

CHAPTER 5 DISCUSSION AND CONCLUSION

5.1 Relationship between species richness and abundance and environmental factors along elevation gradients

This study showed that stream anuran species richness, relative abundance and diversity in the Huai Chang Tai stream was highest at 550 msl and tended to decrease with increasing elevation. Species richness was influenced significantly by stream characteristics (i.e., rate of stream flow), density of vegetation at ground level and elevation. This means that, once the sampling effort is taken into account, higher numbers of species can be expected in sites with slower currents and lower density of vegetation at ground level and at lower elevations. This may be due to streams at lower elevations were wider and slower flowing than at upper elevations, thus high amounts of available water perhaps increased the number of breeding sites (more habitat heterogeneity) (Malonza and Veith, 2012). Bickford *et al.* (2010b) also reported that breeding habitat heterogeneity best explained frog species diversity and abundance. In addition, stream-breeding frogs require streams that are large enough to hold water for a sufficient length of time for their tadpoles to develop (Parris and McCarthy, 1999). Therefore, larger streams can provide more suitable breeding habitats for a greater range of species because higher amounts of water do tend to increase the number of breeding sites and they may contain water for a longer time than smaller streams. Moreover, results from NMDS indicated that there were changes in the composition across the elevations. Specifically, compositions of frogs were significantly different between lower and upper elevations: 550 & 900 - 1100 msl and 700 & 1100 msl. This may be due to the difference in stream size and slope (the steeper the slope, the faster the flowing water) between the lower and upper areas which is consistent with a previous study reported that the composition of frog assemblages in forest streams was correlated with the stream size and stream gradient (Parris and McCarthy, 1999). Parris and McCarthy (1999) also reported that the upstream catchment volume (which was correlated with stream width, permanence of the water and rates of water flow) was positively associated with species richness of frog assemblages in forest streams in southeastern Queensland, Australia. Thus, it can be considered that the low species diversity at higher elevations in my study site was at least in part due to having the

smallest stream size and highest rates of water flow since the lower elevations are flatter and in upper elevations the steeper slope results in faster water flow.

However, with regard to the relative abundance of specific species, there are some complications related to spatial variation. The occurrence of high abundances may depend on a more complicated interplay of factors regarding detectability: not only stream flow and volume, but air relative humidity and temperature. Lower elevations had lower relative humidity and higher air temperatures (drier weather) than upper elevations. In addition, as mentioned above, at lower elevations the stream was wider and slower flowing, and supported greater abundance. Therefore, higher abundances could be expected in areas with slower currents, lower relative humidity and higher air temperatures (drier weather) in the surrounding area resulting in anurans aggregating at the stream, which has more moisture relative to surroundings in order to prevent physiological water loss (Bickford, 2005; Kongjaroen, 2007; Naniwadekar and Vasudevan, 2007; Porter, 1972).

Some previous studies have reported that vegetation can provide increased humidity, shelter and breeding habitats for many species of anurans, thereby increasing the species richness and abundance (Keller *et al.*, 2009; Parris and McCarthy, 1999). However, in my study the density of ground vegetation was negatively correlated to richness and abundance and accounted for a small proportion of the variation in species richness and abundance. This may be due to vegetation acting as a habitat for predators as well, such as the White-lipped pit viper (*Cryptelytrops albolabris*) and Pope's pit viper (*Popeia popeiorum*) (Khonsue, 1996), which I found several times in stream-side vegetation during the survey.

Although 550 msl had the highest richness and abundance, the highest biomass was at 700 msl and the second highest biomass was at 900 msl. This may be due to higher abundance of *Brachytarsophrys carinense*, *Limnonectes taylori* and *Odorrana livida* (female) which have relatively large body sizes (mean SVL = 12.1, 6.6 and 9.5 cm and mean biomass = 180.38, 61.25 and 98.05, respectively). This may indicate that mid elevation might be a key role in anuran productivity and in maintaining community dynamics by coupling aquatic and terrestrial habitats via transfer of biomass and energy in this study area. Because the anuran's life cycle requires both aquatic and terrestrial habitats, thus predators from both habitats can feed on anurans.

