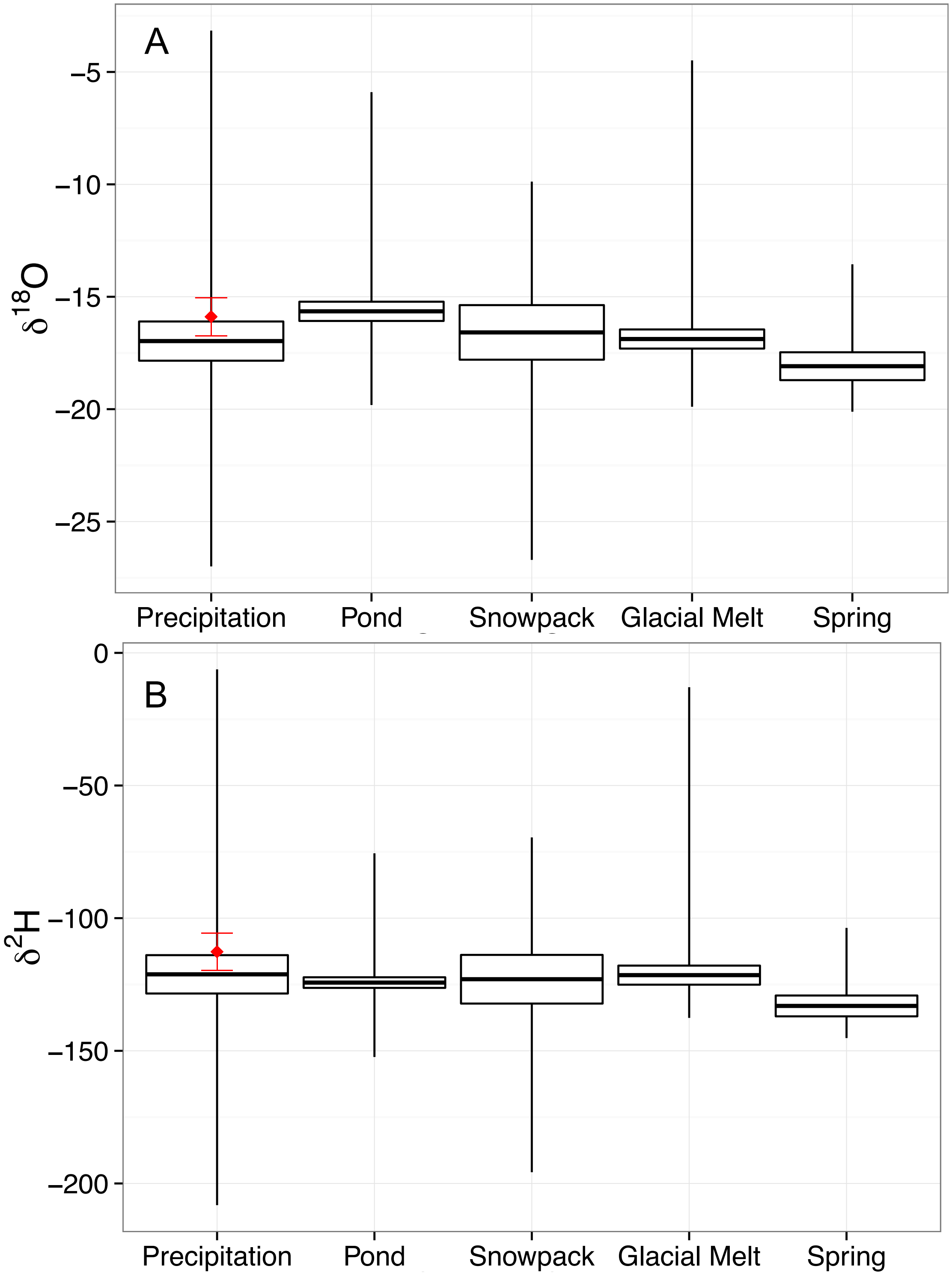


Figure 1.4. The $\delta^{18}O$ and $\delta^{2}H$ values of different water sources (mean ± SE). A) $\delta^{18}O$ and B) $\delta^{2}H$ values of different water sources (black boxes; mean ± SE). Tails represent the spread of data from minimum to maximum values. The red point is the amount-weighted precipitation value (mean ± SE): $^{18}O = -15.89 \pm 0.84$‰ and $\delta^{2}H = -112.65 \pm 7.05$‰.

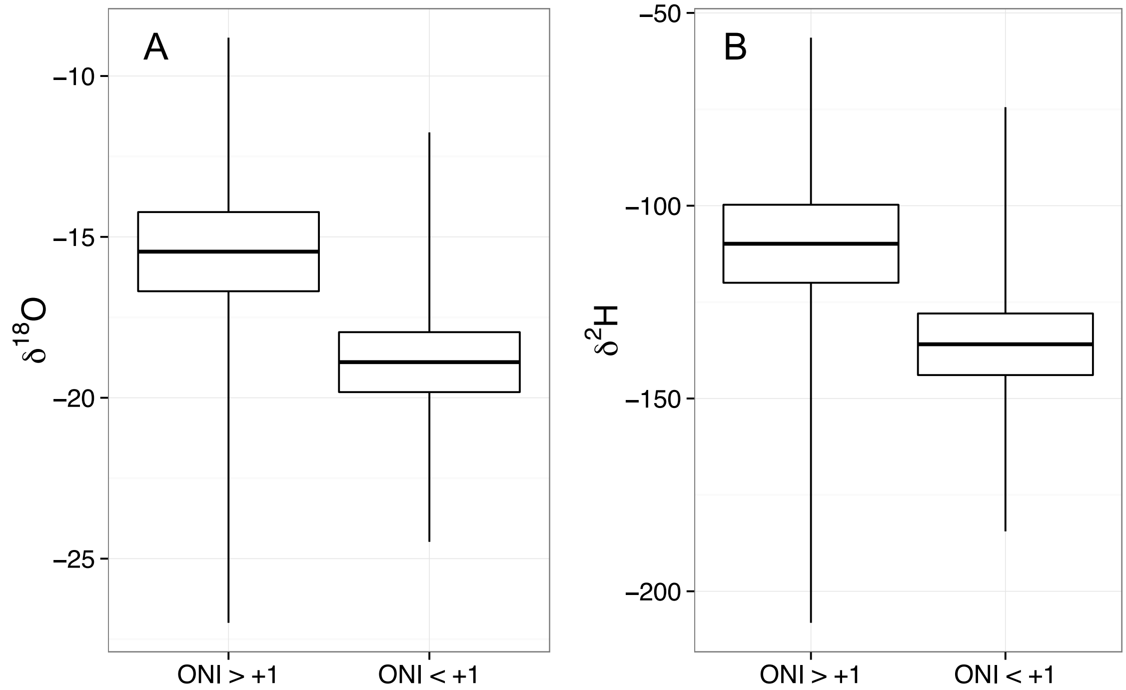


Figure 1.5. Stable isotope values of precipitation in strong 2015/2016 El Niño. A) δ^{18}O and B) δ^{2}H in precipitation collected during the strong 2015/2016 El Niño (ONI > 1) compared to samples from neutral and weak El Niño periods (ONI < 1). All samples are from the wet season. Boxes represent Mean ± SE, tails represent the spread of data from minimum to maximum values.

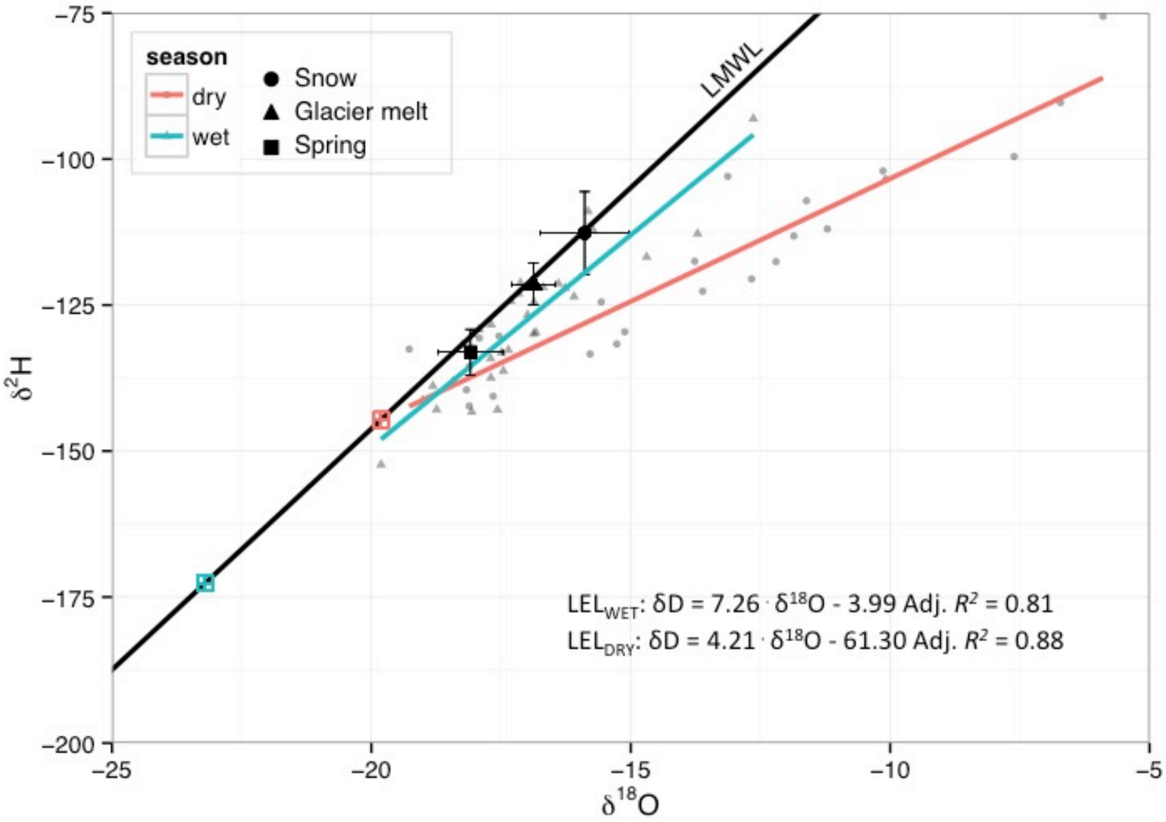


Figure 1.6. Local evaporation lines (LELs) for ponds in the dry and wet seasons. Red and blue squares mark the intersection of LMWL with LEL_{dry} and LEL_{wet}, respectively. Black symbols represent average values (± SD) for amount-weighted precipitation, glacial meltwater collected by surface flow, and spring water.

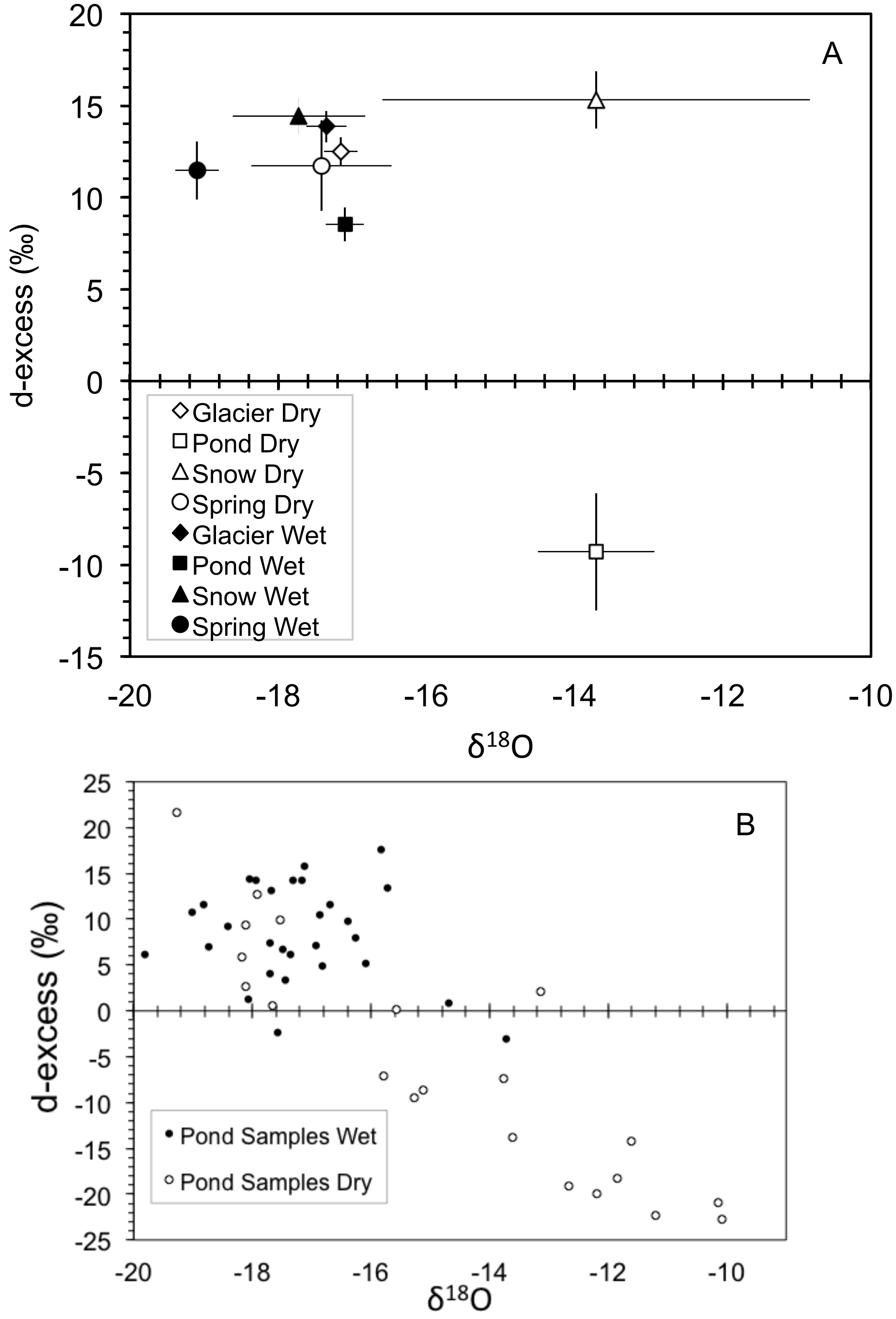
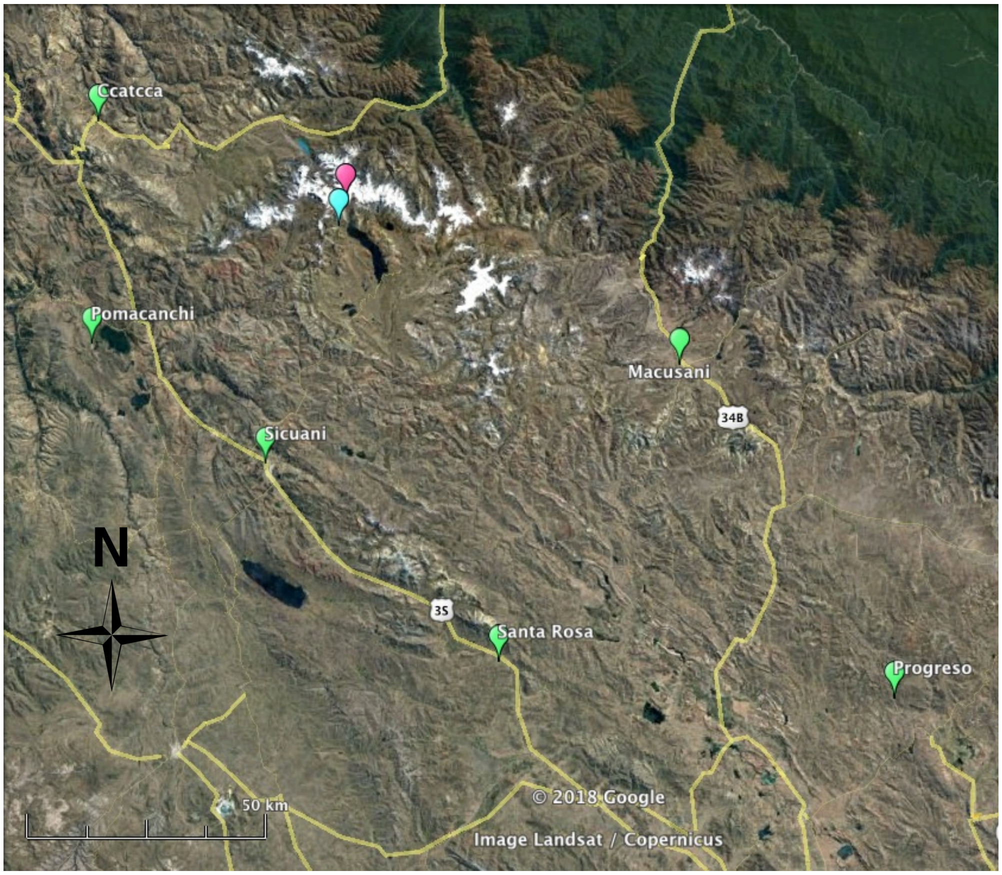


Figure 1.7. The δ^{18}O – d-excess relationship for precipitation, springs, melt, ponds. A) Seasonal differences in the δ^{18}O – d-excess relationship for water sources. Seasonal differences for δ^{18}O are visible for snow (precipitation). Seasonal differences in both δ^{18}O and d-excess are apparent for ponds, likely a result of high evaporative enrichment in the dry season. (B) Comparison of the wet and dry season δ^{18}O – d-excess relationship for pond samples.

APPENDICES



Appendix 1.1. Map of weather stations: SENAMHI stations (green markers), the Appalachian State University weather station at Murmurani Alto (blue marker), and the precipitation stable isotope sampling station used in this study (pink marker). Satellite image from Google Earth.

Appendix 1.2. Stable isotope measurement agreement between observed (mean ± SD) and expected values of standards

	δ^{18}O ‰		δ^{2}H ‰	
Standard	Observed	Expected	Observed	Expected
LGR-4C	-7.64 ± 1.3	-7.94 ± 0.15	-51.09 ± 2.2	-51.6 ± 0.5
LGR-2C	-16.25 ± 0.46	-16.24 ± 0.15	-124.27 ± 2.7	-123.7 ± 0.5
GISP	-24.92 ± 0.49	-24.76 ± 0.09	-189.74 ± 3.0	-189.5 ± 1.2
SLAP2	-55.41 ± 0.17	-55.50 ± 0.02	-427.20 ± 1.1	-427.5 ± 0.3

Chapter II. Amphibian phenology and occupancy in the shadow of melting glaciers, Cordillera Vilcanota, Peru

ABSTRACT

Tropical high-elevation ecosystems are particularly vulnerable to climate change because high elevations are warming faster than low elevations and glacier-fed aquatic habitats will be negatively affected by glacier retreat and disappearance. In the Cordillera Vilcanota in southern Perú, significant changes in temperature, glacier cover, and species distributions have already been observed. Two frog species, *Pleurodema marmoratum* and *Telmatobius marmoratus*, have colonized new aquatic habitat inside the recently deglaciated zone. Their 150-200+ meter upward shift represents one of the largest elevational range expansions observed for any vertebrate.

To understand the effects of climate change on amphibian populations at the edge of the biosphere, we studied pond availability, water sources, and frog reproductive phenology over three hydrologic years. We conducted visual encounter surveys and repeat photography to document amphibian occupancy, breeding activity, and pond hydroperiod variation. We determined d-excess and $\delta^{18}O$ values from the stable isotopes of water to distinguish amphibian breeding ponds receiving glacial runoff versus ponds receiving only precipitation. Reproductive phenology in *Pleurodema marmoratum*, a terrestrial frog with aquatic larvae, was strongly linked to precipitation seasonality, whereas reproduction in the fully-aquatic *Telmatobius marmoratus* occurred throughout the annual hydrological cycle. These divergent strategies for dealing with variable and extreme conditions in high mountain environments leave each species vulnerable to

different threats. *Pleurodema marmoratum* may be threatened by shortened reproductive periods in temporary ponds. El Niño caused a delay in the 2015 wet season and shortened the *P. marmoratum* breeding period in ephemeral ponds. *Telmatobius marmoratus* persistence is vulnerable to the loss of permanent ponds under threat from ongoing glacial retreat. Our study shows that hydrologic alterations from the loss of glacial meltwater, changing precipitation patterns, and El Niño-driven climate variations threaten some of the highest elevation amphibians on Earth.

INTRODUCTION

Understanding how biological communities and ecosystems will respond to climate change is one of the greatest unknowns in conservation biology. Under a changing climate, species must be able to tolerate the changes, move, adapt, or face extinction (Berg et al. 2010). Climate change already is driving significant biological shifts in species distributions, phenology, and ecosystem services (Parmesan and Yohe 2003, Pecl et al. 2017). For example, meta-analyses of species distributions show that species have shifted to higher elevations at a median rate of 11.0 meters per decade, and to higher latitudes at a median rate of 16.9 kilometers per decade (Chen et al. 2011). Vertebrates have, on average, been breeding 2.88 days earlier per decade since 1950 (Cohen et al. 2018).

Rapid climate change in high elevation Andean ecosystems is already having potent impacts on organisms and ecosystem function (Anderson et al. 2011). Tropical montane species are considered particularly vulnerable because high elevations are warming faster than low elevations (Bradley et al. 2006, Pepin et al. 2015), and thermal tolerance theory suggests that tropical species have narrow ranges of thermal tolerance limits because they live in stable thermal conditions (sensu Janzen 1967). However, not all tropical habitats are warm and constant. A large number of tropical montane species are tolerant to a wider range of thermal conditions than previously thought (Navas 2002, Catenazzi et al. 2014, Rueda-Solano et al. 2016, von May et al. 2017).

Despite the focus on the warming aspect of climate change, a suite of vital conditions is changing along with the increasing temperatures. Alterations to water resources, driven by climate change, are becoming recognized as a serious threat to both human populations and ecosystems in mountain environments (Barry and Seimon 2000, Bradley et al. 2006, Kaser et al. 2010, Anderson et al. 2011, Huss et al. 2017, Buytaert et al. 2017). Tropical montane ecosystems are particularly vulnerable to the loss of freshwater reserves in glacial ice that is associated with climate change (Mark et al. 2005, Bradley et al. 2006, Viviroli and Weingartner 2008, Viviroli et al. 2011). Glaciers act as vast stores of freshwater. Reductions to glacial meltwater runoff are associated with impending water crises in mountain regions worldwide, including the Alps, the tropical Andes, central Asia (Milner et al. 2017, Buytaert et al. 2017). As glaciers retreat, they initially increase meltwater inputs to downstream hydrology as they lose mass until they reach their maximum contribution, followed by an abrupt decrease in meltwater inputs to runoff (Braun et al. 2000, Nolin et al. 2010, Baraer et al. 2012, Carey et al. 2014). Many tropical glaciers are already out of equilibrium with the current warming climate and are predicted to disappear within a few decades (Vuille et al. 2008). Recent simulations suggest that small tropical glaciers, especially those nearest to the equator, will disappear, and large glaciers will be severely reduced by 2100 (Vuille et al. 2018).

In watersheds where glaciers are lost or severely reduced, the reduction of glacial meltwater during the dry season alters downstream water availability for both humans and ecosystems (Mark et al. 2005, Vuille et al. 2008, Thompson et al. 2011, Vuille et al. 2018). Glacially fed waters, including streams, wetlands, ponds, and lakes, will be severely affected by glacier retreat and disappearance (Dangles et al. 2017, Polk et al. 2017). Despite the expected wide-scale negative impacts, limited research has addressed links between hydrologic alterations and high-elevation ecosystems. The importance of glacial meltwater to stream contribution is elevated in tropical regions where there is a distinct wet and dry season. In the central Andes, for example, glaciers are the only natural source of water for streams during the pronounced dry season (Mark et al. 2005).

Hydrologic alterations associated with changes in precipitation and glacial retreat affect habitat availability and key ecological characteristics such as life cycles and community assembly in aquatic ecosystems (Jacobsen 2008). Meltwater from glaciers increase the availability and size of wetlands surrounding glacial valleys (Polk et al. 2017). As glaciers retreat and disappear, abrupt changes occur in surface hydrology upon which aquatic assemblages depend (Seimon et al. 2007, Seimon 2010, Jacobsen et al. 2012, Jacobsen and Dangles 2012). Hydrologic changes caused by glacial loss will have negative effects on the high-elevation aquatic ecosystem function and biodiversity and quality of life for humans dependent on those ecosystems (Jacobsen et al. 2012, Quenta et al. 2016).

Aquatic habitats integrate many aspects of the changing environment (e.g., temperature and hydroperiod) in high elevation ecosystems and are critical hotspots of biodiversity. The hydrological gradient from seasonal (i.e., temporary water bodies) to perennial (i.e., permanent water bodies) is a major influence on the abundance and distribution of many organisms. One important consideration for aquatic organisms is availability of appropriate habitat for the larval and adult stages. In addition, for species that use temporary habitats for reproduction, differences in time necessary to complete larval stage are very important. Hydroperiod predictability and variation affects the timing and success of reproduction.

Worldwide, amphibians have colonized many extreme habitats, in part, because some species have a remarkable degree of plasticity in development and behavioral strategies to deal with variable hydroperiods. For example, North American spadefoot toads (Scaphiopodidae) live in dry deserts with unpredictable rainfall. They can complete larval development in ponds with extremely short hydroperiods and *S. couchii* may have the shortest aquatic larval periods of any known amphibian (e.g., 8 days; Pfennig 1992, Newman 1987, 1994; Kulkarni et al. 2011). On the other hand, some anuran species such as American Bullfrogs (*Lithobates catesbeiana*) and European Common Frogs (*Rana temporaria*) remain in the aquatic environment as larvae for multiple reproductive seasons (Walsh et al. 2016).

Organisms in high elevation ecosystems in the tropical Andes must deal with environmental conditions including extreme daily temperature variation, nightly freeze risk, low humidity, high solar insolation, and a pronounced dry season. Because of the strong seasonality of precipitation in the southern tropical Andes (Garreaud et al. 2009, Perry et al. 2014, 2017) changes to precipitation and glacial runoff likely have strong effects on many aquatic organisms. Climate predictions suggest that precipitation regimes will become even more seasonal under future climate than they are today (Urrutia and

Vuille 2009, Buytaert et al. 2010). Increased precipitation seasonality has the potential to extend the dry season and alter the phenology of aquatic organisms, which is an aspect of climate change long predicted to affect amphibians (Donnelly and Crump 1998, Shoo et al. 2011). The tropical Andes are sensitive to the intensity of major El Niño events that are associated with temperature and precipitation anomalies (Hanshaw and Bookhagen 2014, Perry et al. 2014, Thompson et al. 2017).

Many amphibian species are vulnerable to changes in temperature, pond permanence, and water quality (McDiarmid 1994). Droughts and high temperatures associated with climate change and El Niño have contributed to reproductive failure and population declines in frogs (Burrowes et al. 2004, Bickford 2005, Rohr and Raffel 2010). Changes to pond permanence and loss of ponds resulting from warming and negative precipitation trends have had adverse effects on amphibian communities in temperate mountain ecosystems (McMenamin et al. 2008). During the last 16 years, decreasing precipitation and increasing temperatures in warm months have led to a four-fold increase in desiccated ponds in Yellowstone National Park (McMenamin et al. 2008). The loss of ponds has been catastrophic for pond-breeding amphibian populations of several species, and has led to both decreases in the richness and population sizes of frogs that use remaining ponds (McMenamin et al. 2008). In the tropical Andes, the combined threats of climate change, glacier retreat, and severe El Niño can result in a degradation or loss of crucial aquatic habitat (i.e., ponds, lakes, *bofedales* –perennial high-elevation peat wetlands).

There is extensive documentation of the glacier changes occurring in high mountain regions and threats to human water resources from the resulting hydrologic alterations (Huss et al. 2017, Vuille et al. 2018), but there remains little information about corresponding biological changes. Amphibians, in particular, have been understudied despite an ongoing global biodiversity crisis and population declines (Wake and Vredenburg 2008). We studied the breeding phenology of two species of frogs with contrasting life history strategies above 5200 m in the tropical Andes. We examined reproductive phenologies over three hydrologic years. We had three objectives. Specifically, we studied the ways which frog phenology and pond hydrodynamics (i.e., filling and drying) were associated with precipitation seasonality and inter-annual climatic variation, including the strong El Niño in 2015/2016. We used the stable isotopes of water to identify ponds that currently receive glacial meltwater input in the dry season and predict amphibian population persistence under continuing climate change and glacier loss. And finally, we used occupancy surveys and models to examine patterns of upslope migration into habitat created by rapid deglaciation throughout the high-elevation zones of the Cordillera Vilcanota.

METHODS

Study site

Our study took place in the Cordillera Vilcanota (hereafter CV; 13.7°S, 71.0°W) in Cusco Department, southeastern Peru. The CV is located at the western margin of the Amazon Basin, and near the northern extent of the altiplano (2.1). The CV is located within the Amazonas–High Andes Freshwater Ecoregion (Abell et al. 2008) and provides some of the highest elevation source waters for the Urubamba–Ucayali and Inambari-Madre de Dios river systems. During the dry season and drought years, glacial meltwater from the CV contributes significant water resources for domestic use, hydroelectricity generation, agriculture, tourism, and ecosystems (Buytaert et al. 2017).

The climate in the CV is characterized by distinct wet and dry seasons throughout the hydrological year (July to June). Most precipitation falls from October–April, with the peak wet season in December–February (Perry et al. 2014, Seimon et al. 2017). There is little seasonal variation in average air temperature in the tropical high Andes, but the daily temperature variation can be large. Daily air temperature is more variable during the dry season, and average air temperatures are slightly lower in the dry season than in the wet season (Schauwecker et al. 2014).

Two species of frogs have moved upslope into ponds in a recently-deglaciated pass in the CV between 5244–5400 m (Seimon et al. 2007). Elevational records from the recently deglaciated zone in the CV include the highest reported records for both *Telmatobius marmoratus* (Telmatobiidae) and *Pleurodema marmoratum* (Leptodactylidae), as well as the current global elevation record for an amphibian (*P. marmoratum*). The upper limits of their ranges in the CV represent a 150–200 m upward range expansion for the two amphibian taxa (Seimon et al. 2007). Both species have persisted in the recently deglaciated zone since at least 2003, despite extreme environmental conditions associated with the very high elevation and the appearance of the deadly fungal amphibian disease, chtyridiomycosis (Seimon et al. 2017).

Pleurodema marmoratum is a relatively common frog with an aquatic larval stage and terrestrial adult stage (classified as Least Concern despite reported local population declines; IUCN 2015a). *Pleurodema marmoratum* have been regularly observed in the CV deglaciated zone from 2003–2016 (Seimon et al. 2007, 2017). *Telmatobius* *marmoratus* is widely distributed at elevations from 1800 to 5244 m and is aquatic during both the tadpole and adult life stages. The species is classified as Vulnerable because of population declines associated with water pollution, overharvesting, and chytridiomycosis (IUCN 2015b). Tadpoles of *Telmatobius marmoratus* have been observed in the deglaciated zone in 2004, 2005, and 2013 to 2016, with no individuals observed in surveys conducted in 2008, 2009, and 2012 (Seimon et al. 2017). No adults of *T. marmoratus* were observed from 2004 until October 2013, indicating that the species may be in the initial stages of recovering after an outbreak of chytridiomycosis (Seimon et al. 2017).

We focused our study on ponds inside a recently deglaciated corridor from 5230–5390 m between the rapidly-retreating ice fields of Nevado Osjollo Anante (5600 m) and Nevado Hatunrit'i (6100 masl; Fig. 2.1). Many amphibian ponds formed as kettles, or from small pieces of ice that melted on the surface leaving behind a bed of glacial flour that can retain water. Eskers and recessional moraines often delineate the pond edges. Ponds are surrounded by unsorted glacial till with minimal soil development, and the pond substrates are composed of glacial flour, silt, and periphyton. The temporary ponds are mostly singular, isolated ponds, whereas a majority (but not all) of the permanent ponds form linked paternoster lakes. Permanent ponds in the oldest moraines (dated to 1931 by historical aerial photography; Seimon et al. 2007) have a narrow ring of *bofedal* wetlands. *Bofedales* have both lentic and lotic areas that support a high diversity of aquatic organisms, and provide ecosystem services such as carbon sequestration, and water storage, and pasture sites used by high-elevation herding communities (Fonken et al. 2014, Quenta et al. 2016).

Meteorological Data

We recorded shaded surface temperatures from August 2013 to June 2016 at 5250 m near study ponds at the field site (iButton thermochron dataloggers; Maxim Integrated). The iButton loggers were suspended 1-2 cm above the surface inside a rock cairn. We obtained 21-year daily precipitation records from the Servicio Nacional de Meteorológica y Hidrología (SENAMHI). The SENAMHI stations are located near the study area, in Ccatcca (3729 m), Santa Rosa (3986 m), Pomacanchi (3160 m), and Progreso (3980 m). The SENAMHI stations are located at lower elevations than the study area, but encircle the geographic area of the CV and were used as proxies for average meteorological conditions in the study area. Annual precipitation totals during the study and long-term (16–20 years) precipitation averages, excluding incomplete years, for the weather stations are shown in Table 1. No long-term meteorological records are available from the study site, but short-term average precipitation totals for two sites within the Vilcanota (Pukarumi and Murmurani Alto) are available in the literature (Perry et al. 2017). Average annual precipitation totals in Pukarumi (4100 m), a site on the northern slope of the Cordillera Vilcanota, were 1,111 mm for 2011 – 2015. In Murmurani Alto (5100 m), on the southern slope of the Vilcanota, mean annual precipitation from 2010 – 2015 was 732 mm. We summed 24-hour precipitation totals at the four regional stations and calculated the monthly average. We compared conditions during the study period (July 2013 to June 2016) to the baseline conditions from 1996 to 2017 (excluding incomplete years) to determine the monthly average precipitation anomaly.

We classified strong El Niño periods using the Ocean Niño Index (ONI) using sea surface temperature anomaly data from the Niño 3.4 region (Huang et al. 2017). We categorized El Niño samples as those measured during 3-month periods with average sea surface temperature anomalies greater than 1.0, and strong El Niño periods with ONI > 2.

Pond Hydrology

We recorded water temperature from April 2014 to February 2016 in two temporary ponds and three permanent ponds located from 5250 – 5360 m (Tidbit v2 TempLoggers; Onset Computer Corp.). We located temperature loggers where we observed tadpoles, in shallow water (5 – 20 cm depth; N = 4) and deep (~150 cm; N = 1) locations.

We visually assessed pond hydroperiod status in monthly visits for three hydrological years (HY1: July 2013 – June 2014, HY2: July 2014 – June 2015, and HY3: July 2015 – June 2016). We did not visit the ponds in August and September 2014 or August 2015 prior to the onset of the wet season and before most temporary ponds fill with water. We visited 45 ponds in HY1 and a subset of 30 ponds in HY2 and HY3. During each visit we recorded whether the pond held water or was dry, and we used this information to classify ponds according to hydroperiod. We classified ponds as temporary (i.e., held water for only part of the hydrologic year) or permanent (i.e., held water for the entire hydrologic year for all three study years). We also determined the maximum period that each temporary pond held water in each hydrologic year.

