

ECOLOGY AND CONSERVATION OF THE AMPHIBIANS ON MOUNT KINABALU

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DECLARATION

I hereby declare that this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

The thesis has also not been submitted for any degree in any university previously.

Eva Catharina Madelene Karlsson
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SUMMARY

Monitoring of amphibians in Southeast Asia is scarce, however the rates of new species descriptions are substantial. This biodiverse region is undergoing high rates of land clearing, which coupled with a changing climate, is expected to have a major impact on amphibians in the region. Over 40% of amphibian species in Borneo alone are listed in one of the IUCN threatened categories, and for the majority there is limited information available on ecology, population numbers, and life history. This thesis investigates; shifts in distribution, ecology, community composition of the amphibian community on Mount Kinabalu in Borneo, Malaysia, as well as the use of automated acoustic techniques for monitoring these communities.

Species on Mount Kinabalu appear to have shifted their mean altitudinal distribution upwards since 1929, although some species have moved downwards; leading to an overall increase in species richness in the mid-elevation sites that were surveyed. Data also supports that there has been a change in beta diversity, between 1929 and recent survey years, potentially due to a change in the drivers: from nestedness to turnover.

The distribution of an endangered species (*Ansonia platysoma*) was assessed and potential areas with a high chance of the species being present were predicted. These areas should be a priority for future surveys. Altitude and soil were identified as variables that can predict the distribution of the species. However, there were no clear results on what variables affect the density of individuals.

The co-occurrence patterns between amphibian species in Kinabalu Park shows that a few species tend to co-occur strongly, whereas the majority are not non-randomly associated. The amphibian community in Kinabalu Park can be separated into four distinct groups that share similar responses to environmental variables. This indicates that the response to any future changes in the environment will likely be at the community level, rather than the species level and highlights the need to use a community perspective in studies that investigate future responses.

Acoustic monitoring appears to be a useful tool for amphibian surveys, with some caveats. The acoustic activity levels of amphibians vary substantially between species. Most of the species were active during the night, and species with high-frequency vocalisations call more consistently during this period. A few species are diurnal, suggesting that classical night surveys might miss them. Some species have very low chances of being recorded by an acoustic monitoring station, due to several factors such as: that do not vocalise, have low population densities, or are unlikely to enter the detection area of the unit during their active periods (for example canopy living species). The substantial monetary and personnel investment needed for classical surveys, mean that methods like passive acoustic monitoring offer a low-cost alternative for monitoring of vocalising species.

I conclude by identifying the need to implement long-term monitoring programs in Southeast Asia, and I argue that this is starting to become possible with increasingly accessible technology. With relatively low financial investment, crucial information can be collected that can inform not only

management of species, but also the basic science around distribution, ecology and behaviour of species'.

CONTRIBUTIONS

Table 0.1. Details of contributions of data and specific work details.

Data Chapter	Section	Performed by
Altitudinal shifts of amphibian species over 80 years on Mount Kinabalu, Borneo	Fieldwork: 2007 Fieldwork: 2010 Fieldwork: 2016 Analysis Write Up	David Bickford David Bickford Catharina Karlsson Catharina Karlsson Catharina Karlsson
Beta diversity in the amphibian community on Kinabalu Park indicate a shift in driver over the last 80 years and a lag in response to environmental change	Fieldwork: 2007 Fieldwork: 2010 Fieldwork: 2016 Analysis Write Up	David Bickford David Bickford Catharina Karlsson Catharina Karlsson Catharina Karlsson
Habitat and occupancy of <i>Ansonia platysoma</i> on Borneo, Malaysia	Fieldwork: 2016 Analysis Write Up	Catharina Karlsson Catharina Karlsson Catharina Karlsson
Co-occurrence and connectivity in a tropical mountain amphibian community	Fieldwork: 2016 Analysis Write Up	Catharina Karlsson Catharina Karlsson Catharina Karlsson
Acoustic and environmental monitoring using open-source hardware and software	Fieldwork: 2016 Fieldwork: 2017 Analysis Write Up	Catharina Karlsson Catharina Karlsson Catharina Karlsson Catharina Karlsson
Bioacoustics of the amphibian community in Kinabalu Park and the use of passive acoustic monitoring	Fieldwork: 2016 Analysis Write Up	Catharina Karlsson Catharina Karlsson Catharina Karlsson

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INTRODUCTION

New amphibian species are being described at an unprecedented rate: at least 30 new species were described from South Asia in 2014 alone (Biju, Garg, Gururaja, Shouche, & Walujkar, 2014; Biju, Garg, Mahony, et al., 2014; Vijayakumar, Dinesh, Prabhu, & Shanker, 2014). In the last ten years 36 new species have been described from Borneo, amounting to 20% of the 182¹ amphibian species known from the island (Inger, 2017). With the high rate of new descriptions, the number of amphibian species listed as data deficient is increasing (Howard & Bickford, 2014), i.e. for many species there is insufficient demographic and distribution information for a full assessment. At present, at least 30% of amphibian species globally are listed as at risk from extinction on the IUCN red list of threatened species, and at least 25% are listed as data deficient (IUCN, 2009). The high number of species listed as threatened with extinction is attributed to factors including disease, habitat loss, land-use change, pesticides, pollutants and climate change (Blaustein, Romansic, & Kiesecker, 2003; Blaustein et al., 2010; Cothran, Brown, & Relyea, 2013; S. Cushman, 2006; Davidson, Shaffer, & Jennings, 2002; Duarte et al., 2012; Egea-Serrano, Relyea, Tejedo, & Torralva, 2012; Keith et al., 2014; Mann, Hyne, Choung, & Wilson, 2009; Nori et al., 2015; Pyron & Wiens, 2013; Roe, Hopkins, Durant, & Unrine, 2006; Scheele et al., 2014; Spolyarich, Hyne, Wilson, Palmer, & Byrne, 2011; Stuart et al., 2010; Wassens, Hall, Osborne, & Watts, 2010). It is also estimated that extinction risk of data deficient amphibian species is much higher than previously thought, with 63% of currently data deficient species predicted to be listed in one of the threatened categories if the information were available for a full assessment (Howard & Bickford, 2014). Considering both the high number of species listed as threatened

¹ 183 species are reported in the book, however *Rentapia rugosa* has been merged with *Rentapia everetti* (Chandramouli & Amarasinghe 2016).

and the high number of data deficient species estimated to be threatened, the funding and man-power required to collect adequate baseline data, or apply conservation measures, for all amphibian species, is unlikely to be available (Collins, Moore, Church, & Mckay, 2005).

Southeast Asia is a region of particular concern because it has very high biodiversity and is also experiencing many changes with regards to climate and land use (Das, 2006; Giam, Ng, Yap, & Tan, 2010; Hertwig, Schweizer, Das, & Haas, 2013; J. Rowley et al., 2010). Amphibian species in this region are often data-poor with limited information available relating to current distribution, and little information available relating to life history, demographics or the effects of land-use and climatic change (Giam et al., 2010; Howard & Bickford, 2014; Navjot S. Sodhi et al., 2008). The area of amphibian research in Southeast Asia has limited resources available. It would be highly beneficial to the fields of ecology and conservation of amphibians, if there were more directed research into basic ecological questions. Specifically, information regarding the distribution of species, ecological requirements of species, and when and why amphibian species shift their distribution is currently lacking and information in these areas would help to target limited resources.

Shifts in the distribution of species

Species shift their distribution for many reasons: as a response to changes in environmental variables (e.g., climate), changes in interaction pressure from another species, or habitat transformation (e.g., deforestation) (S. A. Cushman, 2006; HilleRisLambers, Harsch, Ettinger, Ford, & Theobald, 2013; Lathlean, McWilliam, Pankhurst, & Minchinton, 2017a; Lenoir et al., 2010; Lindenmayer, 2009). Climate change is of particular concern for amphibians, which are ectothermic and thus expected to respond comparatively fast to changes in temperature to ensure that they remain in physiological optima (I.-C. Chen et al.,

2009; I. C. Chen et al., 2011; Hillman, Withers, Drewes, & Hillyard, 2009). One way to test predictions about the response of amphibian distributions to climate change is to check whether species have already shifted their distribution as a response to changing climate in recent decades. Historical records of species ranges can be problematic, however: they often do not report survey effort or they do not give accurate information on how the data were collected (W. M. Kemp et al., 2005; Kharouba, Algar, & Kerr, 2009a; Stöckli, Wipf, Nilsson, & Rixen, 2011; Szabo, Vesk, Baxter, & Possingham, 2010). This makes it difficult to assess whether differences between historical and recent datasets are due to real change or an artefact of different survey techniques. However, some statistical diversity estimators are available that are independent of survey effort and can be used for direct comparison of species richness, providing that the number of individuals encountered is reported (Chao, Chazdon, Colwell, & Shen, 2004; Chao, Chiu, & Jost, 2016; Chao et al., 2017; Chao & Jost, 2012; Hsieh, Ma, & Chao, 2016). The related field of species distribution modelling seeks to estimate the past, current, future or potential distribution of species. Two main types of species distribution model exist: empirical and mechanistic models. As will be seen in the next section, mechanistic models are ideal but usually impractical; empirical models are more commonly used and can be developed with minimal data.

Species Distribution Models

The geographical distribution of a species should ideally be predicted from a mechanistic model and parameterised with physiological information (Anderson & Raza, 2010; Gerick, Munshaw, Palen, Combes, & O'Regan, 2014; Kearney et al., 2008; Kelly (Letcher) et al., 2013; Moorecroft & Lewis, 2004; X. Morin & Thuiller, 2009), e.g., the maximum temperature a species can tolerate. Such models can produce accurate species

distributions, but the data requirements are usually prohibitive as the necessary physiological response data simply does not exist and it is unfeasible or unethical to collect them.

An alternative is to use empirical species distribution models (henceforth referred to as just “SDMs”), a phenomenological approach whereby known species occurrence points are correlated with environmental variables. The use of SDMs has several advantages for the conservation field but requires a thorough understanding of their inherent limitations and uncertainties (Guillera-Arroita & Lahoz-Monfort, 2012; Marmion, Parviainen, Luoto, Heikkinen, & Thuiller, 2009). This understanding is necessary to ensure that correct conclusions are drawn from the results, especially when recommending conservation and management plans to individuals that might not have scientific training, such as stakeholders and policy-makers (Keith et al., 2014; Kelly (Letcher) et al., 2013; X. Morin & Thuiller, 2009; Musters, Kalkman, & van Strien, 2013).

An SDM uses geographical information system (GIS)-based correlative modelling to find associations between environmental variables, and occurrence records for the species under study (Royle & Dorazio, 2006; Shcheglovitova & Anderson, 2013; Townsend Peterson, 2001; Wiens, Stralberg, Jongsomjit, Howell, & Snyder, 2009). The environmental variables in an SDM are typically abiotic factors (Miguel B Araújo & New, 2007; Elith et al., 2011; Steven J. Phillips, Anderson, & Schapire, 2006; Thuiller, 2003), including climate variables. The model thus creates a bioclimatic envelope model that depicts the niche of the species (Miguel B Araújo & New, 2007; Elith, Kearney, & Phillips, 2010; Elith et al., 2011). This niche model is then used to examine the geographic space and establish areas where the species of interest is likely to occur under the given environmental conditions (Kharouba, Algar, & Kerr, 2009b; Segurado & Araújo, 2004; Shcheglovitova & Anderson, 2013; Zeng, Low, & Yeo, 2016). One limitation of SDMs, as typically implemented, is that the predictor variables do not extend beyond abiotic variables to biotic variables. For some species, this

may lead to inaccurate predictions because biotic effects of interactions with other species (e.g., via competition or predation) are often fundamental determinants of species' distributions.

Recently presence–background and presence–absence SDMs have gained popularity (Elith, 2014; Gormley et al., 2011; Steven, 2009). Presence-background models are appealing because of the limited data required to run a model: a set of occurrence points. However, a set of occurrence points alone may not yield accurate predictions: accurate predictions require appropriate training conditions (Elith et al., 2010; Kramer-Schadt et al., 2013). The training conditions can have a large impact on model predictions (Anderson & Raza, 2010; Elith & Graham H., 2009; Elith et al., 2010; Peterson, 2003; S. J. Phillips & Elith, 2013). For example, when running a presence-background SDM in MaxEnt (widely used software for SDMs), the software generates pseudo-absence points from a background area, and this background area should correspond to the area in which searches for the species have been conducted.