5.1.1 Temporal variation

Regarding temporal variation, in this study, species richness and abundance during the dry season were higher than in the rainy season which is consistent with previous studies in evergreen forests in Thailand, including the river at Chachoengsao Wildlife Research Center, Khao Aung Rue Nai Wildlife Sanctuary (Khonsue, 1996), the Lam Ta Kong river at Khao Yai National Park (Kongjaroen, 2007), the Nam San Noi of Phuluang Wildlife Sanctuary (Phochayavanich, 2007) and Khao Sip Ha Chan National Park (Danaisawat, 2009). This may be due to higher air temperatures, lower relative humidity and minimum rainfall resulting in fewer water bodies and drier surrounding areas. Thus, water bodies were limited to only some streams that still contained water during the dry season (Figure 5.1). Moreover, locations with natural check dams along streams (see Figure 5.2) were likely to increase the diversity of breeding habitats such as streamside pools and stagnant or slowly-flowing areas for some species (such as *Microhyla heymonsi*, *M. pulchra*, *Hylarana nigrovittata*) (Chan-ard, 1987). As the environment dries out, individuals may stay closer to water sources (Bickford, 2005). Thus, not only aquatic frogs, but more terrestrial frogs (such as *Microhyla fissipes*, *M. heymonsi*, *M. pulchra*) also aggregated along the stream. All of these factors probably resulted in higher numbers of species and observed frogs, particularly during the end of the dry season. In contrast, during the rainy season if there is heavy rain which causes strong flows of water (or floods) (see Figure 5.3), adults are more likely to move away. In addition, there are likely to be more breeding habitats such as temporary rain pools and higher relative humidity in the surrounding forest to allow anurans to disperse farther from the stream (Khonsue, 1996; Kongjaroen, 2007). It has been noted for some time that seasonal rainfall and topography interact to create moisture gradients that may be important in determining the patterns of abundance and distribution for amphibians and reptiles (Vonesh, 1998). The same may well be true at the Huai Chang Tai stream as indicated by the significant effect of elevation for anuran abundance distribution regarding the season during the study period. This may also explain why my intercept—at 550 msl during the dry season—had a particularly strong effect on anuran abundance.



Figure 5.1 Stream characteristics at one checkpoint at 550 msl during the dry (left) and wet (right) season.



Figure 5.2 The presence of a natural check dam at one checkpoint at 550 msl during the dry season (right) compared to the wet season (left).



Figure 5.3 Strong flows of water along stream segment at 900 msl during the rainy season.

5.1.2 Species-specific patterns and species of concern

Concerning the altitude distribution of species, from this study it was observed that relatively few species were typically highland species (such as *Xenophrys major*, *X. parva*). I suppose that this is because the year-round lower temperatures, higher relative humidity and flowing water at the highest elevations are suitable for only some species, such as members of the family Megophryidae (Chan-ard, 1987; Kongjaroen, 2007) which are stream breeders and have long tadpole development (Chan-ard, 1987; Kongjaroen, 2007). This may also imply more risks for highland species such as these from climate change (Donnelly, 1998).

Overall, species responses to elevation gradients probably depend greatly on the particular species' reproductive strategy (Malonza and Veith, 2012). For example, *L. taylori* is an aquatic frog and is stream-dependent and a non-seasonal breeder requiring stream water to lay its eggs (Chan-ard, 1987; Simchareon and Duangchantrasiri, 2002). Simchareon and Duangchantrasiri (2002) reported that its nesting site characters are flowing water with rocky bottoms which were found throughout the study stream. Thus, this species dominance may be due to its ability to find suitable breeding habitats across a wide range of elevations, its ability to breed year round and inhabit areas nearby streams. Several studies have noted this species as having a wide distribution in evergreen forests (400 - 1650 msl) (Chan-ard, 1987; Frost, 2013; Kongjaroen, 2007; Stuart *et al.*, 2006).

O. livida, which is described as Data Deficient by IUCN, was also relatively widespread in my study area. It also has a relatively wide distribution in forest streams (400 - 1150 msl) (Bain *et al.*, 2003; IUCN, 2013; this study), usually found on rocks (Bain *et al.*, 2003), in particular on rocks of waterfalls (from this study) or shrub branches along the streams due to its greatly expanded toe pads that allows climbing on rocks, waterfalls and vegetation (Bain *et al.*, 2003), which were found throughout the study stream.