We also compared relative pond area during monthly pond visits by repeat photography of ponds from the same location and angle. We calculated pixel area in the repeat photos in ImageJ and converted area for each pond to a relative scale from 0 (completely dry) to 1 (the maximum fill for that pond). We compared visible water coverage in the photos in the wet and dry seasons, by year, and prior to and during El Niño.

We investigated the water stable isotope signatures of ponds for which we had both wet and dry season samples. We collected water samples of ponds and glacial meltwater (at springs or surface runoff near glacial margins) over three days in December 2013 and February 2014 (wet season), and May and June 2014 (dry season) for Liquid Water Isotope Analysis of stable isotopes of oxygen ($\delta^{18}O$) and hydrogen (δD). We collected precipitation stable isotope samples opportunistically over the course of the study at 7 am from a flat collecting plate at 5235 m. Water samples were filtered through 0.45 µm syringe filters into acid-washed 30 ml HDPE sample bottles or 8 ml borosilicate vials rinsed 2–3 times prior to filling. Bottles and vials were filled completely to minimize headspace. Sample lids were tightly sealed and wrapped with Parafilm (Bernis NA, Wisconsin, USA) to prevent evaporation. We calculated the Deuterium excess (d-excess or 'd') of water sample as:

$$\mathrm{d} = \delta D - 8\delta^{18}\mathrm{O} \quad (\mathrm{Eq.\,1})$$

following Dansgaard (1964). Deuterium excess is caused by kinetic isotopic fractionation during evaporation or condensation and, in precipitation, can be used to distinguish different air trajectories and moisture sources (Gat 1996). When d-excess = 10, the sample is located on the global meteoric water line (GMWL). Samples with values < 10 plot below the GMWL and signal a deviation from equilibrium fractionation conditions, i.e., evaporation has occurred in the sampled water (Dansgaard 1964).

Negative d-excess values signal excessive evaporation. Changes in d-excess in surface water over time can reveal information about the relative influence of evaporation in pond hydrology. We used d-excess as an index to distinguish between evaporated and non-evaporated pond water sources in the wet and dry seasons.

Amphibian Phenology and Occupancy

We conducted diurnal amphibian surveys in HY1 – HY3 to detect breeding activity in study ponds. Observers surveyed for frogs in the water, vegetation, and rocks around pond perimeters for a standardized amount of time (i.e., 1, 5, 10, or 20 minutes depending on pond size). Typically, one or two observers sampled each pond. When two observers conducted surveys at the same time, they did not share information about frog encounters during the surveys and were given no information about expected or prior survey results to maintain observation independence. In addition, surveys were initiated from different locations every month, and ponds were sampled in a different order to ensure sampling did not occur under the same conditions (i.e., temperature). Ponds less than 40 m apart were usually sampled as one site, unless site covariates were very different on a smaller spatial scale (e.g., if one pond hydroperiod was permanent it was considered separately from adjacent ephemeral ponds).

At such high elevations, ponds experience surface freezing to some degree on a nightly basis in the coldest, dry season months (May – September) and any time after large precipitation events. The ice layer typically melts by mid-morning. We avoided sampling ponds with extensive ice cover, so our first surveys were often delayed until mid-morning. Perimeter surveys of small, ephemeral ponds often included the entire pond, whereas they were effective for ~2 m from the water's edge of large ponds, leaving a higher proportion unsampled area in large ponds. Therefore, we present information on the proportion of ponds with observations and survey-specific presence-absence data. Our sampling method was effective even for large ponds in detecting presence-absence because tadpoles aggregate in shallow, warm water around the pond perimeters (Seimon et al. 2007). Observers recorded the presence-absence of reproductive activity (i.e., egg masses, frogs in amplexus, and calling frogs), and later life history stages (i.e., tadpoles, metamorphs ≥ Gosner Stage 42; Gosner 1960, and emerging juveniles).

We also conducted amphibian presence-absence surveys in previously-unexplored recently-deglaciated areas in the CV. Survey locations in and near recently-deglaciated areas were identified using satellite imagery available in Google Earth. We surveyed ponds from the edge of the glaciers, through the recently-deglaciated zone, to the surrounding areas that have remained ice-free. Recently deglaciated zones were identified by comparing historical imagery in Google Earth to recent images, and by biogeomorphological traits that indicate recent glacier cover (visible both in satellite images and in situ). Recently deglaciated areas appear light in color in satellite images. In situ, the color difference between old and recently exposed moraines is clear, and exists because unconsolidated moraines of unsorted, minimally weathered, bare rock dominate the recently deglaciated zones. In contrast to the areas that were not recently covered by permanent ice, the recently deglaciated areas have very little soil development, extremely sparse plant cover, and scarce lichen cover (Seimon et al. 2007). In several locations we located a distinct line separating bare, recently disturbed moraines and lichen-covered, older moraines (Fig. 2.2). Previous studies have linked this ecotone with the extent of permanent ice cover since the beginning of the current period of rapid glacial retreat at the end of the Little Ice Age (~1880 in the region; Thompson et al. 1992, Rabatel et al. 2005, Seimon et al. 2007).

We visited glacier-edge sites only once because they were remote, difficult to access, and resurveying the sites was prohibitively expensive. However, for a large subset of single-visit ponds, we conducted multiple timed surveys using independent observers to estimate presence-absence and account for imperfect detection in a single site visit.

Statistical Analyses

All statistical analyses were completed in R version 3.2.1 (R Core Team 2015). We fit Linear Mixed Effects Models ("LMEs") in the package lme4 (Bates et al. 2014) to examine seasonal trends in precipitation anomalies and annual differences in pond hydroperiods (temporary pond duration in months). In the analysis of monthly average precipitation anomalies, we specified each unique month-year combination as the fixed effect and meterological station (N = 4) as a random effect. In the pond hydroperiod analysis, we specified hydrologic year (N = 3) as the fixed effect and pond (N = 12) as a random effect to account for repeated observations.

We used Generalized Additive Mixed Models ("GAMMs") in the package mgcv to evaluate relative pond area changes over time (Wood 2011). Generalized Additive Mixed Modeling produces non-parametric smoothed curves that identify a relationship between predictor variables, which may include a mixture of smoothed, non-parametric and linear, parametric variables, and a response variable. The GAMMs also accept random effects, which can accommodate a hierarchical sampling design (i.e., repeated site visits). We analyzed relative pond area for temporary and permanent ponds with mean monthly precipitation, mean precipitation with a one-month lag, sea surface temperature anomaly, 30-day precipitation anomaly, and month as smoothed terms. We included the parametric factor hydrologic year, and random effects (month and pond) to account for repeated sampling.

We measured the body size (snout–urostyle length) of juveniles that had recently emerged from ponds and compared size between temporary and permanent ponds. The comparison model was a mixed LME with size as the response variable, pond permanence as the fixed factor, and pond name as a random blocking factor. We fit Occupancy Models ("OMs") in the package unmarked (Fiske and Chandler 2011) to evaluate patterns of presence-absence of *P. marmoratum* across the landscape while accounting for imperfect detection. We used a single-season model using survey results from HY2 (N = 104 sites) to estimate occupancy and detectability with different combinations of covariates (MacKenzie et al. 2002). We fit OMs only for months during the peak *P. marmoratum* tadpole developmental season to meet the assumption that occupancy should not change between site visits. We ranked OMs using Akaike's Information Criterion (AIC), and assessed goodness of fit for the most well-supported models using the R package AICcmodavg (MacKenzie and Bailey 2004, Mazerolle 2017). We did not analyze *T. marmoratus* occupancy or detection probability because they were detected in very few ponds (N = 4).

RESULTS

Meteorological Data

Mean hydrologic year precipitation totals averaged over available stations were similar; HY1 = 723.5 ± 91.6 mm (N = 4 stations), HY2 = 778.8 ± 107.8 mm (N = 4), and HY3 = 722 ± 176.4 mm (N = 3) to long-term averages (Table 2.1). All values hereafter are presented as mean ± 1SD unless otherwise noted. Annual precipitation followed a well-defined wet (October–April) and dry (May–September) seasonal pattern, with the onset of wet conditions delayed until November in 2015 (Fig. 2.3). There were positive and negative departures from average monthly conditions in every hydrologic year of the study, with notable dry periods in early 2014 and March 2016 (Fig. 2.3).

Pond Hydrology

Mean pond temperature was 5.7 ± 0.4°C at dataloggers in deep water (range = 4.7–7.3°C), and 7.8 ± 1.7°C (range = 1.2– 20.6°C) in the shallow locations. Temporary ponds (N = 13) held water for 8.0 ± 0.9 months in HY1, 8.8 ± 0.7 months in HY2, and 6.5 ± 1.0 months in HY3. The difference in mean pond duration was significant in each hydrologic year (Table 2.2).

Relative pond area was greatest in the wet season and smallest in the dry season, and temporary ponds completely dried during each year of the study (Fig. 2.4). The top model for temporary pond area was a GAM-GLM hybrid that included significant terms of lagged monthly precipitation, precipitation anomaly, month, and hydrologic year (Table 2.3, Fig. 2.4). The top model for permanent ponds included the significant terms monthly precipitation, precipitation anomaly, and hydrologic year (Table 2.4, Fig. 2.4).

The GAM analysis revealed that pond area was significantly larger in HY1 for both permanent and temporary ponds, and significantly smaller in HY 3 for permanent ponds (Tables 2.3, 2.4).

The d-excess of weighted precipitation did not differ among wet season months (one-way anova: $F = 1.03$; $df = 4, 20$; $p = 0.42$). Deuterium excess also did not differ between the wet and dry season for precipitation, springs, and surface meltwater (Fig. 2.5). Pond d-excess was significantly higher in the wet season compared to the dry season (t-test assuming unequal variances: $t = -5.35$, $df = 26.84$, $p = 0.00001$). All sampled ponds had d-excess values similar to those of precipitation and other sources (i.e., meltwater) in the wet season. The d-excess value of many, but not all, ponds was extremely low in the dry season. A subset of ponds maintained high d-excess values in the dry season (Fig. 2.6). The subset of ponds with high d-excess values in the dry season also remained relatively depleted in ^{18}O.

Amphibian Phenology and Occupancy

Pleurodema marmoratum. We observed egg clutches of *Pleurodema marmoratum* very early in the onset of the wet season each of the three hydrologic years. We observed breeding activity from September–December. Reproductive activity in the greatest number of ponds occurred from October–November. Egg laying ended in December, before the onset of peak wet season (December–February).

In HY2 we were unable to determine if frog reproduction began in September, as it did in HY1 and HY3, because we did not visit the site in September 2014. During our first visit (October 2014) we found only egg masses and no tadpoles, which indicates that reproduction began in October. However, because of imperfect detection of early-stage tadpoles, it is possible that reproduction began prior to our first observations in October 2014.

Variation in the dates of first observations of eggs depended on the onset of precipitation, which was strongly affected by climatological conditions. During the El Niño breeding period, September to December 2015, temporary ponds filled later and eggs were recorded for the first time on 17 November 2015, one month after we first observed eggs in permanent ponds (17 September 2015, Fig. 2.7).

Tadpole encounters concluded by May or June of each year, which indicates *P. marmoratum* typically completes metamorphosis within one hydrologic year (Fig. 2.8). In July 2013, we observed one larval *P. marmoratum* (~Gosner Stage 36) in each of two connected permanent ponds. The observation suggests that *P. marmoratum* might occasionally remain in ponds over the dry season, but this was the only time we observed *P. marmoratum* tadpoles in ponds during the dry season.

We observed juveniles emerging from temporary ponds in March to June 2014, March to June 2015, and in April of 2016. In HY1 and 2, we observed juveniles emerging several months earlier from permanent ponds than in the temporary ponds. We observed emergence from permanent ponds from January to July 2014, February to July of 2015, and April to June of 2016. The body size of juvenile *P. marmoratum* emerging from temporary ponds was significantly smaller than that of juveniles in permanent ponds (estimate = -0.2056774, SE = 0.06253314, df =16, t = -3.28909, p = 0.0046).

We analyzed occupancy information from 104 ponds. The best model, both in terms of parsimony and lowest AIC value, was: $\rho(.)\psi(site + bofedal)$. Site acts as a blocking factor because our sampling effort was uneven and survey sites were not always determined randomly at every location (e.g., ponds at sites D–F ponds were part of the long-term study, whereas other sites were sampled haphazardly and only once). There was a significant effect of *bofedal* vegetation on tadpole occupancy (Fig. 2.9).

Telmatobius marmoratus. Occupancy was limited to only three connected permanent ponds within Site D (D19, D20, and D21) and one permanent pond below Site D in the same watershed. We observed tadpoles in Cohort A in D21, and subsequent cohorts were found in D20 and a seasonal impoundment along the seasonally-flowing stream connecting D21 and D20. We never observed eggs of *T. marmoratus*. We heard a male calling on one occasion (27 November 2014). We observed the addition of early Gosner Stage tadpoles into the population at Site D and tracked tadpole cohorts over HY1– HY3. We observed four distinct cohorts (Table 2.5). We observed the appearance of early-stage tadpoles (i.e., a new cohort) and metamorphosis in two of the cohorts, which had remained as tadpoles for approximately one year. In contrast to *P. marmoratum*, *T. marmoratus* remained in ponds as tadpoles through the dry seasons of 2013–2015.

DISCUSSION

Climate change and deglaciation are changing water availability throughout the Andes. Our study is the first to establish a clear link between the threats to water availability and amphibian habitat in the high Andes. Reproductive phenology in

Pleurodema marmoratum, a terrestrial frog with aquatic larvae, was strongly linked to precipitation seasonality. In contrast, *Telmatobius marmoratus* reproduction occurred throughout the annual hydrological cycle and tadpoles were present in permanent water bodies in both wet and dry seasons. These two species demonstrated divergent strategies for dealing with highly variable and extreme conditions in high mountain environments, which may lead to differential ability to persist in the highest elevation zones under continued deglaciation.

We encountered amphibians in ponds with contrasting hydroperiods (i.e., permanent and temporary) in the recently deglaciated zone. Ponds whose water levels rely mostly on precipitation input typically fill early in the wet season (September – October) and dry down early in the dry season (May – June). We found that reproductive activity of *P. marmoratum* is linked with annual precipitation seasonality. Fossorial adults appeared to remain underground until the onset of the wet season. Breeding begins in the early wet season (September) and peaks in October – November, prior to the maximum wet period in December – February. The reproductive phenology of *P. marmoratum* was highly seasonal in both temporary and permanent ponds and differed only during the strong 2015/2016 El Niño, when the filling of temporary ponds with snowmelt was delayed by approximately one month. Tadpoles of *P. marmoratum* completed metamorphosis beginning in January and ending by June–July in permanent ponds, and May– June in temporary ponds.

Our study provides evidence that *Pleurodema marmoratum* have expanded upwards to colonize habitat created by rapid deglaciation on a broad regional scale.

Because *P. marmoratum* can utilize either permanent or temporary ponds, they appear to benefit from rapid deglaciation more than they are threatened by it. However, the high-elevation Andean wetlands associated with high occupancy probability for *P. marmoratum* are also threatened by loss of glacial meltwater. Recent changes to high-elevation wetland area in the Andes of central Peru have been linked to changes in glacier area and runoff (Polk et al. 2016). While the formation of new aquatic habitat followed by a transition to a reduced hydrological state is part of the natural succession that occurs in a deglaciating environment (Carey et al. 2014), the rate of change is now extremely fast. Loss of glaciers at these rates are likely to outpace the dispersal and growth of glacier-fed aquatic ecosystems and their ecosystem services (such as carbon and water storage), connectivity along elevational gradients, and result in the loss of biodiversity. *Bofedales* are particularly threatened by the loss of glacial meltwater. There is growing evidence that *bofedales* play important roles both in local and global ecosystem processes including carbon sequestration, water regulation and purification, support pastoral production and economic activity in highland communities, and act as oases of biodiversity, among other ecosystem services (Squeo et al. 2006, Fonken 2014, Yager 2015, Cooper et al. 2015), yet the survival of *bofedales* is linked to the availability of water from glacier melt.

Pleurodema marmoratum are likely to persist in the highest habitable zones even if permanent ponds shift towards temporary hydroperiod ponds. However, an increased frequency or severity of El Niño leading to shortened hydroperiods may lead to failure to complete metamorphosis, or in species with developmental plasticity, tradeoffs in body size at metamorphosis that may be detrimental to later survivorship (Werner 1984, Smith

1987). Body size of recently metamorphosed juvenile *P. marmoratum* was significantly smaller in temporary ponds compared to permanent ponds. Shortened hydroperiods during El Niño could reduce body size at metamorphosis. Combined with resource limitations in the high, bare temporary ponds, El Niños and altered precipitation patterns have the potential to cause changes in the population size structure and survivorship.

Precipitation patterns in the CV show a marginally negative year-round trend (using 1965 – 2009 baseline data), with a significant decrease in seasonal precipitation in SON (Salzmann et al. 2013). Our study shows that, for frogs, increasingly negative precipitation anomalies in SON, in the early wet season, could harm reproductive success of temporary-pond-breeding amphibians like *P. marmoratum*. The severity and frequency of El Niño events, timing of the onset of the wet season, duration of the wet season, and precipitation trends that impact the early wet season could have particularly strong effects on temporary-pond-breeding amphibians in the high Andes as stored water from glaciers is diminished. *Pleurodema marmoratum* likely has developmental plasticity and behaviors that allow them to be successful in highly variable conditions but the limits to this plasticity are unknown. For example, tadpoles aggregate in shallow, warm water presumably with the result of accelerating their rate of development. Tadpoles have a limited time for development in temporary ponds and we observed post-metamorphic juveniles every year, but we also observed dead tadpoles in two temporary ponds that dried before tadpoles were able to complete metamorphosis in June 2015 and May 2016. To determine the extent of vulnerability to shortened hydroperiods and El Niño, additional information on the developmental plasticity of *P. marmoratum* and what levels and durations of recruitment failures populations can withstand.

In contrast to *Pleurodema marmoratum, in our study, Telmatobius marmoratus* were found in the recently deglaciated zone only in a small subset of three permanent ponds and the seasonally flowing streams and one temporary pond that link them. *Telmatobius marmoratus* relies on permanent water bodies because both adults and larvae use aquatic habitat. Although we never encountered egg clutches, we observed early-stage tadpoles enter the population in distinct cohorts. We did not determine the exact time to metamorphosis, because we could not effectively mark or identify individuals. However, it is clear that tadpoles spent more than one hydrologic year in ponds and did not time breeding to coincide with the onset of the rainy season.

In watersheds undergoing deglaciation, hydrology and aquatic habitat will become increasingly dependent on precipitation as the role of glacier meltwater is reduced (Mark and Seltzer 2003). We used the stable isotopes of water to show that glacial meltwater-dominated and snowmelt-dominated amphibian ponds differ in $\delta^{18}O$ and in the signal of evaporative enrichment in the dry season. We were able to show, for the first time, the relative influence of glacial meltwater at the level of individual ponds used by frogs for breeding. All ponds received regular runoff from snow on the surface or shallow groundwater in the wet season, minimizing the isotopic enrichment effect of evaporation, so the wet season d-excess signature was similar among ponds and the $\delta^{18}O$ signature was relatively depleted. The d-excess values of temporary ponds indicated closed (evaporating) systems that received little to no input in the early dry season; these ponds were supplied by precipitation only. Permanent ponds D13 and Low Ice Cave Lake had similar d-excess values in the wet and dry season and they remained relatively depleted in ^{18}O in the dry season, so we infer that they received substantial glacial meltwater input in the dry season. We assume that the only source of water in the dry season is glacial meltwater runoff from old ice or snowpack built up during the wet season. Our data from Pond D13 source spring was the most isotopically-depleted water we sampled anywhere in the study area, suggesting that glacier meltwater was indeed the source for D13 pond. Our stable isotope method cannot distinguish between glacial ice and recent snowpack, but for our purposes that distinction is not necessary because new snowfall melts away within hours of falling (i.e., there is no ground accumulation of snow through the wet season; accumulation occurs only on top of the ice).

A compelling source of evidence for changing hydrological conditions in the study ponds comes from the vegetation changes described by Seimon et al. (2017). Vegetation shifts over a decade suggest that at least one pond utilized by *P. marmoratum* has been altered from permanent to temporary, and that two permanent ponds used by both anuran species are in the process of transitioning to temporary ponds (Seimon et al. 2017). The transitioning ponds are two of only three permanent ponds where *T. marmoratus* have been found over more than a decade. Crucially, the permanent ponds that have already experienced *bofedal* die-offs (D21, D20, and parts of D19) are also the ponds that have likely lost their connection to glacial meltwater inputs. The changing vegetation observed by Seimon et al. (2017) and the stable isotope evidence presented here suggest these ponds are undergoing a transition from glacial meltwater ponds to ponds receiving only precipitation.

In our study *Telmatobius marmoratus* were found in the recently deglaciated zone only in a small subset of three permanent ponds and the seasonally flowing streams that link them. *Telmatobius marmoratus* relies on permanent water bodies because both adults and larvae depend on permanent aquatic habitat. Although we never encountered egg clutches, we observed early-stage tadpoles enter the population in distinct cohorts. We did not determine the exact time to metamorphosis, because we could not effectively mark or identify individuals. However, it is clear that tadpoles spent more than one hydrologic year in ponds and did not time breeding to coincide with the onset of the rainy season. We anticipate that *T. marmoratus* is unlikely to survive in the highest ponds if continued ice retreat reduces dry season meltwater input to the few permanent ponds where *T. marmoratus* are found. Although deglaciation facilitated the expansion of *T. marmoratus* to these ponds, continued deglaciation will likely proceed to subsequently eliminate the species from the same sites, making for an ephemeral elevational expansion. Other montane species may migrate upslope tracking suitable climate and water availability, but topography and future water limitations may trap species in isolated, eventually-unsuitable habitats.

Current occupancy by *T. marmoratus* is limited to ponds on only one side of Site D. They may be able to escape if their current ponds continue drying, and utilize the remaining ponds in site D that are connected to dry season water inflow. For example, we observed *T. marmoratus* tadpoles move out of D21, the most severely desiccating pond, as soon as it was reconnected via seasonal surface flow to lower ponds following a strong precipitation and pond area anomaly in the dry season of 2013. *Telmatobius marmoratus* tadpoles were not observed again in D21 for the duration of the study. They moved into D20.5 and D20. The next pond in the chain, D19, links to the other side of the pond system in the D watershed with ponds D14 and D13. Our stable isotope results suggest that D13 has a connection to dry season water inputs, so dispersal there via the surface streams might allow *T. marmoratus* to persist. It seems unlikely that *T. marmoratus* will be able to utilize new ponds forming near the retreating glacial margins. Other permanent ponds, e.g., F1 and Lower Ice Cave Lake, are located near (<50 m) from the retreating ice margin and have stable isotope signatures indicating year-round connection to glacial meltwater, but have no surface outflow linking them to ponds where *T. marmoratus* currently occurs. Because of their aquatic lifestyle, it seems unlikely these frogs have the ability to disperse between ponds not linked by surface flow in the harsh, high-elevation environment. Additional research is needed to determine what cues signal dispersal to new ponds and terrestrial dispersal abilities.

If shrinking pond size and availability confine *Telmatobius marmoratus* to fewer or less desirable habitats, then there could be synergistic impacts of climate change, disease, and predators. The amphibian pathogen *Batrachochytrium dendrobatidis* occurs throughout the region and has been associated with mass mortality events in the past (Seimon et al. 2007). Seasonal reductions in pond size and stream flow are associated with spikes in disease prevalence and infection intensity in *Telmatobius* spp. (Catenazzi et al. 2013). In a drying landscape, anurans may be exposed to more stress and crowding which could increase density-dependent effects of disease, competition, and predation (Burrowes et al. 2004). Introduced aquatic predators such as *Onchyrynchus mykiss* (rainbow trout) already threaten *Telmatobius* populations throughout the Andes (Angulo 2008). Reductions in habitat area may lead to crowding that can affect anuran immune systems, altering their susceptibility to disease (e.g., *Batrachochytrium dendrobatidis*, *Bd*), or increase disease transmission between tadpoles and adults (Lloyd-Smith et al. 2005, Seimon 2010, Catenazzi et al. 2013). The potential interactions between water scarcity and disease dynamics are particularly worrisome for *T. marmoratus* throughout the high Andes. The pathogen *Bd* has been associated with wide-scale mortality events and population declines of *Telmatobius* species in Peru (Seimon et al. 2007 von May et al. 2008, Catenazzi et al. 2011) and the extirpation and apparent extinction of the three *Telmatobius* species in Ecuador (Merino-Viteri et al. 2005).

Conclusions – Our results from the edge of the biosphere highlight the broad-scale importance of diminishing glacial meltwater and changing precipitation patterns for ecosystems in the central Andes. The central Andean region in particular is increasingly expected to face severe future water scarcity, with consequences for biodiversity and ecosystem function in addition to human populations (Neukom et al. 2015, Buytaert et al. 2017). The Vilcanota is a hotspot of glacier contribution to stream flow throughout the highlands, especially in the dry season and during drought years (Buytaert et al. 2017). Our research highlights changes in aquatic communities that are likely to occur as permanent aquatic habitat shifts towards precipitation-driven ephemeral habitat. In addition, we demonstrate that climatological phenomena that affect the timing and duration of the wet season, such as El Niño, are likely to become increasingly important for many species whose phenology is tied to the onset and duration of the wet season. Our research is one of the first studies to link hydrologic changes to organismal changes in the high Andes. As climate change continues, the wet season will likely continue to become shorter with more intense, less predictable precipitation events. More research is needed on a broad scale to evaluate the ecological effects of changes to precipitation patterns, and loss of glacial inputs to high elevation biodiversity, ecosystem services, and connectivity between low elevations and high elevations.

As increasing temperatures in the lowlands drive many species upslope, and highland human populations grow, the lack of water will become increasingly problematic. Water scarcity in the highlands may lead to increased human-wildlife conflicts as water that once sustained aquatic habitat dries up or is diverted for other uses. In addition, human populations will likely look towards replacing glaciers with water storage in reservoirs, and there is little available information about the impacts of changes to the natural flow regime and necessary environmental flows in high elevation aquatic ecosystems. To escape such conflicts, scientists and conservation organizations may be able to form collaborative partnerships with local communities to meet the water needs of both human communities and ecosystems. The Cordillera Vilcanota provides an opportunity to focus conservation efforts on high-elevation waterscapes, including high elevation lakes, streams, and *bofedales*. Highland communities have long directed water to manage wetlands (Yager 2009), and supporting effective strategies may help to engineer futures (sensu Shoo et al. 2011) for humans, wildlife, and high elevation ecosystems under rapid climate change and the coming water crisis in the high Andes.

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TABLES

Table 2.1. Regional precipitation characteristics during study period

Location	Hydrologic year precipitation totals (mm) 2013-2014	2014-2015	2015-2016	Mean Annual Precipitation* (mm)
Ccatcca	778.9	755.6	541.3	681 ± 132
Progreso	610.5	646.8	733.7	639 ± 102
Pomacanchi	814.3	905.8	893.7	852 ± 110
Santa Rosa	690.4	806.8	NA	782 ± 87

Note: *Ccatcca long-term average from: 1996–2015; Progresso: 1996 – 2013, 2015 – 2017; Pomacanchi: 1996 – 2016; Santa Rosa: 1998 – 2014

Table 2.2. Results of linear mixed effects model on temporary pond hydroperiods.

Fixed Effect	Estimate	Std. Error	t-value
HY1 (2013-14)	8.0221	0.2410	33.29***
HY2 (2014-15)	0.8462	0.2056	4.12***
HY3 (2015-16)	-1.4615	0.2056	-7.11***

Note: ***p<0.001

Table 2.3. Hybrid GAM-GLM results of temporal variation in permanent pond area.

Parametric terms	Estimate	SE	t
HY1 (2013-14)	0.8981	0.049	17.809***
HY2 (2014-15)	-0.011	0.015	-0.801
HY3 (2015-16)	-0.047	0.015	-3.155**

Smooth terms	Ref. DF	F
Monthly precip total	4.033	26.719***
Precip anomaly	3.635	2.635*

Note: *p<0.05; **p<0.01; ***p<0.001

Table 2.4. Hybrid GAM-GLM results for temporal variation in temporary pond area.

Parametric terms	Estimate	SE	t
HY1 (2013-14)	0.285	0.108	2.632**
HY2 (2014-15)	-0.032	0.033	-0.967
HY3 (2015-16)	-0.06	0.033	-1.707

Smooth terms	Ref. DF	F
Monthly precip total	2.000	1.464
Lagged precip total	5.708	7.613***
Precip anomaly	6.804	8.940***
Month	8.000	5.818***

Note: *p<0.05; **p<0.01; ***p<0.001

Table 2.5. *Telmatobius marmoratus* tadpoles cohorts observed during the study. Each cohort is designated by a different shade. Blank rows in the table correspond to months we were unable to sample.

	Developmental Stages																				
Month	26–30					31–41											42–46				
9 2013											▓				▓						
10 2013											▓				▓						
11 2013															▓						
12 2013																	▓	▓	▓		
1 2014																			▓	▓	
2 2014	▒	▒																			
3 2014	▒	▒																			
4 2014		▒																			
5 2014																					
6 2014																					
7 2014																					
10 2014			█																		
11 2014			█																		
12 2014			█	█	█	█										▒	▒	▒			▒
1 2015				█	█														▒	▒	
2 2015		█	█	█	█															▒	▒
3 2015			█		█	█															
4 2015					█																
5 2015		█		█	█																
6 2015			█		█	█															
7 2015			█	█	█																
9 2015																					
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11 2015											█										
12 2015																	█				
1 2016	░																█				
2 2016	░																█	█			
3 2016	░																				
4 2016	░																		█		
5 2016	░				░	░										█			█	█	█
6 2016					░																

FIGURES

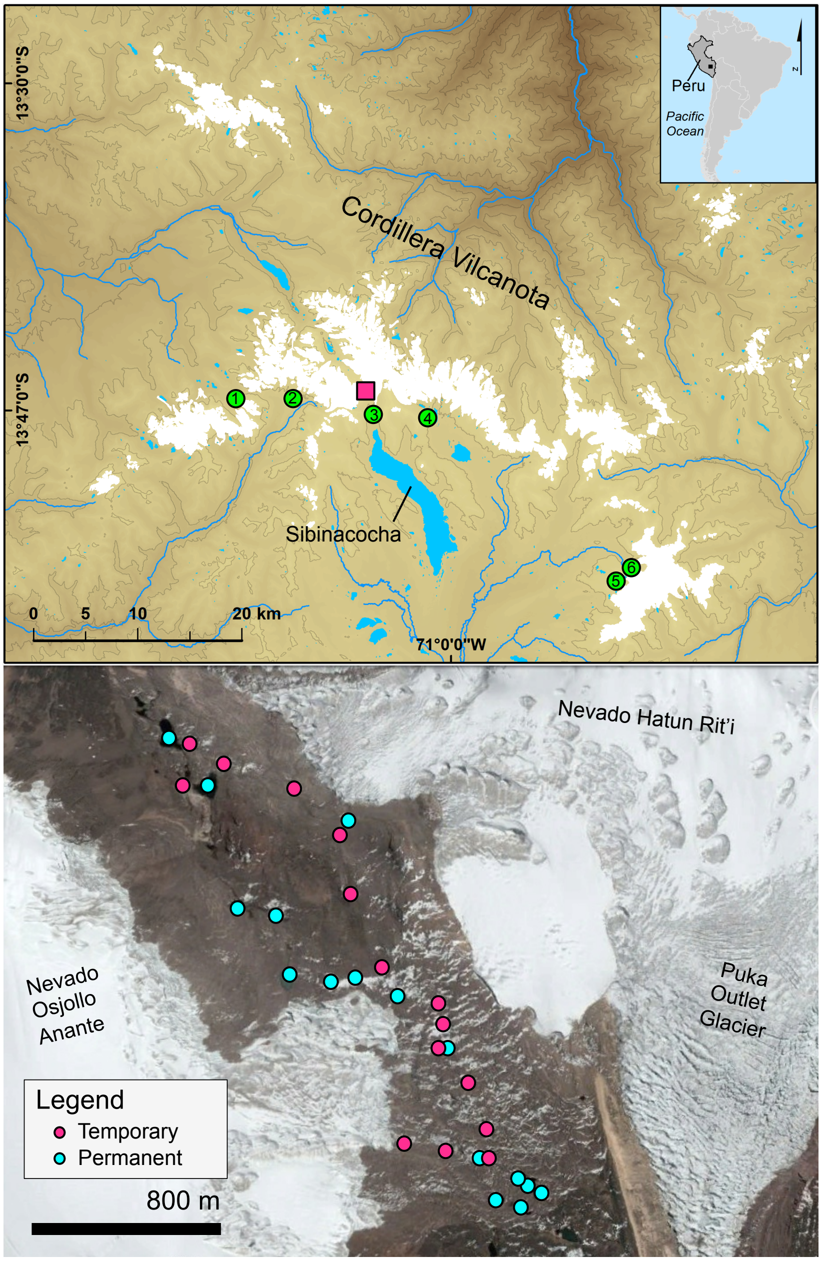

Figure 2.1. Map of main study site and occupancy survey sites in the Vilcanota. Top panel shows occupancy survey sites and main study site (pink box). Inset shows the location of study area within Peru. Study sites (green circles) from west to east: 1 = Pachaspata, 2 = Pico Tres, 3 = Rincon, 4 = Hatun Ñañupunta, 5 = Challpa, 6 = Qori Kalis. Bottom panel shows temporary (red circles) and permanent (blue circles) pond locations at main study site where reproductive phenology study took place.