In view of these considerations, presence-background SDMs for amphibian species in Southeast Asia can be beneficial for directing search efforts to areas where particular species' are more likely to occur (Peterman, Crawford, & Kuhns, 2013; Jérôme Sueur, Pavoine, Hamerlynck, & Duvail, 2008). However, these predictions may not be sufficiently accurate for other purposes (e.g., range-shift predictions), because (i) the prior information required to set up correct training conditions is often missing, i.e., information on search effort in specific areas; and (ii) the predictor variables of the SDMs will usually, by necessity, be restricted to abiotic variables (this latter restriction applies also to most presence-absence SDMs).

Joint Species Distribution Models

The distribution of species is limited by the environment as well as interactions with other species (Hutchinson, 1957; Pironon et al., 2017). However, as noted above, most SDMs incorporate only abiotic variables (Hertzog, Besnard, & Jay-Robert, 2014; Zeng et al., 2016). On the other hand, community ecology modelling of co-occurrence patterns often incorporates only biotic interactions (Gotelli, 2000; Veech, 2013). Some SDM work has attempted to incorporate species interactions by linking or limiting the distribution of a species based on that of other species (Gavish et al., 2017; Henderson, Ohmann, Gregory, & Roberts, 2014; Schmitt, Pouteau, Justeau, de Boissieu, & Birnbaum, 2017), but these models have assumed the biotic interaction is unidirectional, when in fact it is often bi-directional (HilleRisLambers et al., 2013; Kissling et al., 2011; Laurindo, Gregorin, & Tavares, 2017). Recent studies have started to combine co-occurrence and environmental modelling into joint species distribution models (JSDMs) (Leach, Montgomery, & Reid, 2017; Pollock et al., 2014; Segurado & Araújo, 2004).

These JSDMs still suffer from several issues inherent to SDMs in general, e.g., they typically take only presence-absence points as inputs and thereby ignore abundance, which can limit a model's predictive power if species interactions are mediated strongly by their abundances. There is also an issue with zero inflation as both abundance and presence-absence data tend to have a high number of zeroes. Zero inflation is challenging to deal with statistically because a recorded absence can be due to either true absence or low density of the species (Clark, Gelfand, Woodall, & Zhu, 2014; Clark, Nemergut, Seyednasrollah, Turner, & Zhang, 2017). It is particularly challenging to design models in which abiotic variables drive both presence-absence and abundance of a species. Generalised joint attribute modelling (GJAM) accommodates several of these issues: it can accommodate discrete abundances and can also handle data of different scales (for example percentage cover and

discrete abundance at the same time). It also solves the zero-inflation issue by using an explicit zero class for presence-absence, leading to a presence-absence distribution that is not binomial. These Zero-inflated models (ZIM) assume that abundance is conditional on the species being present in the first place. The GJAM also uses an inverse prediction to model simultaneously the co-occurrence between species and their responses to abiotic variables (Clark *et al* 2011; Clark *et al* 2013). JSDMs are becoming more sophisticated and showing great promise as tools for inferring the response of species to one another and the environment. The main issue remains, though, the lack of available data. Southeast Asia is under-surveyed and under-funded; thus, the issue of data availability remains. A possible solution is to incorporate technological advancement into surveys and monitoring of amphibian species, to gather data more rapidly.

Acoustic Monitoring

A potential new avenue for detection surveys of vocalising species, including amphibians, is the application of acoustic monitoring(Digby, Towsey, Bell, & Teal, 2013) (Aide et al., 2013; Digby et al., 2013; Pieretti et al., 2015; Riede, 1993). All traditional data collection methods for amphibians have several things in common: they are expensive, require a substantial investment of time, are spatially limited, have difficulties recording the many amphibian species that live high up in trees, and are reliant on the expertise level of the recorder (Andreassen, Surlykke, & Hallam, 2014; Bedoya, Isaza, Daza, & López, 2014; Digby et al., 2013). These difficulties have led to a lack of permanent records of species presence and absence, abundance and the effect of stochastic population fluctuations, making it difficult to assess changes in amphibian populations over time due to environmental changes over recent decades including the effects of climatic change, habitat modifications and release of pollutants into the environment (Aide et al., 2013). Recently, acoustic

monitoring of amphibians has been used extensively to great benefit, because acoustic recording stations can be left out in a survey area, minimising the human impact on the area and increases the amount of data that can be recorded (De Solla, Fernie, Barrett, & Bishop, 2006; Heinermann et al., 2015; Ospina, Villanueva-Rivera, Corrada-Bravo, & Aide, 2013; Jérôme Sueur et al., 2008). However, manual sorting of the spectrograms is time consuming, and this has in the past limited the utility of acoustic data (Gasc, Pavoine, Lellouch, Grandcolas, & Sueur, 2015; Jérôme Sueur, Farina, Gasc, Pieretti, & Pavoine, 2014; Jérôme Sueur et al., 2008; Towsey, Wimmer, Williamson, & Roe, 2013; Towsey et al., 2014; Towsey & Zhang, 2014). Recent developments include new automatic classification techniques that cut down the amount of time needed for sorting, and reduces data storage requirements (Bedoya et al., 2014; Digby et al., 2013; Frommolt & Tauchert, 2014; Marques et al., 2013; Pellet & Schmidt, 2005).

Classification algorithms automate the process of species identification in acoustic files. Several automated acoustic classification algorithms that are in use will only search for species they have been trained on (Aide et al., 2013; Araya-Salas & Smith-Vidaurre, 2017; Crump & Houlahan, 2017), and the training itself requires many hours of manual work to identify species in the sound files and create a classification library (Bedoya et al., 2014). However, in Southeast Asia, acoustic classification has multiple issues: many species are undescribed; complete species lists do not exist for many areas; the call of a species is often not described; and there is a lack of available sound files of calls to create a training library. There is a need for algorithms to be developed that can deal with unknown species in datasets. Potential algorithms are network analysis (Kingsbury, Sainath, & Soltau, 2012; Maas, Hannun, & Ng, 2013; X. Zhang, Trmal, Povey, & Khudanpur, 2014) and artificial intelligence algorithms such as Aureas (Bedoya et al., 2014). Aureas is based on a voice recognition algorithm as well as fuzzy logic, facilitating flexibility and allowing the software

to deal with dialect variations and identifying unknown species in datasets. This software has so far been tested only on data recorded on directional microphones that block noise coming in from the sides. For passive acoustic monitoring purposes, omni-directional microphones are needed, although in this case the recordings often contain a high amount of background noise that must be filtered out.

Acoustic monitoring as a field suffers from several standardisation issues. Most notable is the lack of methods to estimate the detection area of a unit (Darras, Pütz, Fahrurrozi, Rembold, & Tscharntke, 2016), making comparisons between units and studies dubious. Moreover, the distance calls travel differs across species (Marten & Marler, 1977). Despite this, studies continue to make inferences on call travel distance for multiple species based on information from a single species with very different call characteristics (Aide et al., 2013), or do not attempt to estimate detection area or call distance at all (Andreassen et al., 2014; Ospina et al., 2013).

The rise of acoustic technology has been facilitated by increasing computing power: several methods that were previously too data intensive to use are now feasible (Bedoya et al., 2014; Kingsbury et al., 2012; Pieretti et al., 2015). The use of these techniques in amphibian ecology and conservation has so far been limited, despite their potential benefits in the context of rapid population declines and limited resources available for surveying and training of new experts.

Aims and Research Objectives

The most urgent need for amphibian conservation in Southeast Asia is to collect baseline data for the many species that lack such data. To do this, new datasets need to be collected. Already existing datasets need to be analysed in new ways, and new data-collection techniques need to be incorporated in the tropics. This thesis uses datasets on the amphibian

community in Kinabalu Park in Malaysian Borneo. The thesis aimed to address the above-stated issues by 1) assessing whether the amphibian species have shifted their distributions over the past century; 2) assessing whether the amphibian beta diversity has changed over time; 3) performing a species-level analysis of habitat effects on occurrence and density; 4) investigating the co-occurrence interaction network and co-occurrence community structure and the effect of environmental variables on the amphibian community; 5) trialling an open-source acoustic and environmental monitoring station; and 6) using acoustic monitoring data to assess the acoustic ecology of the amphibian community in Kinabalu Park and assessing how it compares to classical survey techniques.

CHAPTER 1. Altitudinal shifts of amphibian species over 80 years on Mount Kinabalu, Borneo

Introduction

The recent rate of change in global climate is a challenge to biodiversity because of the time needed for species to evolve adaptations for persistence (Parmesan, Yohe, & G, 2003; Peterson, 2001, 2003; Walther et al., 2002). Climate change is predicted to have direct impacts around the world, and there is especially high concern for fragile mountain ecosystems. The impacts on tropical mountain species are expected to be high due to narrow thermal tolerance of many tropical species (La Sorte & Jetz, 2010; Wilson, Gutiérrez, Gutiérrez, & Monserrat, 2007). Ectothermic organisms will be especially vulnerable as they cannot thermoregulate to offset rising temperatures, but must instead adjust microhabitat preferences and behaviour, or migrate upslope (La Sorte & Jetz, 2010, 2012; Ruiz-Labourdette, Nogués-Bravo, Ollero, Schmitz, & Pineda, 2012; Wilson et al., 2007).

Tropical mountainous regions have very high levels of amphibian diversity and endemism (Raxworthy et al., 2008; Sergio & Pedrini, 2007). Mountains present an environmental gradient for temperature, precipitation, wind speed, and UV radiation (Hodkinson, 2005), all of which affect the climate envelope of amphibian species. This makes any changes to the environment on tropical mountains especially concerning, considering the narrow thermal tolerance that most tropical species have. Most amphibians are dependent on water to complete their life cycle (Becker, Fonseca, Haddad, & Prado, 2010), making them especially vulnerable to changes in precipitation and evaporation (Jiménez-Robles, Guayasamin, Ron, & De la Riva, 2017; Ospina et al., 2013). Additionally, the physiological impact of increased temperature is expected to be greater for ectotherms

than endotherms (Bickford, Howard, Ng, & Sheridan, 2010; Blaustein et al., 2010; Bonetti & Wiens, 2014; Duarte et al., 2012; Scheffers, Edwards, Diesmos, Williams, & Evans, 2014).

Amphibians are already in sharp decline due to habitat loss and degradation, emerging infectious diseases, and environmental pollutants, as well as synergistic effects of all the above (Allentoft & O'Brien, 2010; M. B. Araújo, Thuiller, & Pearson, 2006; Blaustein et al., 2010; Hamer & Mahony, 2007; Hamer, Makings, Lane, & Mahony, 2004; Hossack, Lowe, Ware, & Corn, 2013; Howard & Bickford, 2014; Rohr, Halstead, & Raffel, 2011). Climate change is expected to exacerbate these declines. Currently around 40% of extant amphibians are threatened with extinction and 60% of data deficient species have been predicted to also be threatened, making amphibians the most endangered terrestrial taxonomic group (Howard & Bickford, 2014; Scheffers et al., 2013; Navjot S. Sodhi et al., 2008).

These issues are of particular concern in Southeast Asia, where—in common with other tropical regions—amphibian diversity is very high (Raxworthy et al., 2008; Sergio & Pedrini, 2007), and where description of species is still ongoing (J. Rowley et al., 2010), large geographic areas having never been surveyed and most areas have little or no continuous monitoring (Navjot S. Sodhi et al., 2010). Monitoring data are essential for assessing changes to amphibian communities over time. Comparing current datasets with historical datasets can aid in establishing any shifts in the distribution of species and whether these shifts are consistent with observed climate change (Van der Putten, Macel, & Visser, 2010).

Mount Kinabalu in Malaysian Borneo is the highest mountain in Southeast Asia at 4095 m above sea level (asl), and has high species diversity and endemism across a variety of taxa (Kitayama, 1992; J. M. . Smith, 1980). In this study, I compared historical survey data from Mount Kinabalu in 1929 with surveys conducted by myself and others, roughly 80 years later, to investigate whether there had been any change in the occurrence and overall species richness of amphibian species on the mountain. Specifically, I aimed to address the

following: 1) if there has been any shift in the mean altitude of the amphibian species on Mount Kinabalu and 2) if there has been any change in species richness and, 3) if there has indeed been any change, whether these changes follow the patterns expected under climate change over the last century.

Methods

Study site

This study was conducted on Mount Kinabalu (Figure 1.1), Sabah, Malaysia on the island of Borneo (Lat: 6.157, Long: 116.637) between 900 and 3300 m asl. The study site was designated a National Park in 1964 and a UNESCO world heritage site in 2000, and thus has received minimal human disturbance.