In contrast, a relatively uncommon species, also described as a near threatened species, *Limnonectes blythii*, is a stream breeder (Chan-ard, 1987). It had a narrow distribution, found only at 550 - 600 msl, which is consistent with a previous study by Chan-ard in 1987 in HKK, which reported this species to roughly occur between 300 - 600 msl. The abundance estimates from my study may be slightly lower than the past HKK study; however, this may be due to my study transect starting at 550 msl, perhaps near the end

of its elevational range. Another relatively uncommon species in this study, *X. parva*, showed a narrower range at upper elevations of only between 900 - 1150 msl while the past HKK study reported its ranges to be between 600 to above 1000 msl. Although this species has been noted to have a wide elevational range (500 - 2500 msl elsewhere) (IUCN, 2013; Meren Ao *et al.*, 2003), it is unclear why I did not detect it at 700 msl. However, I hypothesized that it may be 1) due to Thailand being near the end of its distribution range, being nearer to the equator than other countries, thus the suitable climate is at a higher elevation and 2) there might be an elevational shift occurring (Raxworthy *et al.*, 2008). While *Ingerophrynus parvus*, *B. carinense* and *Leptotalax pelodytoides* had relatively similar elevational ranges as recorded from a previous study (Chan-ard, 1987).

This study supports previous studies that suggested that suitable breeding habitat plays an important role for anuran distribution (Hofer *et al.*, 1999; Zimmerman and Bierregaard, 1986). This may be due to the difference in microhabitat requirements of each anuran species and their physiological tolerance limits (Hofer *et al.*, 2000). Overall, the results partly support my hypothesis that anuran species richness and abundance is greater at lower elevations and in slower moving water from this study stream located in evergreen forests. This can be seen from the negative relationship between anuran species richness and abundance with elevation gradients and rate of stream flow and for models with elevation and rate of stream flow. However, vegetation characteristics seem to have relatively weak effects on anuran communities in this study. This may be because of the presence of predators that feed on amphibians. Although vegetation characteristics presumably are not important for all species, they are generally assumed to be important for riparian frogs (Keller *et al.*, 2009; Parris and McCarthy, 1999). In this case, however, relatively few species here used vegetation (1 - 2 m) as calling sites compared to other studies, such as *O. livida*, which was found on rocks of waterfalls or shrub branches along the streams and *Raorchestes parvulus*, which is typically found in the forest away from streams, but was also found nearby the stream transect.

Many studies (Afonso and Eterovick, 2007; Danaisawat, 2009; Keller *et al.*, 2009; Khatiwada, 2011; Khonsue, 1996; Kongjaroen, 2007; Naniwadekar and Vasudevan, 2007; Parris and McCarthy, 1999; Phochayavanich, 2007) have shown that the environment is an important factor in considering amphibian community; therefore,

in particular areas, changes in local conditions result in differences in environment, such as habitats and/or microhabitats. This means that there are differences in streams and differences in characteristics and environments along an elevational gradient. Thus, patterns from this study may not hold true for particular areas, e.g., streams or in other forest types. Thus, elsewhere it is expected to have different patterns due to the reasons noted above. In studying amphibians, it is important to consider the fine scale, i.e., local conditions such as microhabitats in the particular area due to amphibians having particular constraints such as permeable skin, reliance on external heat to regulate their body temperature, physiological tolerance limits — such as having a narrow range of temperature and moisture tolerances (Duellman and Trueb, 1986; Hofer *et al.*, 2000; Terborgh, 1971). Moreover, microhabitat data could be useful for further study, for example, to build habitat suitability models (Tantipisanuh *et al.*, 2014); Tantipisanuh *et al.* (2014) also suggested more of this kind of data on amphibians.

My results provide comparative baseline data across an elevation gradient which I believe will be useful for future monitoring, particularly in the face of climate change (Raxworthy *et al.*, 2008). Raxworthy *et al.* (2008) also reported that collecting details of habitat or microhabitat data would be useful because it might allow the actual mechanisms of distribution shifts to be better explored, and this would be useful as I suspect for elevational shifts from *X. parva*. However, further study is needed in HKK and other protected areas of the region, for example, the elevational distributions of at least eight species remain unknown in the area due to low samples. This may be due to some species are terrestrial frogs and/or some species are lowland species, so my study transect started perhaps near the end of their elevational range. This also may explain some species such as *Ingerophrynus parvus*, which was found to be restricted at only 550 msl. I suspect habitat destruction and hunting are probably not big threats in remote areas of at least some protected areas like HKK, but climate change was shown to have significant effects on amphibians in the tropics, with montane areas being particularly vulnerable (Raxworthy *et al.*, 2008; Sala *et al.*, 2000). In this regard, I found two species (*X. major* and *X. parva*) at only higher elevations and *X. parva* had narrower ranges than found in HKK approximately 27 years prior to this study (Chan-ard, 1987). I also suggest more study streams to see if the observed patterns hold true, including additional long-term elevational surveys to monitor potential changes in anuran distributions and more survey efforts to estimate baseline populations and other

information such as breeding habits of the data deficient *L. doriae*, *L. limborgi* and *O. livida*. Long-term ecological study of the near-threatened *L. blythii* which appears to be vulnerable to poaching and over-consumption by local people (Chan-ard, 2003; IUCN, 2013), is also recommended in relatively undisturbed areas such as HKK to understand its natural patterns of abundance and population dynamics relative to heavily hunted populations. Overall, given the importance of stream characteristics, I suggest taking a community approach to managing forest streams and stream-side vegetation across broad ranges of elevation in protected areas as this is likely to protect the greatest richness and abundance of species.