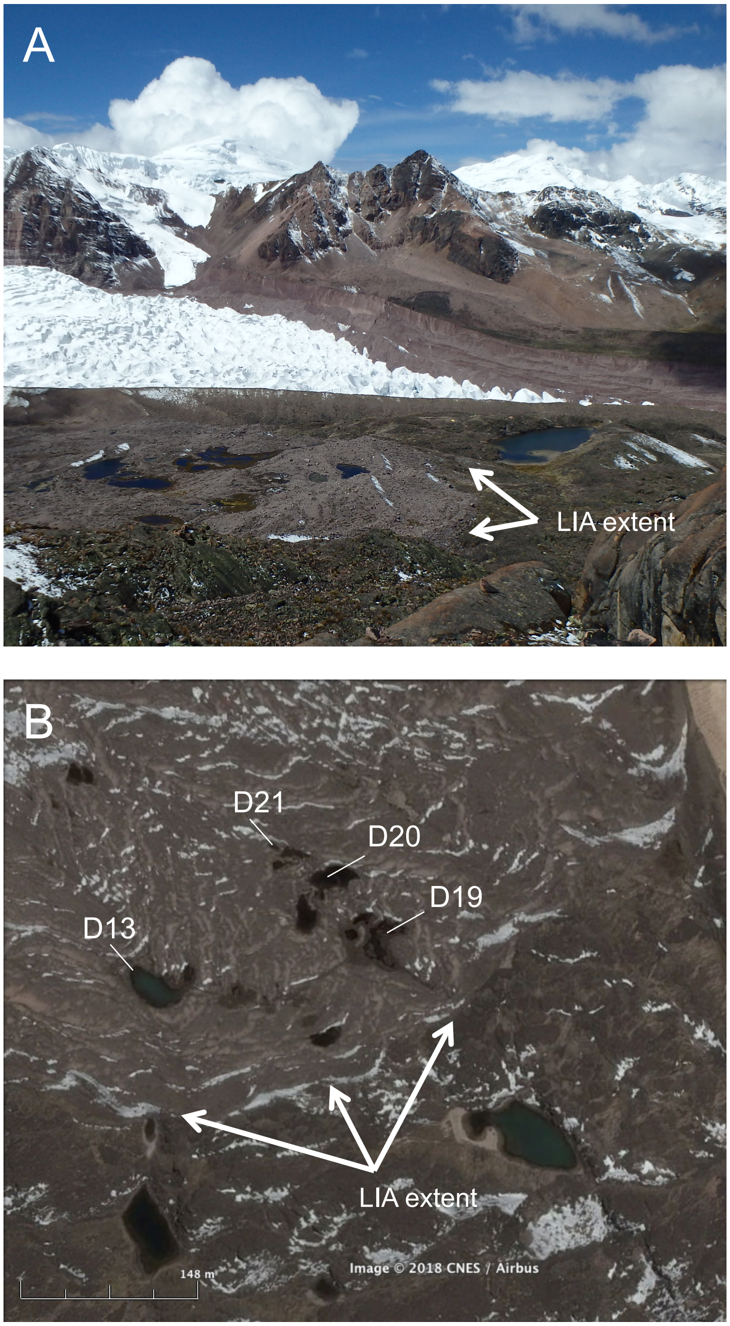

Figure 2.2. Method for identifying the ecotone between the recently deglaciated zone and areas that were not ice-bound during the Little Ice Age (LIA). A) In situ color differences between old and newly exposed moraines and B) satellite imagery available via Google Earth. Numbered ponds are referred to in the text.

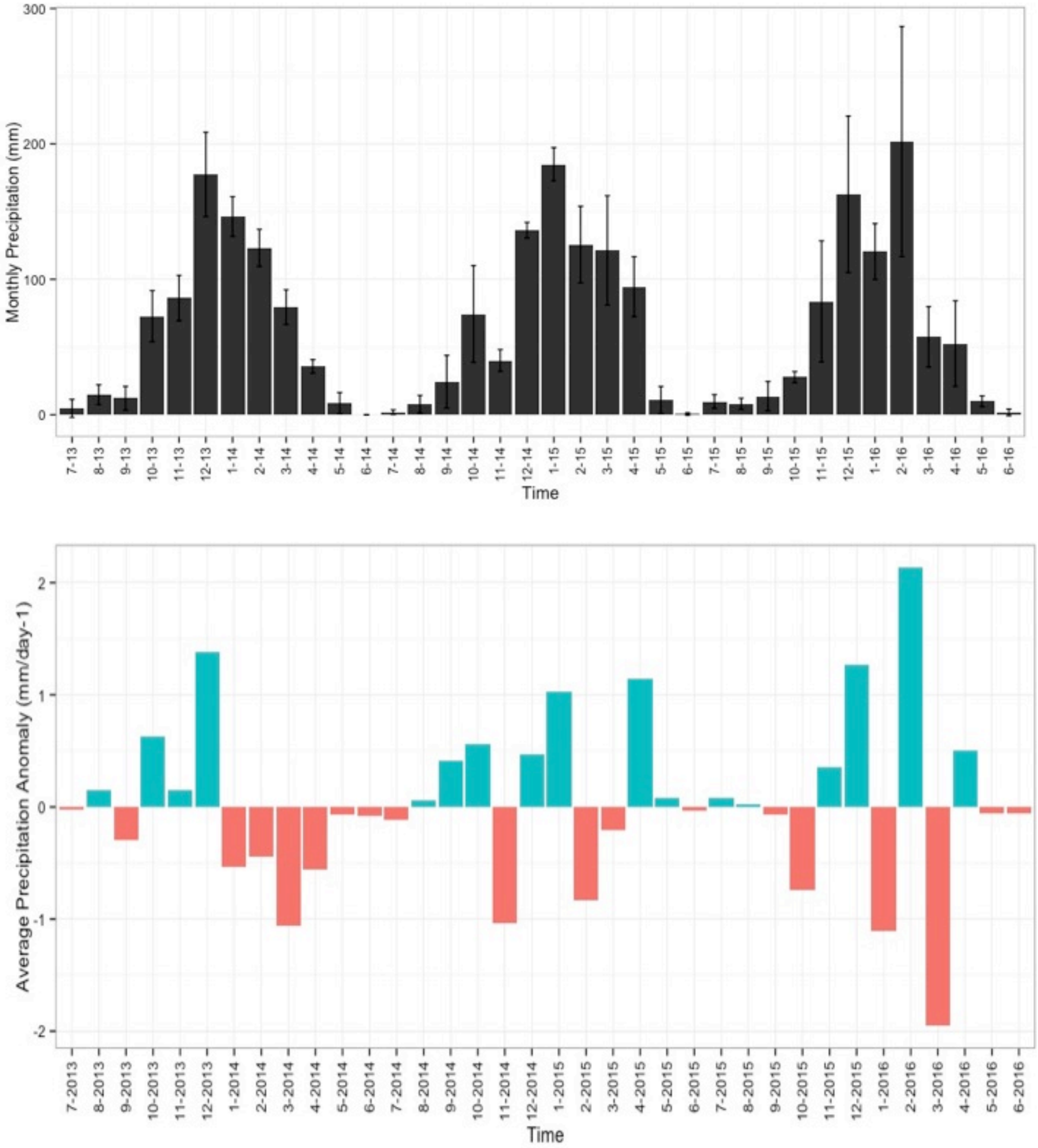

Figure 2.3. Precipitation patterns and precipitation anomaly. Precipitation patterns (top) and precipitation anomaly (bottom) based on records from four SENAMHI weather stations during the study period. Precipitation anomaly is based on a 1996–2017 reference period.

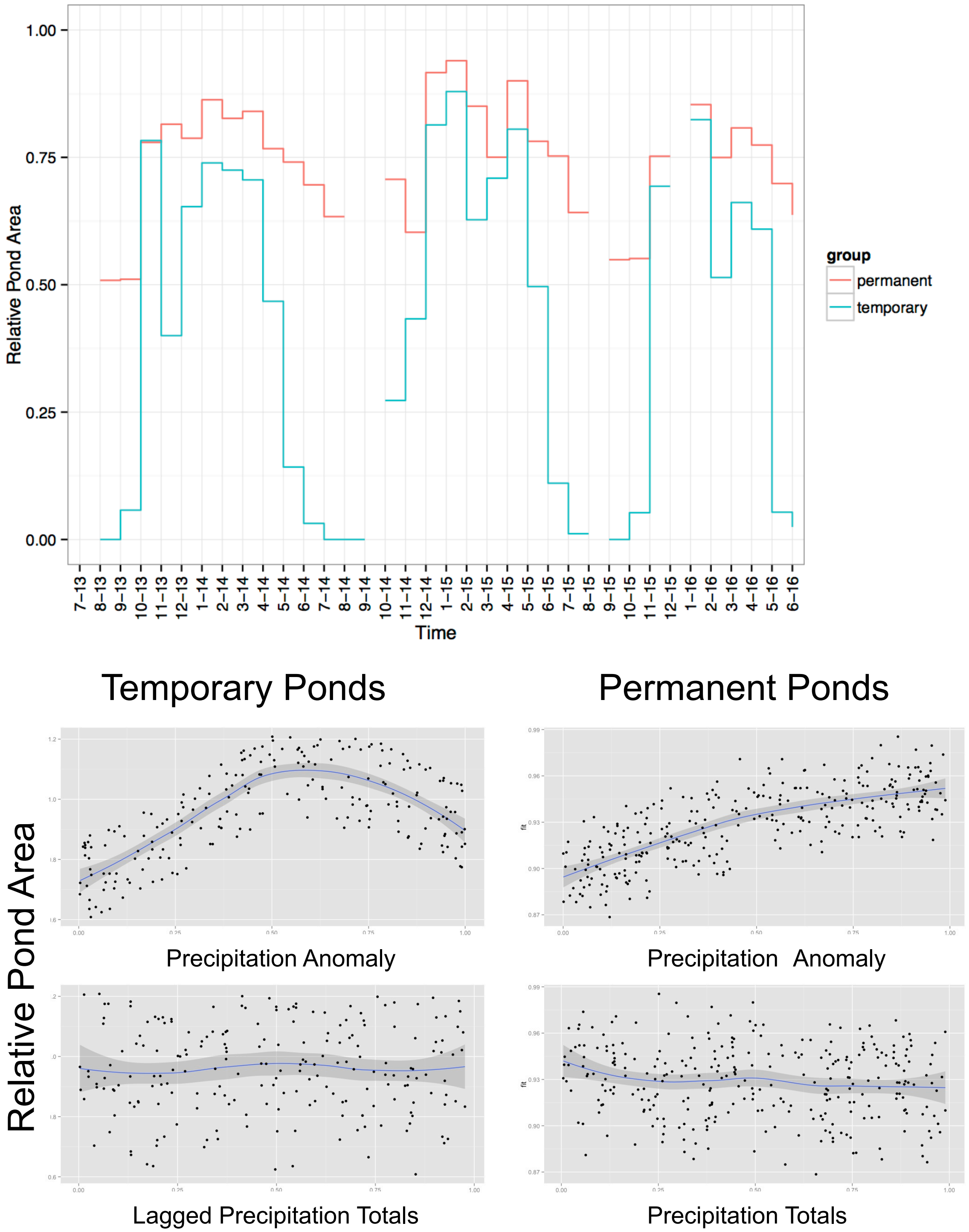

Figure 2.4. Variation in relative pond area during study period. Top – Variation in relative pond area during study period. Bottom – Predicted pond area (with 95% CI) from GAMM analysis showing relationship between nonparametric terms in the top model and relative pond area for both temporary and permanent ponds.

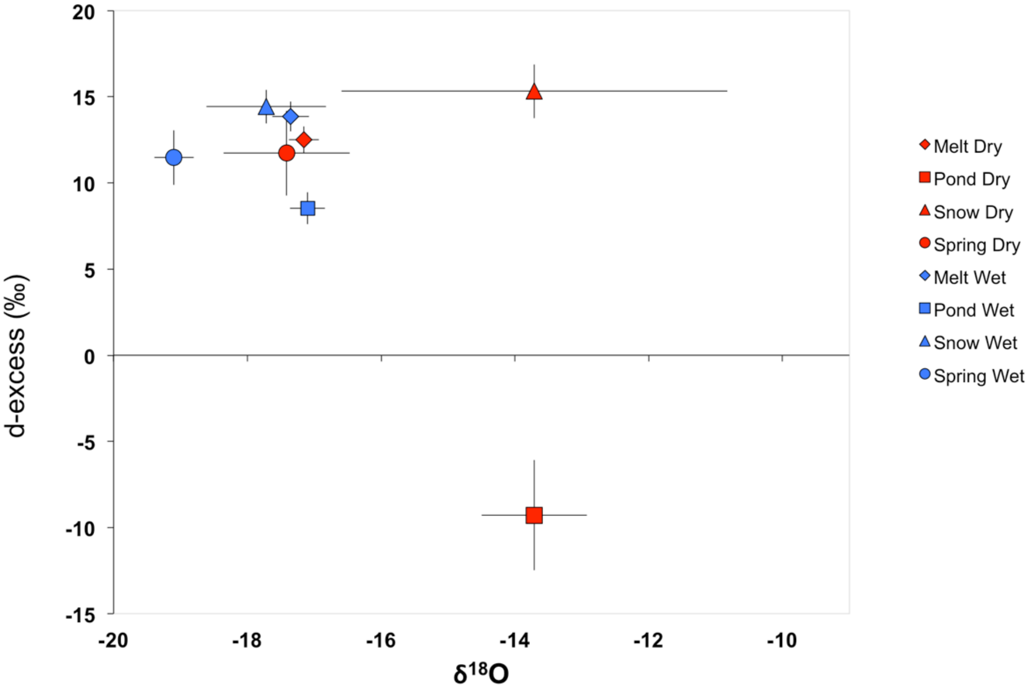

Figure 2.5. Relationship between d-excess and δ^{18}O (Mean ± SD) for different meteoric waters in the Cordillera Vilcanota.

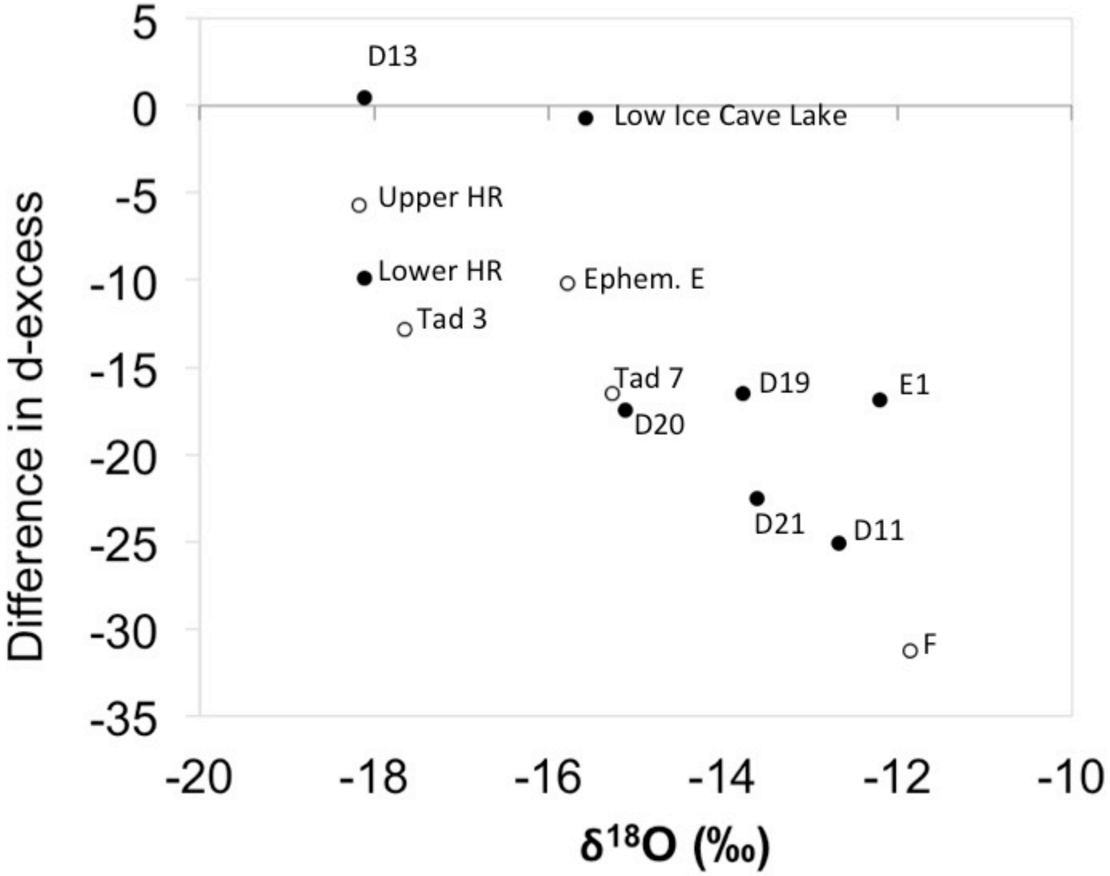

Figure 2.6. Relationship between the difference in d-excess (wet-dry season values) and δ^{18}O for ponds measured in both seasons. Open circles indicate seasonal ponds and closed circles represent permanent ponds.

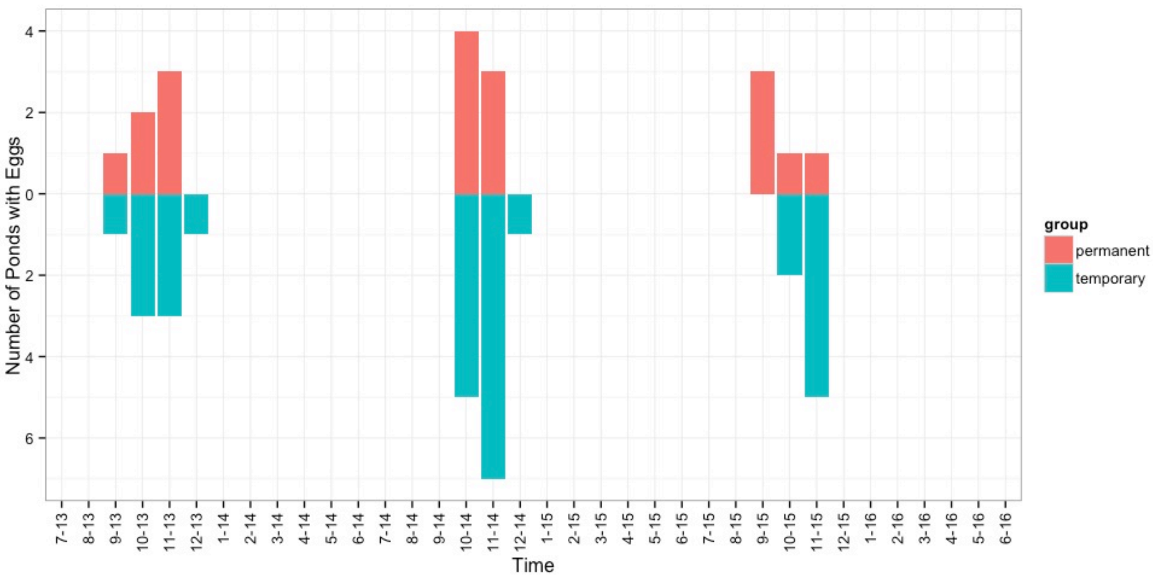

Figure 2.7. Timing of egg deposition for *Pleurodema marmoratum.*

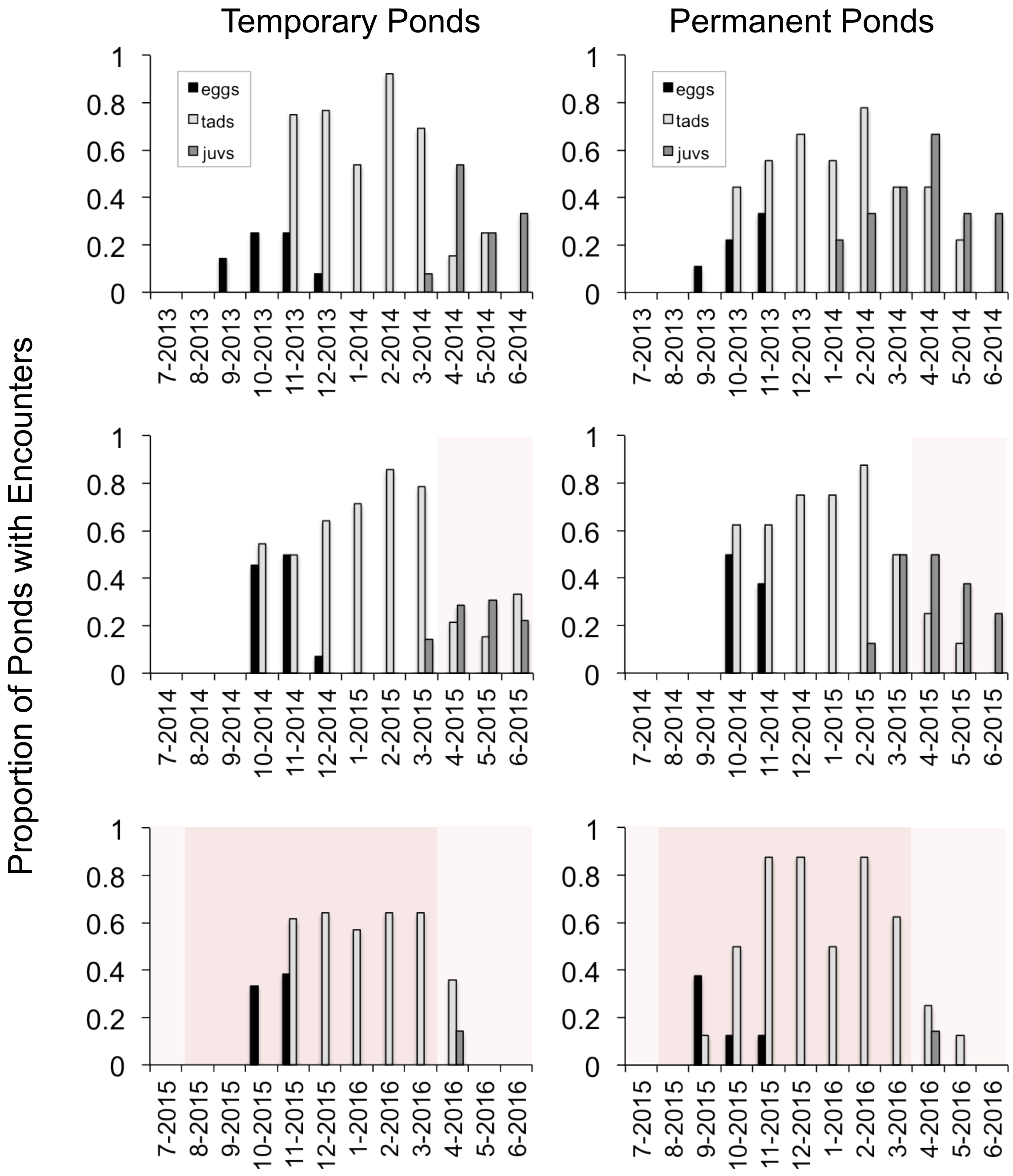

Figure 2.8. Reproductive phenology of *Pleurodema marmoratum*. Each row represents observations from one breeding season. Red shading indicates severity of El Niño. Light red shading is ONI > 1.0, dark red shading is ONI > 2.0.

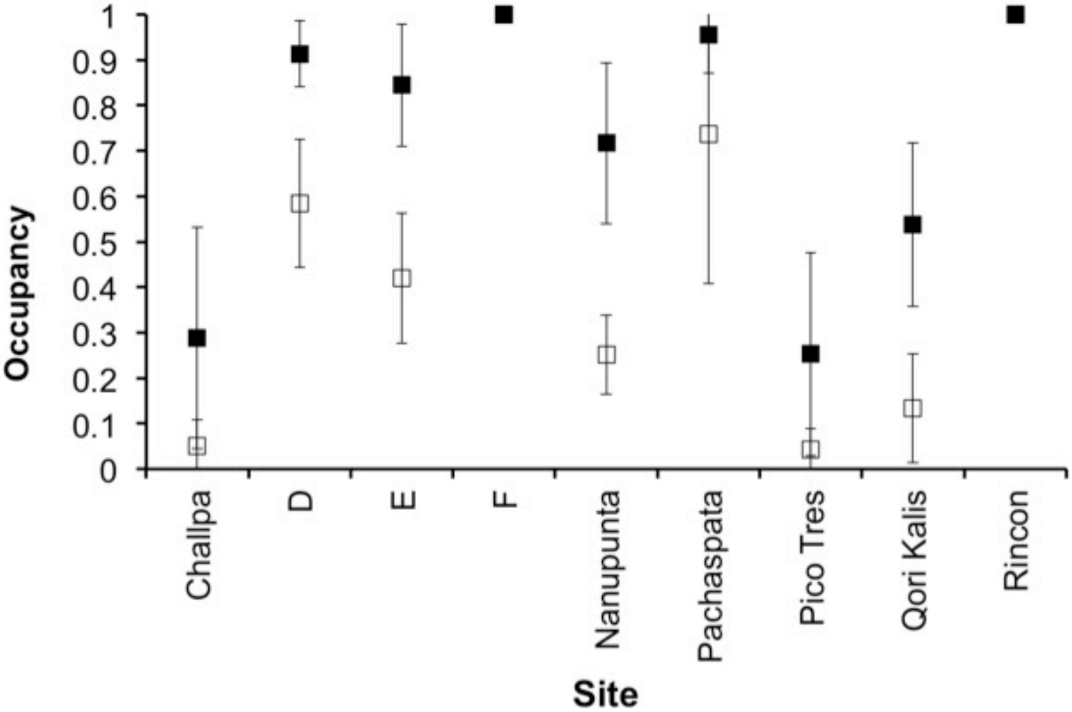

Figure 2.9. Occupancy estimates (estimate ± SE) for *Pleurodema marmoratum.* Site occupancy was significantly higher where there were *bofedal* wetlands (closed squares) compared to sites without *bofedal* wetlands (open squares).

Chapter III: Amphibian adaptations to extreme freeze-thaw cycles at 5250 m elevation in the tropical Andes of Peru

ABSTRACT

High elevation tropical environments are characterized by extreme conditions including high solar insolation, rapid environmental temperature fluctuations, and daily freeze-thaw cycles. The thermal environment in high mountain areas is of special concern because climate change is causing temperatures to increase most rapidly at high elevations. Temperature plays a key role in the physiology and ecology of ecotherms, including anurans, which are found at higher elevations in the tropics than anywhere in the temperate zone. In the Cordillera Vilcanota in southern Peru, significant changes in temperature and glacial cover have allowed two anuran species to expand their elevational ranges upslope by 200+ meters, making them some of the highest recorded amphibian populations on the planet. These anurans, *Pleurodema marmoratum* and *Telmatobius marmoratus*, occur at the same elevation and biome, yet experience contrasting thermal environments because of their use of terrestrial or aquatic microhabitats. *Telmatobius marmoratus* escapes extremes of the daily temperature fluctuation by utilizing thermally buffered aquatic habitat at all life stages. *Pleurodema marmoratum* has one of the highest CT_{max} values (>32° C) reported from the tropical Andes of Peru and is frost-tolerant. The wide thermal tolerance breadth reflects the extreme daily temperature variation *P. marmoratum* encounter in the high-elevation habitat. We recorded frog operative temperatures ranging from -3.5 to 44° C in a single day. Our finding expands knowledge of frost-tolerance in amphibians to the widespread

Neotropical family Leptodactylidae. Our study provides strong support for the role of physiological plasticity as one of the key traits allowing species to migrate upslope because of climate change, and as a key adaptation for life in extreme high-elevation tropical environments. As climate change results in species range shifts, other taxa may encounter novel thermal environments, and insights gained here may help us mitigate negative effects of species range shifts needed for future conservation efforts.

INTRODUCTION

Research over the last several decades has revealed that a surprising diversity of life exists in Earth's most extreme environments, from extreme ocean depths (Lonsdale 1977), to altitudes of 20 km in the atmosphere (Smith et al. 2010), and from extreme heat (Brock 1978, Sissons et al. 1987) to cold environments including deserts and the poles (De Maayer et al. 2014). Research on organisms in extreme environments helps to determine the limits of habitability on Earth. Most research in extreme environments focuses on microbial and plant life. However, some vertebrates, particularly ectotherms, have been highly successful at adapting to extreme environments (Rothschild and Mancinelli 2001, Costanzo and Lee 2013).

Amphibians have adapted to live in nearly every habitat on Earth and frequently inhabit seemingly inhospitable climates despite physiological limitations associated with ectothermy, their permeable skin, and, in many cases, a dependence on water for one or more life history stages. Freeze tolerance has been found in several frog, salamander, and reptile species (Storey and Storey 1986, Costanzo et al. 1992b, Voituron et al. 2002) and has nearly always been understood in terms of surviving long, cold, high-latitude winters.

For example, Siberian newts (*Salamandrella schrenckii* and *S. keyserlingii*) can survive being frozen for 5 months with minimum temperatures of -35°C (Berman et al. 2010). Alaskan wood frogs (*Lithobates sylvatica*) can survive being frozen for 7 months with minimum temperatures below -18°C (Larson et al. 2014). However, polar regions are only one of the extreme environments where amphibians are found. Reviews examining freeze tolerance in ectotherms have not considered that this trait can also be found in South American taxa (Voituron et al. 2009, Costanzo and Lee 2013, Storey and Storey 2017), but many species of montane tropical amphibians appear to be cold-tolerant (Navas 1997). Because high-elevation tropical environments have air temperatures that fall below freezing every night, freeze tolerance in tropical high-elevation frogs has been hypothesized (Carvajalino-Fernández et al. 2011), but has not been tested. Unlike frogs in temperate zones where freeze tolerance is ecologically relevant in terms of hibernation on a seasonal scale, tropical frogs must be able to freeze on a diurnal scale, withstanding freeze-thaw cycles every night potentially year-round.

Although they have received less attention than polar regions, high-elevation environments can generate stressors that are more severe than high-latitude environments because of low atmospheric pressures, high ultraviolet radiation and irradiance, low humidity, and daily freeze-thaw cycles (Hedburg and Hedburg 1979). Tropical high-elevation environments integrate conditions typically associated with other extreme environments like deserts (e.g., wide nocturnal-diurnal temperature fluctuation, low humidity) and high latitudes (e.g., low temperatures) with extreme conditions that result from altitude (e.g., low atmospheric pressure and oxygen saturation; Korner 2003, Nagy and Grabherr 2009). One of the unique and most ecologically-relevant climatic variables in the high-elevation tropics is that daily fluctuations in temperature are more extreme than are the seasonal fluctuations in temperature (Halloy 1991, Schmidt et al. 2009). Tropical high-elevation organisms must survive daily cycles of high temperatures during the day and freezing temperatures at night, throughout the year.

Temperature is one of the most critical abiotic aspects of the environment for amphibians because they are ectotherms. Temperature controls almost all aspects of physiological function, affects population structure, and may be one of the most limiting factors in the elevational distributions of amphibian species (summarized by Hillman et al. 2009). Body temperatures outside the thermal tolerance limits impair function, increase stress, and can lead to death. Tropical ectotherms are predicted to be severely affected by warming temperatures (Deutsch et al. 2008, Tewksbury et al. 2008).

Thermal threats to tropical amphibians are of particular interest in high-mountain environments for three reasons. First, temperatures are increasing because of climate change more rapidly at high elevations compared to lower elevations (Bradley et al. 2006, Urrutia and Vuille 2009, Pepin et al. 2015). Second, tropical montane regions are of special concern because they are engines and epicenters of biodiversity (Hoorn et al. 2010, Graham et al. 2014). In the Peruvian Andes, highly biodiverse amphibian communities have experienced rapid declines of species richness and abundance (Catenazzi et al. 2011). Finally, thermal stress has a negative effect on amphibian immune responses (Raffel et al. 2006, Rollins-Smith 2017), potentially intensifying the effects of other stressors, such as disease (Ribas et al. 2009, Greenspan et al. 2017b). Understanding thermal tolerance limits of high elevation amphibians is of considerable interest to conservation planners who wish to predict species vulnerability in the context of climate change and disease (von May et al. 2017), yet a significant proportion of research effort on thermal tolerance limits in the tropics has been focused on lowland wet forests. Nearly all research on amphibians has focused on populations living below the treeline (i.e., <4000 masl).

The climate variability hypothesis (CVH) predicts that organisms will have wide thermal tolerance limit ranges in thermally variable environments (Janzen 1967, Stevens 1989). The CVH has historically been understood as predicting narrow thermal range limits of species in thermally stable environments present at low latitudes. Thermal limit breadths are predicted to be narrow in the tropics and increase in width with increasing latitude. Within the tropics, increasing elevation is predicted to result in narrower thermal limits. However, these assumptions may be a result of a research bias towards species in select tropical ecosystems. Much of the generalized understanding of physiological tolerance limits in the tropics is derived from observations of species in closed-canopy ecosystems, which can be relatively stable thermal environments (Ghalambor et al. 2006) but, even there, considerable temperature variation is present at different canopy levels (Scheffers et al. 2013). Differences in thermal variability among tropical biomes can be extreme, particularly across ecotones that separate closed and open canopy habitats. For example, thermal regimes across the forest-puna ecotone in the high Andes results are very different (Catenazzi et al. 2014), likely because of surface heating by high insolation levels at high elevations in the tropics. Over-generalizing the thermal stability of tropical environments can lead to poor predictions for species distributions and survival as the thermal environment that tropical ectotherms experience changes (e.g., Payne and Smith 2017). Assuming that most tropical habitats are uniformly consistent thermal environments may lead to underestimating the ability of many tropical ectotherms to tolerate a wide range of thermal conditions and to utilize behavioral thermoregulation to avoid extreme events (Navas 1996b, 2004, 2013; Sunday et al. 2014). Not all tropical habitats are warm and constant, and not all tropical taxa have narrow thermal tolerance limits. Many tropical montane amphibian and reptile species appear to have greater tolerance to low and high temperatures, and wider thermal tolerance breadths than low-elevation species (Navas 1996a,b, 2013; Catenazzi et al. 2014; Rueda-Solano et al. 2016; von May et al. 2017).

Tropical high-elevation amphibians provide unique opportunities to study different strategies for life in extreme habitats and for understanding how species respond to climate change. Understanding adaptations to the thermal environment and species' thermal tolerance limits is key for understanding how they respond to climate change including predicting which species can tolerate the physical environment at elevations higher than where they currently live and which species currently living at high elevations may be forced off (i.e., be extirpated from) the mountaintop as climate change progresses. As organisms respond to climate change, they must migrate to different elevations or latitudes, adapt to the new conditions, or perish (Miles 1994, Aitken et al. 2008). In addition, the high thermal variability of high-elevation habitats provides a model system for understanding the importance of thermal microhabitat variation, thermoregulation, and optimal temperatures (Navas et al. 2008). Thermal microhabitat variation has important implications for species persistence and future distributions under climate change, limits to vertebrate life in extreme habitats, and also for interacting effects of temperature preferences and susceptibility to pathogens (Greenspan et al. 2017a).

Studies of adaptations to the extreme environment of tropical high-elevation organisms are scarce. Globally, nearly all research takes place below 3500 meters above sea level ("masl"; Jacobsen and Dangles 2017). However, the high ice line on tropical mountains allows the biosphere to extend higher on tropical mountains than in the temperate zone, and tropical mountains support diverse ecosystems at higher elevations than do temperate mountains (Nagy and Grabherr 2009, Jacobsen and Dangles 2017). Climate change is causing montane ecosystems globally to increase in extent, especially in the tropical Andes where glaciers are rapidly disappearing (Bradley et al. 2006, Thompson 2011). In the tropical Andes, the limits of the biosphere now extend well beyond 5000 masl (Seimon et al. 2007). The elevational bias in research effort results in the exclusion of high elevation zones where climatic warming is occurring most rapidly and organisms and ecosystems are already responding to these changes (Anderson et al. 2011, Pepin et al. 2015).

The tropical Andes constitute the majority (i.e., 94%) of the world's tropical high elevation land area (Jacobsen 2008). In the Cordillera Vilcanota in southeastern Peru, the second most heavily glaciated tropical mountain range, at least three anuran species have expanded their elevational ranges by 150-200 meters upslope within the last century (Seimon et al. 2007). Two of those frog species have colonized a recently deglaciated corridor above 5200 masl, providing one of the best examples of climate change directly facilitating range expansion of vertebrate species. As a result of upslope migration, *Pleurodema marmoratum* (marbled four-eyed frog) holds the global elevational range record for any amphibian species (at nearly 5400 masl; Seimon et al. 2007, 2017). Another species, *Telmatobius marmoratus* (marbled water frog), is found in the recently deglaciated zone up to 5244 masl (Seimon et al. 2007).