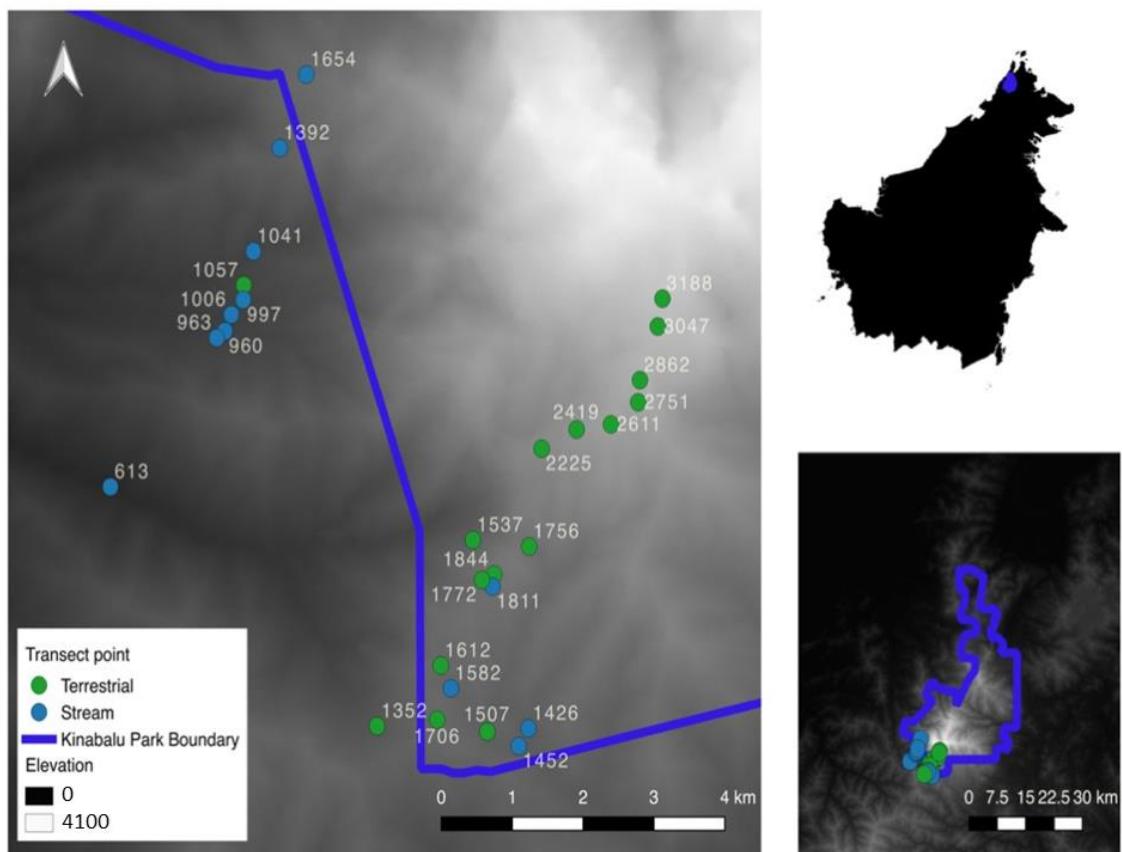


Figure 1.1. Kinabalu Park in Borneo. Green dots indicate terrestrial sites. Blue dots indicate stream sites. Elevation for each site is given in white text. The elevation gradient goes from 4100 m (white) to -1 m (black). The park boundary is shown by the blue line.

We compared data from April-May 1929 (M. a. Smith, 1931) with data collected in 2007, 2010 and 2016 to determine whether amphibians species have shifted their elevational ranges. Data from 1929 include the names of eight sites, the elevation of each site, species encountered, and number of individuals encountered of each species (M. a. Smith, 1931).

Search effort was not reported in 1929, likely because the amphibian occurrence data were secondary to the main objective of the surveys. Data from recent (2007, 2010, and 2016) surveys include names of 15–28 sites, the elevation of each site, species encountered, number of individuals of each species encountered, and search effort (Table 1.1). Because the exact locations of the 1929 surveys could not be determined with 100% accuracy (only site name and altitude were reported; (see Appendix 1.1), multiple sites around each of the 1929 localities visited were visited in the recent surveys (Table 1.1). In 2007, a total of 15 sites were sampled over 17 surveys from 10 to 28 May; in 2010, 18 sites were sampled over 38 surveys from 5 June to 13 July, and in 2016, 28 sites were sampled over 84 surveys from 2 February to 25 May (Figure 1.1). In 1929 the surveys were conducted between April and May.

Table 1.1. The eight sites that were surveyed in 1929 and the number of sites at similar altitudes surveyed in recent years, which were down-sampled to match the 1929 sites in analysis. The altitude reported in 1929 is given after each site name.

1929	2007	2010	2016	Average elevation (m asl)
Kiau (914)	2	3	3	845 (range: 613 – 963)
Kenokok (1005)	2	2	4	1025 (range: 997 – 1057)
Lobang (1219)	1	1	1	1537
Tenompok (1432)	2	5	7	1520 (range: 1352 – 1706)
Marei Parei (1524)	2	4	2	1523 (range: 1392 – 1654)
Lumu Lunu (1676)	2	2	4	1796 (range: 1756 – 1844)
Kamborangah (2194)	2	2	4	2502 (range: 2225 – 2751)
Pakka (3108)	2	2	3	3032 (range: 2862 – 3188)

Visual and auditory encounter surveys

For recent surveys, similar methods were used in 2007 and 2010, with modifications made for the 2016 surveys. In 2007, one to two Visual and auditory encounter surveys

(VES) at each of the eight sites were conducted and in 2010, four to seven VES transects were conducted. Searches began at 19:00 hrs shortly after sunset and were conducted by three or four personnel for one hour (i.e., 3-4 person-hours of total search effort). Surveyors walked along forest and stream transects at a slow to moderate pace, visually searching for amphibians along the transect. Every individual amphibian detected was captured if possible, sexed, measured (snout-vent length, SVL), and released at the point of capture. Voucher specimens were deposited in the Sabah Parks Museum.

The people that performed the surveys were not the same between the 2007 and 2010 surveys and the most recent survey in 2016. However, there was a one-month training process in detection and species identification for surveyors in 2016 to reduce the effect of varying expertise levels. Surveys where one or two of surveyors were thought to not be following the survey protocol properly were also removed from analysis (a total of seven surveys in 2016). In 2016, VES were conducted between 18:30 and 01:00 hrs by two personnel. As in 2007 and 2010, surveyors walked along forest and stream transects, but for 2016 the surveys covered a fixed distance each time. Terrestrial surveys were conducted along a 500 m transect, including 2 m to either side, and stream transect surveys were conducted along a 100 m transect, covering the stream bed plus 1 m up the stream bank on both sides. Terrestrial and stream surveys were both conducted up to a height of 2 m. . Surveys in 2016 were conducted for a set active search time of 60 min for terrestrial surveys, to account for handling time of individuals and to ensure standardisation of search time between sites. At the start and end of each survey, relative air humidity and air temperature were recorded, and stream surveys also included water temperature and pH measurements at the start and the end of each survey. Amphibians were recorded both visually and by aural detection. In 2016 every individual amphibian detected was sexed, measured (snout-vent length, SVL), the substrate and height off the ground were recorded, and verification photos

were taken laterally and from the side, if the individual was required to be caught it was released at the point of capture.

For analysis, sites were assigned to one of three altitude bands, reflecting shifts in habitat (Malkmus, Manthey, Vogel, Hoffmann, & Kosuch, 2002). All sites from 900-1300 m asl (the transition between lowland dipterocarp to lower mixed Fagaceae forest with a forest canopy height of 30-35 m) were termed “low”, sites from 1300-1800 m asl (mixed Fagaceae forest with a canopy height of 15-18 m) were termed “mid”, and sites from 1800-3300 m asl (upper montane to sub-alpine forest with a canopy height of 5-12 m) were termed “high”. Because Kinabalu Park has been protected since 1967, and was not logged before then, and because there have been no records of vegetation shifts, the vegetation is considered unchanged since 1929.

Statistical Analysis

To assess whether species were conforming to prior predictions, the temperature change on Mount Kinabalu since 1929 was first estimated. The Global Historical Climatology Network (GHCN: <https://www.ncdc.noaa.gov>) was accessed. There are temperature records from a station in the city of Kota Kinabalu. The earliest temperature record is from 1956, when the yearly average was 30.3°C compared to the average yearly temperature in the decade before 2016 which is 31.8°C. However, the GHCN also reports annual climate anomalies, that is temperatures that differ from the established baseline from 1961-1990. Temperature anomalies in the 5° cell that cover the area of Kinabalu Park for the decade leading up to 1929 (1920-1929) were used. The difference in temperature recorded in the decade leading up to 2007 (1998-2007) and the decade leading up to 1929 was a positive increase of 1.36°C. The average of temperatures recorded in 2007, 2010 and 2016 was used as a baseline for recent temperature values along the attitude gradient of the mountain. The

ancient temperature was thus back-predicted from the recently known values using the expected shift in temperature of 1.36°C.

The weighted elevation mean was calculated (I.-C. Chen et al., 2009; I. C. Chen et al., 2011) for 1929, and all recent survey years. To account for the higher sampling intensity in recent years, the data were then also sub-sampled on the recent survey years to match the number of survey sites in 1929 and the weighted elevation mean was calculated from these sub-sampled data (this procedure was repeated 1000 times). The weighted elevation mean from the sub-sampled data was then used as a response variable in a mixed effects model with species as a fixed effect and area and year as random effects, weighted by species abundance. The effect of reproductive mode and body size on the raw altitude shift was also assessed using a linear model. Body size measurements used for each species were the maximum female size and the maximum male size (Malkmus et al., 2002). Ten models were created with all combinations of the three explanatory variables and they were compared using the Akaike information criteria (AIC).

Changes in species richness were assessed using iNEXT (Chao et al., 2015, 2016, 2017; Chao & Jost, 2012; Hsieh et al., 2016). The estimation of sample coverage using iNEXT is independent of survey effort and leads to an estimate of the number of species from the community detected in the sample. The iNEXT software also uses a rarefaction estimate, to account for uneven numbers of sample sites (Chao et al., 2017; Hsieh et al., 2016). Rarefaction curves were calculated using Hill number zero, i.e. species richness. All statistical analyses were conducted in R (R Core Team, 2017).

Results

A total of 19 species were found both in historical and in at least one of the recent surveys. Thirteen species were found in all recent years, with two species found only in two of the recent years, and four species found only in one of the recent years (Appendix 1.2). Six species shifted their average elevation out of their previous habitat band (e.g., high to mid). In 1929 there were nine species in the low habitat band, five in the mid habitat band and five in the high habitat band. In the recent surveys, seven species were found in the low habitat band, eight in the mid habitat band and four in the high habitat band. This suggests that there has been an increase in species richness in the mid habitat band and a reduction in species in the low and high habitat bands (Appendix 1.4).

The predicted change in mean elevation of species in Kinabalu Park, assuming species are perfectly tracking temperature change is 200 m (Figure 1.2). Using all data, 14 species demonstrated an upward shift and five species had a downward shift from 1929 to recent surveys (Figure 1.3). The mean elevation change across all species from 1929 to recent years was +49 m (CI = [-131 m ,228 m]; SD = 399 1 m ; range= [-1062 m, 583 m]). Species that shifted upwards had a mean elevation shift of 245 m (range= [68 m, 583 m]) and species that shifted downwards had a mean shift of -500 m (range= [-1062 m ,-196 m]). If *Leptobrachella baluensis* is removed, on grounds that it was missed at lower elevations in 1929 surveys and appears to be an outlier, the weighted mean elevation shift becomes 111 m (CI = [-90 m , 311 m].6; SD = 303 m; range = [-519 m , 583 m]).