5.2 *Bd* detection

During the past decade in Thailand, researchers from universities, government offices (i.e., DNP), and organizations (e.g., ZPO) have been surveying for *Bd* and many research projects are still ongoing (Kamolnorrant *et al.*, 2010). In 2010, the ZPO found *Bd*, but only in non-native specimens from a zoo (Kamolnorrant *et al.*, 2010). As noted above, in 2012 *Bd* was then reported from one out of six sampled individuals of wild *Ingerophrynus parvus* (a species common to southern Thailand) using real-time PCR methods (Vörös *et al.*, 2012). However, in my study, all samples were negative for the presence of chytrid fungus in this relatively undisturbed forest in this one stream. Additionally, during my one year study at HKK, I had 2399 observations of frogs and did not detect any symptomatic frogs, dead or alive. Even the infected specimen detected by Vörös *et al.* had a low infection intensity, and did not display the most common clinical signs of infection (Vörös *et al.*, 2012). However, as also noted above, there is new genetic evidence of an ancestral *Bd* haplotype that might be endemic to Asia (Goka *et al.*, 2009). Thus, *Bd* is possibly present in natural areas at very low levels, but yet undetected. The conventional PCR assay is a convenient and quick method for detecting a target DNA region (Annis *et al.*, 2004); however, current protocols with real-time PCR can detect as few as one zoospore (Kriger *et al.*, 2006). Therefore, it is possible that the fungus was present in my study area in minute concentrations which I could not detect with conventional PCR. Currently, I am working on re-examining these samples using real-time PCR to determine whether they contain trace amounts of naturally occurring *Bd*.

5.3 Conclusion

The stream anuran community in the Huai Chang Tai stream at HKK was primarily structured by abiotic factors, in particular stream flow and elevation. The results from this study indicate that the occurrence of amphibians along the spatial (elevation) and time (season) gradient were quite different. Therefore, long-term monitoring of amphibian populations in the future should take into consideration both the spatial and temporal factors that influence species occurrence and abundance.

In this study, *Limnonectes blythii* was found (currently classified as near threatened by IUCN (2013)). It is threatened by poaching for consumption by local people due to its large body size (Chan-ard, 2003; IUCN, 2013). To protect this species and others, there needs to be a clearer framework for identifying priority areas for amphibian conservation that focus on natural sites. Additional surveys of other protected areas are urgently needed to develop a greater understanding as to whether the diversity and abundance observed here in HKK is relatively high and therefore merits high priority as a baseline reference or there are many sites elsewhere with higher richness and abundance that require greater attention.

Particular species (e.g., *Xenophrys major*, *X. parva*) were found only at higher elevations during this study which means these species might be restricted to relatively narrow elevation ranges which is also consistent with Phochayavanich *et al.* (2010) from elsewhere in Thailand. As climate change has been shown to effect amphibians in the tropics, with montane areas being particularly vulnerable (Raxworthy *et al.*, 2008; Sala *et al.*, 2000) and with many amphibian species distributions and abundances largely driven by rainfall, temperature and moisture (Bickford *et al.*, 2010a; Duellman and Trueb, 1994), their breeding phenology and physiology may be directly affected by global warming (Navas and Otani, 2007). Therefore, I highlight the importance of conserving the tropical montane forest for these species and encourage the preservation of these areas as important reservoirs of a diverse range of amphibians in the region. Results of this study may also serve as baseline data critical for future comparisons for amphibian monitoring in the region.

Finally, my research also highlights the need of further comprehensive follow-up studies on the distribution of *Bd* in Southeast Asia to understand whether it occurs naturally and if so, why it has not caused the same levels of infection seen in other

biogeographic regions. Also, more surveys are needed in order to monitor the status of areas where positives are detected to see if prevalence and infection intensity rise. More data on the distribution of the fungus will help researchers obtain a better understanding of the threat of *Bd* to native amphibians within the region. Such data may also help authorities and concerned citizens mitigate this hazard.