We studied the thermal environment of *P. marmoratum* and *T. marmoratus* at 5250 m in the Cordillera Vilcanota of southern Peru. We had three specific goals. First, we determined the range of ecologically relevant environmental temperatures (T_e) and operative temperatures that these two frog species are likely to experience in the extreme environment at the edge of the cryosphere. We used dataloggers and biophysical frog models placed in the terrestrial environment (for *P. marmoratum*) and dataloggers in the aquatic environment (for *T. marmoratus*), allowing us to compare the thermal environment experienced by these two frogs with contrasting life histories. Second, we assessed the thermal tolerance limits and warming tolerance for *Pleurodema marmoratum* and we evaluate the limitations of this standard metric in the extreme thermal environment that characterizes our study site. Finally, we conducted the first experimental test of frost tolerance in a tropical anuran. Our study extends knowledge of amphibian thermal limits by at least 1400 vertical meters to the current upper limit of the Andean biosphere.

METHODS

Study Location

Our study took place in the Cordillera Vilcanota (hereafter "CV"; 13.7°S, 71.0°W) in Cusco Department, southeastern Perú, at the western margin of the Amazon Basin (Fig. 1a). The climate in the CV is characterized by a distinct wet (peak:

December, January, February) and dry season (peak: June, July, August), with most precipitation falling from October-May (Perry et al. 2014, 2017). Daily air temperature is most variable during the dry season, and average air temperatures are slightly lower in the dry season than in the wet season (Schauwecker et al. 2014). Ground temperatures varying from -11 to 25°C have been recorded at 5220 m at the site (Schmidt et al. 2009)

From 1980-2009, minimum and maximum air temperatures in the CV changed by –0.2°C and 0.23°C respectively (Salzmann et al. 2013). Tropical glaciers are sensitive to small changes in temperature because they exist at the melting point year-round (Vuille et al. 2008). These small temperature changes have caused reductions in CV glaciers by ~30% in area, and 40-45% in volume between 1985 and 2006 (Salzmann et al. 2013). The combination of warming temperatures and rapid glacial retreat has opened an ice-free corridor from 5200-5400 masl north of Lake Sibinacocha. The ice-free corridor is located between the rapidly retreating ice fields of Osjollo Anante (~5500 masl) and Nevado Hatunrit'i (6106 masl; Fig. 1b). Within the ice-free corridor, rapid deglaciation has resulted in the formation of glacial meltwater and snowmelt ponds that have been colonized by three amphibian species (Fig. 2a; Seimon et al. 2007). Two amphibian species are currently found within the ice-free corridor (Seimon et al. 2017). The region is recognized as a high-priority area for long-term population studies of amphibians in Perú (Catenazzi and von May 2014).

Study Species

Pleurodema marmoratum is a pond- and wetland-breeding species with an aquatic tadpole and terrestrial adult stage (Fig. 2b). Adults and juveniles are always found under cover rocks (with the exception of recently metamorphosed juveniles undergoing their initial dispersal from pond to rocks along the perimeter), presumably to utilize the more humid microclimate compared to the arid conditions above rocks (Reider, personal observation). The species has a wide geographic distribution in the central Andes and an elevational range of 3200–5400 masl (IUCN 2015a). Another species, *Telmatobius marmoratus*, is aquatic at all life stages and is found in streams, ponds, and *bofedales* (high-elevation Andean peatlands) (Fig. 2c). *Telmatobius marmoratus* also has a wide elevational range, 1800–5244 masl, and has suffered population declines throughout its range (IUCN 2015b). Repeated sampling over the last 15 years indicates that *Pleurodema marmoratum* and *Telmatobius marmoratus* have maintained populations inside the recently deglaciated zone at our field site since at least 2002 (Seimon et al. 2017).

Environmental Temperatures

Air Temperature. We recorded air temperature (T_a) from August 2013 to June 2016. We placed pairs of iButton thermochron loggers (Maxim Integrated Products, Sunnyvale, California, U.S.A) recording every 20 or 30 minutes in a plastic bag inside a shaded, ventilated rock cairn next to our study ponds from 5230–5250 masl. We compared a subset of data from both concurrently-recording thermochrons to ensure they were measuring the same temperature (within 0.5 °C). Thermochrons were replaced approximately every 4 weeks. The memory for each iButton lasted approximately 3.2 weeks, leading to gaps in our air temperature record. We report daily air temperature (averaged across three years, or two years when there were gaps) and daily maximum and minimum air temperatures.

Frog Thermal Environment. At such high elevations, relative humidity is low, solar irradiance is high on clear days, and surface temperatures can be extremely variable despite low air temperatures. Therefore, we placed 4-5 groups of iButton thermochrons recording environmental temperatures (T_e) every 30 minutes inside of plastic bags at different depths in *Pleurodema marmoratum* habitat around ponds. In each group of ibuttons we placed one logger on the surface under a cover rock and at 5-10 cm depth intervals (to 50 cm below the surface; $T_{surface}$, T_{-10}, T_{-20}, etc.). Note that $T_{surface}$ under cover is relevant to frogs in the high-elevation environmentbut is not representative of exposed temperatures on the surface of cover rocks. We dug holes in the glacial till and loosely refilled them after placing iButtons at the desired depths. We limited the logger locations to within 20 m of study ponds where we had previously encountered frogs and used randomized cover rocks at different aspects and exposure to sun throughout the day.

Frog Temperatures and Thermal Limits

Frog Operative Temperatures. One method used to predict the risk of additional climate warming on amphibian populations is comparing the thermal environment experienced by the organisms to the physiological thermal tolerance limits of the organisms. Metrics that have been applied to montane amphibians include warming tolerance (WT) and operative warming tolerance (OWT). The WT is the difference between the average upper physiological thermal limit for a species (i.e., the critical thermal maximum or "CT_{max}") and the average maximum air temperature (Deutsch et al. 2008). The OWT is the difference between CT_{max} and the operative temperature, or the temperature a frog is likely to be at in its habitat. A large WT value suggests that individuals in that location have a high thermal safety margin for warming temperatures (Duarte et al. 2012). However, the average air temperature is a poor indicator of temperatures individual organisms actually experience in high elevation environments and OWT is a preferred method in variable environments (Navas and Araujo 2000, Catenazzi et al. 2014). The OWT is particularly relevant for inactive frogs (Navas 1996), and likely most relevant for frogs that live under cover rocks. Both WT and OWT may underestimate the temperatures that frogs actually experience in environments with extreme daily thermal amplitudes, so the extremes of OWT are also critical (Camacho et al. 2015).

Frog body temperatures are strongly affected by substrate temperature (Navas et al. 2013), but are also affected by evaporative water loss ("EWL"). At such high elevations, air humidity is low and high rates of EWL likely reduce frog body temperatures. We encountered many frogs in relatively wet microhabitats, near seeps and springs, and EWL may have been reduced in these moist refugia. We did not quantify humidity or EWL in frog microhabitats. However, ignoring the effect of EWL on frog operating temperatures might lead to higher temperature measurements than actual frog temperatures would be. Therefore, we chose not to model frog operative temperatures at equilibrium (T_e) directly from T_a or $T_{surface}$ as others have done (e.g., Catenazzi et al. 2011), and instead used paired permeable and semi-permeable agar models (Rowley and Alford 2010). The paired semi-permeable and permeable models bracket the range of likely T_e for inactive frogs under the same conditions (Navas and Araujo 2000). Typically, agar models yield an estimate of EWL for the period they are deployed. However, in our study the models experienced freeze-thaw cycles and precipitation that may have affected estimates of EWL, so those data are not presented here. We chose locations for agar models under rocks where we had encountered frogs during surveys. On 1, 3, and 4 July, 2014, we placed 10-11 pairs of proxy frog models made from 3% agar under rocks in frog habitat for 24 h. Each agar model had an iButton thermochron logging every 30 minutes embedded inside it.

Telmatobius marmoratus is a fully aquatic species, and EWL is not a concern in the water. Therefore, we used Hobo Tidbit dataloggers to document the possible range of operative temperatures (T_e) *Telmatobius marmoratus* experiences in the water. We placed dataloggers in shallow water (~10 and 20 cm deep; N = 2) and in deep water (1.3–1.5 m deep; N = 1) in two ponds where we observed *T. marmoratus* tadpoles and adults.

Frog Body Temperature. We searched for *Pleurodema marmoratum* under rocks around four study ponds on 29 June, 3 July, and 4 July 2014. We used a rapid-read thermometer (Taylor Precision Products Waterproof Digital Thermometer) calibrated in boiling water (82° C at 5250 masl) and ice water (0° C). We gently pinned the frog using a flexible ruler and placed the probe tip in contact with the frog to measure body temperature (T_b). The thermometer took ~ five seconds to stabilize. During the five-second measuring interval we kept the frog in its original position on the substrate and protected it from wind, sun, and snow to minimize temperature changes. We sterilized the ruler and thermometer probe with 90% EtOH after every measurement. We recorded substrate temperature (T_s) under the frog immediately following the measurement of T_b. We used a linear regression model (call lm in R) to compare the relationship between T_a and T_s to T_b.

Critical Thermal Maximum. We used a standard metric, the loss of righting response (Navas et al. 2007), to measure CT_{max} in *Pleurodema marmoratum* from 5230-5250 masl (N = 69 frogs). Loss of righting response is the first easily observed response to temperature stress. Other experimental endpoints (e.g., onset of muscle spasms) have been recommended to test thermal limits (Lutterschmidt and Hutchinson 1997), but can lead to death, which we wanted to avoid. We tested frogs in both the warm-wet and cold-dry seasons. We allowed frogs to acclimate to ambient conditions at the same elevation for 24 hours before testing. We placed frogs in plastic cups with a thin layer of water, and cups were immersed ~3 cm deep in a water bath by placing a stone on top of the cup. We preheated water to a starting temperature close to 15° C. We slowly increased the temperature of the water bath from approximately 15 to 36° C at a rate of approx. 0.4-0.8° C/min. We used a general linear model to examine the relationship between warming rate, frog body size, and month on CT_{max}.

We measured the frog temperature in the thin layer of water every 1-2 minutes with a non-contact instant-read infrared (hereafter: IR) thermometer (accuracy ±2%; emissivity = 0.95; Nubee NUB8500H). We assumed surface temperature was close to core temperature for these small frogs (mean size ± SD: 1.8 ± 0.7 cm; Navas et al. 2007). Measuring area of the IR thermometer was approximately 0.4-0.5 cm in diameter at 5 cm distance, so the device could have integrated the temperature of a small amount of water area in the measurement. We assume frog surface temperature and water temperature immediately around the frog were very similar because of the small size and thermal inertia of the frogs. We compared temperature readings from the IR thermometer with a quick-reading thermometer using linear regression (y=1.00x-0.07; R^2=0.997; Supporting Information Appendix 3.1).

We inverted each frog onto its dorsum after a two-minute heating interval and stimulated the righting response by gently touching the frog with a probe. When a frog was unable to right itself after 5 seconds, we recorded the final temperature measurement as CT_{max}, and removed the frog from the water bath. We moved frogs to a cool water to prevent additional heat absorption and later returned them to their capture location.

Frost Tolerance. We allowed individual *Pleurodema marmoratum* (N=42) to acclimate to the same thermal conditions at 5230 m for 24 hours before exposing them to ambient freezing temperatures on five separate nights in the dry season (May-June 2016). We placed frogs individually into 500 ml plastic freeze chambers. Total exposure on each night was intentionally limited to prevent cell damage and death, and the chambers were insulated from the ground and protected from the wind to prevent rapid cooling. Each chamber had a frog-sized corral with a HOBO Type-T thermocouple (Onset Computer Corp.) recording every 10 seconds and positioned in contact with the frog abdomen. We dried surface moisture from all frogs and placed them in the chambers at 1600 hrs.

We included a 1 cm x 2 cm strip of moistened cellulose filter paper in each chamber under the frog to provide an ice nucleation source to prevent rapid freezing and cell damage. We removed frogs from the experiment when they were visibly frozen. We were unable to record a crystallization temperature because the thermocouples did not detect an exothermic reaction. The thermocouple sensors may not have been positioned correctly to be constantly in contact because frogs were moving in the chamber until just prior to freezing. Instead, we report the average and lowest temperatures recorded for each frog during the period between when movement ceased and when the frog was visibly frozen. While the freezing period was ~60 minutes for most individuals, we tested a subset of frogs for a longer (6 hr) duration. After a recovery interval of 12 h we tested frog survival using a 1 min locomotory test of hopping performance. We compared the hopping ability of frogs that froze and frogs that did not freeze using Walsh's t-test. Frogs were placed in a plastic bin and stimulated to hop with a probe. Only frogs that demonstrated locomotory ability were considered survivors.

RESULTS

Environmental Temperatures

Average air temperature for the entire study period, September 2013 – June 2016, was 1.49°C ± 3.79 °C (mean ± SD). Daily temperature variation was much greater than seasonal temperature variation and was greatest in the dry season (Fig. 3). Air temperature extremes were more pronounced during the dry season than the wet season (Table 1). Freezing air temperatures occurred throughout the year (Fig. 3).

Surface temperatures in frog refugia (i.e., under cover rocks) were more variable than ground temperatures (Fig. 4). The range of surface temperatures was greater in the dry season (42 °C in May-June 2015) compared to the wet season (33.5 °C in December 2015-January 2016; Fig. 4). Minimum night surface temperatures were lower in the dry season compared to the wet season (Fig. 3). Temperature variation decreased with depth (Fig. 3). From 20 to 50 cm below the surface, ground temperatures were a nearly constant 5°C and never dropped below freezing.

Surface temperatures were very similar to air temperature at night, but during the day surface temperatures were often much higher than air temperature (Fig. 5). Because of the large thermal amplitude of surface temperatures, average temperatures were much lower than maximum temperatures (Fig. 5a-b).

Frog Temperatures and Thermal Limits

We encountered multiple *Telmatobius marmoratus* tadpoles, juveniles, and one adult in shallow water (< 1 m deep). These individuals were occasionally observed free-swimming, and frequently observed sequestered under submerged rocks. We observed one adult *T. marmoratus* swimming in deep water (> 1 m deep). During the dry season, we rarely observed *T. marmoratus* of any size because pond water levels were reduced and the frogs could not access the gently sloping pond edges with submerged cover rocks. Water operating temperature (T_e) in *T. marmoratus* ponds was much more variable in shallow water compared to deep water indicating the presence of a strong thermocline. In shallow water, average maximum T_e was 17.4 ± 2.86 °C, and ranged from 12.57 – 23.64 °C. In deep water, average maximum T_e was 6.29 ± 2.86 °C, and ranged from 5.47 – 8.06 °C (Fig. 3b-c).

We encountered nearly all *Pleurodema marmoratum* adults and juveniles under cover rocks. Three recently metamorphosed juveniles were observed dispersing from their pond across the surface of a *bofedal* and rocks surrounding the pond. *Pleurodema marmoratum* T_b measurements under rocks fell tightly within the thermal range recorded by frog models. Frog T_b was better predicted by T_s than by T_a ($F_{2,23} = 18.26$, $R^2 = 0.6136$, $p < 0.001$; Fig. 6, Table 2)

Agar frog models recorded variable average maximum operating temperatures (T_e) of 14.8 ± 7.6 °C during daylight hours (0700 – 1800; Fig. 7). We excluded nocturnal measurements from the determination of average maximum T_e because of the large variation from night to day thermal maxima. The maximum extreme of operative temperature recorded by agar models was much higher than the mean maximum OT (Fig. 7a, Table 2). Average minimum T_e was less variable than maximum T_e, both during the day and at night. Most agar models experienced freezing temperatures every night from 2200h until local sunrise around 0700h. Air temperatures during the agar model experiment did not reach higher than 15°C during the day (Fig. 7b).

The mean CT_{max} was 32.56 ± 1.88 °C. The CT_{max} general linear model was significant (full model: $F = 4.522_{6,58}$, Multiple $R^2 = 0.3187$, $p = 0.0008$; Fig. 8) and revealed differences among CT_{max} measured in June and July (dry season) compared to March, November, and January (wet season; Table 3). Warming rate and frog size did not have a significant effect on CT_{max}. The warming rate for a subset (~15%) of our trials exceeded the desired range, but excluding values from these trials did not have a significant effect on average CT_{max}.

The difference between CT_{max} and maximum annual air temperatures (i.e., Warming Tolerance, WT) was 24.54 ± 2.05 °C. The WT was lower in the dry season and higher in wet season months. The difference between CT_{max} and the maximum average temperature frogs are exposed to in the environment (OWT; data from agar models) was 22.69 ± 8.1 °C. Average OWT during the day (0700 to 1800) was lower, 14.8 ± 7.6 °C (Table 2), and during the hottest part of the day in frog refugia as measured by dataloggers, 1100-1500 hr, was 10.49 ± 1.73 °C (Fig. 6).

Eighty-one percent of frogs exposed to natural freezing conditions survived (Table 4a). The mean temperature tolerated by frogs that survived freezing was -3.04 ± 1.16°C (N= 26; Fig. 7). Frogs were active up to freezing, when T_b may have been below 0 °C. Eleven frogs did not freeze (mean temperature -1.522 ± 0.797 °C) and five frogs froze but did not survive (including one frog that revived after thawing but failed the locomotory test; Table 4b). There was not a significant difference in locomotory ability between frogs that froze (N = 27; mean number of hops = 30.4) and frogs that did not freeze (N = 11; mean = 25.2; $t = 1.2878$, $df = 23.5$, $p = 0.21$).

DISCUSSION

Our study fills a critical knowledge gap, extending understanding of anuran operating temperatures and warming tolerance by 1400 vertical meters to 5250 masl in the Andes of southern Peru. The habitat occupied by these anurans is located within one of the most extreme biomes on Earth: in ponds and wetlands fed primarily by glacial meltwater within recent glacial moraines deposited by the rapidly retreating ice sheets. One of the most challenging features of tropical high-elevation environments above the treeline, including paramo and puna, is the wide daily variation in surface temperatures, ranging from very hot to below freezing. The wide thermal amplitude in high-elevation tropical environments resembles "summer every day and winter every night" (Hedburg and Hedburg 1979). Our study shows that two frog species that coexist at the edge of the cryosphere have adapted to the extreme thermal environment above 5200 masl by utilizing different microclimatic niches within the high-elevation landscape. The terrestrial species, *Pleurodema marmoratum*, experiences a remarkably wide daily variation in operative temperatures and possesses an important adaptation in the form of a wide thermal tolerance range, from at least -3.036 ± 1.16 °C to 32.56 ± 1.88 °C. In addition, *P. marmoratum* can tolerate short durations of freezing. In contrast, the use of aquatic habitat by *Telmatobius marmoratus* likely confers an important advantage in the extreme thermal environment at high elevations. *Telmatobius marmoratus* is buffered by the thermal diffusivity and inertia of water and avoids the extremes of the daily thermal cycle. For example, water temperature at a depth of ~1.5 m was 5.76°C with a range of 4.61–8.06°C. In contrast, average temperature in terrestrial habitat under cover rocks was approximately 1.24°C with a range from -7.0–35.5°C.

Understanding the range of microclimatic variation within habitats is a major requirement for accurately forecasting species responses to climate change, land-use change, and predicting their future distributions, especially in ectotherms (Laurance et al. 2011, Duarte et al. 2012, Nowakowski et al. 2017). Research on microclimatic refuges has predominantly been understood in terms of habitat complexity in ecosystems such as tropical rainforests with large structural components. However, the concept of microhabitat refugia is important in structurally-simple ecosystems without a forest canopy in the high Andes where steep topography and small landscape features create large differences in microclimates on scales relevant to amphibians. Microclimates alter biotic responses to climate change, and serve as refuges during climatic extremes (Scheffers et al. 2014). Identification and engineering of microhabitat refuges has been proposed as a conservation strategy for amphibians undergoing climate change (Puschendorf et al. 2009, Shoo et al. 2011, Hannah et al. 2014). In addition to the focus on climate shifts and extreme climate events in the context of global climate change, understanding microhabitat variation can improve our understanding of the elevational limits of life on tropical mountains. Climate change is expected to drive upslope migrations of organisms in montane regions (Chen et al. 2011). Upslope shifts may expose species to novel thermal regimes, so understanding the thermal tolerances of species at the expanding edge of their ranges is critical for understanding the limits of upslope range expansions.

Along tropical elevational gradients, amphibians tend to have a narrower range of thermal operating temperatures at mid-elevations within the cloud forest canopy compared to species living in biomes above the treeline (Navas et al. 2013, von May et al. 2017). Measurements of warming tolerance or critical thermal limits for amphibians typically have taken place from the lowlands up to a maximum of 3700 masl. The wide thermal tolerance range of high-elevation anurans reflects their adaptation to a thermal environment characterized by wide temperature amplitude. A growing body of research suggests that high-elevation tropical ectotherms are not as sensitive to thermal variation and warming temperatures as previously thought. High-elevation frogs may have a low sensitivity to warming temperatures because of climate change, which would benefit these high-elevation pioneers in an environment where temperatures are expected to increase at least twice as fast as in the lowlands (Pepin et al. 2015).

Our study highlights the urgent need to include high-elevation populations of amphibians in studies of thermal physiology. Predicting thermal limits for high-elevation species using measurements made on cloud-forest species at lower elevations is likely to lead to underestimation of the breadth of thermal tolerance limits. For example, the linear relationship expected between anuran CT_{max} and elevation does not extend very well to species in biomes above the treeline (Navas et al. 2013, Catenazzi et al. 2014). Above the treeline, amphibian body temperatures and CT_{max} are higher than predicted by elevation alone.

The two species considered in our study live in the same biome at the same elevation, yet they occupy sharply contrasting thermal environments. The wide thermal amplitude in surface temperatures at high elevation is modulated in aquatic habitat by the thermal inertia of water. The fully aquatic life history characteristic of species in the genus *Telmatobius* may be a key adaptive trait among these high-elevation specialists. The thermal environment available to *T. marmoratus* at depth is relatively stable and cold, but includes warm, shallow areas suitable for thermoregulation particularly during the wet season when pond water tables are high enough to give frogs access to the shallow, gently sloping perimeter of ponds. In addition to seasonal differences in the aquatic thermal environment, the thermal tolerances and preferred temperatures within a species can vary by life stage. The optimal thermal conditions for aquatic eggs and larvae of frogs may be different from those experienced by adults and deserve more attention.

Pleurodema marmoratum living at 5250 masl appear to have a very large WT of 24.4 °C, and thus should be protected from extreme climate events and warming. However, the relevance of a simple metric like WT is limited in variable environments such as high elevations. We found that air temperature is a poor surrogate for anuran body temperature, as others have noted at lower elevation sites in the Andes (Catenazzi et al. 2014). The range of body temperatures that frogs experience on a regular basis can be significantly wider than air temperatures because surface water and surface rocks are heated by high insolation levels. Operative warming tolerance is expected to be larger than WT (Catenazzi et al. 2014), but at high elevations OWT is lower than WT. Extreme insolation results in surface temperatures that are much higher, and much more variable, than air temperatures. To improve the OWT estimate, we took the non-standard approach of calculating average maximum T_e using only data recorded during local daylight (0700 – 1800). Estimates of central tendency (i.e., mean, median) reveal little about the extreme variation in the high-elevation environment (Sunday et al. 2014, Camacho et al. 2015), which means that standard metrics like WT and OWT may underestimate warming sensitivity of populations at such extreme elevations.

The extremes of operative temperature (EOT) represent the full range of daily temperature variation, which has important consequences for amphibian microhabitat selection (Camacho et al. 2015). It remains unclear whether *P. marmoratus* experiences the full thermal amplitude of its tolerance limits on a daily basis. Although other high-Andean species have been observed actively basking to increase body temperatures (Sinsch 1989, Lambrinos and Kleier 2003), at such extreme elevations individuals likely thermoregulate while under sun-warmed rocks to maintain water balance by staying protected from the high insolation and low humidity. However, model frog temperature exceeded CT_{max} by >10°C under at least one cover rock (Fig. 7), suggesting that frogs must actively thermoregulate by moving deeper underground or into crevices to avoid daytime sites that will exceed their thermal maximum. In addition, frogs that move towards the surface late in the day to take advantage of warm substrate likely need to migrate downwards at night to avoid locations near the surface that approach freezing. The thermal inertia of the ground shows that frogs that prefer to maintain the highest possible temperature would stay near the surface under a suitable cover rock from just after local sunrise to sunset. At sunset (1700 – 1800 hrs), they would migrate down to ~10 cm, and by ~2100 hrs they would migrate down to ~20 cm. Our results suggest that behavioral thermoregulation is critical for amphibians utilizing the extreme thermal environment at high elevation.

The absolute maximum temperatures experienced by agar models show that it is possible for frogs to select a rock where maximum temperature dramatically exceeds CT_{max}. Although we did not determine the upper lethal temperature of *Pleurodema marmoratum*, passing CT_{max} by >10°C could result in muscular spasms and cause death. We encountered dead, dessicated adult and juvenile *Pleurodema marmoratum* (N = 7) under rocks in the study area. The cause of these deaths was undetermined, but the extreme temperatures recorded by agar models suggest thermal stress is one possibility. Several of the dead were infected with the amphibian pathogen *Batrachochytrium dendrobaditis,* which can reduce the CT_{max} of frogs by up to 4°C (Greenspan et al. 2017a). Daily extreme temperature variability combined with additional future warming could limit the availability of suitable microhabitat refuges especially during the dry season, when the daily thermal variation is greatest. As thermal extremes are becoming more common throughout the tropics (Buckley and Huey 2016), amphibians that live in environments where extreme temperatures are already common may be affected despite their wide thermal tolerance limits.

Pleurodema marmoratum exhibits frost tolerance. Our study is the first recorded test of frost tolerance in a tropical amphibian and extends evidence of frost tolerance to the family Leptodactylidae. Because tropical environments experience air temperatures below 0°C on a daily basis at elevations higher than 3500 masl, freezing risk has been postulated for anurans (Navas 1996b, Carvajalino-Fernández et al. 2011, Navas et al. 2013). We found that just 10 cm below the surface, ground temperatures almost never fall below freezing even in the coldest hours of the night, or the coldest part of the year. By choosing specific nighttime refuges below the surface, individual frogs may dramatically reduce their risk of freezing.

Tolerance to short periods of freezing might be more common than currently recognized throughout the tropical Andes. A better understanding of the lower thermal limits of anurans could help determine the upper elevational limits anurans could reach as climate change continues. The ability to tolerate cold temperatures may be one of the most important factors limiting upper elevational range limits for many species (von May et al. 2017). *Pleurodema marmoratum* has a wide latitudinal range that extends from the tropical Andes in Peru to the subtropics in northwestern Argentina. Freeze tolerance has been reported in subtropical populations of adult *Rhinella spinulosa* (Halloy and Gonzalez 1993, Sanabria et al. 2015), which is also found in the Cordillera Vilcanota up to ~5000 masl (Seimon et al. 2007). Freeze tolerance has been observed in tadpoles of *Rhinella spinulosa* and *Pleurodema bufonium* from high latitudes in southern Patagonia (Navas et al. 2010). If freeze tolerance is highly conserved phylogenetically, we would expect to see freeze tolerance in the tropics in species closely related to those that have ranges that extend into higher latitudes. However, freeze tolerance has evolved separately multiple times in anuran lineages (Voituron et al. 2009), so it is possible to have evolved separately in tropical lineages as well.

Pleurodema marmoratum likely utilizes both freeze avoidance through supercooling and frost tolerance to survive low temperatures in the high Andes.

Combining the two strategies is reportedly uncommon in nature (Costanzo and Lee 2013) but might instead be underrepresented in the literature because most studies have focused on high-latitude species that must tolerate long winters. Utilizing both freeze avoidance and freeze tolerance would be particularly advantageous where temperatures are relatively mild, unpredictable, and highly variable: precisely the thermal environment terrestrial high-elevation frog experience every 24-hr period. Some frogs in freezing trials did not freeze when others did under very similar conditions. It is possible that temperatures varied between freeze chambers within 30 cm of each other, and that some frogs utilized supercooling to avoid freezing.

Our frog biophysical models confirm that temperatures of 0°C and below occurred for a period of 9 hours (from 2100 hrs to 1000 hrs) in June and July, and surface freezing occurs throughout the year. The lowest temperatures, and likely the highest freezing risk, occurred just before local sunrise. There was a small but biologically important seasonal variation in temperature minima, which were lowest in the dry season (June – August).

High-elevation tropical frogs likely have year-round adaptations to freezing, possibly including the use of cryoprotectants. Glucose is one of the most common cryoprotectants (Storey and Storey 2004), and elevated glucose levels have been detected in response to cold exposure in a congeneric anuran, *Pleurodema thaul,* from subtropical Chile at 1000 masl (Hernandez Cifuentes 2016). Additional tests under controlled conditions are necessary to determine the degree of frost tolerance, i.e., show if *P. marmoratum* can survive freezing in a biologically significant quantity of body fluids, and to determine the minimum survivable temperatures for these high-elevation tropical frogs. Future research should also investigate the utilization of cryoprotectants to determine if these frogs have converged on the strategy of utilizing glucose as a cryoprotectant, or if they are using novel cryoprotectants.

Pleurodema marmoratum may meet several qualifications for true freeze tolerance (including freezing of 50% or more of total body water, penetration of ice into all extracellular spaces, and the interruption of all vital functions including breathing, circulation, nerve and muscle activity; Storey and Storey 2004). Tropical frogs will never meet the duration criteria for freeze tolerance specified by Storey and Storey (1992) because freezing periods longer than a few hours do not occur in the tropical environment. Therefore, we conservatively use the term "frost tolerance" but we propose that nightly freezing in the tropics is ecologically relevant to amphibian species at high elevation. We are confident these small frogs exposed to 1-6 hours of freezing experienced freezing in skin and skeletal muscles. We carefully observed experimental frogs during freezing and thawing. Frozen frogs had visible ice crystals on their skin, their eyes dulled indicating freezing of the lens, and they were completely hard to the touch. During recovery, we observed the thawing of skin and muscles, the eye, and a delay of several minutes before respiration resumed, presumably as core tissues also thawed.

We do not think our average low temperature or 6-hour freeze duration reflects the extreme limit of frost tolerance or lower thermal limits for *P. marmoratum*. When freezing occurs too quickly it causes damaging rates of ice formation and may inhibit the use of possible cryoprotective measures which can lead to cell damage and death (Storey and Storey 2004). We utilized a moist strip of filter paper in freeze chambers to initiate freezing at a relatively high temperature, with the intention of reducing mortality. The addition of ice nucleators mimics natural conditions; in the field these frogs would be in contact with environmental ice in the substrate, which likely represents the principle mode of ice nucleation in moist frog habitats (Storey and Storey 2004). Differences in ambient conditions and freezing rates are one possible explanation why we observed mortality during the 1-hour freeze trials despite no mortality in the 6-hour trials. We rule out an effect of body size because the range of body sizes of frogs that died were similar to those of the frogs that survived. Non-survivors experienced a slightly lower minimum temperature than survivors. Additional information is necessary to understand why some of our study frogs died when exposed to freezing while others survived and recovered. Our experiment was conducted in situ, so we had less control over minimum temperatures and cooling rates than would have been possible in a laboratory setting.

In addition to understanding the role of thermal adaptations in the context of climate change, it is essential to understand the role that amphibian thermoregulation and thermal tolerance limits play in amphibian susceptibility to infectious diseases, like chytridiomycosis (*Batracochytrium dendrobaditis*, *Bd*). The *Bd* pathogen grows well and is highly pathogenic at cool temperatures (Piotrowski et al. 2004, Andre et al. 2008). Tropical montane amphibian assemblages have a high infection prevalence and high susceptibility to *Bd*, leading to extinctions and severe declines in many assemblages (Lips et al. 2006, Catenazzi et al. 2014, Hirschfield et al. 2016). Some strains of *Bd* may be able to survive freezing and grow at very low temperatures (Voyles et al. 2017), which may explain how Bd has expanded its elevational range to frog populations living above 5000 masl in the Andes (Seimon et al. 2017). Studies show that short exposure to high temperatures and thermoregulation (i.e., behavioral fever) within the range of temperatures we found occur at our field site can inhibit *Bd* infections (Woodhams et al. 2003, Greenspan et al. 2017b). Future research should directly investigate the possibility of extreme diurnal temperature variation and behavioral thermoregulation to inhibit *Bd* susceptibility.

Although high-elevation biomes occupy a relatively small land area, tropical high-elevation environments are home to a much greater species diversity than cold temperate high-elevation or high-latitude ecosystems (Navas 1997). Tropical high-elevation grasslands and tropical alpine wetlands are home to at least 186 species of anurans, including a high proportion of endangered and critically endangered species (IUCN Red List 2017). Our study provides strong support for the role of physiological plasticity as one of the key traits allowing species to migrate upslope because of climate change, and as a key adaptation for life in extreme high-elevation tropical environments.. One of the greatest unknowns in understanding the ecological effects of climate change is how communities will respond to climate change. Increasing average temperatures are expected to drive or facilitate species upslope migrations but, so far, limited evidence exists for upslope migration of anurans in the tropical Andes (Catenazzi et al. 2014). Upslope migrations have not only been observed at our field site, the our site represents the most extreme elevations globally where amphibians have been found (Seimon et al. 2007). Freezing temperatures are more likely than high temperatures to limit upward migration by tropical anurans to high elevation (von May et al. 2017). As the frost line shifts upwards, more species may have the opportunity to expand their elevation ranges upward. Our research suggests that *Pleurodema marmoratum* is adapted to remarkable temperature variation found at very high elevations. Additional research is necessary to understand how the thermal tolerance limits of other tropical anurans might allow or prevent them from utilizing habitat at the highest elevations.

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TABLES

Table 3.1. Range and means of shaded air, ground, and water temperatures. Data from 5230–5250 masl.

Location	Temperature (°C)	
Air		
	Dry Season	Wet Season
Mean ± SD	1.24 ± 0.85	1.76 ± 0.66
Mean maximum	14.46 ± 4.64	11.90 ± 2.12
Maximum	23.12	17.0
Minimum	-9.5	-5.5
Ground		
	Surface	-10 cm
Mean	4.51 ± 8.26	4.49 ± 3.68
Mean maximum	12.60 ± 12	9.52 ± 3.88
Maximum	35.50	15.50
Minimum	-7.0	-0.50
Water		
	Shallow	Deep
Mean	8.21 ± 1.61	5.76 ± 0.48
Mean maximum	17.41 ± 2.86	6.29 ± 0.64
Maximum	23.64	8.06
Minimum	-3.21	4.61

Table 3.2. Temperatures relevant to the thermal biology of *Pleurodema marmoratum* at 5230 masl.

Category	Temperature (°C)
Body Temp. (mean ± SD)	9.78 ± 2.32
T_e (mean daytime maximum ± SD)	14.8 ± 7.6
T_e maximum	44.0
T_e minimum	-3.5
CT_{max} (mean ± SD)	32.56 ± 2.16
Operative warming tolerance	12.32 – 23.2
Extreme warming tolerance	-13.6 – -9.28

Table 3.3. General linear model of variation in *Pleurodema marmoratum* CT_{max}.