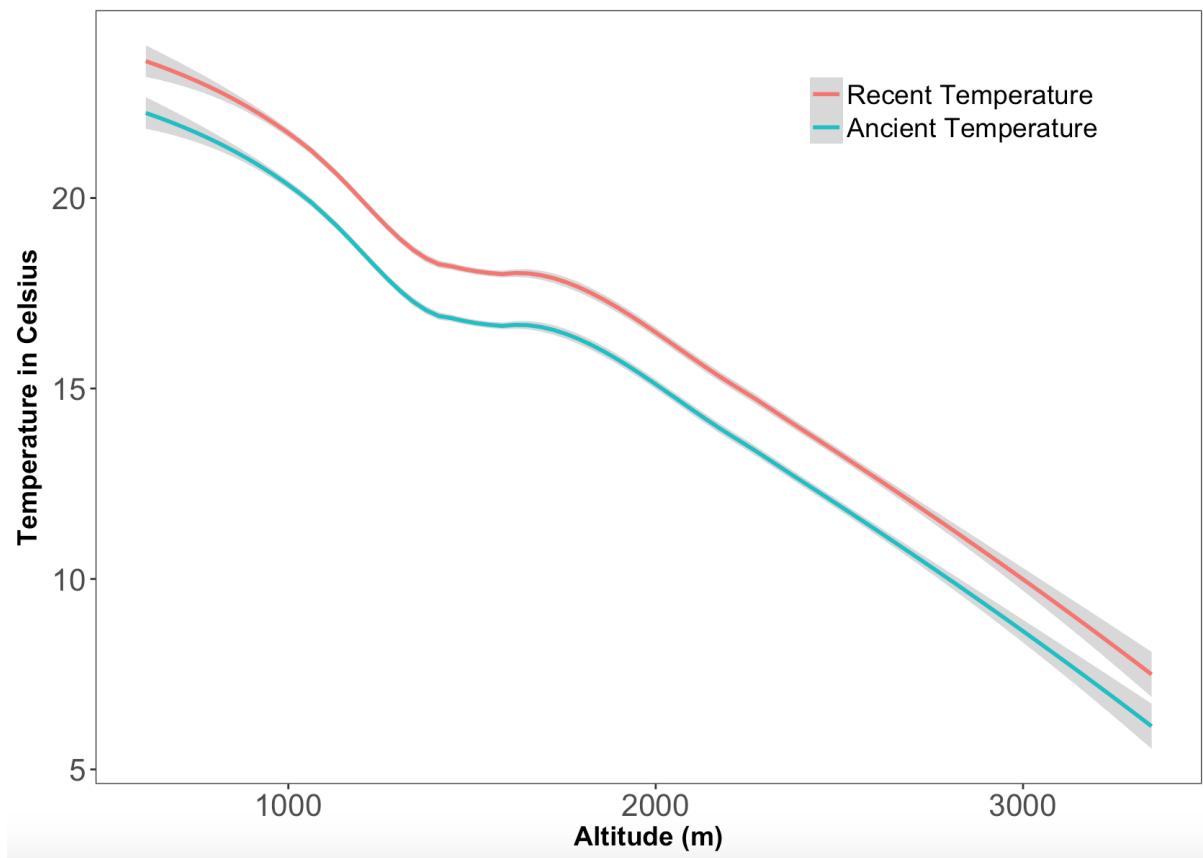


Figure 1.2. Temperature plotted against altitude for modern and ancient years. The 95% confidence intervals are in grey. See Methods section for details regarding estimate of ancient temperature.

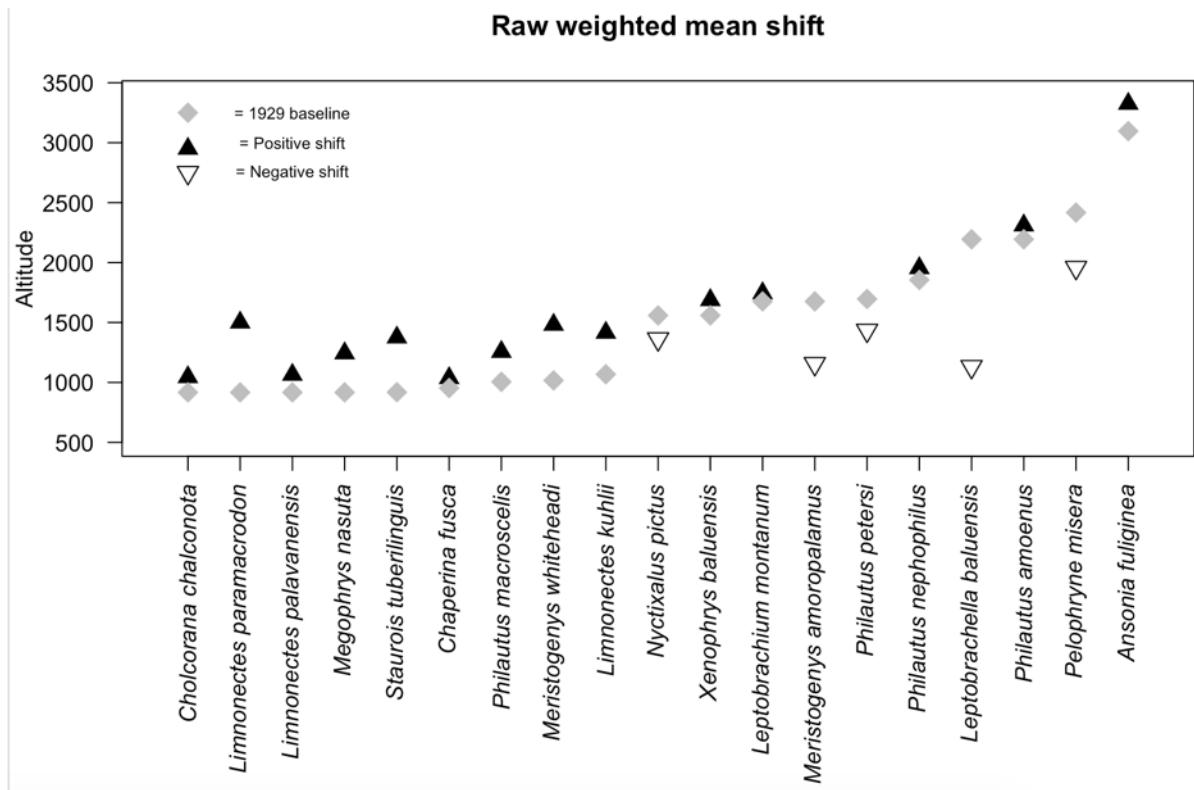


Figure 1.3. Weighted mean shift in the species' elevation. Grey diamonds represent the baseline of centred mean elevation in 1929. Triangles represent centred mean elevation in recent years: filled black triangles indicate positive shifts: open triangles indicate negative shifts.

Using sub-sampled data, to account for differences in survey effort across sample periods, 13 species had an upward shift and six species had a downward shift from 1929 to recent surveys. Mean elevation change from sub-sampled data was +72 m (SD= 542 m ; CI = [-171 m , 315 m]; Mean = 1575 m) (Figure 1.4). The mixed effects model shows a statistically significant positive shift in elevation from 1929 to the recent years (Table 1.2).

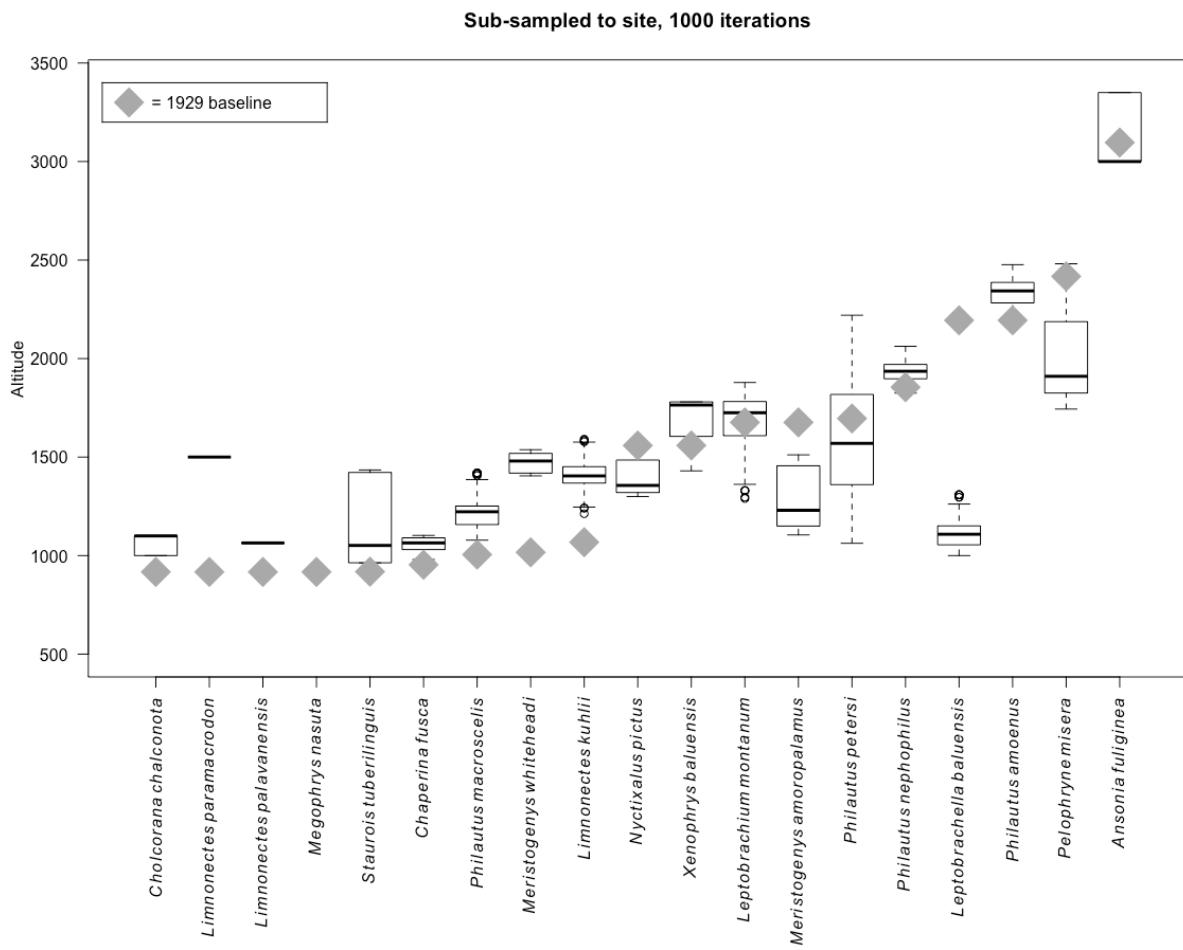


Figure 1.4. Weighted mean elevation from 1929 (grey diamonds) and the recent surveys (boxplots). The recent data were subsampled to match the number of sites in 1929 (with 1000 repeats of the subsampling procedure for each year).

Table 1. 2. Mixed effect model results, with species as a fixed effect and area and year as random effects and weighted by species abundance.

Model	Estimate	Std. error	t-value	P-value
Altitude Shift	65.175	28.029	2.325	0.022

Species appear, on average, to have broadened their elevation ranges in recent years since 1929 (Figure 1.5). The species are thus found over a larger elevational range, regardless of whether if the mean elevation shift is negative or positive.

Body size, but not reproductive mode, was statistically associated with altitude shift. The five downward shifting species represented a mix of reproductive modes: two stream breeders, one tree-hollow breeder, one ground/puddle breeder and one direct developer. Model selection retained three models that were within three AIC points of each other: one with both male and female reproductive mode (AIC: 281.4, female P-value: 0.415, female T-value: 0.836, male P-value = 0.883, male T-value = -0.150); one with only female body size as an explanatory factor (AIC: 279.4, P-value: 0.013, t-value: 2.778); and one with only male body size as an explanatory factor (AIC: 280.2, P-value = 0.019, T-value = 2.389). Fits of the two latter models are shown in Figure 1.6.

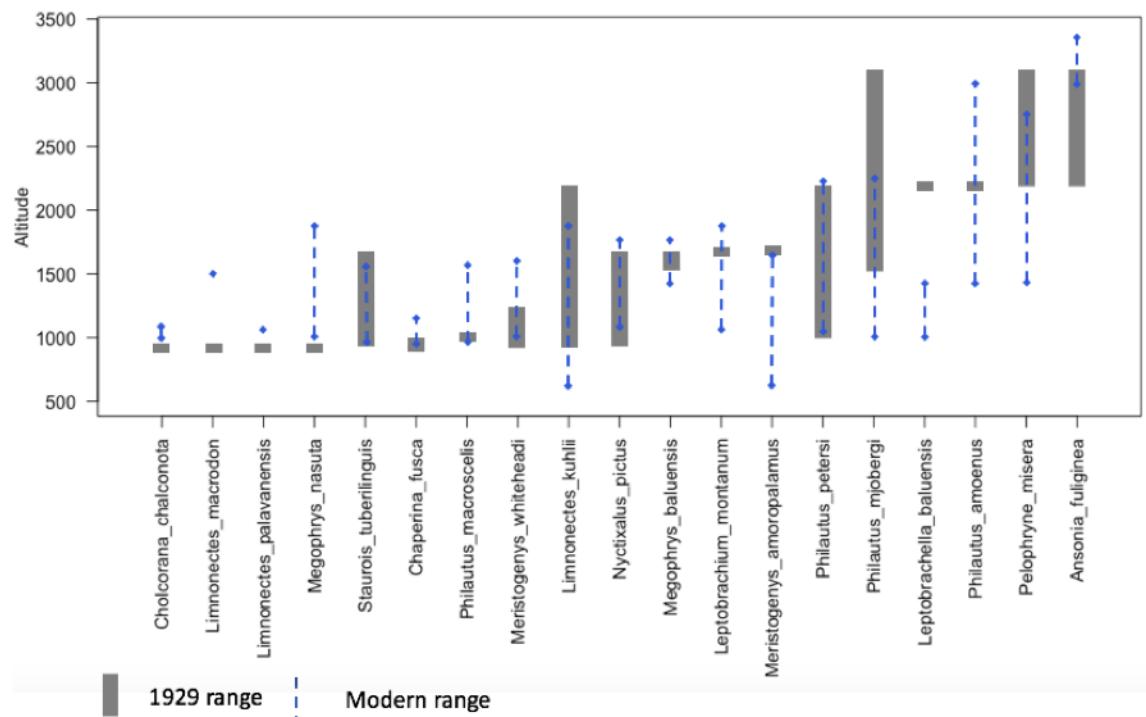


Figure 1.5. Shift in species ranges over time. Grey bars represent species' elevation ranges in 1929 and the blue dashed lines represent recent elevation range.

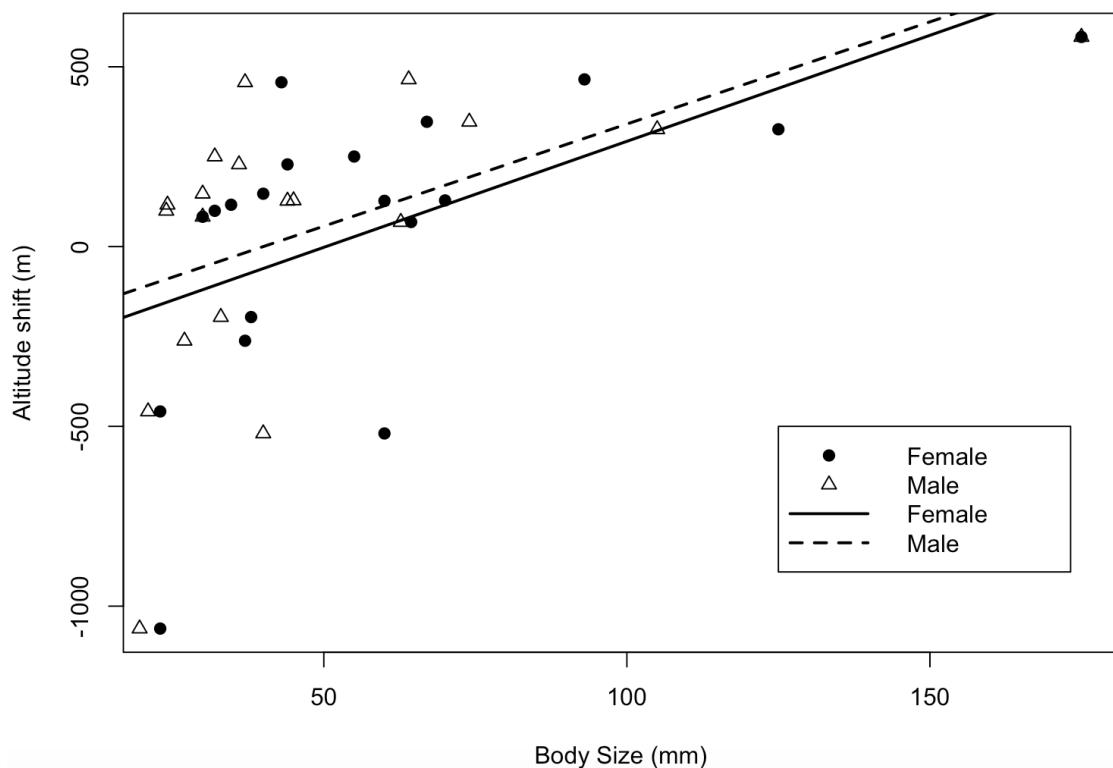


Figure 1.6. Linear model results of the effect of body size on altitude shift for both sexes (solid line fitted to solid points for female body size; dashed line fitted to open triangles for male body size).

Rarefaction estimates (Figure 1.7) indicate that estimated species richness as a function of sampling effort plateaued in all years for the lower altitude (900-1300 m asl). The mid-altitude curves (1300-1800 m asl) indicate saturation of the sample curves for 1929 and 2016, but not 2007 and 2010, indicating that the sampling might not have been adequate in those years. In the high-altitude sites (1800-3300 m asl) the rarefaction curve shows evidence of saturation for 2016 only.

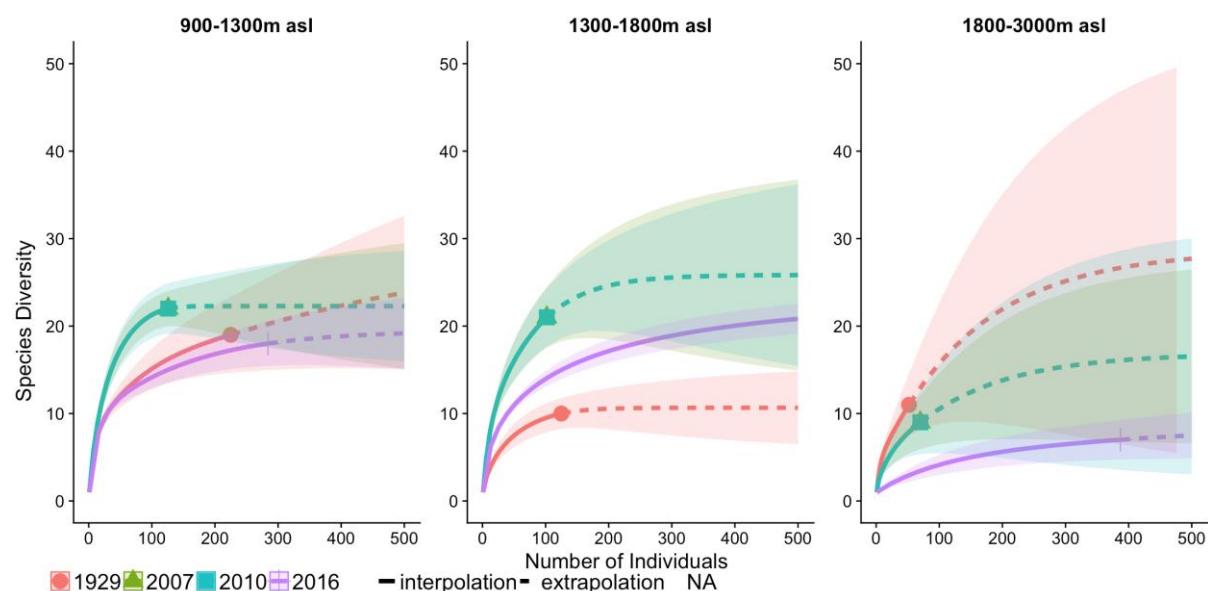


Figure 1.7. Rarefaction estimates using Hill number zero (species richness) for all four sampling years.

The sample coverage-based estimates indicate that species richness has decreased in high-elevation habitat, from 11 species in 1929 to five in 2007 and 2010, and one in 2016. Species richness in the low elevation habitat is estimated at 18 species in 1929, 23 in 2007 and 2010 and 16 in 2016. Species richness in the mid elevation habitat band was estimated as 7 in 1929, 18 in 2007 and 2010, and 11 in 2016 (Figure 1.8).

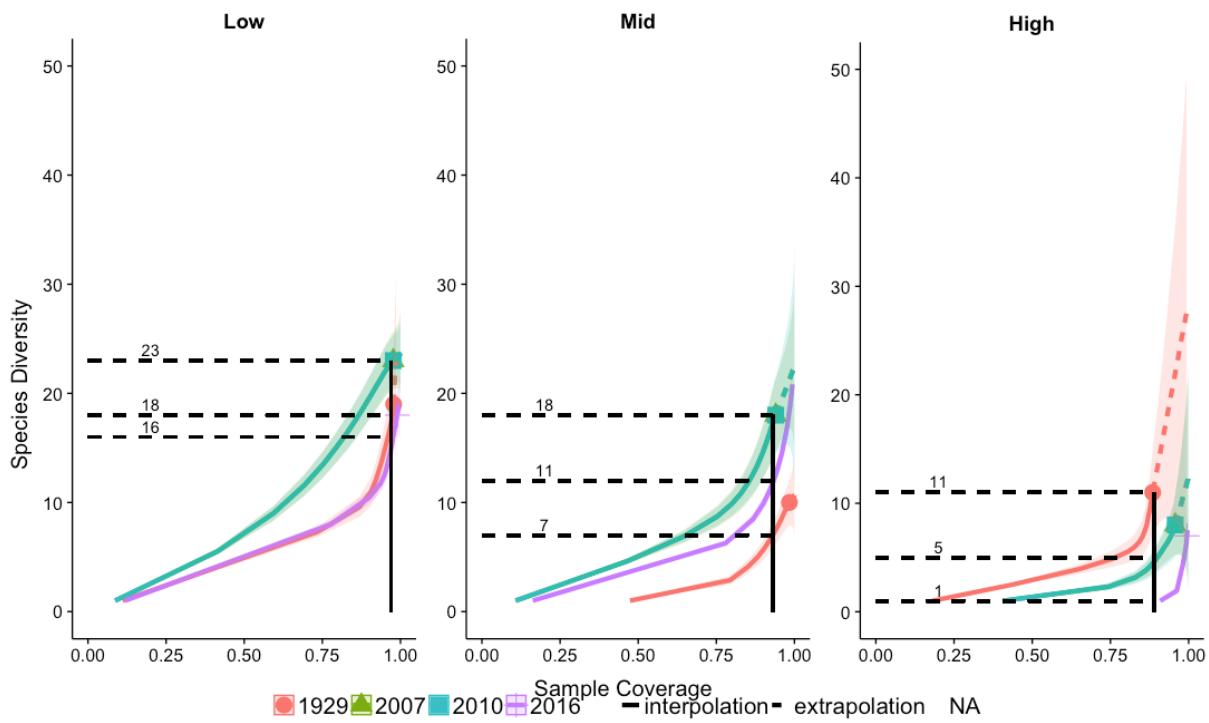


Figure 1.8. Sample coverage estimation from iNEXT over the four survey years between the different habitat bands. The solid vertical line indicates the cut-off point for accurate comparison between survey years based on sample coverage. Dashed horizontal lines represent the point at which species diversity values can be compared. Numbers above horizontal dashed lines give the corresponding species diversity (richness values). The horizontal dashed lines for 2007 and 2010 overlap almost completely in all habitat bands and only one is shown in each case.

High altitude sites in Kinabalu Park (above 2200 m asl) host two related species, *Philautus saueri* and *P. amoenus*, that are very difficult to distinguish due to their similar morphological features and high intraspecific variability in colour and patterns. For this study, the separate records of these two species from 2016 (the only year where the two were distinguished) were combined for the analyses. Verification photos are available upon request.

Discussion

Based on observed mean temperature change, one would predict the amphibians in Kinabalu Park to have shifted upwards by approximately 200 m since 1929. The analysis of the survey data indicates that, in fact, the species have on average shifted upwards by 48 m in the raw data or 72 m in the sub-sampled data. This shows that even though the species are shifting their distribution, they are not perfectly tracking temperature change. Since a lag in response to environmental change is highly plausible, this is not an unexpected result.

Caution is still advised when interpreting these results as the sampling effort in 1929 is unknown. Another ectothermic group (geometrid moths) has exhibited an average increase in mean altitude of 67 m over just 42 years on Mount Kinabalu (I.-C. Chen et al., 2009) and when the whole order of Lepidoptera was analysed it exhibited a faster shift in the upper limit than the lower one on Mount Kinabalu (I-Ching Chen et al., 2011), indicating that the mean elevation shift of ectotherms happens faster in the extreme upper limits of a species. Since moths and butterflies have a faster generation time than amphibians, are not as dependent on specific water bodies or humidity conditions for breeding, and are able to fly, they would be expected to track a temperature change more closely, consistent with the slower per-year shifts observed in my amphibian data than in the moth data.