Term	Estimate	Std. Error	*t*-value	*p*-value
Intercept	31.888	0.699	45.621	<0.000 ***
Warming rate	1.640	0.914	1.793	0.078
Size (SUL)	0.056	0.030	1.828	0.073
July	-1.830	0.759	-2.410	0.019 *
June	-1.768	0.471	-3.754	<0.000 ***
March	-0.469	0.540	-0.868	0.389
November	-1.106	0.647	-1.708	0.093

Note: * $p < 0.05$ *** $p < 0.001$

Table 3.4. *Pleurodema marmoratum* frost exposure survival. A) *Pleurodema marmoratum* survival after one and six hours of exposure to freezing conditions. B) Comparison of average minimum temperature and body size (Snout–Urostyle Length) for frogs that survived freezing and frogs that did not survive.

a.

Duration	N	% Survived
1 Hour	25	81
6 Hours	6	100

b.

Outcome of Frost Exposure	N	Average Minimum Temperature °C ± SD	Size (SUL) cm ± SD
Survived	26	-3.036 ± 1.16	1.48 ± 0.33
Did Not Survive	5	-4.717 ± 1.00	1.38 ± 0.09

FIGURES

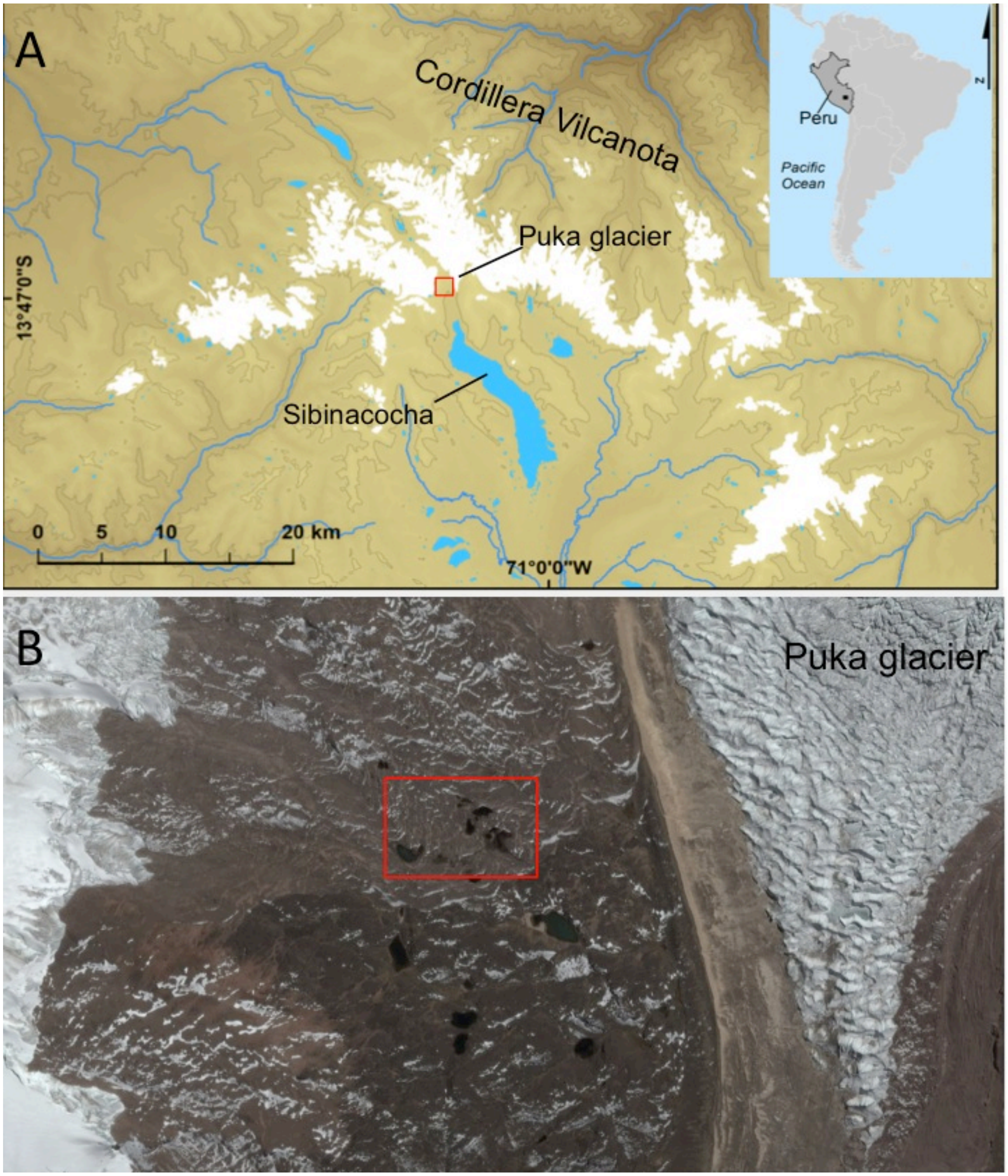


Figure 3.1. Map of the Cordillera Vilcanota in Cusco, Perú. A) Location of study site (red box) and inset showing location within South America. b) Satellite image of study ponds (inside red box) at 5230–5250 masl.

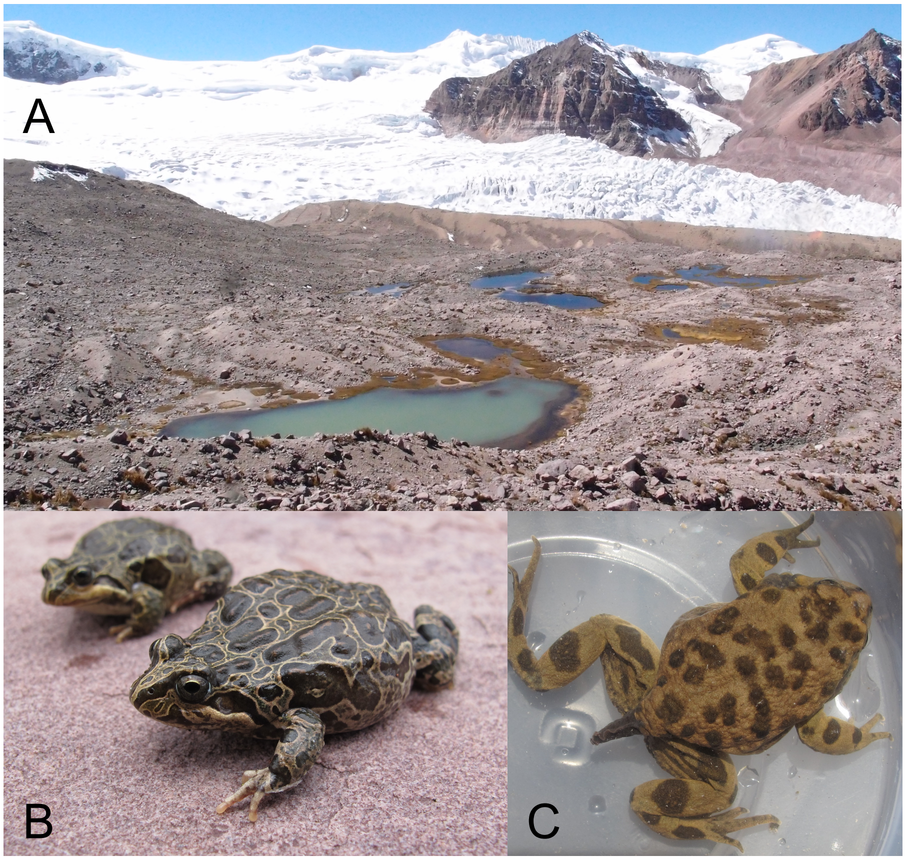


Figure 3.2. Photos of study ponds and study species. a) Photo of study ponds at 5230 masl, with Puka outlet glacier in the background. b) *Pleurodema marmoratum* adults (Snout-urostyle length "SUL": 3.1 cm) c) *Telmatobius marmoratus* late-stage metamorph (SUL: 3.8 cm)

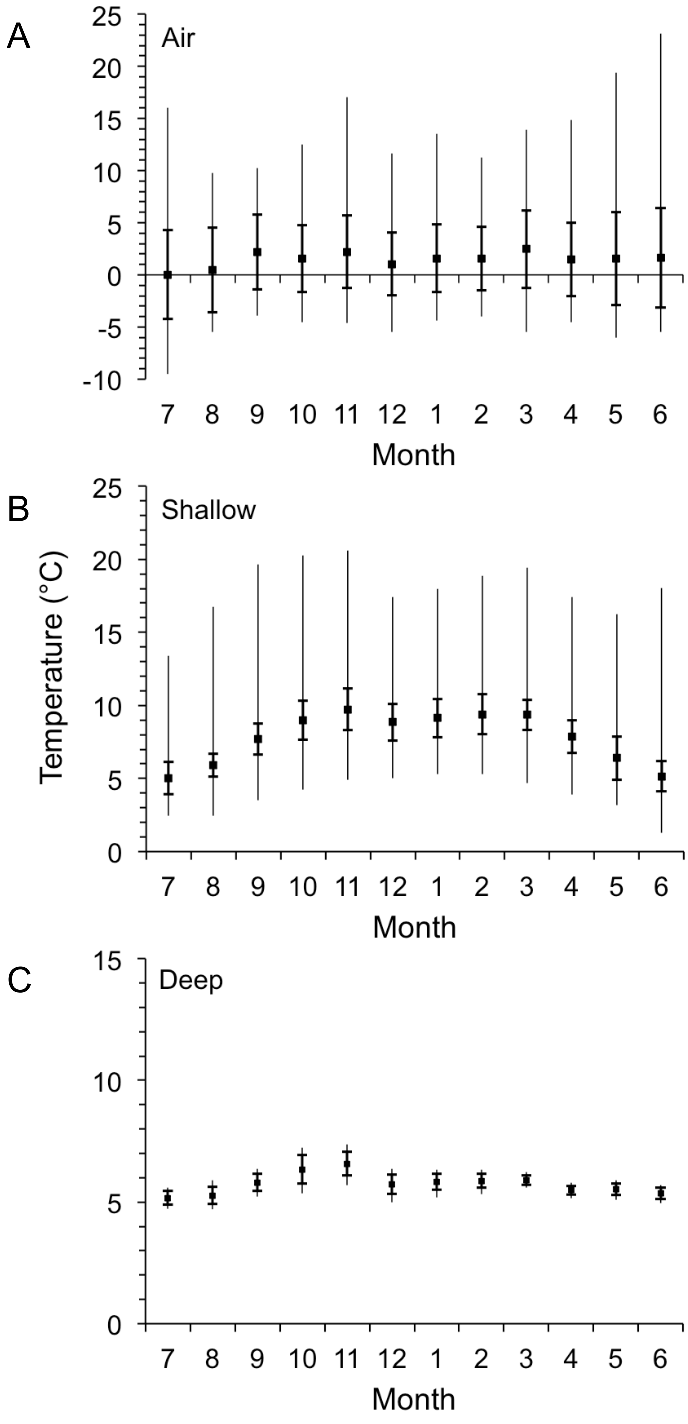


Figure 3.3. Water and air temperatures during study period. a-c) Dot and error bars are mean (°C) ± SD. Black line is maximum–minimum range of recorded temperatures. a) Shaded air temperature for each month of the hydrologic year (July to June). b) Average of monthly shallow water temperatures recorded at ~10 and 20 cm depth in two permanent ponds. c) Monthly deep-water temperature recorded at 1.3–1.5 m depth in one permanent pond.

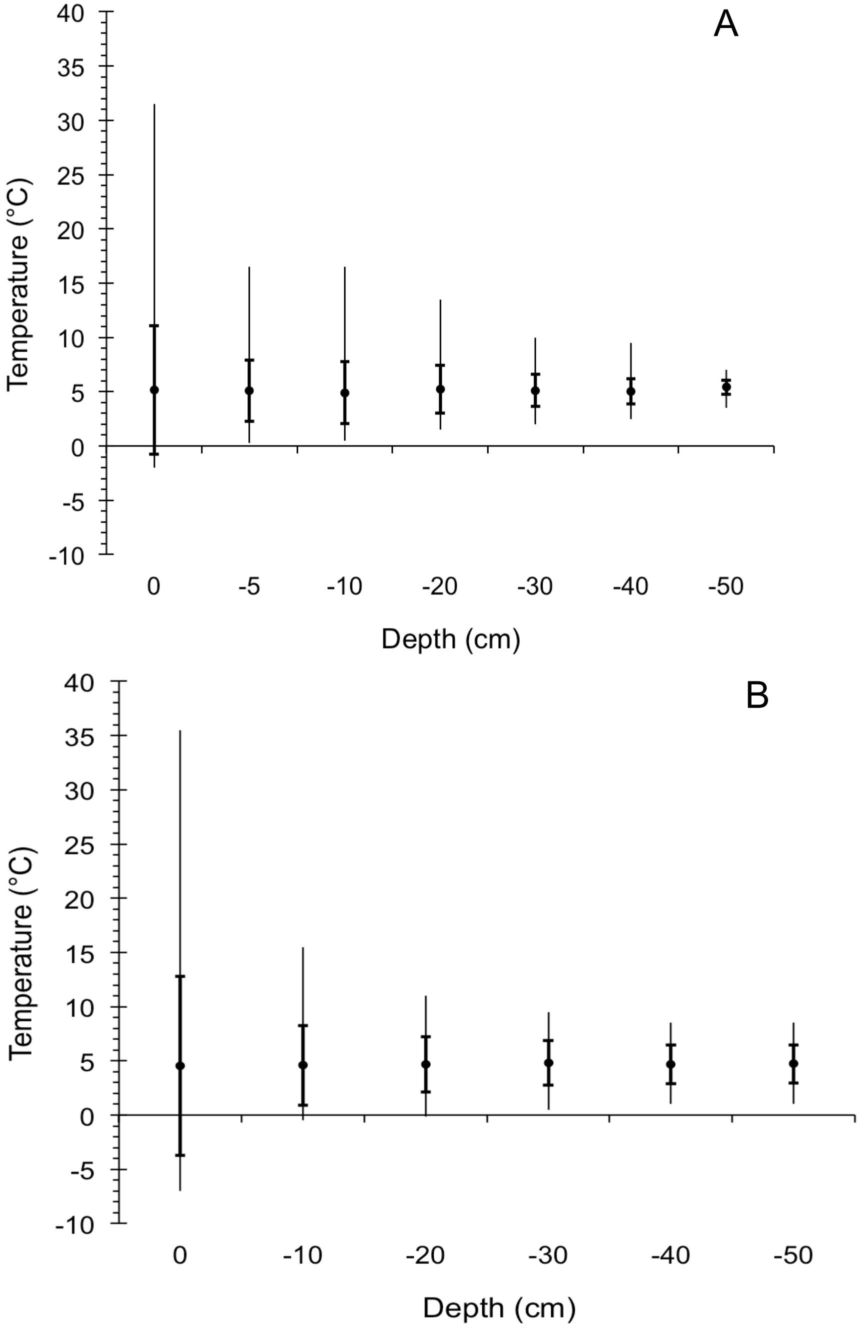


Figure 3.4. Ground temperature at different depths in the wet and dry seasons. Dot and error bars are mean (°C) ± SD. Full line at each ground depth represents the range of recorded temperatures (maximum-minimum). a) 17 December 2015 to 16 January 2016 (wet season). b) 14 May 2016 to 12 June 2016 (dry season)

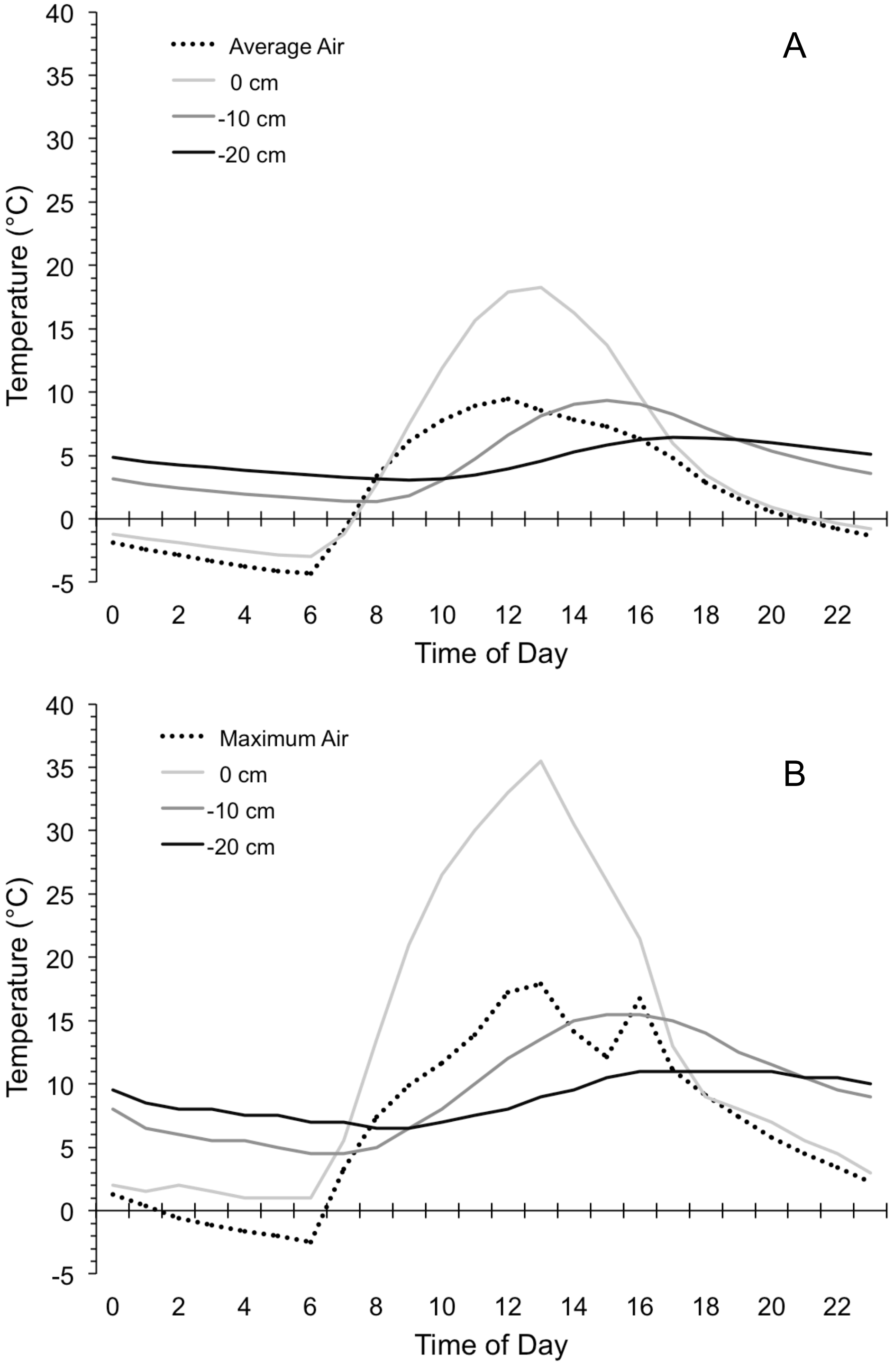


Figure 3.5. Daily variation of air, covered surface, and ground temperature (°C). May 14, 2016 to June 12, 2016. A) Mean temperature B) Mean maximum temperature

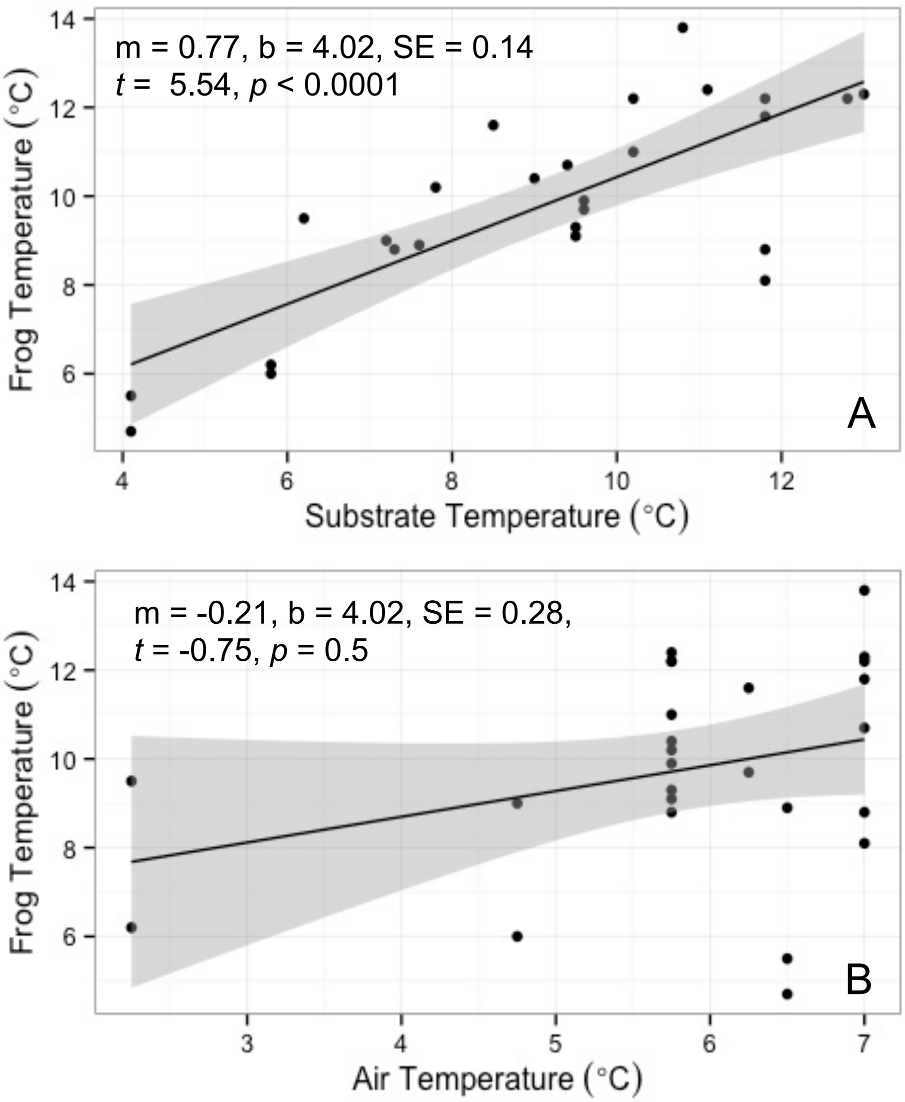


Figure 3.6. Results of multiple linear regression showing the relationship between substrate and air temperature, and frog body temperature.

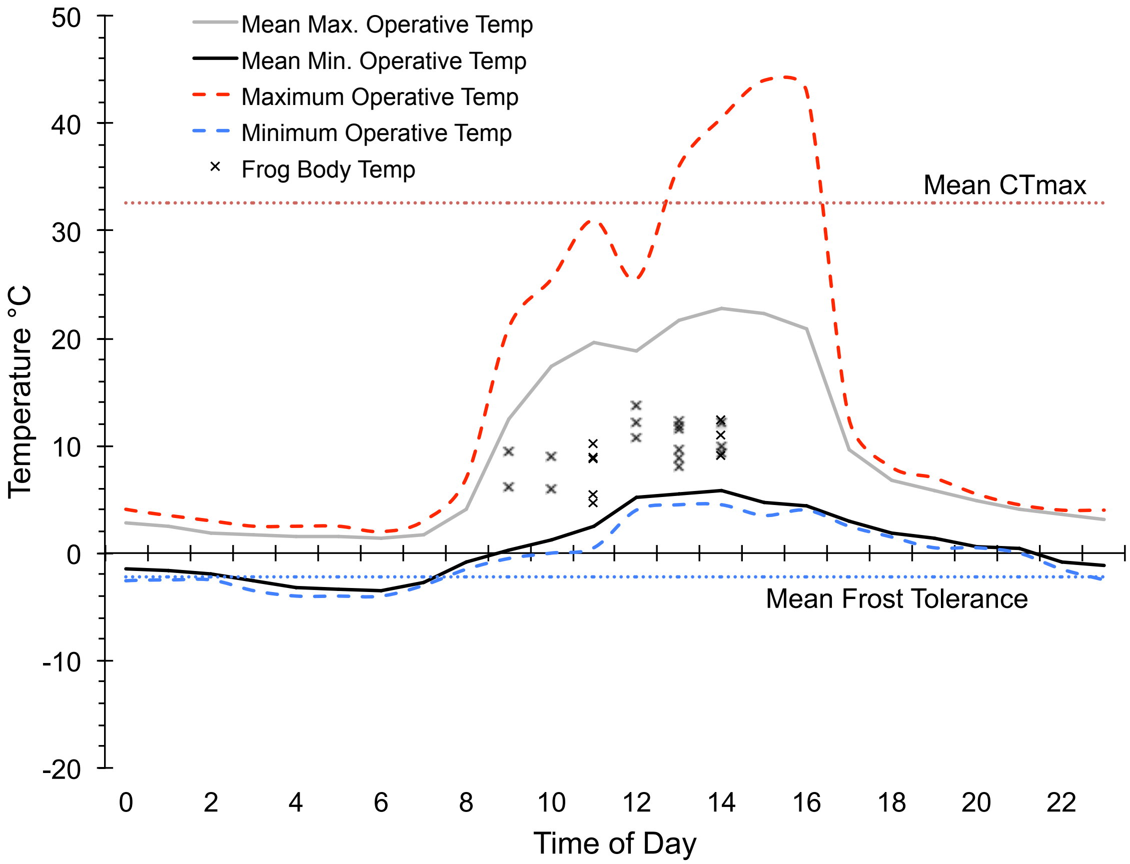


Figure 3.7. OWT, OT of agar biophysical models, and frog T_b. Operative temperatures (OT) recorded by *Pleurodema marmoratum* agar biophysical models, and frog body temperatures (black X). Solid lines are mean maximum OTs,and represent the range of body temperatures a frog under equilibrium conditions is likely to experience. Maximum recorded OT are dashed lines, and model the full range of possible body temperatures a frog under the same rock could experience. Mean CT_{max} and the mean frost temperature survived by frogs are dotted lines. The area between CT_{max} and mean OT_{max} is the Operative Warming Tolerance (OWT). The OWT approaches and can exceed CT_{max} during the hottest part of the day.

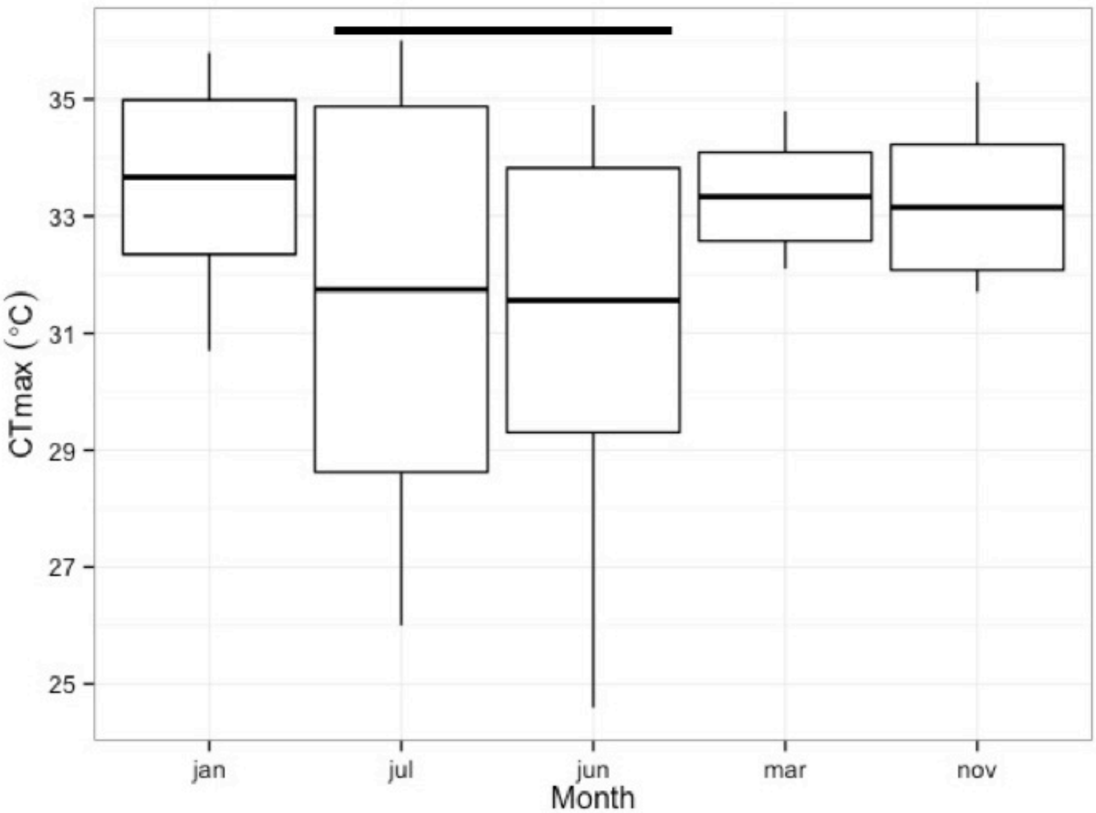
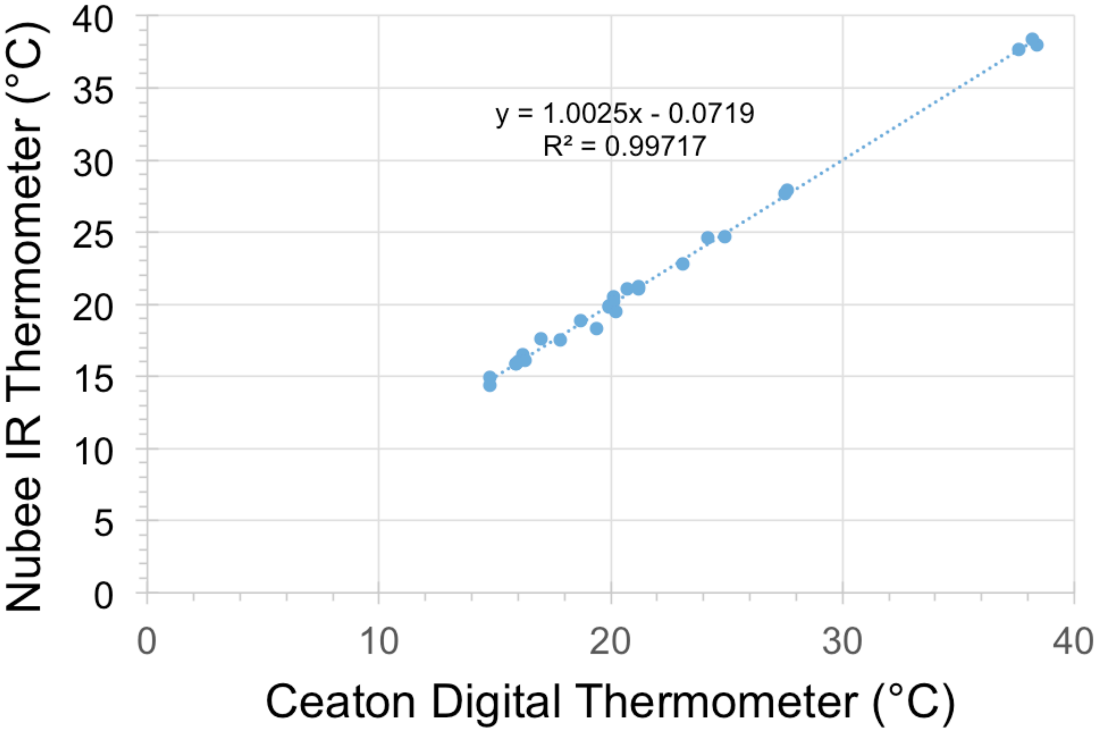
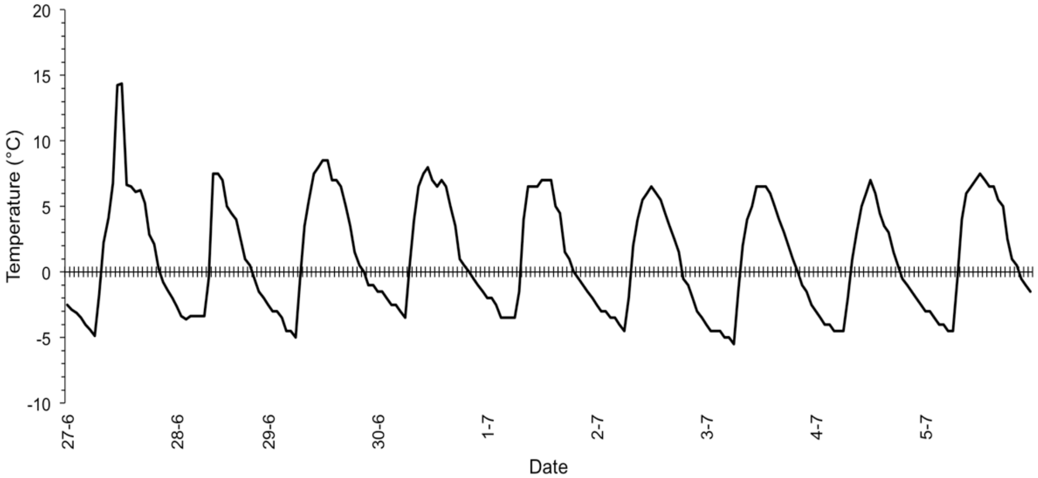


Figure 3.8. Variation in *Pleurodema marmoratum* CT_{max} (boxes are mean ± SD). Bar indicates months with significantly lower CT_{max}.



Appendix 3.1. Results of linear regression comparing thermometer accuracy



Appendix 3.2. Range of average shaded air temperatures during agar biophysical model experiment to determine frog operative temperatures.

Chapter IV. Survival at the Summits: Post-Epizootic Amphibian Disease Dynamics in the High Tropical Andes

ABSTRACT

The deadly amphibian disease, chytridiomycosis, caused by *Batrachochytrium dendrobatidis* (*Bd*) has caused declines and extirpations around the world. Some 10-20 years after the declines, *Bd* is shifting towards enzootic disease dynamics. Various populations are rebounding and some species are being rediscovered after being presumed extirpated. More than a decade after a *Bd* epizootic in the Andes of southern Peru, we investigated *Bd*-host disease dynamics in amphibian communities across several high-elevation sites in the Cordillera Vilcanota. The widespread geographic distribution of *Bd* throughout the Vilcanota range indicates a role of climate change in the spread of this important wildlife disease to recently deglaciated zones. Host-pathogen dynamics appear to have stabilized at our sites, and we investigate the recovery of a *Bd*-susceptible species, *Telmatobius marmoratus*, which belongs to a vulnerable lineage that has suffered *Bd*-associated decline across much of the Andes. We investigated enzootic disease dynamics from a population of *Telmatobius* frogs that suffered severe *Bd*-related declines. We studied the seasonal and ontogenetic-driven patterns of *Bd* dynamics in *Telmatobius marmoratus*, and compared them to patterns from another species, *Pleurodema marmoratum*. We found that *Bd* prevalence and infection intensity are highest for *T. marmoratus* in the dry season, but mortality was linked to ontogenetic development rather than to seasonal dynamics of pathogen prevalence. Infection intensity in *P. marmoratum* adults was higher in the dry season than in the wet season, but this trend was reversed in the juvenile size class. We demonstrate that the different thermal niches used by these two species give them very different opportunities for protective thermoregulatory behaviors. We also documented the presence of *Bd* in populations of *P. marmoratum* throughout the region and in newly-developed habitats recently created by retreating glaciers. Our study suggests that thermal mismatch between *Bd* and its amphibian hosts may play an important role in disease dynamics in the extreme, high-elevation environment.