One interesting result is that the five species that have moved downwards. In particular, *L. baluensis*, appears to have shifted downwards over 1000 m. The species is quite distinct so the record from 1929 is reliable. It is a small species (with an average snout to vent length of 20 mm) that is found on the ground and breeds in streams. However, it is relatively small and lives on the forest floor, and thus could easily have been missed at lower elevations. This species has thus in reality potentially not experienced a range shift but a range contraction. It is possible that the shift in other species has increased the competition in

streams to a point where *L. baluensis* cannot compete any longer. Indeed, many of the species that have shifted upwards are stream breeders.

For the other four downward shifting species, the results appear to be the opposite of what is expected under general climate change theory. However, other studies have also reported species exhibiting a downwards movement in response to climate change (I.-C. Chen et al., 2009; Franco et al., 2006; Lenoir et al., 2010; Peh, 2016; Wilson et al., 2007). My data indicate that on Mount Kinabalu around 26% of amphibian species are moving downwards, a number similar to that reported from other studies (Lenoir et al., 2010). At least one other study of tropical amphibians, from Madagascar, has reported downward shifts. At least 10-15% of amphibian species in Madagascar shifted downwards between 1993 and 2003 (Raxworthy et al., 2008), but few studies have examined long-term elevation range shifts. The study in Madagascar was also over a narrower elevation range (1400-2876 m asl) than the surveys on Mount Kinabalu. Considering that the Madagascar data cover only ten years, it is possible that the prevalence of downwards shifts would be similar to that at Mount Kinabalu if the analyses could be performed over a longer period. I propose that one explanation for the downslope shift at Mount Kinabalu might be that species that have shifted downwards are generalist species that originally existed over multiple habitat bands, and that at higher altitudes they are now outcompeted by species that are specialised for the very specific climatic and habitat conditions at higher altitudes.

The body size results show that, in general, the larger-sized species are the ones that are shifting upwards whereas the smaller ones are contracting their range and moving downwards. Considering that amphibians have been variously reported to either follow Bergman's rule (the positive correlation between temperature and body size), show an inverse pattern, or no pattern at all (Cvetković, Tomašević, Ficetola, Crnobrnja-Isailović, & Miaud, 2009; Liu, Feng, Jin, Mi, & Zhou, 2018; Sheridan, Caruso, Apodaca, & Rissler,

2018), interpretation of these results is difficult. There are currently no studies on any of the individual species in our dataset to estimate within-species patterns of body size against temperature gradients. However, it is plausible that larger-sized species simply track a temperature shift better whereas smaller-sized species rely on microhabitat climates.

As the iNEXT results are not dependent on sampling effort, the results they provide are more robust than the centre-weighted means. They show that species are shifting towards the mid-altitude domain, with higher altitude species moving down, and some of the lower altitude species moving up. Despite this the lower altitude band still has a similar level of species richness to 1929. I propose that this is because species are moving up from even lower elevations as my “low” altitude bands lower limit is 900 m asl. Overall, the iNEXT results lend credibility to the estimated shifts of the species on the mountain over the last 80 years, because they control for survey effort, which was not recorded in 1929.

Biotic interactions have been indicated by several authors as one of the major important factors in shifts in distribution as well as community structure (Brooker, Travis, Clark, & Dytham, 2007; Hillerislambers, Harsch, Ettinger, Ford, & Theobald, 2013; Levine & HilleRisLambers, 2009; Van der Putten et al., 2010). If the species shifted purely due to temperature change the response should be cleaner with only an upwards shift. However, the results indicate that the picture is more complicated. It is plausible that temperature shifts are causing changes to the biotic interactions. Biotic interactions might aid in stabilising the ecosystem (Ives, Dennis, Cottingham, & Carpenter, 2003), and changing the interaction strengths could cause major and unexpected changes to the diversity and community structure.

Management implications within the park are currently minor. Primarily because species that have shifted out of certain habitat bands were previously also present in the habitat type they are now concentrated in. However, as the shift is expected to continue, it is

imperative to continue to monitor these communities. As the indications are that lowland communities are starting to move up into higher altitude areas, there is a need for these areas to be preserved. There is also a need to ensure connectivity between areas to facilitate movement between currently existing primary forest fragments. The potential management implications are mostly confined to areas outside of the park. Several of the sites surveyed are currently outside the park boundary, and these privately-owned areas are currently primary forest that are connected to the park. Considering their high conservation value, it is imperative that the owners are supported in maintaining them. There are several amphibian species detected in these surveys that are endemic to Mount Kinabalu, or to Borneo, and are of especially high conservation concern. Current knowledge of most of the species in the park is low with limited information on populations, life history and behaviour. In addition, vast areas of Mount Kinabalu are highly inaccessible and are likely to hold a high number of currently unknown species. Considering that biodiversity data degrade over time (Tessarolo, Ladle, Rangel, & Hortal, 2017), there are not only a need to collect data on a long-term basis but also to ensure they are analysed from multiple angles.

Conclusions

The amphibians on Mount Kinabalu are on average moving upwards, but some species are instead shifting downwards. This has caused an increase in species richness in the mid-altitude habitat band whereas richness in the high and low altitude habitat bands has decreased. It appears that the species are shifting on the mountain and the community structure is now different from 1929. It also appears that species with larger body size are more likely to shift upwards. Since these shifts are expected to continue there is a need to ensure continuous monitoring of the populations, especially since the response of the species to ongoing climate change might not be easily predictable if they have moved out of their

original habitat bands. Protection of lowland habitat close to the park is potentially of a high priority, especially to improve connectivity between primary forest fragments. I propose that future studies focus on biotic interactions between species, how these change over time, and how changes to the interaction network coupled with climate change affect the shift in the species' distributions.

CHAPTER 2. Beta-diversity of the amphibians on Mount Kinabalu indicates a shift in drivers over the last 80 years and a lag in community response to environmental change

Introduction

Beta diversity measures the variation in species diversity between sites and is computed as the ratio between the regional and local species diversity. Mean beta diversity is the diversity of species within a site (also known as alpha-diversity). Beta diversity can be due to either nestedness or turnover of species (Baselga, 2010; Dobrovolski, Melo, Cassemiro, & Diniz-Filho, 2012; Kraft et al., 2011; Schmera & Podani, 2011). Nestedness occurs when the species at some sites are subset of the species at other sites, whereas turnover occurs when sites have very distinct species from one another (Baselga, 2010). The mechanistic drivers of nestedness versus turnover can be difficult to disentangle. The degree of turnover across taxa appears to be influenced by the environment, dispersal ability, geography (including latitude) and spatial scale (Soininen, Heino, & Wang, 2017).

Amphibian beta diversity is a topic that has received little research attention, but a recent study showed that amphibian diversity is mainly characterised by turnover, independently of spatial scale (Melchior, Rossa-Feres, & da Silva, 2017). In the neo-tropics, turnover among amphibians has been strongly related to reproductive modes and altitude (Jiménez-Robles et al., 2017). It is possible that the effect of environmental variables (e.g., turnover) on beta diversity varies locally for amphibians—this was the case in a recent study on reptiles (Virgilio, Laffan, Ebach, & Chapple, 2014).

Turnover in species across sites can be due to environmental filtering, whereby the environment drives the species present at a site, or to competition between species (Dobrovolski et al., 2012). There have been indications that environmental filtering is a

driver of species diversity in general at higher elevations whereas competition between species increases in importance with decreasing altitude (Hoiss, Krauss, Potts, Roberts, & Steffan-Dewenter, 2012; Machac, Janda, Dunn, & Sanders, 2011). Environmental filtering (referred to as δ) can occur in extreme environments where the number of adaptations that are viable is reduced (Hawkins, Rueda, Rangel, Field, & Diniz-Filho, 2014; Swenson & Enquist, 2007) and can be assessed by calculating how much of the climate space is occupied by the community. In situations where recent environmental change has occurred, an additional driver of beta diversity can be environmental disequilibrium (referred to as λ), which is when a species' lag in their response to events such as a shift in temperature (Devictor et al., 2012; La Sorte & Jetz, 2010, 2012). For reasons described in Chapter 1, environmental change is predicted to have major impacts on amphibian species, and this will likely be reflected in changes to beta diversity drivers and patterns.

There is a need for studies looking at amphibian species diversity and changes to communities over time. It is important to disentangle whether nestedness or turnover are the main drivers of beta diversity for amphibians, and to assess whether environmental filtering or competition are the main drivers of beta diversity (Blonder et al., 2015; Hoiss et al., 2012). In this study I aimed to answer the following: 1) if beta diversity in the amphibian community has changed over the past 80 years; 2) if the drivers of beta diversity have changed over the past 80 years; 3) if there is evidence of environmental filtering; and 4) if there is evidence of environmental disequilibrium.

Methods

Study Site

This study was conducted on Mount Kinabalu in Kinabalu Park, Sabah, Malaysia on the island of Borneo (Lat: 6.157, Long: 116.637) between 900 to 3300 m asl. Kinabalu Park has been designated as a National Park since 1964 and a UNESCO world heritage site since year 2000. See Chapter 1, Figure 1.1 for a map of site localities.

As a baseline, a historical dataset from 1929 collected between April and May was used (M. a. Smith, 1931). In 2007 a total of 15 sites were sampled over 17 surveys between 10 May and 28 May; in 2010 a total of 18 sites were sampled over 38 surveys between 5 June and 13 July; in 2016 a total of 28 sites over 84 surveys were sampled between 2 February and 25 May (Figure 1). For specifics on data collection for visual and auditory encounter surveys (VES), refer to the methods in Chapter 1.

Statistical Analysis

The dataset was first analysed with an ordination technique to assess directionality and collinear structures between the variables for each of the recent survey years. The vegan package in R (Oksanen et al., 2015) was used for distance based redundancy analysis (dbRDA) to assess the ordination of the community in 2007, 2010 and 2016. PERMANOVA was run using beta diversity as a function of the parameter z of the Arrhenius model. In the Arrhenius model the number of species is dependent on the size of the sample area. The parameter z can then be calculated by assessing how many species are shared between two sites and how many are unique. The explanatory variables used were temperature, altitude and humidity. The sample size from 1929 was not large enough and it was excluded from the

dbRDA and the PERMANOVA. PERMANOVA assess the mean beta diversity, the diversity of species in each specific site also known as alpha diversity.

For the beta diversity analysis three different indices were calculated. The Sørenson index of dissimilarity (β_{SOR}) assesses the compositional variation between the species assemblages and takes both nestedness and species turnover into account. The pairwise Simpson index of dissimilarity (β_{SIM}) was also used because it can discriminate between nestedness and turnover, where a low value indicates higher levels of nestedness. The index β_{SIM} measures the turnover component of beta diversity. The index β_{SNE} , which is the difference between β_{SOR} and β_{SIM} measures the nestedness component of beta diversity (Baselga, 2010). The package comclim (Blonder et al., 2015) was used to calculate the level of environmental filtering and environmental disequilibrium between the years 2007, 2010 and 2016 using 1000 replicates. This is assessed by comparing the climate values of the community across time. All current climate data recorded from the sites were scaled. Extreme climate represents the expected limit of possible climate change, since the current climate record were all scaled the extreme climate was set at -1. The current response of the community is tested against this extreme limit. The data were standardized for survey effort for the years 2007, 2010 and 2016. All statistical analyses were conducted in R (R Core Team, 2017).

Results

The dbRDA did not include 1929 ass data for temperature and humidity are lacking for this year. The results (Figure 2.1) indicate that altitude, humidity and temperature all influence beta diversity but in different directions. Responses seem to be more clustered for 2007 and 2016 than for 2010. All three variables were kept for the PERMANOVA analysis.

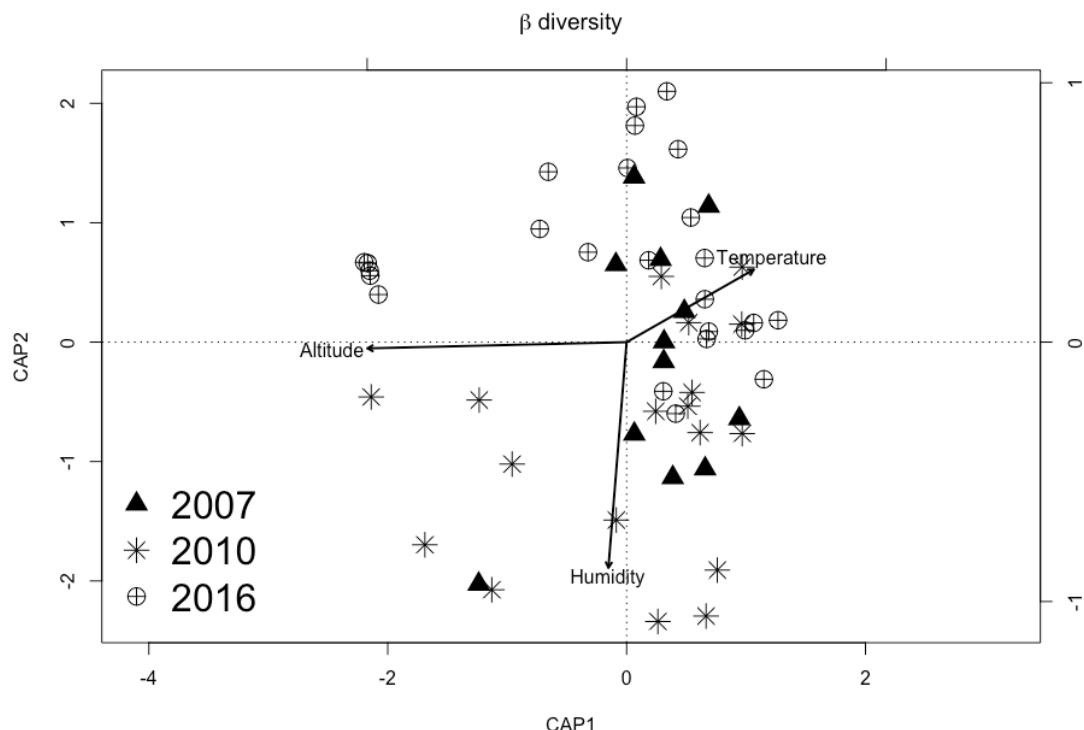


Figure 2.1. Distance-based redundancy analysis for β diversity using Bray-Curtis distance for survey years 2007, 2010 and 2016 with the variables temperature, altitude and humidity. Each point represents the average from that specific site.

PERMANOVA analysis of the dataset using altitude and year ($N = 64$) shows that year of sampling as well as altitude has a significant effect on beta diversity. When PERMANOVA was extended to include temperature ($N = 56$) and humidity ($N = 55$) (the year 1929 was excluded again here due to lack of data), it was found that temperature on its own does not have a significant effect on beta diversity but there was a significant interaction

effect between temperature and year. Humidity does not appear to affect beta diversity at all (Table 2.1).

Table 2.1. PERMANOVA models on mean beta diversity using z from the Arrhenius model.

Model (N = 64, df = 60)		
Beta diversity ~ Altitude + Year		
<i>Variable</i>	<i>R</i> ²	<i>P value</i>
Altitude	0.115	0.005*
Year	0.043	0.005*
Model (N = 56, df = 49)		
Beta diversity ~ Altitude + Year + Temperature + Temperature:Year + Temperature:Altitude		
<i>Variable</i>	<i>R</i> ²	<i>P value</i>
Altitude	0.133	0.005*
Year	0.045	0.005*
Temperature	0.012	0.602
Year:Temperature	0.029	0.035*
Altitude:Temperature	0.033	0.029*
Model (N = 55, df = 45)		
Beta diversity ~ Altitude + Year + Temperature + Humidity + Temperature:Year + Temperature:Altitude + Temperature:Humidity + Altitude:Humidity + Year:Humidity		
<i>Variable</i>	<i>R</i> ²	<i>P value</i>
Altitude	0.136	0.005*
Year	0.045	0.005*
Temperature	0.011	0.801
Humidity	0.027	0.055
Year:Temperature	0.027	0.039*
Altitude:Temperature	0.032	0.019*
Temperature:Humidity	0.016	0.706
Altitude:Humidity	0.014	0.547
Year:Humidity	0.010	0.831

The three beta diversity indices, split over habitat bands (Figure 2.2), show a potential shift in the drivers of the community. The indices in the year 1929 show that the community had a mixed structure with beta diversity driven by a combination of nestedness and turnover,

and with a variability in relative contributions of these across habitat band. In the modern survey years, however, the beta diversity is predominantly attributable to turnover.

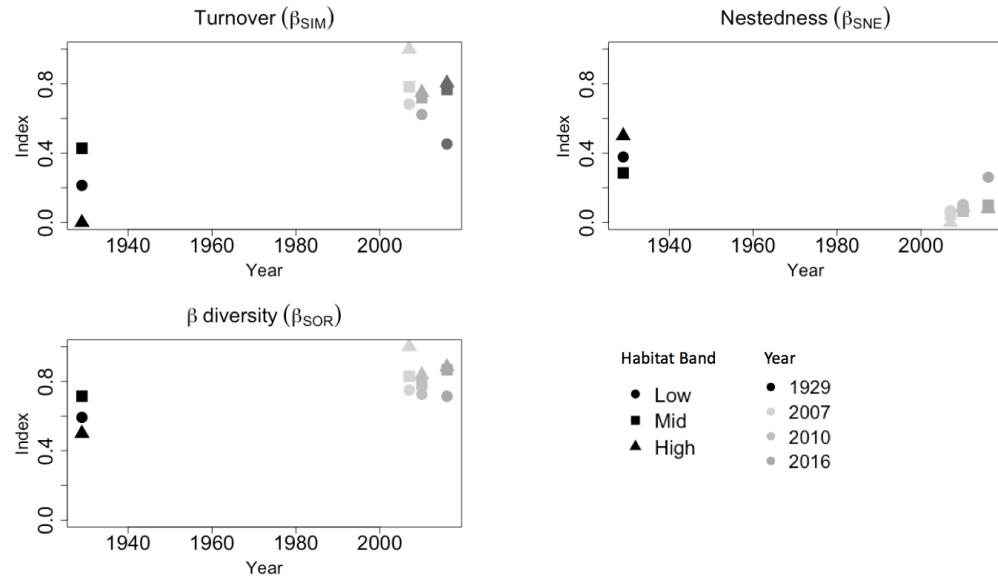


Figure 2.2. The indices β_{SIM} , β_{SNE} and β_{SOR} for each survey year and each habitat band (“low”, “mid” and “high”). β_{SIM} measures turnover, β_{SNE} measures nestedness, and β_{SOR} , the sum of β_{SIM} and β_{SNE} , measures overall beta diversity.

As there is variation in the number of sites sampled across the years, particularly in the high-altitude habitat band which only had two sites sampled in 1929, the beta diversity values were also calculated over all sites in each year (Figure 2.3). The indices in this case again indicate that a combination of turnover and nestedness drove beta diversity in 1929, but that in the modern years the driver was predominately turnover. The results also indicate that the overall beta diversity (β_{SOR}) of the amphibian community has not undergone a major shift in the last 80 years.

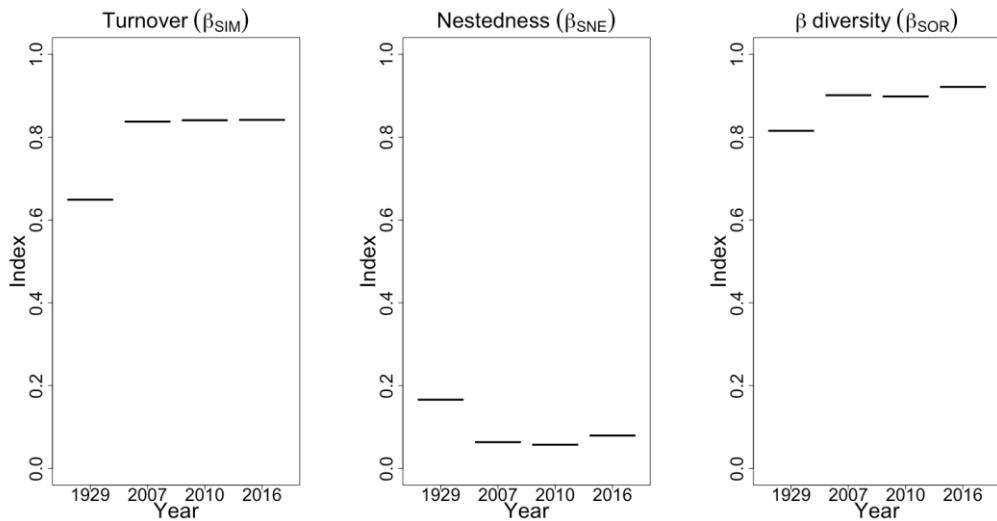


Figure 2.3. β_{SIM} , β_{SNE} and β_{SOR} index for each survey year. β_{SIM} indicates the level of turnover, β_{SNE} indicates levels of nestedness and β_{SOR} is the additive value of β_{SIM} and β_{SNE} giving an indication of overall beta diversity. Values are combined over all sites.

The community climate analysis (Table 2.2) indicate that the community is mainly structured by environmental filtering with generally negative δ values. The mid-elevation results for 2010 are an exception, where the δ value is positive. The environmental filtering in 2016, though negative, also appears to be weaker than in other years. Environmental disequilibrium (λ) also structures the community, except for the low elevation community in 2010 where the λ value is negative. Environmental filtering also appears to be weaker in 2016 than in other years.

Table 2.2. The δ and λ values from community climate analysis for the years 2007, 2010 and 2016. The δ values below zero indicate environmental filtering. The λ values above zero indicate disequilibrium in the community. The data are divided into habitat bands of low (900-1300 m asl), mid (1300-1800 m asl) and high (1800-3300 m asl).

Environmental Filtering (δ)				Environmental Disequilibrium (λ)			
Year	Low	Mid	High	Year	Low	Mid	High
2007	-0.998	-1.328	-2.734	2007	1.408	1.193	0.568
2010	-0.551	0.691	-0.789	2010	-0.464	2.424	1.572
2016	-0.330	-0.518	-0.020	2016	0.619	0.367	0.445

Discussion

There was a strong effect of altitude on the amphibian diversity on Mount Kinabalu, consistent with the results reported by other studies (Jiménez-Robles et al., 2017). The literature also reports that the drivers of diversity can follow different patterns at different spatial scales and can vary across sites (Melchior et al., 2017; Soininen et al., 2017). The spatial scale in this study is not large, but variation across altitudinal bands in both the drivers of beta diversity and the community climate analysis was observed.

The PERMANOVA analysis indicates that beta diversity change has been high over the years, between-year variance is thus relatively high. Temperature does not appear to have driven these changes. The temperature differences between these years may not have been substantial enough to affect the diversity, in which case variation in species numbers and in beta diversity in modern years could be due to other reasons, such as natural stochastic fluctuations in population numbers.

In 1929 the beta diversity appears to be driven both by species nestedness and turnover, whereas in modern years the driver is mainly turnover. This would be consistent with species shifting their locations. Turnover, where the communities within a local region are not subsets of each other, would be expected if the communities are unstable and individual species have started to shift their location. Given that the original situation in 1929 was one of relatively high nestedness, one might predict nestedness to persist into the future even if all species are shifting – but this assumes that all the species can shift their location at the same speed. However, there are inherent differences between species in physical adaptations, physiological adaptations, and demography, and thus they are expected to track environmental change at different rates.

Community climate analysis also reveal some interesting results, despite the necessary restriction to recent years (because of lack of climate data from 1929).

Environmental filtering is expected to be the main driver of community structure in extreme environments such as a mountain, and that is indeed the case in this dataset. More surprising is that environmental disequilibrium is also driving the community structure. This suggests that the community is currently not perfectly matched to the environmental conditions. As amphibians are ectothermic, the impact of not tracking temperature shifts, in terms of the physiological and metabolic effects, could be substantial (Hillman, 2009). The observed environmental disequilibrium is likely attributable to the inability of most species to track an environmental shift perfectly. A lag effect is expected in the response of most species. In line with this, it is plausible that the reason the amphibian community on Mount Kinabalu exhibits environmental disequilibrium is due to an imperfect response to shifting temperature. This is also supported by the results in Chapter 1, which showed that individual species were not tracking the temperature shift perfectly.