INTRODUCTION

Emerging infectious diseases have become significant threats to biodiversity worldwide. Fungal pathogens have emerged as a cause of population declines, the collapse of ecological communities, and species extinctions in bats, bees, corals, amphibians, and snakes (Fisher et al. 2012). *Batrachochytrium dendrobatidis* (hereafter *Bd*), one of the most pervasive and highly virulent fungal pathogens of wildlife, infects amphibians and can cause the lethal disease chytridiomycosis (Berger et al. 1998, Longcore et al. 1999). Amphibians are a highly diverse group of vertebrates with nearly 7,900 described species (Frost 2018), yet amphibian population declines and species extinctions have occurred on a global scale and resemble a major extinction event (Stuart et al. 2004, Skerratt et al. 2007, Wake and Vredenburg 2008). Chytridiomycosis has been detected in over 500 species of amphibians, primarily anurans, and is linked to extinctions and ongoing global declines of amphibian species (Skerratt et al. 2007, Fisher et al. 2009, Olson et al. 2013, James et al. 2015).

The effects of chytridiomycosis epizootics appear to be particularly severe in montane habitats. The disease has been implicated in population declines and even extinctions in high-elevation amphibian communities in the Sierra Nevada of North America (Fellers et al. 2007, Vredenburg et al. 2010), montane wet forests in Australia (Woodhams and Alford 2005, Alford 2010, Gillespie et al. 2015), the Pyrenees in Europe (Bosch et al. 2001, 2013; Rosa et al. 2013), volcanic mountains in Cameroon (Hirschfeld et al. 2016), the Cordillera Central in Central America (Berger et al. 1998, Brem and Lips 2008, Crawford et al. 2010), and the Andes in South America (Ron and Merino 2000, Merino-Viteri et al. 2005, Seimon et al. 2007, Catenazzi et al. 2011). The impact of chytridiomycosis is one of the principal conservation concerns for high-elevation amphibians (Woodhams et al. 2011).

Studying *Bd*-infected populations in montane ecosystems has provided significant advances in our understanding of host-pathogen disease dynamics. For example, the studies on the mountain yellow-legged frog in the California Sierra Nevada allowed researchers to unravel population density and high infection intensity as mechanisms driving *Bd* epizootics and mortality (Vredenburg et al. 2010). The same system also demonstrated the importance of life history, i.e., the role of long-lived tadpoles in *Bd* transmission from disease reservoirs as mechanisms driving chytridiomycosis enzootic *Bd* dynamics (Briggs et al. 2010). Studies of *Bd* occurrence in amphibians along elevational gradients revealed greater impacts of *Bd* at mid and high elevations compared to low elevation (Brem and Lips 2008, Woodhams et al. 2011, Catenazzi et al. 2014). In the Sierra Nevada of North America and the Andes of South America studies demonstrated that the pathogen could cause disease in cold, high-mountain areas despite the hypothesis that strong preferences of *Bd* in pure culture for warm temperatures would limit the disease to mid-elevations (Piotrowski et al. 2004, Pounds et al. 2006, Seimon et al. 2007, Knapp et al. 2011). The identification of *Bd* as a cause of population declines in the high Andes of southern Peru revealed the role that global climate change can have in determining the geographic range of *Bd* and extending the known elevational limits of *Bd* pathogenicity to nearly 5400 m (Seimon et al. 2007).

Temperature strongly affects both the growth and virulence of *Bd*, as well as amphibian immune responses (Woodhams et al. 2008, Ribas et al. 2009, Forrest and Schlaepfer 2011, Voyles et al. 2012, Voyles et al. 2017, Rollins-Smith 2017). In culture, *Bd* grows optimally at cool temperatures, 13-25 ºC (Stevenson et al. 2013). However, at temperatures below the optimum, *Bd* grows more slowly but may be more virulent and long-lived than within its optimal range (Woodhams et al. 2008, Voyles et al. 2012). Although amphibians appear to be susceptible to *Bd* infection in thermally variable and cold temperatures (Raffel et al. 2006, Savage et al. 2011), short heat pulses may increase amphibian survival by creating environmental checks on pathogen growth (Daskin et al. 2011, Greenspan et al. 2017). Cold temperatures also inhibit the antifungal benefits of symbiotic skin bacteria (Daskin et al. 2014), but cutaneous symbiotic bacteria with anti-*Bd* properties have been isolated from high-elevation amphibians in the Andes (Burkart et al. 2017). High elevation species are presumably better adapted to cold and wide daily temperature variation, so their defenses and immune systems might not be as sensitive to cold and variable temperatures as animals living at low elevations. Amphibian hosts living in thermal environments that are more favorable to them than they are to *Bd* should be more likely to survive infection and persist (Catenazzi et al. 2014, Nowakowski et al.

2016). While temperature could be important, pronounced precipitation-driven seasonality in the high Andes has also been shown to correlate with *Bd* prevalence in tadpoles (Catenazzi et al. 2013).

The long-term impacts of *Bd* on amphibian populations are highly unpredictable (Scheele et al. 2017). Epizootic events of highly virulent strains of *Bd* cause mass mortality (i.e., die-offs) and declines in susceptible populations (Lips et al. 2006, Vredenburg et al. 2010). Years after the die-offs, some populations have begun to reappear, suggesting a shift in the host-pathogen dynamics. Surviving individuals (e.g., remnant or resistant populations) may continue to experience the effects of *Bd* infection as an enzootic pathogen (Briggs et al. 2010, Knapp et al. 2017, Scheele et al. 2017). In the enzootic stage, even some highly-susceptible species can persist with *Bd*, and some species have reappeared after not being detected for decades following epizootics (Woodhams and Alford 2005, McDonald et al. 2005, Rodriguez-Contreras et al. 2008, Puschendorf et al. 2013, Chaves et al. 2014, Whitfield et al. 2017, Seimon et al. 2017). Understanding the relationship between hosts and pathogens in these rebounding populations may be highly informative for understanding the mechanisms allowing populations to persist or recover when they were so strongly affected by *Bd* initially. Few studies have examined host-pathogen dynamics from populations recovering from extirpation.

In the Andes, *Bd* has been found in southern Peru since 2002 (Catenazzi and von May 2014). Studies up to 3800 masl in the Manu National Park biodiversity hotspot describe the extirpation of several anuran species infected with *Bd* (Catenazzi et al. 2011). Ten years after the *Bd*-epizootic collapse, some species had disappeared, while others survived as the host/*Bd* system transitioned to an enzootic state (Catenazzi et al. 2017). The *Bd* pathogen has been found at even higher elevations than in Manu National Park in the southern Andes of Peru. In the Cordillera Vilcanota, a heavily glaciated mountain range ~80 km from the upper reaches of Manu National Park, two species of anurans have colonized ponds created by snow and glacial meltwater at 5244–5400 masl (Seimon et al. 2007). At such high elevations, organisms must cope with low atmospheric pressure, high ultraviolet radiation, and perhaps most importantly for host-*Bd* dynamics, extreme daily temperature fluctuations (Nagy and Grabherr 2009).

There has been over a decade of amphibian and *Bd* monitoring in the Cordillera Vilcanota (Fig. 4.1) but, until the current study, none have examined intra-annual patterns of disease dynamics. The high pass north of Lake Sibinacocha is the focus of a long-term amphibian monitoring since 2003, the same year that *Bd* infection and chytridiomycosis was first documented in two frog species from this site (Seimon et al. 2007, 2017). One species, *Pleurodema marmoratum* (Marbled Four-eyed Frog), appeared to persist without population declines throughout the epizootic, but another species, *Telmatobius marmoratus* (Marbled Water Frog), suffered mass mortality events between 2003 and 2004 and was not detected despite repeated sampling efforts between 2008 and 2012 (Seimon et al. 2017). *Telmatobius marmoratus* was once again documented in 2013, suggesting this species may be recovering (Seimon et al. 2017). However, it remained unclear if the rediscovered *T. marmoratus* population remains susceptible to *Bd*. Other amphibians have been shown to persist with *Bd* after declines while still suffering from the fungal disease (Longo and Burrows 2010a, Catenazzi et al. 2017). The mechanisms leading to differential outcomes and post-decline recovery for species, especially among susceptible species are not well understood. Understanding host-pathogen disease dynamics in the context of extreme environmental conditions at very high elevations may provide new insights into the mechanisms leading to amphibian population recovery and persistence with enzootic *Bd*.

The rediscovery of *T. marmoratus* at very high elevation is particularly interesting because the genus *Telmatobius* includes 63 species (Frost 2018) that historically were found in montane and high-elevation habitats from 1000–5244 m throughout the central and southern Andes (De la Riva and Harvey 2003, Seimon et al. 2007). However, the genus has been extirpated from Ecuador, and declines and extirpations have occurred throughout Peru, Bolivia, Chile, and Argentina without clear evidence of post-decline population rebounds in this broad region (Merino-Viteri et al. 2005, Berenguel et al. 2016, Barrionuevo and Mangione 2006, Catenazzi et al. 2011, Burrowes and De la Riva 2017a). Although additional factors like overharvesting, introduction of non-native trout, etc., have had a negative impact on *Telmatobius*, the wide-scale declines are associated with *Bd* (Angulo 2008). The genus appears to be highly susceptible to severe declines from *Bd* infection, much like *Atelopus* (another highly-susceptible montane anuran genus).

We hypothesized that by the beginning of our study in 2013, more than a decade after the die-offs, host-*Bd* dynamics had transitioned to a more stable enzootic state rather than *Bd* outbreaks associated with epizootic mass die-offs (Briggs et al. 2010, Vredenburg et al. 2010, Catenazzi et al. 2013). We investigated demographic and seasonal influences on *Bd* prevalence and infection intensity, and expanded the geographic extent of pathogen surveys to data-poor regions of the Cordillera Vilcanota, including the several unexplored valleys in the northern part of the range and the Quelccaya Ice Cap. We had three specific objectives. First, to examine the effects of season and ontogeny on pathogen dynamics, we sampled *Bd* infection in two species of frogs from study ponds over two years. Second, we compared patterns of *Bd* prevalence and infection intensity for a *Bd*-susceptible and less-susceptible species. Lastly, we examined the geographic scale of the upward expansion of the amphibian chytrid fungus in areas that were until recently covered by permanent ice. We surveyed *Bd* infection in frogs from recently developed glacial meltwater ponds and wetlands throughout the mountain range. Our study provides new insight into amphibian population disease dynamics at the elevational limit of known *Bd* pathogenicity.

METHODS

Study Site. Our study sites are located in the Cordillera Vilcanota (13.7°S, 71.1°W), approximately 100 km ESE of the city of Cusco, Peru, in the area bordered by highways 3S (Cusco–Puno Highway) and the Interoceanic Highway (Cusco–Puerto Maldonado). Precipitation in the Vilcanota is highly seasonal, with most precipitation falling in the wet season, from October to April, and a pronounced dry season from May to September (Perry et al. 2014).

We sampled frogs in the wet and dry seasons at our primary study site in a recently deglaciated pass between the high elevation lakes Sibinacocha and Singrinacocha (Site 8 in Fig. 4.2). Two species of frogs breed in high elevation snowmelt and glacial runoff ponds in the Vilcanota above 5000 masl (Fig. 4.3). *Pleurodema marmoratum* is a relatively common frog with an aquatic larval stage and terrestrial adult stage (classified as Least Concern despite local population declines; IUCN 2015a; Fig. 4.3a). *Pleurodema marmoratum* individuals have been regularly observed in the deglaciated zone from 2003 to 2016 (Seimon et al. 2007, 2017). *Telmatobius marmoratus* is widely distributed at elevations from 1800 to 5244 masl and is aquatic during both the tadpole and adult life stages (Fig. 4.3b; IUCN 2015b, Seimon et al. 2007). The species is classified as Vulnerable because of population declines associated with water pollution, overharvesting, and chytridiomycosis (IUCN 2015b).

We recorded water temperature to illustrate the range of possible *T. marmoratus* body temperatures. We used a HOBO Tidbit datalogger (Onset Data Corp.) in shallow water (~10 cm) and deep water (~100 cm) in a pond at Site 8 where we observed both *T. marmoratus* and tadpoles of *P. marmoratum*. We recorded substrate temperature under frog cover rocks at the same pond to estimate substrate temperatures in terrestrial microhabitat used by *P. marmoratum* using iButton thermochron loggers (N=3 or 4 loggers were deployed each month; Maxim Corp.). We obtained climate data from the Peruvian National Weather Service (SENAHMI 2018), from weather stations located in the Cordillera Vilcanota region. There are no weather stations at the field sites we sampled, but these weather stations capture regional trends such as negative precipitation anomalies and positive temperature anomalies during El Niño. We used daily maximum temperature from three weather stations (Progreso, Ccatcca, and Pomacanchi) and 24-hr precipitation totals from four weather stations (Progreso, Ccatcca, Pomacanchi, and Santa Rosa) to calculate temperature and precipitation anomalies for the study period compared to the reference period of 1999-2017. Anomalies were then averaged across the stations.

In addition to the repeated visits to the primary field site (Site 8), we also made a single visit to 11 additional sites in remote valleys (Fig. 4.2). We collected *Bd* samples from frogs to determine how widespread the fungus is in areas that were, until recently, covered by permanent ice and snow. On the basis of previous work demonstrating that frogs and *Bd* have expanded into recently deglaciated areas at Site 8 in the Vilcanota (Seimon et al. 2007, 2017) we hypothesized that we would find widespread evidence of *Bd* in addition to the sites that have been monitored repeatedly over the last ~15 years. We identified study ponds and wetlands in recently deglaciated zones within the Vilcanota using satellite imagery available from Google Earth. We used the visual differences of lichen-covered and nearly lichen-free moraines to distinguish between recently ice-covered moraines and moraines that have been exposed. Lichen cover has been previously used to date locations covered by permanent ice around the end of the Little Ice Age (~1880) from areas that were ice-free at that time (Rabatel et al. 2005, Seimon et al. 2007, Schmidt et al. 2009).

Disease Sampling. Swab samples were collected using BBL sterile rayon tipped culture swabs (Fisher Scientific, Pittsburgh, PA, USA). Each frog was gently swabbed on the underside of the thighs for 5 strokes, hind feet for 5 strokes, and the abdomen for 10 strokes for a total of 30 strokes per frog. Tadpoles were swabbed 20 times on the mouth. Swab samples were air dried, sealed and stored at ambient temperature, and then transferred to a freezer where they were stored until analysis. We captured each frog in a clean plastic bag and used new non-powdered nitrile gloves for each animal to prevent cross contamination. *Pleurodema marmoratum* found under the same rock were sometimes placed in the same bag, but in this case only one frog from a bag was swabbed; otherwise frogs were placed in separate bags. We collected dead frogs in good condition that were encountered during surveys and stored them in 90% ethanol for histology and Ranavirus detection by PCR (Appendix 4.1). Footwear, nets, and rulers were sanitized with 90% EtOH between sampling sessions within a study site, and equipment was disinfected with bleach between study sites (Phillott et al. 2010).

PCR Bd testing. We extracted *Bd* from swabs or tissue using Prepman Ultra (Life Technologies). We analyzed the extracts using real-time quantitative PCR amplification of the internal transcribed spacer (ITS1) and 5.8S rDNA region using standard methods (Boyle et al. 2004, Seimon et al. 2015, 2017). The *Bd* assay compares each sample to a standard curve using samples of known zoospore or plasmid concentrations to quantify ITS1 copy number on each swab. We report *Bd* infection intensity ("Z_{swab}") as the estimated number of ITS1 copies in the DNA extract. We also report a value of Z_{swab} that has been adjusted to account for the fact that ITS1-5.8S is a multi-copy region of the *Bd* genome and copy number varies considerably (range of 10–144 copies with an average of 77 copies per zoospore) between different *Bd* strains (Longo et al. 2013). Because we do not know what *Bd* strain or strains exist in the Vilcanota (or the copy number of ITS1-5.8S), we divided Z_{swab} values by the average of 77 copies of ITS1-5.8S per zoospore to estimate an adjusted multi-copy Z_{swab} range (as in Seimon et al. 2015). We categorized samples as *Bd*-positive when $Z_{swab}>0$ and as *Bd*-negative when $Z_{swab}=0$. We calculated *Bd* prevalence by size class and season by dividing the number of *Bd*-positive frogs by the total number of samples. There were differences in the details of the qPCR methods we used for samples collected at the first half and second half of the study. Detailed methods for samples collected from the earlier period of July 2013 to July 2014 have been previously published in Seimon et al. (2015, 2017), and methods for the samples collected from February–December 2015 can be found in Catenazzi and Ttito (2016). We used results of an inter-laboratory ring test to standardize zoospore loads determined using the two analytical methods (Appendix 4.2).

Statistical Analyses. All statistical analyses were performed in R Version 3.2.1 (R Core Team 2015). All *Bd* analyses were conducted separately for *P. marmoratum* and *T. marmoratus*. We used the R package Hmisc to calculate the Wilson 95% Confidence Interval for *Bd* prevalence proportions.

We analyzed *Bd* prevalence using a binomial generalized linear mixed effects (glmer) model with logit link in the lme4 package of R. We first built models for *P. marmoratum* that included fixed effects factors of Breeding (i.e., was *P. marmoratum* actively breeding, coded as yes or no), Season (i.e., wet or dry), and Frog Size-Class (i.e., juvenile or adult). We designated samples taken in September–December as Breeding yes, and January – August as Breeding no. We considered swabs collected in October – April as wet-season samples; swabs taken in May–September were considered dry season samples. We classified juvenile *P. marmoratum* as individuals with Snout-Urostyle Length (hereafter "SUL") up to 1.6 cm, and adults as individuals with SUL greater than 1.6 cm. We included Pond nested within Year as a random effect to account for ponds at the main field site that were sampled more than once. We used the model.sel function in the MuMin package to guide model selection using Akaike's Information Criterion with a small sample size correction (AICc; Anderson and Burnham 2002). We did not consider models that included the biologically uninformative interaction between Breeding and Season (the breeding season includes a subset of samples from the early wet season). Because the sample size for post-metamorphic *T. marmoratus* was very small, we included only tadpoles in the analysis. We checked final models for overdispersion with the blmeco package in R.

To analyze infection intensity, we $\log_{10}$-transformed Z_{swab} to meet the model assumptions. We built candidate models using linear mixed effects models (lme) in the R package nlme. We used Akaike's Information Criterion with a correction for small sample sizes (AICc) to select the final model (Anderson and Burnham 2002) for *Bd* infection intensity (i.e., Z_{swab}) in *P. marmoratum* and *T. marmoratus* using the MuMin package. We compared the two top models (ΔAICc < 2) using ANOVA and selected the model with the lowest AICc. In both analyses we included Pond as a random effect to account for repeated visits. We also compared the density distribution of Z_{swab} by Season and Breeding using a non-parametric bootstrap method. We used sm.density.compare in the R package sm for the tests of equality.

RESULTS

Main Site

We obtained samples from *P. marmoratum* adults and juveniles in both the wet and dry seasons. Samples from post-metamorphic *T. marmoratus* were obtained only in the wet season. We only encountered four adult *Telmatobius marmoratus* (SUL Range: 5.1-6.9

cm) over the entire study despite more than 70 person/hours of search effort in ponds where *T. marmoratus* tadpoles and juveniles were found.

Prevalence of *Bd* was higher in the dry season than the wet season for *P. marmoratum* adults and juveniles and *T. marmoratus* tadpoles (Table 4.1). Infection intensity (i.e., Z_{swab}) was highly variable within each size class of *P. marmoratum* and *T. marmoratus* (Table 4.2). In *P. marmoratum* adults, density distributions of log-transformed Z_{swab} were statistically equal in the dry and wet seasons, and in and out of the breeding period (Fig. 4.4a,c). In juveniles of *P. marmoratum*, the density distribution of Z_{swab} was significantly shifted towards higher infection intensity in the dry season compared to the wet season, and in the breeding period compared to outside the breeding period ($p < 0.05$; Fig. 4.4b,d). The density distribution of log-transformed Z_{swab} in *T. marmoratus* tadpoles was significantly shifted towards higher infection intensity in the dry season ($p < 0.05$; Fig. 4.5).

The highest-supported models for *Bd* prevalence and infection intensity in *P. marmoratum* are presented in Table 4.3. The two top models for *Bd* infection intensity in *P. marmoratum* had very similar support. Both models fell within the ΔAICc > 2 guideline for model selection (Anderson and Burnham 2002) and were not significantly different by ANOVA (chi-square = 1.96, *df* = 1, *p* = 0.16). We selected the infection intensity model with the full set of biologically-relevant explanatory variables, and the lowest AICc. The explanatory variables in the final glmer model for *Bd* prevalence and final lmer model for infection intensity in *P. marmoratum* were the fixed effects of Breeding, Season, Size, and the interaction terms Breeding × Size and Season × Size

(Table 4.3). The models for prevalence and infection intensity in *T. marmoratus* included only Season.

Bd prevalence. The differences in *P. marmoratum Bd* prevalence by season, breeding period, and size class were not statistically significant (Table 4.4). The interaction term Season × Size was not statistically significant (Table 4.4, Fig. 4.6a). The interaction term Breeding × Size was significant, with juvenile *P. marmoratum* having higher *Bd* prevalence during the breeding season than adults during the breeding season (Table 4.4, Fig. 4.6b). Juvenile *P. marmoratum Bd* prevalence was higher in the breeding period than outside the breeding period (Fig. 4.6b). Prevalence of *Bd* in larval *T. marmoratus* was significantly higher in the dry season than in the wet season (Table 4.4; Fig. 4.7a.).

Bd infection intensity. Infection intensity in *Pleurodema marmoratum* was significantly higher in the dry season compared to the wet season. There were also significant interactions between Breeding × Size and Season × Size (Table 4.5). The effect of breeding period alone on infection intensity was marginally non-significant (p = 0.06; Table 4.5). Adult and juvenile *P. marmoratum* have different seasonal patterns of *Bd* infection intensity. Infection intensity in adults and juveniles did not differ during the dry season, but adult infection intensity was higher in the dry season compared to the wet season (Fig. 4.8a). Infection intensity in juveniles was high in both the dry and the wet seasons (Fig. 4.8a). Infection intensity was also similar in adults and juveniles in the non-breeding period, but adults and juveniles diverged during the breeding period. Adult infection intensity was lower during the breeding period than outside the breeding period, whereas juvenile infection intensity was higher during the breeding period (Fig. 4.8b). *Telmatobius marmoratus* tadpoles had significantly higher *Bd*-infection intensity in the dry season than in the wet season (Table 4.5; Fig. 4.7b).

Bd-associated Mortality. Between August 2013 and March 2015 we encountered nine dead *Pleurodema marmoratum* during field surveys at Site 8. Two (of N = 5) of the dead adults tested positive for *Bd* by PCR (Z_{swab} range = $10^{1.34} – 10^{4.14}$). All dead juveniles tested positive for *Bd* by PCR (N = 4; Z_{swab} range = $10^{3.06} – 10^{5.20}$). Three of the juveniles and one adult that had tested positive for *Bd* by PCR were negative by histology, i.e., had no lesions associated with the disease (Appendix 4.1). None of the individuals tested positive for Ranavirus by PCR or histology (Appendix 4.1).

We surveyed *Telmatobius marmoratus* in a connected set of ponds at Site 8 in April 2013, and again from July 2013 to June 2016 (Fig. 4.3c). We observed four distinct cohorts, designated here as Cohorts A-D, indicated by the appearance of tadpoles in developmental stages 25-28 (following Gosner 1960; Table 6). New cohorts often overlapped temporally and spatially with advanced developmental stages (Table 4.6, Fig. 4.9). Although we do not know when the eggs were deposited or hatched, Cohort B took ~12 months to go from early larval stages (Gosner 26) to the last observation of metamorphs (Gosner 45-46). Cohort C took more than twice as long, ~ 29 months, to do the same.

We observed mortalities in *T. marmoratus* as two of the cohorts completed metamorphosis. None of the dead frogs had missing limbs or wounds of any kind that could be associated with predation. In February and March 2014 we encountered five dead juvenile *T. marmoratus* (Fig. 4.9a) from Cohort A. One dead juvenile was not tested because it was in an extremely advanced state of decay. Of the remaining four dead *T. marmoratus* that were tested for *Bd*, one was in good condition with gross abnormalities consistent with chytridiomycosis (i.e., sloughing of skin). The animal tested positive for *Bd* using the standard PCR methods ($Z_{swab} = 10^{3.48}$). The three remaining dead *T. marmoratus* juveniles were negative by PCR, but these individuals were in an advanced state of decomposition (i.e., leeches had consumed almost all internal structures leaving compromised skin, which appeared to be sloughing). For these individuals, we extracted DNA from samples of skin from the ventral region and toe rather than from a swab. In January 2015 we observed two late-stage metamorphs from Cohort B with sloughing skin that tested positive for *Bd* by PCR (Table 4.6). In April and May 2016 we observed a second *T. marmoratus* mortality event. We encountered five dead *T. marmoratus* juveniles, likely from Cohort C, that had recently undergone metamorphosis (Fig. 4.8b). We did not collect *Bd* swabs from these individuals because our permits did not allow us to do so. All five dead juveniles exhibited sloughing of skin associated with chytridiomycosis.

Climate Trends and Temperatures. The El Niño that occurred in 2015/2016 was a very strong event (Ocean Niño Index > +2.0; Huang et al. 2017). The El Niño is characterized in the data as a series of strong positive temperature anomalies (Fig. 4.10). Precipitation patterns fluctuated between strong positive and negative precipitation anomalies, indicating that precipitation during the 2015/2016 wet season was highly variable (Fig. 4.10).

The temperatures logged in deep water were relatively stable compared to the shallow-water logger (Fig. 4.11). Deep water was 5.73 ºC ± 0.7 ºC (mean ± SD) and shallow water averaged 8.13 ºC ± 2.5 ºC. Because of the extreme daily temperature variation at such high elevations, the monthly average range and full range of temperatures are presented in Fig. 4.11. Terrestrial microhabitat temperatures were also highly variable (Fig. 4.12). Although the average substrate temperature was 5.5 ± 0.9 ºC, the average monthly temperature variation was 37.5 ± 7.9 ºC.

Regional *Bd* Surveys

We surveyed 11 previously unsampled high-elevation sites 5–28 km from Site 8 (the main field site). Our surveys occurred along elevational gradients from the valley bottoms, which were often occupied by high-elevation peat wetlands known as *bofedales*, to the rocky moraines at the margin of the retreating glaciers. We found *Bd*-infected *Pleurodema marmoratum* at five of the new sites (Fig. 4.2; Table 4.6). We encountered *P. marmoratum* infected with *Bd* inside the recently deglaciated zones at sites 2, 3, 4, 6, and 11. We encountered *P. marmoratum* that were *Bd*-positive outside the recently deglaciated zone at site 10. We encountered *P. marmoratum* tadpoles in the valleys below sites 7 and 12, but nowhere along transects into the recently deglaciated zone. Tadpoles found in the valleys were not tested for *Bd*. We found no frogs at site 5. Tadpoles and frogs encountered at sites 1 and 9 were *Bd*-negative.

DISCUSSION

We investigated host-pathogen disease dynamics and regional prevalence of *Bd* in amphibian communities living in the high Andes above 5000 masl. While *Bd* has been recorded at these elevations in the high southern Andes for over a decade (Seimon et al. 2007, Catenazzi et al. 2011, Catenazzi and Ttito 2016, Seimon et al. 2017), we present the first detailed study to consider the role of ontogeny, breeding activity and seasonality play a role in host/pathogen disease dynamics at the upper elevational limit of known *Bd* occurrence, This also is the first study to investigate enzootic disease dynamics from a population of *Telmatobius* frogs that suffered severe *Bd*-related declines over a decade ago.

The rebound of *T. marmoratus*-supports the hypothesis that susceptibility alone does not explain which species survive after an epizootic disease outbreak. Our study supports previous reports of high *Bd* prevalence in Andean *Telmatobius* (Seimon et al 2007, Catenazzi et al. 2011, Catenazzi et al. 2013, Seimon et al. 2017). Post-epizootic recovery has been documented in multiple anuran species a decade or more after mass die-offs for species that went through severe declines (e.g., Knapp et al. 2016, Scheele et al. 2017, Whitfield et al. 2017). However, to our knowledge, this is the first example of rebound or stabilization of a population of *Bd*-susceptible *Telmatobius* frogs.

Telmatobius frogs were once widespread throughout cloud forests in the Andes–Amazon region, but have since declined from much of their range. For example, less than 100 km away from the Vilcanota and 1500 m lower in elevation, *Telmatobius* disappeared completely from the Kosñipata Valley following the early 2000s *Bd* epizootic (Catenazzi et al. 2017). Future research focusing on understanding the mechanisms causing differential outcomes of host-pathogen dynamics in Andean frogs could dramatically advance our understanding of the risk that *Bd* poses to other amphibian communities and help guide appropriate conservation management strategies, and may lead to conservation solutions for vulnerable species. For example, the survival and recovery of *T. marmoratus* at very high elevation but not at lower elevations might be affected by environmental differences along the wide elevational gradient (e.g., host-pathogen thermal mismatch), variations in *Bd* pathogenicity or transmission pathways, or differential host resistance (e.g., through symbiotic skin bacteria as other studies have shown in montane Andean marsupial frogs; Puschendorf et al. 2013, Catenazzi et al 2014, Nowakowski et al. 2016, Burrowes and De la Riva 2017a, Burkart et al. 2017). Continued monitoring of these high elevation anuran populations is necessary to determine if host-pathogen dynamics return to an epizootic state in *Telmatobius* populations.

Contrary to the hypothesis that *T. marmoratus* would build up high infection intensities and experience epizootic mortality events, the die-offs we observed did not coincide with dry season peaks of *Bd* infection intensity or prevalence. Climate variability could have affected host-pathogen dynamics at our site. Both mortality events coincided with positive temperature anomalies, but strong positive anomalies occurred throughout the study without additional mortality events. We only observed mortality in recently metamorphosed juveniles. In February and March 2014 we encountered five dead *T. marmoratus* at Site 8. These dead individuals were part of a cohort (A) that had recently completed metamorphosis (beyond Gosner stage 45; Gosner 1960). We tracked Cohort A in repeated surveys over several months. It is noteworthy that these tadpoles were likely from the same cohort we observed when we "rediscovered" *T. marmoratus* at Site 8 in April 2013. Only one of the four tested carcasses was *Bd*-positive, but there is a strong possibility that the extremely poor condition of the carcasses resulted in false negatives because of DNA degradation. Carcasses exhibiting such an advanced state of decay are often excluded from PCR assays (e.g., Kolby 2014). Pathogen detection through PCR is often combined with histology in these cases, but we were unable to preserve the nearly liquefied carcasses for examination.

We observed a new cohort (B) in the study ponds at the same time as the mortality event in Cohort A, and we repeatedly surveyed ponds to determine if there would be a mortality event in subsequent dry seasons. As Cohort B approached metamorphosis in January 2015, we encountered two late-stage metamorphs that tested positive for *Bd* and exhibited symptoms of clinical chytridiomycosis (i.e., sloughing of skin; Fig. 4.9c). Early-stage metamorphs (Gosner stages 42-43), likely from Cohort C, were encountered for the first time during sampling in January 2016. For a second time, as Cohort C began to go through metamorphosis, we observed five dead post-metamorphic *T. marmoratus* juveniles between April-May 2016.

There is a well-documented pattern of *Bd*-associated mortality at the onset or completion of metamorphosis that fits the pattern of die-offs we observed (Garner et al. 2009). Amphibian larvae are susceptible to infection but are able to carry high infection loads without mortality, while chytridiomycosis in post-metamorphic juveniles and adults can be fatal (Garner et al. 2009). The fungus affects only the keratinized tissues of amphibians, which transitions from being localized in the mouthparts to the skin during metamorphosis, increasing the amount of substrate for infection (Marantelli et al. 2004, McMahon and Rohr 2015. The ontogenetic effect occurs because *Bd* infects the skin of post-metamorphic amphibians, causing hyperkeratosis and thickening of the epidermis. Also during metamorphosis, amphibians experience stress and depressed immune function (Rollins-Smith 2017), possibly reducing the immune response to infection.

Our findings resemble the enzootic pathogen-host dynamics described for *Bd* in *Rana mucosa* (Yellow-legged Frog) in the Sierra Nevada of California, USA. In *R. mucosa*, adults can survive across multiple years with *Bd* infection but the timing of *Bd*-induced mortality around metamorphosis causes low metamorph survival. The abundance of *R. mucosa* remains low because of limited recruitment to the juvenile size class (Briggs et al. 2010). In contrast, other studies have shown that *Bd*-susceptible populations can suffer mortality from *Bd* (e.g., of up to 90% of post-metamorphic individuals; Tobler and Schmidt 2010), without having a negative effect on the population (Tobler et al. 2012, Sapsford et al. 2015). Although negative effects of *Bd* do not always translate from the individual to the population level the high potential for stochastic population fluctuations in high mountain environments combined with the potentially small breeding population and long developmental time of *T. marmoratus* mean that limiting recruitment to the reproductive adult size classes would likely have a negative impact on these populations.

We caution that the future persistence of *T. marmoratus* at the upper limits of its range is far from certain. The timing of mortality events at metamorphosis suggests that *Bd* probably still negatively affects the *T. marmoratus* population in the region despite rebounding to detectable levels. We lack pre-decline frog abundance estimates before *Bd* was detected at this site, so we are unsure how recent *T. marmoratus* abundance compares to pre-decline levels. We encountered only four adult frogs and four reproductive events throughout the course of the study, which suggests the presence of a small or cryptic breeding population. If the breeding population is as small as it appears to be, founders effects, disease, and hydrologic alterations from ongoing rapid climate change may eventually result in extirpation of the highest-recorded *Telmatobius* populations.

Two traits hypothesized to confer increased chances of post-decline stabilization or population recovery are high recruitment rates and the presence of environmental refugia (Scheele et al. 2017). Since *T. marmoratus* appears to have limited reproductive effort and the population likely has low recruitment, we consider the environmental suitability of temperatures at our site and the possibility that there exist environmental refugia that limit *Bd* growth. Environmental suitability for *Bd* is a key determinant of population-level responses to ongoing *Bd* infection (Puschendorf et al. 2009). Seimon et al. (2007) suggested that high solar insolation may be responsible for creating a thermal environment conducive to *Bd* despite the low average temperatures associated with high elevation environments. At low temperatures *Bd* may be more virulent and have higher fecundity and longevity than at optimal temperatures (Woodhams et al. 2008, Voyles et al. 2012). We now know that *Bd* can grow and cause disease along a wider thermal range than previously thought. In fact, some *Bd* strains have high growth rates at low temperatures (2-3°C), and remain viable after 24 h freeze (-12°C) and heat shock (28°C; Voyles et al. 2017). Our thermal data from deep water, shallow water, and terrestrial frog refuges under rocks establish that temperatures within the optimal growth range of *Bd* of 13-25°C (Stevenson et al. 2013) occur year-round, even at 5250 m. Our observations support the Seimon et al. (2007) hypothesis that in the high Andes, low temperatures and even freezing do not limit *Bd* growth and pathogenicity.

Although the thermal environment in our study area appears conducive to *Bd*, high solar insolation may also create opportunities for protective thermoregulation allowing frogs to self-limit infections. Behaviors and thermal tolerance limits that allow body temperatures to exceed the range of pathogen performance or increase immune function can clear or reduce the intensity of existing infections (Woodhams et al. 2003, Richards-Zawacki 2010, Rowley and Alford 2013, Roznik et al. 2015). The possibility of behaviorally clearing or reducing infection loads is related to the thermal tolerance mismatch hypothesis: infection risk is reduced when the host occupies microenvironments that are more unfavorable for the pathogen than to itself (Nowakowski et al. 2016). In the high-elevation tropics there may be brief diurnal periods when the thermal mismatch between host and pathogen allows the persistence of populations in locations otherwise suitable for *Bd* (sensu Longo et al. 2010b).

Frogs living in the high tropical Andes above the forest ecotone appear to have a much larger thermal mismatch with *Bd* than frogs living downslope in the montane or lowland forests (Catenazzi et al. 2013). In the high tropical Andes above the treeline, daily temperature variation is extreme, and frogs can be exposed to a wide range of body temperatures (Catenazzi et al. 2013, Navas et al. 2013). Frogs and tadpoles in aquatic habitat may utilize shallow, warm areas in ponds and terrestrial adults may select sun-warmed rocks to thermoregulate and minimize existing infections below a lethal level.

Short daily heat pulses (26°C) may be able to help frogs reduce *Bd*-infection loads and slow the rate that infections reach lethal limits (Greenspan et al. 2017).

We show that the breadth of thermoregulatory options available to frogs living even at the same elevation (5244 m) depends on their microhabitat and life history. In the terrestrial environment of *P. marmoratum*, substrate temperatures below cover rocks reached or exceeded the 26°C heat-pulse limit that could allow daily thermoregulatory behaviors that limit *Bd* growth and frog mortality (Greenspan et al. 2017). In contrast, neither average maximum or absolute maximum temperatures in deep water ever reached the 26°C heat-pulse limit (Greenspan et al. 2017). In shallow water, the absolute maxima reached 26°C few times in our 2.5 year record. Fully-aquatic *T. marmoratus* have a narrow range of thermoregulatory options that fall almost entirely within a range that seems conducive to *Bd* growth. We frequently encountered *T. marmoratus* metamorphs and juveniles in the shallow, warmer areas around pond perimeters. We observed that as pond surface area contracted during the dry season, shallow, rocky areas that were warmed by the sun were no longer accessible to frogs as the water levels dropped. Reduced access to warm microhabitats in the dry season might further affect host's ability to behaviorally limit infections, and could influence the observed peak of *Bd* infection intensity and prevalence in the dry season. During the dry season, being limited to cold temperatures could limit immune responses and *Bd* virulence (Rollins-Smith 2017), and reduced water levels could increase *Bd* transmission and persistence (Catenazzi et al. 2013, Seimon et al. 2017).

In the terrestrial high-elevation environment, temperatures may reach the extremes of the thermal range for *Bd* on a daily basis year-round. In shallow aquatic high-elevation habitats, *Bd* also experiences wide daily thermal variation. A pathogen that can grow well under such variable conditions will likely be problematic for amphibian hosts with limited critical thermal limits or opportunities for protective thermoregulation in high-Andean habitats. Aquatic *Telmatobius* probably have less opportunity for protective thermoregulation than terrestrial stages of *P. marmoratum*. However, *Bd* may exhibit growth limitations at a different temperature limit at such high elevation, and if frog immune systems perform better under the highly variable daily temperatures than the pathogen, the frogs could benefit from a form of host-pathogen thermal mismatch.

Demographic density effects may also play a role in disease dynamics at our high-elevation sites because the mortality events coincided with periods of overlapping tadpole cohorts. Although tadpoles typically do not die from chytridiomycosis, multi-year larval periods and overlapping tadpole cohorts can act as reservoirs for the disease (Bosch et al. 2001, Rachowicz and Vredenburg 2004, Briggs et al. 2010, Vredenburg et al. 2010, Catenazzi et al. 2013). Other environmental reservoirs and potential non-amphibian vectors of *Bd* have been identified in groups that we observed syntopically in high Andean ponds. For example, *Bd* is able to survive on bird feathers, bird feet scales, on arthropod exoskeletons and inside their digestive tracts, and *Bd* has been identified from museum bird specimens from the Andes (Garmyn et al. 2012, McMahon et al. 2013, Burrowes and De la Riva 2017b). Additional studies on transmission pathways, host resistance, and host-pathogen thermal mismatch are needed to understand fully host-*Bd* dynamics in the high Andes.

The seasonal effect on *Bd*-infection intensity in *P. marmoratum* differs from that of *T. marmoratus*, which is likely because of differences in natural history and *Bd* susceptibility between the two species. Although overall *Bd* infection intensity in *P.*

marmoratum was higher in the dry season compared to the wet season, this pattern was driven by results from adult frogs. Our study reveals an unexpected reversal of seasonal disease dynamics among juvenile *P. marmoratum*. Our highest-ranking models suggest that for both *Bd* prevalence and infection intensity, juvenile *P. marmoratum* exhibited high levels of *Bd* infection in the breeding season. The breeding season typically occurs in the early part of the wet season between October and early December (K. Reider, unpublished data). High prevalence and infection intensity of *Bd* in juveniles in the breeding season was surprising for several reasons. First, we expected *Bd* prevalence and infection intensity in juvenile *P. marmoratum* to be highest in the dry season, when the range of temperatures individuals experience in aquatic habit is limited to colder temperatures, and because the majority of tadpoles in our study ponds complete metamorphosis at the beginning of the dry season (late May-June). Cold temperatures, variation in temperature, and metamorphosis are known to reduce the amphibian immune response to infection (Raffel et al. 2006, Garner et al. 2009, Rollins-Smith 2017).

Additionally, a reduction in water levels in the dry season may affect *Bd* transmission by concentrating aquatic amphibians and zoospores (Catenazzi et al. 2013, Seimon et al. 2017). Second, we included the breeding category in models to test for an expected increase in prevalence and infection intensity in the breeding season for adult *P. marmoratum*, rather than juveniles. We expected to see an effect on adults because during the breeding season adults may have increased exposure to infected individuals and environmental reservoirs of zoospores in ponds (Briggs et al. 2010) and are likely to have depressed immune responses from the stress and energy expenditure required for breeding (Rollins-Smith 2017). One possible explanation for the pattern of increased *Bd* in *P. marmoratum* juveniles is that breeding adults could transport zoospores from breeding ponds back to refugia shared with juveniles, facilitating frog-to-frog or terrestrial transmission pathways. The pathogen may persist in saturated soils under rocks near seeps and springs favored by *P. marmoratus*. It has been suggested that one reason high-elevation soils are dominated by aquatic chytrids (although only *Bd* is a parasite of amphibians) is that these soils are so saturated under the snow that they function as aquatic habitats (Freeman et al. 2009).

We demonstrate that *Bd* is widespread throughout the high Andes of southern Peru, even in several recently deglaciated areas, extending the findings of prior surveys that established that *Bd* has been present for over a decade at these elevations (Seimon et al. 2007, 2017). In addition to the main study site, Site 8 (Fig. 4.2), five of the new locations where we identified *Bd* in *P. marmoratum* were near glacial margins and exhibited signs of being covered by permanent ice until recently (i.e., unconsolidated moraines with very little plant or lichen cover). We assume *Bd* is not present in glacial ice (*Bd* was not among the chytrid fungi identified from recently deglaciated soils in the Vilcanota; Freeman et al. 2009), so the widespread geographic prevalence of *Bd* throughout the region indicates a clear role of climate change in the regional upward expansion of an emerging wildlife disease to additional recently deglaciated zones.

Our widespread *Bd* surveys throughout the region revealed *Bd*-positive samples at 75% of sites where we found frogs (six out of eight survey locations where we found frogs) despite site remoteness and low search effort. Even at sites with the highest search effort, our sample sizes were low, reflecting the difficulty of finding frogs at very high elevations. Because we were able to detect *Bd* even with small sample sizes, sometimes swabbing only a single individual, we surmise population-level prevalence in these locations could be relatively high. The negative *Bd* results from sites 1 and 9 are more likely to reflect failure to detect infected frogs rather than true negatives. For example, at one of the few sites for which there were additional data, 10% *Bd* prevalence was observed less than two km ("Site G" in Seimon et al. 2017) from Site 9. Given limitations on finding frogs in high Andean habitats, future studies might benefit from surveying for environmental presence of *Bd* (as in Schmidt et al. 2013).

The widespread occurrence of *Bd* across high elevations in the Andes is important for several reasons. First, although we are encouraged by the possible rebound of *Telmatobius marmoratus* at our main study sites we caution that *Bd* has likely already had a dramatic effect on anuran populations. Current distribution patterns in the highest elevations probably reflect the widespread historic population declines caused by the *Bd* epizootic as well as limitations to dispersal and pond colonization. In all of our sampling across the region, we only located one species, *Pleurodema marmoratum*, in the highest elevation zones except for at Site 8. The *Bd* epizootic of the early 2000s could have affected the course of additional anuran populations that underwent, or could have undergone, upward range shifts, and could still be depressing population levels of hard-to-detect species around the Vilcanota to below-detectable limits. If additional *T. marmoratus* populations are recovering, they are likely to require rigorous sampling effort to detect them. Second, our results suggest that as temperatures continue to warm and glacial retreat progresses, anurans as well as *Bd* are likely to continue expanding upward and across high-elevation zones to into new habitat. Upward expansion of *Bd* is likely to occur in mountain ecosystems wherever *Bd*-infected amphibian populations exist, but is particularly concerning in the tropical Andes. The northern and central Andes make up 94% of all high-elevation tropical land area on Earth (Jacobsen 2008), which means a large proportion of high-elevation land lies within the elevational range of chytrid. Finally, high-elevation ponds and streams are often connected by water flowing from high to low elevation. The inflow of *Bd* zoospores from high elevations has been shown to influence *Bd* prevalence in frog populations at lower elevations (Sapsford et al. 2013). The glaciers that feed Lake Sibinacocha also feed the lower Amazon ecosystem, and their waters flow though some of the most highly biodiverse amphibian communities in montane forests. The presence of widespread *Bd* may create opportunities for transportation of *Bd* zoospores to lower elevations.

Our results have important implications for amphibian conservation, especially in tropical high mountain environments, which are among the least-studied ecosystems on Earth (Jacobsen 2008). An overwhelming proportion of research on *Bd*-host disease dynamics in tropical Andean environments has taken place below the treeline where amphibian declines resulting from chytridiomycosis have been severe and have resulted in the ongoing absence of *Telmatobius* from montane cloud forests (Catenazzi et al. 2011, Burrowes and de la Riva 2017a). In contrast, in the extreme high-elevation environment above the treeline, several species of *Telmatobius* have persisted with *Bd* (Seimon et al. 2017, Burrowes and De la Riva 2017a). Chytridiomycosis is still a major threat to amphibian biodiversity and our study reveals that enzootic *Bd* is still affecting amphibian populations living at the edge of the cryosphere. Disease-induced mortality at metamorphosis is likely to have persistent negative impacts on *Telmatobius* populations through reduced recruitment and the pathogen may be regulating anuran populations throughout the region. Different mechanisms likely govern the host-pathogen dynamics of the two species at our site. Our investigation of the thermal environment of *P. marmoratum* and *T. marmoratus* revealed that these two species likely have widely divergent degrees of host-pathogen thermal mismatch in post-metamorphic life stages, despite co-occurring in habitats at the same elevations. Continued monitoring of these high-elevation anuran populations is necessary to determine if host-pathogen dynamics return to an epizootic state.

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TABLES

Table 4.1. Prevalence of *Bd* infection in the Cordillera Vilcanota

	Dry Prevalence (95% CI)	*N*	Wet Prevalence (95% CI)	*N*
Pleurodema marmoratum				
Adults	66.7 (43.7-83.7)	18	42.9 (30.0-56.7)	49
Juveniles	54.9 (45.2-64.2)	102	24.4 (16.6-34.5)	86
Telmatobius marmoratus				
Adults	-	-	25.0 (1.3-69.9)	4
Juveniles	-	-	57.1 (32.6-78.6)	14
Tadpoles	47 (29.2-67.0)	23	11.7 (5.7-22.2)	60

Table 4.2. *Batrachochytrium dendrobaditis* infection intensity.

Group	N	Median Z_{swab}	Z_{swab} Range	Multi-Copy Range*
Pleurodema marmoratum				
Adults	33	$10^{2.87}$	$10^{-0.31} – 10^{5.86}$	$10^{-2} – 10^{3.98}$
Juveniles	77	$10^{3.36}$	$10^{0.40} – 10^{6.68}$	$10^{-1.52} – 10^{4.79}$
Tadpoles	4	$10^{1.03}$	$10^{0.71} – 10^{1.63}$	$10^{-1.15} – 10^{-0.26}$
Telmatobius marmoratus				
Adults	1	$10^{1.65\S}$	NA	NA
Juveniles	8	$10^{2.52}$	$10^{1.35} – 10^{5.76}$	$10^{-0.54} – 10^{3.87}$
Tadpoles	18	$10^{3.28}$	$10^{-0.31} – 10^{5.52}$	$10^{-2} – 10^{3.63}$

* Multi-copy Z_{swab} range: Upper and lower Z_{swab} values are divided by 77 to reflect the estimated (range of 10–144, average of 77) ITS1-5.8S copies per zoospore (Longo et al. 2013)

§ The Z_{swab} value for the single *Bd*-positive *T. marmoratus* adult

Table 4.3. Model comparison: *Bd* prevalence and infection intensity.

Rank	Breed	Season	Size	Breed :Size	Season :Size	df	Log Likelihood	AICc	ΔAICc
Bd prevalence									
model1	+	+	+	+	+	7	-121.5	257.6	0
model2	+	+	+			5	-124.6	259.6	2.0
Bd infection intensity									
model1	+	+	+	+	+	8	-170.9	359.3	0
model2	+		+	+		6	-173.6	360	0.7
model3	+	+	+	+		7	-173.5	362	2.7

Table 4.4. Results of binomial mixed effects models for *Bd* prevalence. Models consider *P. marmoratum* (adults and juveniles) and *T. marmoratus* (tadpoles).

Model Terms	Estimate	SE	*z-value*	*p-value*
Pleurodema marmoratum				
(Intercept)	0.60	0.71	0.85	0.40
Season =wet	-0.45	0.76	-0.60	0.55
Breed =yes	-0.81	1.08	-0.75	0.45
Size =J	-0.88	0.72	-1.22	0.22
Season =wet, Size=J	-1.23	0.87	-1.41	0.16
Breed =y, Size =J	2.53	1.21	2.10	0.04
Telmatobius marmoratus				
(Intercept)	-0.65	1.02	-0.64	0.525
Season =wet	-2.13	0.69	-3.06	0.002

Table 4.5. Results of linear mixed effects models for infection intensity (log-transformed Z_{swab}) in *P. marmoratum* (adults and juveniles) and *T. marmoratus* (tadpoles)

Model Terms	Estimate	SE	df	t-value	p-value
Pleurodema marmoratum					
(Intercept)	3.89	0.38	88	10.2	0.000
Breeding =yes	-0.83	0.44	88	-1.87	0.064
Season =wet	-1.00	0.42	88	-2.36	0.021
Size =Juvenile	-0.65	0.41	88	-1.57	0.119
Breeding =yes: Size =J	1.72	0.55	88	3.09	0.003
Season =wet: Size =J	1.14	0.53	88	2.13	0.036
Telmatobius marmoratus					
(Intercept)	4.59	1.18	15	3.89	0.001
Season =wet	-2.76	0.91	15	-3.02	0.009

Table 4.6. *Telmatobius marmoratus* tadpole cohorts and mortality at Site 8. Developmental stages follow Gosner (1960).

Month	Limb Buds Develop		Toes develop				Metamorphosis				Metam. complete
	26-27	28-30	31-33	34-36	37-40	41	42	43	44	45	46
9-2013				A	A						
10-2013				A	A						
11-2013					A						
12-2013							A	A	A		
1-2014									A	A	A
2-2014	B	B								A*	A†*
3-2014	B									A	A†*
4-2014	B										
5-2014											
6-2014											
7-2014											
-											
-											
10-2014		C									
11-2014		C									
12-2014		C	C			B	B	B			
1-2015		C							B*	B*	
2-2015	C	C								B	
3-2015		C	C								
4-2015											
5-2015	C	C									
6-2015		C	C								
7-2015		C									
-											
9-2015											
10-2015											
11-2015				C							
12-2015							C				
1-2016	D						C				
2-2016	D						C	C			
3-2016	D										
4-2016	D								C		C†*
5-2016	D	D	D			C			C	C	C†*
6-2016		D									

† Dead juvenile *T. marmoratus* present
* Gross abnormalities associated with chytridiomycosis (i.e., sloughing of skin) present

Table 4.7. Regional *Bd* infection prevalence in *Pleurodema marmoratum*. Site numbers follow Fig. 1.

Site	Site Name	Date	Season	Elevation masl	Juv & Adult Prevalence (N)	Tadpole Prevalence (N)
1	Sinacara	12-2015	Wet	4885-4935	-	0 (4)
2	Paqopampa	12-2015	Wet	4662-4752	-	67% (3)
3	Qolqepunku	12-2015	Wet	4690-4922	0 (2)	14.2% (7)
4	Armaqocha	4-2015	Wet	4705-4782	0.1% (11)	-
5	Qolqe Cruz	4-2015	Wet	4791-4994	-	-
6	Huarurumiqocha	4-2015	Wet	5050-5181	100% (3)	-
7	Hatunuma	1-2015	Wet	4849-5041	-	-
8	Main Site	7-2013 – 12-2015	Wet & Dry	5244-5395	44% (226)	13.8% (29)
9	Hatun Ñañupunta	2-2015	Wet	4989-5022	0 (5)	-
10	Aqoyo	6-2015	Dry	4494-5126	100% (1)	-
11	Qori Kalis	2-2015	Wet	4930-5050	62.5% (8)	-
12	Challpa	2-2015	Wet	5159-5208	-	-

FIGURES

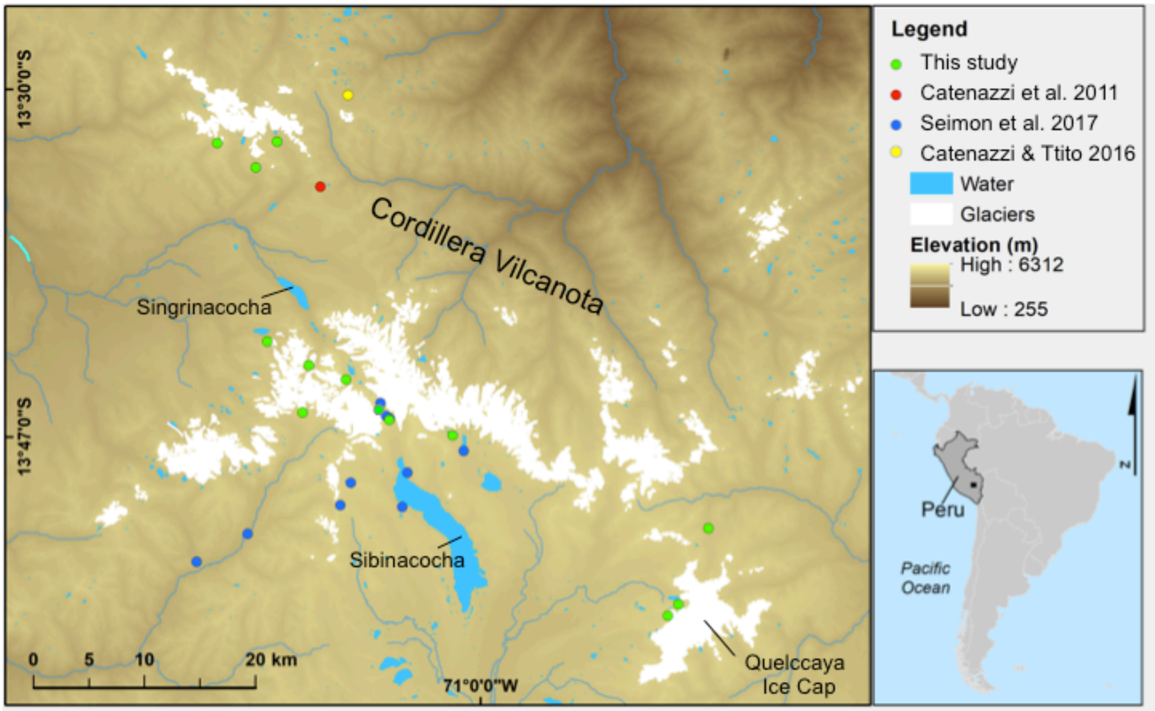

Figure 4.1. Overview map showing *Bd* sampling sites visited during this study and previous research efforts in the Cordillera Vilcanota, Peru.

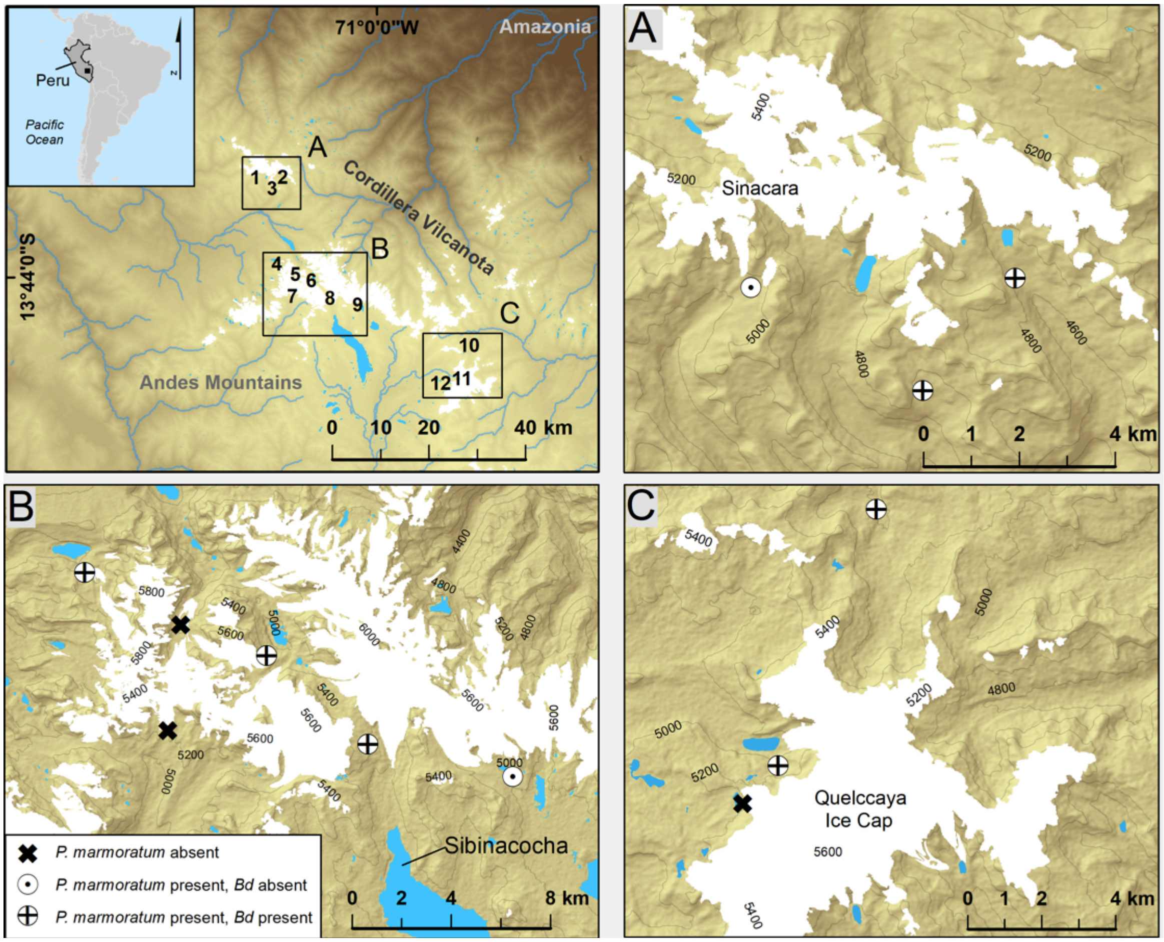

Figure 4.2. Map of study sites in the Cordillera Vilcanota, Peruvian Andes. The main panel inset (left) indicates the location of Peru, and the study area is outlined with a rectangle. Panels A – C are expanded views of the study area showing sampling sites. Glaciers are shown in white. White circles with plus signs indicate presence of *Pleurodema marmoratum* infected with *B. dendrobatidis*. White circles with dots indicate presence of *P. marmoratum* that were not infected. Black × symbols indicate sites where no *P. marmoratum* were found or tested for *Bd*. Site 8 was the only location where we sampled both *P. marmoratum* and *T. marmoratus*.

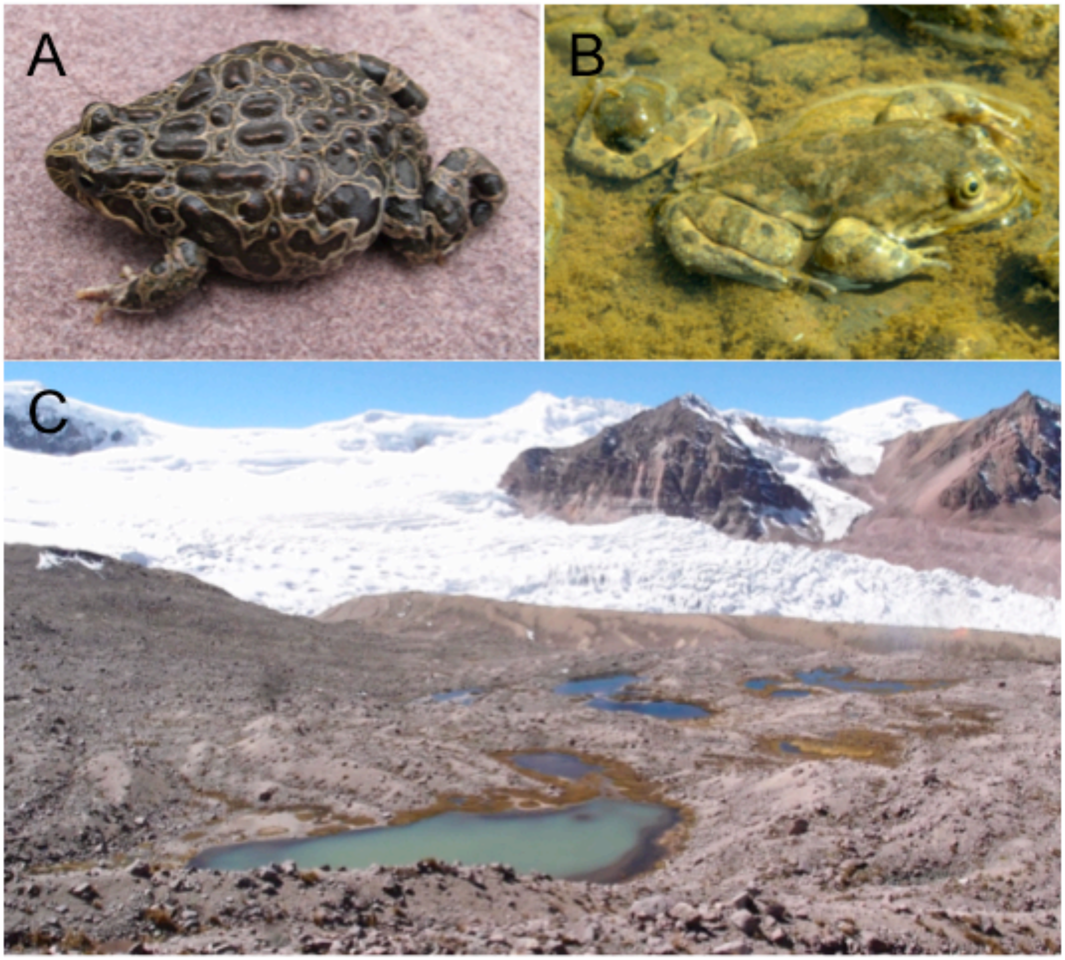

Figure 4.3. Photos of study sites and species in the Cordillera Vilcanota. A) *Pleurodema marmoratum* (Snout-Urostyle Length = 3.1 cm; photo: K. Reider). B) *Telmatobius marmoratus* (Snout-Urostyle Length = 6.9 cm; photo: A. Catenazzi). C) Glacial meltwater ponds at Site 8 (main field site) where we studied co-occuring *P. marmoratum* and *T. marmoratus* infected with *B. dendrobatidis* (Photo: K. Reider)

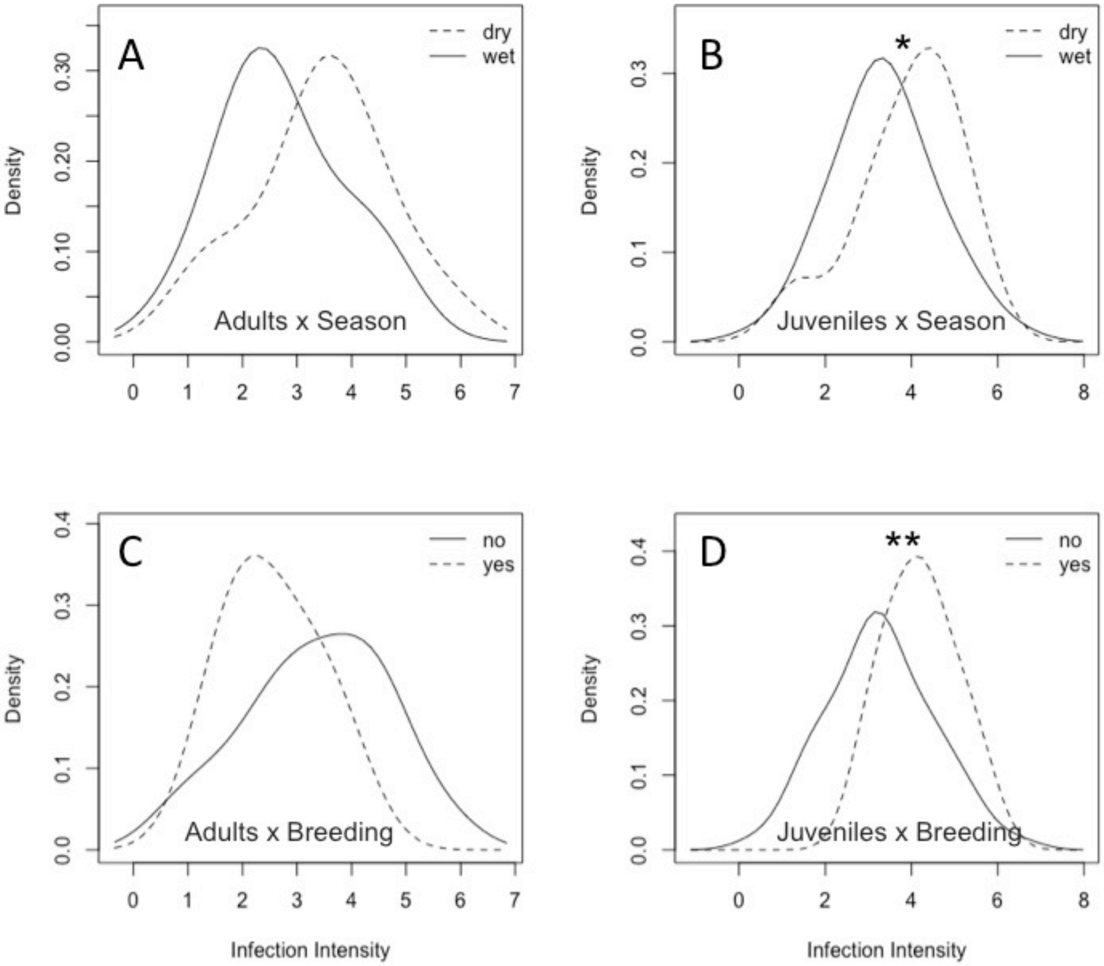

Figure 4.4. Density distribution of *Bd* infection intensity in *Pleurodema marmoratum*. Asterisks indicate statistically significant differences in the distributions (* $p < 0.05$, ** $p < 0.01$).

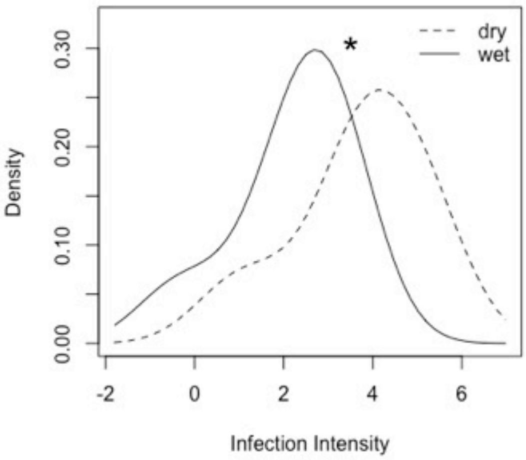

Figure 4.5. Density distribution plots of *Bd* infection intensity in *Telmatobius marmoratus* tadpoles. Asterisks indicate distributions are not equal (* $p < 0.05$)

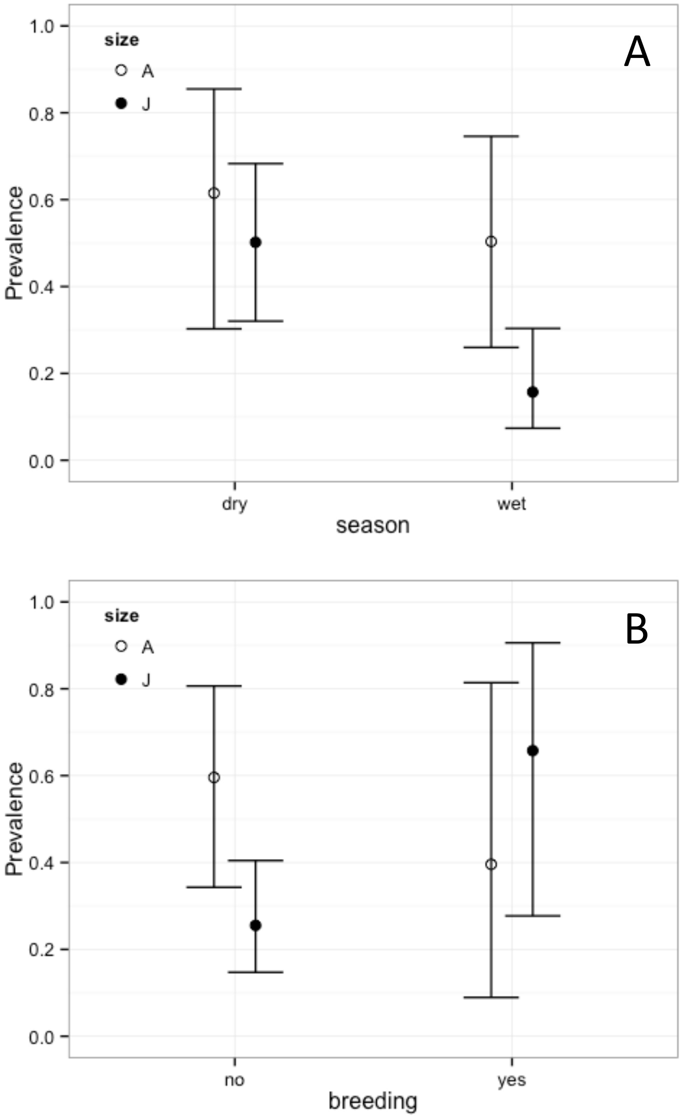

Figure 4.6. Effect plots of *Bd* infection prevalence in *P. marmoratum*. Effect size with 95% CI in size classes (A = Adult, J = Juvenile) in a) the wet and dry seasons and b) during and outside of the *P. marmoratum* breeding season.

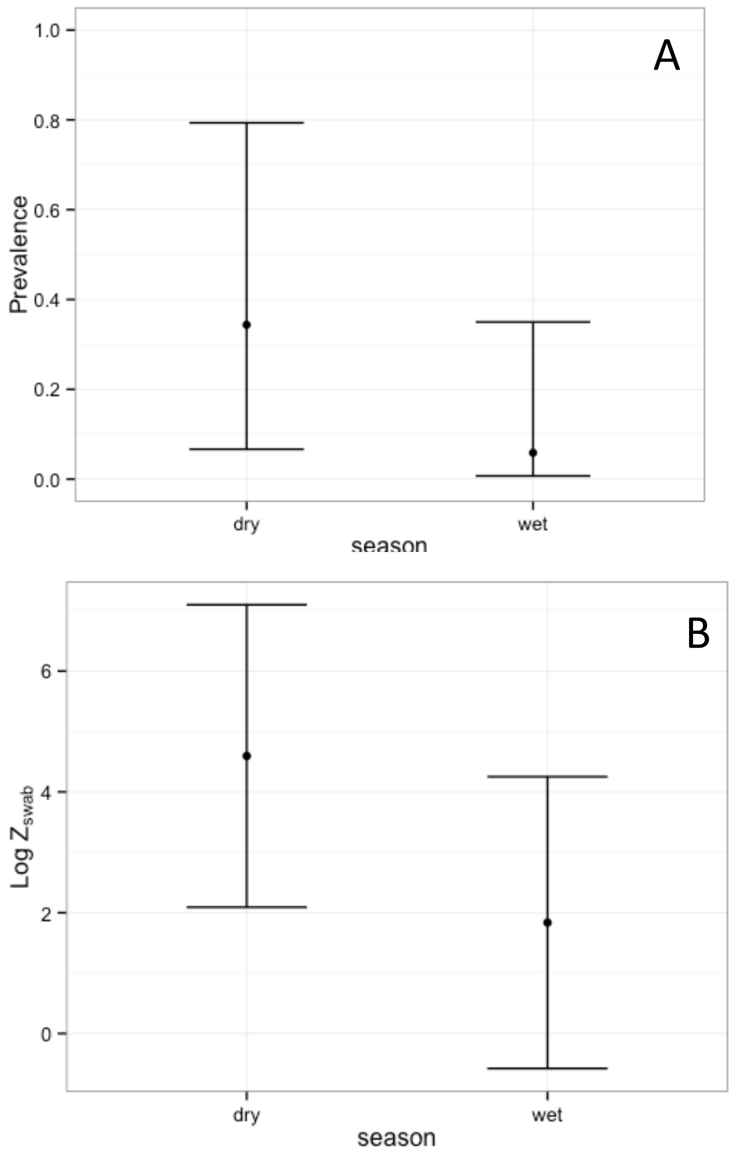

Figure 4.7. Seasonal patterns in *Bd* infection in *Telmatobius marmoratus* tadpoles. Dots indicate means with 95% Confidence Intervals.

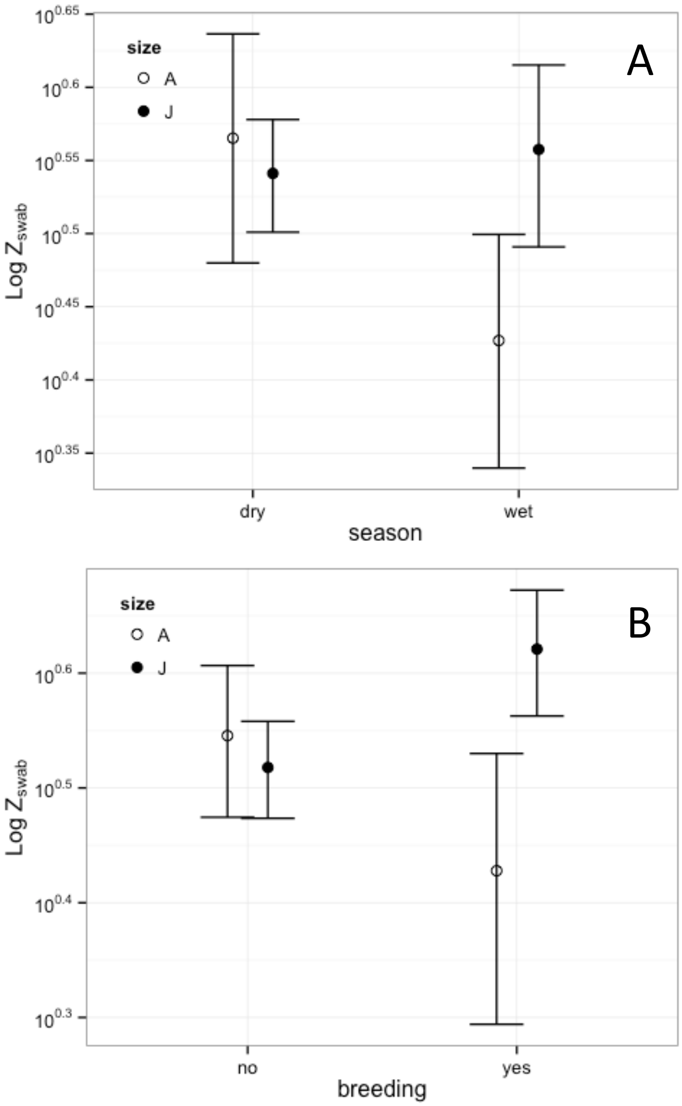

Figure 4.8. Seasonal patterns in *P. marmoratum Bd* infection intensity. Z_{swab} ± 95% CI in adult and juvenile size classes in a) the wet and dry seasons and b) in and outside of the breeding season.

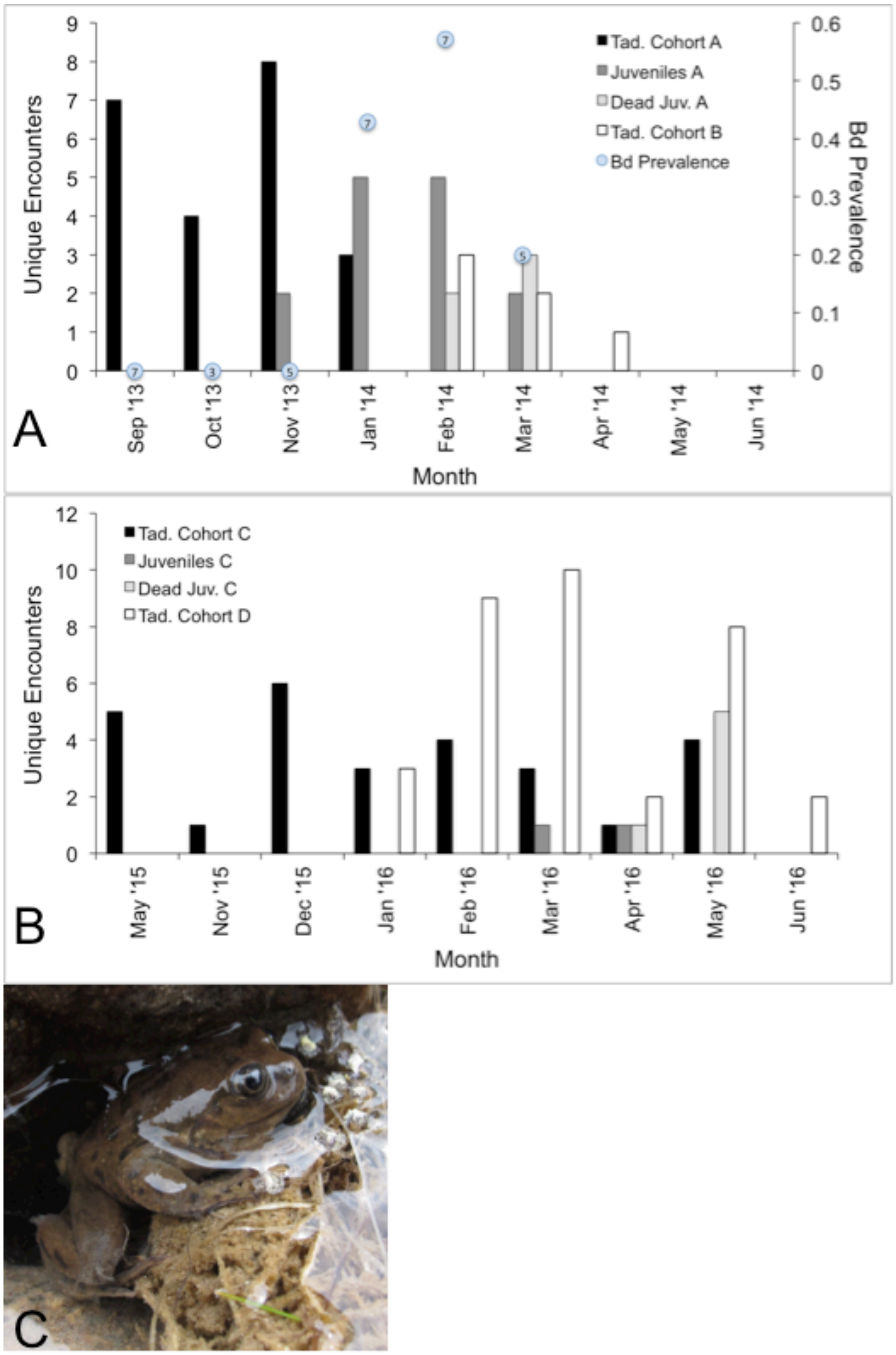

Figure 4.9. *Telmatobius marmoratus* encounters and mortality. A) *Telmatobius* Cohort A growth from tadpoles (black bars) to juveniles (dark gray bars). There were no *T. marmoratus* encounters during surveys in May or June 2014. B) Repeat surveys of Cohort C tadpoles (black bars) revealed mortality of Cohort C juveniles (light gray bars) that coincided with the encounters of recently metamorphosed juveniles (dark gray bars). A new cohort, D, was first observed in January 2016. C) *Bd*-positive *T. marmoratus* juvenile (SUL=3.3 cm) exhibiting sloughing of skin associated with chytridiomycosis in February 2014.

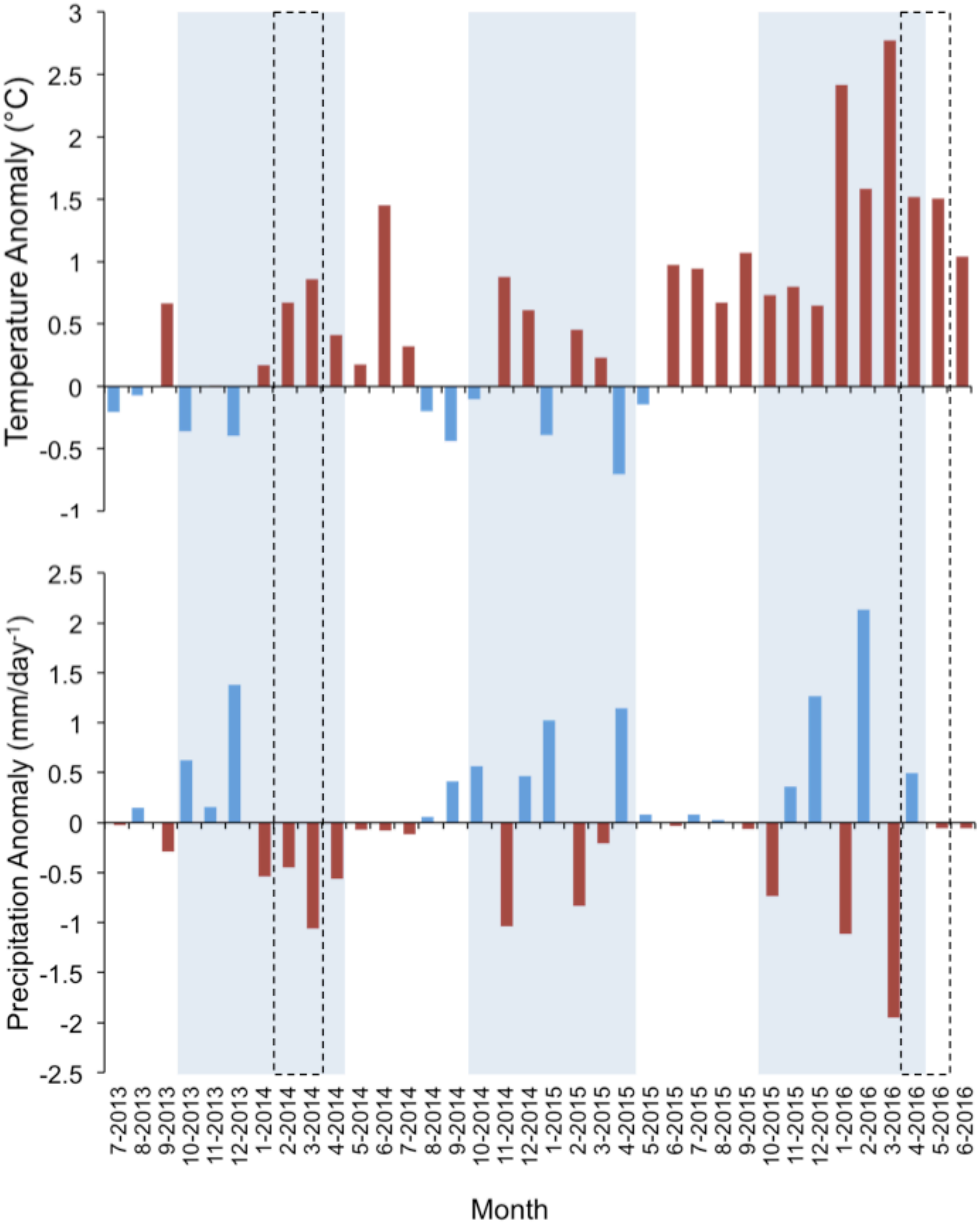

Figure 4.10. Maximum temperatures and precipitation anomalies. A) Temperature B) Precipitation. Data are from 24-hr precipitation totals and maximum temperature recorded at four weather stations near the Cordillera Vilcanota. Shaded areas show wet season months. Dashed lines indicate months with observations of *Telmatobius marmoratus* mortality.

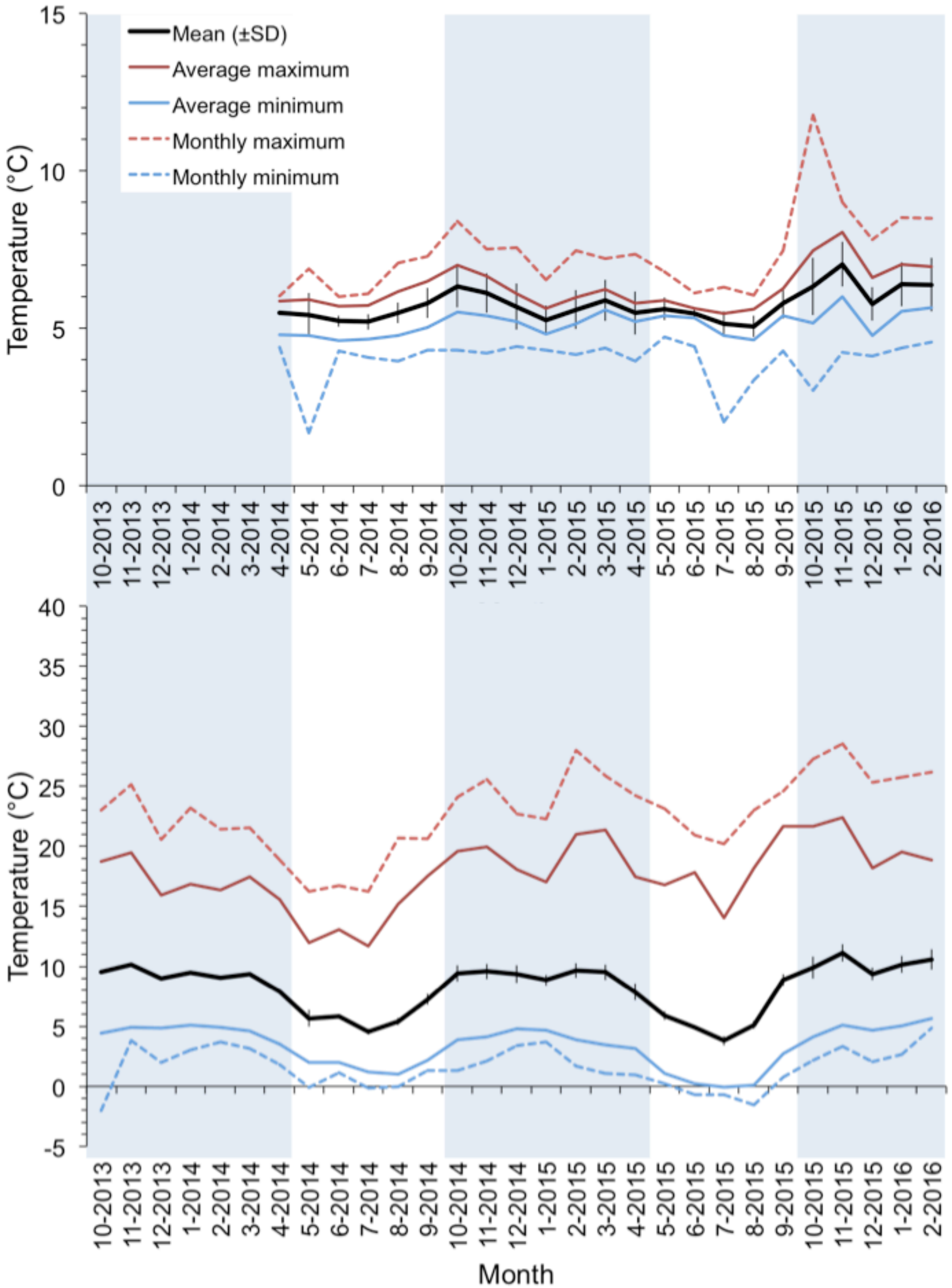

Figure 4.11. Deep and shallow water temperature at 5245 masl. Water temperature at a deep (top; >1 m) and shallow (bottom; <10 cm) location in study pond at 5240 masl with *T. marmoratus* and *P. marmoratum*. Wet season months are shaded. Solid lines are averages. Broken lines are the maximum and minimum recorded temperatures.

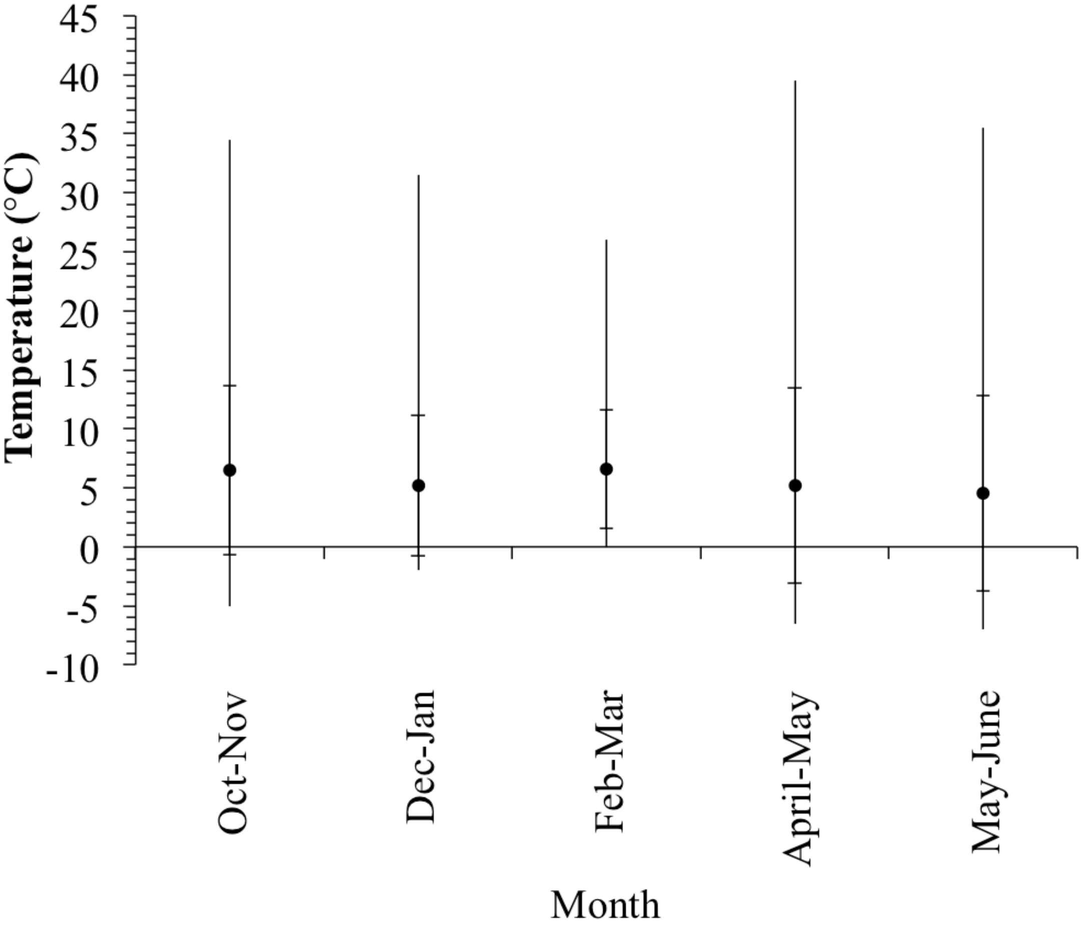

Figure 4.12.Daily variation in substrate temperature under cover rocks. Data are from 2015–2016. Circle and cross-bars are mean ± SD; vertical line illustrates full range of temperatures.

APPENDICES

ID	Species	Location	Animal age	Sample type	Bd PCR (NT= not tested)	Ranavirus PCR (NT= not tested)	Histol. Bd	Histol. Ranavirus
1	PLEMAR	Area E	Adult	Skin, toe	NEG	NT	NEG	NEG
1	PLEMAR	Area E	Adult	Liver	NT	NEG		NEG
1	PLEMAR	Area E	Adult	Kidney	NT	NEG		NEG
2	PLEMAR	Area E	Adult	Skin, toe	NEG	NT	NEG	NEG
2	PLEMAR	Area E	Adult	Liver	NT	NEG		NO LIVER
3	PLEMAR	Area E	Adult	Skin, toe	NEG	NT	NEG	NEG
3	PLEMAR	Area E	Adult	Liver	NT	NEG		NEG
4	PLEMAR	Area D	Juvenile	Skin, toe	POS	NT	NEG	NEG
5	PLEMAR	Area D	Juvenile	Skin, toe	POS	NT		NEG
6	PLEMAR	Area D	Juvenile	Skin, toe	POS	NT	POS	NEG
6	PLEMAR	Area D	Juvenile	Liver	NT	NEG		NEG
7	PLEMAR	Area D	Juvenile	Skin, toe	POS	NT	SUSPECT	NEG
8	PLEMAR	Area D	Adult	Skin, toe	POS	NT	NEG	NEG
9	PLEMAR	Area B	Adult	Skin, toe	POS	NT	NEG	NEG
9	PLEMAR	Area B	Adult	Liver	NT	NEG		NO LIVER
9	PLEMAR	Area B	Adult	Kidney	NT	NEG		NEG
10	TELMAR	Area A	Adult	Skin, toe	POS	NT	POS	NEG
10	TELMAR	Area A	Adult	Liver	NT	NEG		NEG
10	TELMAR	Area A	Adult	Kidney	NT	NEG		NEG

Appendix 4.1. We tested nine dead *Pleurodema marmoratum* (species code = PLEMAR) and one *Telmatobius marmoratus* (species code = TELMAR) for *Bd* and Ranavirus both by PCR and histology. Ranavirus is another important emerging pathogen of amphibians (Johnson et al. 2008) and has been recently identified co-infecting Andean anurans along with Bd (Warne et al. 2016). Ranavirus histology and PCR were conducted as described in Gilbert et al. (2012). Individuals 1-9 were from Site 8. Location codes follow areas within Site 8 sampled by Seimon et al. (2007). Individual 10 was collected at coordinates -13.859104, -71.197782. Histology examinations were performed by a certified pathologist (Dr. D. McAloose of WCS) following methods described in Seimon et al. (2015).

Provided Copy Number	Copy Number: Seimon et al. 2017 method	Copy Number: Catenazzi and Ttito 2016 method	Fold-Difference
1300000	4,943,580	712,210	6.941183078
130000	555,705	83,089	6.68806942
13000	49,469	11,214	4.411360799
1300	4,377	1,594	2.745922208
130	659	155	4.251612903
13	47	18	2.611111111
1.3	6	1	6
1.3	5	1	5
15000	50,796	13,435	3.78087086
15000	46,726	20,418	2.288470957

Appendix 4.2. Potentially important differences between the two *Bd* PCR methods used in this study: the dilution factors in the PCR reaction tube, the PCR system used to amplify genetic material, and the ITS1 copy number in the standards that were used to estimate Z_{swab}. We accounted for dilution and subsampling differences by recording volumes on a sample-by-sample basis. We calculated ITS1 copy number per swab by taking the qPCR copy number per reaction from the standard curve, and then multiplying that number times by its relative proportion in the DNA extract, and then multiplying by the necessary dilution factor.

To correct differences associated with variation between machines and standards, we obtained independent results from qPCR reactions using both the Seimon et al. 2017 and Catenazzi and Ttito 2016 techniques on blind samples of known copy numbers. The samples used for this comparison were part of an independent quality control program run by the Canadian Wildlife Health Cooperative (M. Forzán et al., unpublished data). Both methods agreed perfectly in the differentiation of *Bd* positive from *Bd* negative samples. When comparing results from the two techniques for zoospore equivalent values commonly encountered in infected animals, infection intensity ("Z_{swab}") was very similiar ($R^2 = 0.9996$). However, infection intensity values obtained using the earlier qPCR method yielded values 4.212-fold higher on average compared to the second method. Therefore, we applied a correction factor of 4.212 to all Z_{swab} values obtained using the first method.

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CONCLUSIONS

My dissertation examined the ecology of anurans in very high-elevation proglacial environments in the tropical Andes. The frogs I studied are some of the best examples of vertebrates shifting upslope in response to climate change and provide one of the clearest examples of climate change directly influencing the upward expansion of amphibian chytridiomycosis to the limits of known pathogenicity for this significant wildlife disease (Seimon et al. 2007). Tropical proglacial environments are home to some of the youngest and least studied ecosystems on Earth, yet they are undergoing accelerated environmental changes because of elevation-dependent warming (Diaz et al. 2014, Pepin et al. 2015).

The frog community I studied exists at the currently reported global elevation limits for amphibians. At such elevations, the impacts of climate change are clearly visible both on the landscape and in community composition. The retreating glaciers leave meltwater ponds in their wake, increasing the habitat for aquatic organisms. My dissertation and a growing body of research throughout the high Andes suggest that this increase in meltwater and corresponding increase in aquatic habitat may be short-lived. Glaciers in the tropical Andes have been retreating for several decades, imperiling water resources for ecosystems and human populations along the wide and highly biodiverse elevational gradient from the Andes to the Amazon (Diaz et al. 2014). Glaciers in the semiarid southern tropical Andes are particularly important from a water resources perspective because of the harsh dry season when meltwater from snowpack and glacial ice are the only sources of water to downstream ecosystems (Buytaert et al. 2017). Continued deglaciation may cause a loss of or downward shift in the upper elevational ranges of many aquatic organisms while thermal pressures drive other species upward. Future water scarcity in regions undergoing rapid deglaciation is a major threat to biodiversity and ecosystem services.

Understanding the upper elevational limits of life on Earth has important implications in the context of climate change. In the high Andes, climate change has facilitated upward range expansions of frogs and other taxa (including mollusks and plants) to global elevation records (Seimon et al. 2007). Within the Andes, there are likely anurans besides the three species of frogs (i.e., *P. marmoratum, T. marmoratus*, and *Rhinella spinulosa*) found at or above 5000 m in the Cordillera Vilcanota. Andean frogs are also not the only amphibians to reach such extreme elevations. These three Andean frogs join species from the Tibetan Plateau in the Himalaya as the highest reported amphibian species globally (Seimon and Seimon 2015). In the Himalayan range and Tibetan Plateau, several high elevation frogs have been reported including *Nanorana parkeri* (reported elevation range: 2,850 –5,000 m, Liang et al. 2004a), *Scutiger boulengeri* (reported elevation range: 2,200–5,270 m; Ohler et al. 2004a, Subba et al. 2015), *S. nepalensis* (3,000–5,000 m; Ohler and Shrestha 2004), *S. nyingchiensis* (3,000–5,000 m; Liang et al. 2004b), and *S. sikimmensis* (2,700–5,000 m; Liang et al. 2004c).

How animals from different phylogenetic lineages solve the problem of high elevation life will occupy research efforts for decades. High elevation sites, especially in the tropics, are largely unexplored and these dynamic environments offer new ecological opportunities for amphibians and challenges for amphibian researchers working near the edge of the cryosphere. There are likely additional frog species at very high elevation yet to be reported, simply because few herpetologists venture into the thin air looking for frogs. Vertical range expansions, both upward and downward, in response to changing environmental conditions can also change elevational records over time (Seimon and Seimon 2015; Seimon et al. 2007, 2017).

Climate change is causing a global redistribution of species (Pecl et al. 2017) and threatens species undergoing range shifts with extinction if they are unable to adapt to new conditions under climate change or the environmental conditions in their new range (Aitken et al. 2008). Understanding the evolutionary processes that occur during adaptation to novel environments may factor critically in understanding the upper elevational limits of life on Earth and how species will respond and adapt to climate change. In the case of anurans already living at very high elevations in the Cordillera Vilcanota, the only limit to their upper elevations may be the glaciers themselves. In a future without permanent ice and under reduced precipitation regimes, water scarcity may become an increasingly important determinant of species distributions and human-wildlife conflict. In the case of species currently living below the treeline in the relatively cool, stable environment of montane forests, the extreme temperature variation, cold nighttime temperatures, and lack of appropriate breeding habitats may prevent them from moving upslope unless the forest ecotone moves up too. In the Andes of southern Peru, that upward shift does not appear to be happening for a variety of possible reasons (Rehm and Feeley 2015).

In Chapter 1, I presented the first survey of water stable isotopes from the tropical Andes at very high elevation in the Cordillera Vilcanota. The stable isotopes of water have long been used as tracers to distinguish source waters and discern hydrologic processes, but d-excess has been underutilized compared to ^{18}O in this regard. I show

how seasonal differences in d-excess, along with depleted values of ^{18}O, can provide useful insights into surface water sources, especially in high-elevation tropical mountain areas where evaporation rates are high.

In Chapter 2, I discovered that *Pleurodema marmoratum* has expanded its range into recently deglaciated zones throughout the Vilcanota. The species was recorded up to 5400 m previously in the new ice-free pass north of Sibinacocha (Seimon et al. 2007), and I extend those findings to show that the species is present throughout the region in areas that were until very recently covered by permanent ice. *Pleurodema marmoratum* has a wide geographic range, and along with other anuran species, may be expanding their elevational ranges throughout mountain regions. I demonstrated the use of my stable isotope technique to gain insights into hydrologic alterations occurring because of deglaciation in high elevation tropical aquatic ecosystems. Although deglaciation led to the creation of new aquatic habitats, future diminutions of glacial runoff may limit aquatic species to those that are not tied to permanent water bodies. Chapter 2 also underscores the importance of high elevation wetlands, grouped here under the term *bofedales*, for anuran populations. These oases of high elevation biodiversity and ecosystem services are very vulnerable to water scarcity and may require significant conservation effort (Yager 2009).

In Chapter 3, I found that *Pleurodema marmoratum* has adapted to summer every day, winter every night daily temperature fluctuation by having a wide thermal tolerance range, one of the widest reported for amphibians along a broad elevational gradient in the southern Andes of Peru (Catenazzi et al. 2014). I also found that the species tolerates short-term freezing, which appears to be the first record of anuran freeze tolerance within the tropics.

Finally, in Chapter 4, I surveyed amphibians for Bd at sites spanning 60 km (straight line). *Pleurodema marmoratum* were infected with *Bd* at 75% of sites. The amphibian chytrid fungus has been monitored in the Cordillera Vilcanota for over a decade (Seimon et al. 2017), yet this is the first time repeated sampling within hydrologic years has allowed an analysis of seasonal and ontogenetic variation in host-pathogen disease dynamics. It is particularly worrisome that the changing temperature regimes may shift possible thermal mismatches in favor of the pathogen so continued disease monitoring in the high Andes is absolutely critical.

In conclusion, high-elevation tropical ecosystems are undergoing some of the most rapid climate change-driven alterations on the planet. The Cordillera Vilcanota exists in a unique biogeographic position, spanning the altiplano to some of the biologically richest forests on the planet on the eastern slopes of the southern Andes of Peru (Swenson et al. 2012) and is home to some of the highest peaks in Peru. Unique among high mountain areas in the tropics, the Cordillera Vilcanota has a multidisciplinary baseline of data collected over decades on a variety of spatial scales. The Vilcanota could become a global model for protecting connectivity along the full elevational gradient of the Andes-Amazon if given conservation priority. Previous efforts to initiate large-scale conservation efforts have failed to gain traction, pointing to a need for collaborations with local communities and transdisciplinary research aimed at guiding conservation efforts. There is already significant habitat alteration including loss of forest cover and contamination from mining at a variety of scales (from the upper elevations of the Vilcanota to the lowlands) along the Interoceanic highway, so conservation actions to ensure connectivity are urgent. The Cordillera Vilcanota is a microcosm of the impacts of climate change, changes to freshwater resources, land-use change, and coupled socio-ecological systems affecting mountain regions worldwide. Protecting a wildlife corridor from the highest peaks of the Cordillera Vilcanota to the lowland forests, or connecting a protected area of the Vilcanota with the Manu National Park or Los Amigos Conservation Concession, for example, could create one of the most resilient protected areas in the world in the face of climate change.

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