The beta diversity results in this chapter are interesting in the context of the results from Chapter 1, which indicated that the amphibian species are moving altitudinally on the mountain, that the larger species are more likely to have moved upwards, but that the species overall are not tracking environmental change completely. One explanation for why smaller species have not moved upwards at the same rate as larger species, is that the smaller species are using different microhabitats and have less need to move. However, smaller species are also more likely to be missed in surveys, even by experts, providing a potential confounding effect. It is interesting that altitudinal shift did not appear to be related to in Chapter 1. The relationship between reproductive traits and beta diversity was not explored in the present chapter, but in other amphibian studies reproductive traits have been identified as showing a relationship with beta diversity, turnover and altitude (Jiménez-Robles et al., 2017), and so this would be an interesting avenue to investigate in relation to the amphibians on Mount Kinabalu.

Climate has been shown to be a strong predictor for beta diversity in plants. This effect is potentially going to be less extreme for animal communities as they are thought to be affected by the structural properties of the environment that are not directly related to climate. If an animal species is not dependent on a specific plant species, the extinction of plants in the environment might have a minor effect on the animal if the structural complexity remains similar (Zellweger, Roth, Bugmann, & Bollmann, 2017). Assessing the structural properties of the environment and their effect on animal requires knowledge of the structural components a species is dependent on, such as tree hollows. Unfortunately, this approach is difficult for amphibian communities, especially in under-surveyed regions such as Southeast Asia, because information related to habitat and structural complexity requirements are severely lacking. Evaluation of space use of the amphibian communities over time would be an interesting avenue for further studies.

Conclusions

The amphibian community on Mount Kinabalu shows major changes across the altitudinal gradient. The observation that diversity varies statistically significant with year in the analyses is worrying as it indicates that sampling across years, even when the years are close to each other, is highly variable. This could be an effect of variation in search effort but is more likely due to stochastic variation in the amphibian populations. The drivers of beta diversity on the amphibian community have changed over time, from a mixture between nestedness and turnover in 1929 to mainly turnover in modern years indicating that the community structure is changing. The community also appears to be structured by environmental filtering as well as disequilibrium, indicating an effect of extreme environmental conditions and a lag effect in tracking changes to the environment. This could present an issue for persistence of the amphibians in the future if changes to the environment

strongly outpace the ability of some of the species to either adapt to it or track it. Overall, the results here are intriguing but highlight the need for continuous monitoring of amphibian populations to ensure that any management decisions are made based on real effects and not stochastic between-year variation.

CHAPTER 3. Habitat and occupancy of *Ansonia platysoma* on Borneo, Malaysia

Introduction

Ecosystems around the world have been, and currently are being, altered at an alarming rate, affecting their stability as well as increasing the risk of extinction and threat of population decline for numerous species (Ceballos & Ehrlich, 2002; Chapin III et al., 2000; Foley et al., 2005). Monitoring occupancy and distribution of species is essential for assessing effects of environmental changes in the landscape as well as promoting effective conservation actions (Jones, 2011). Tropical areas contain more species and biodiversity hotspots than temperate areas of the same size (Dirzo & Raven, 2003). Unfortunately, areas in the temperate zone are better studied and understood, with a higher number of active monitoring projects. Clearly, tropical areas need more priority and investment from both the scientific and conservation communities as well as local participation, to increase the available information about these areas (Danielsen et al., 2003; Rodriguez, 2007; Rodríguez, 2003; N S Sodhi & Ehrlich, 2010).

Southeast Asia contains four of the world's 25 biodiversity hotspots (Wallacea, Sundaland, Indo-Burma, and the Philippines) and displays very high amphibian biodiversity. Remaining tropical forests in the region are being rapidly cleared and many species are threatened with extinction (Bickford et al., 2010; J. Rowley et al., 2010; Navjot S. Sodhi et al., 2008, 2010). There is a recognized need for conservation actions related to the amphibian species of Borneo in particular, where the rates of deforestation are high due to logging for hardwood, land-conversion for oil-palm plantations and urbanization (Navjot S. Sodhi et al., 2010). This high rate of landscape modification causes population fragmentation and has major impacts on community structure of amphibians in matrix plantations (Das, 2006;

Gillespie et al., 2012; J. Rowley et al., 2010). Direct impact of fragmentation on species is often unknown and is a further impediment to the development of effective conservation plans since baseline information regarding population numbers, ecology, and life history traits is non-existent (Das, 2006; Gillespie et al., 2012; B. H. Kueh & Maryata, 2005; BH Kueh & Mohamed, 2005; Boon-hee Kueh, Kiruba-devi, Chew, & Maryati, 2007; Matsui, 2006).

There are currently 182 species (Inger, Stuebing, Grafe, & Dehling, 2018) of amphibians described from Borneo. Of these, 42 (23%) are listed in one of the threatened categories, 45 (25%) are listed as “data deficient” or have not been evaluated, only 55 (30%) are listed as “least concern” and the remaining 40 (22%) are listed as “near threatened”. The “not evaluated” category contains 34 species (20%), all of which were described in the last ten years, indicating that rate of new species descriptions in Borneo is still high. Considering the extraordinary lack of ecological knowledge of Borneo amphibian species, there is an urgent need to collect basic ecological data. This is true especially for species currently listed in threatened or data deficient categories by the International Union for Conservation of Nature (IUCN). This to ensure that any conservation actions necessary are implemented correctly and in a timely fashion (Collen, Ram, Zamin, & McRae, 2008; B. H. Kueh & Maryata, 2005; BH Kueh & Mohamed, 2005; Jodi Rowley & Alford, 2007). There is also a heightened need for further information to be collected for mountain species (Alford & Richards, 1999; Bickford et al., 2010; Navjot S. Sodhi et al., 2008), considering their increased risk of negative impacts from climate change and the lack of areas to which montane species can move to (Alford & Richards, 1999; Bickford et al., 2010). The extreme climatic conditions and habitats that exist in mountain areas promote high levels of endemism and specialist adaptations (Dirzo & Raven, 2003).

On the island of Borneo, there are 13 species in the genus *Ansonia*, consisting of

small to medium-sized (snout-vent length less than 65 mm) toads that mostly inhabit forest streams. Six of these are known from Kinabalu Park, three others have uncertain record localities (Inger et al., 2018; Malkmus et al., 2002) and *Ansonia platysoma* is the only species listed in one of the threatened categories by the IUCN (endangered). Very little is known about the life history of this species and it is currently reported from four disjunct locations: Kinabalu Park (Malkmus, 2002), Gunung Mulu National Park (Nishikawa 2013, unpublished), Crocker Range National Park (L. Lee Grismer, 2006; Larry Lee Grismer, 2016) and Ula Temburong National Park (Keller, Rödel, Linsenmair, & Grafe, 2009). The basis of the species being listed as endangered, despite all four locations being protected, is that it has only been found in primary forest areas, and primary forest areas are being rapidly cleared in Borneo. The disjunct populations indicate that each population is only able to interbreed within itself. This is putting the species at risk of genetic inbreeding. There are no known monitoring projects for this species, population status for each locality is unknown, and the specifics of habitat preferences are also unknown. Since the species was last reported from Kinabalu Park in 2010 (field surveys performed by Bickford. D. P. *unpublished*), our study objectives here were to determine a) if the species was still present in the known locations in Kinabalu Park, b) the effect of habitat variables on occupancy of this species and, c) the predicted distribution across the island of Borneo to facilitate future work on this species in other locations.

Methods

This study was conducted in Kinabalu Park (see Figure 3.1), Sabah, Malaysia on the island of Borneo (Lat: 6.157, Long: 116.637) from 900 to 1800 m asl. *A. platysoma* is only known to occur between 600 to 1300 m asl. Streams in elevations above the known elevation range were also surveyed to confirm lack of presence. Terrestrial transects were also performed in these areas to assess occupancy of the species outside of streams. The habitat on the site consists of cloud forest divided into clear vegetation zones (Malkmus, 2002): lowland dipterocarp forest (0-900 m asl); mixed dipterocarp and Fagaceae forest (900-1300 m asl); Fagaceae forest (1300-1800 m asl); cloudy moss forest (1800-3000 m asl); and sub-alpine hard-leaved plants (3000-3700 m asl).

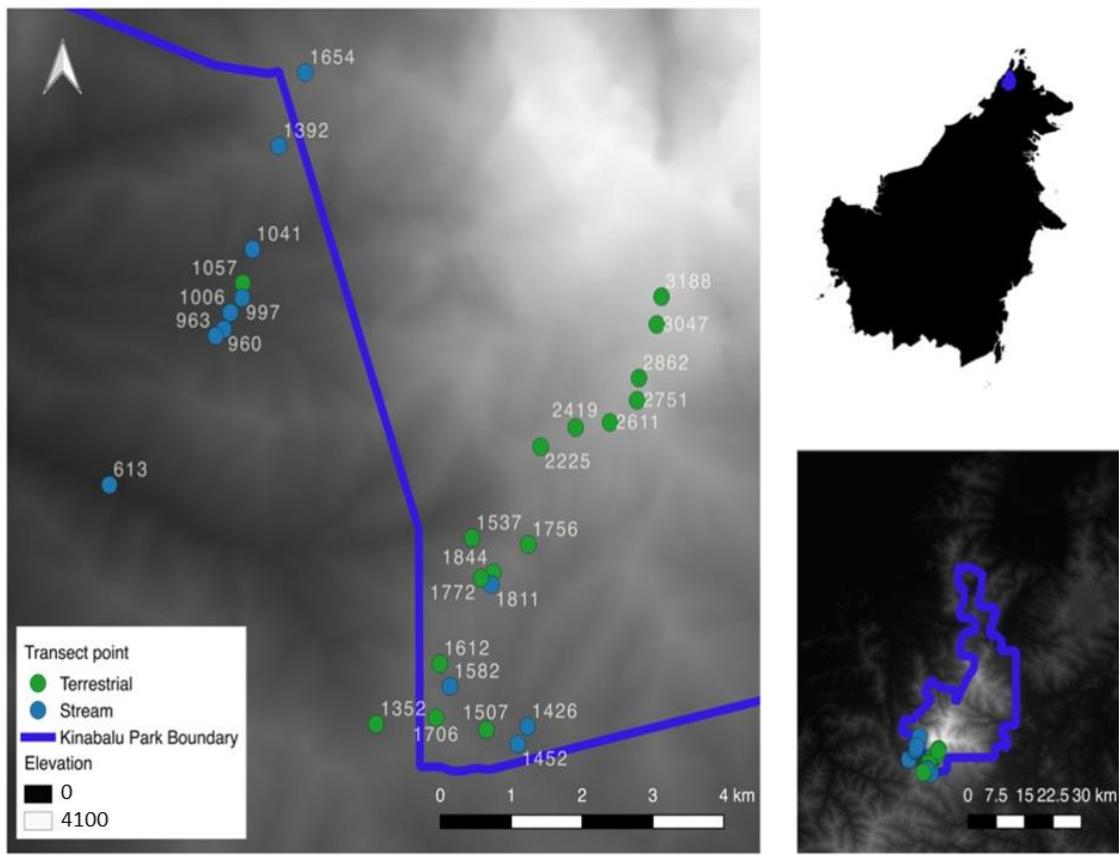


Figure 3.1. The island of Borneo (top-right panel); the location and boundary of Kinabalu Park (bottom-right panel); and a close-up of the area of the park (left panel) with locations of terrestrial sites indicated as green points, locations of the stream sites indicated as blue points and elevation given in white text next to each point. Elevation ranges from a gradient from 0 m (black) to 4100 m (white).

Between January and March 2016, 12 stream sites and 16 terrestrial sites were established. Terrestrial sites consisted of a 500 m transect that was divided into 10 sections of 50 m (Figure 3.2). At each 50 m interval, soil conductivity, soil pH, leaf litter cover (%), leaf litter depth, and herbaceous vegetation cover up to 1 m (%) for three individual 1 m² quadrats (one in the middle of the transect and two to the left of the transect), were recorded and these values were then averaged. In streams, 100 m transects were established. The shorter length of stream versus terrestrial sites was due to the higher complexity of stream sites and issues with accessibility. Streams have a higher level of spatial complexity in comparison to terrestrial sites as they contain water as well as a stream bed which, in Kinabalu Park, can

have varied substrate ranging from small pebbles up to large boulders. The stream transects were divided into 10 m sections (Figure 3.2). At every 10 m, average water depth, stream flow, width of water (the actual body of water in the stream bed), width of stream bed (the width of the stream including dry and wet areas from bank to bank) were recorded. Stream flow was measured a stopwatch by assessing how long it took a stream flow device to move over a set distance. This was performed three times at each point, giving a total of 33 measurements that were then averaged.

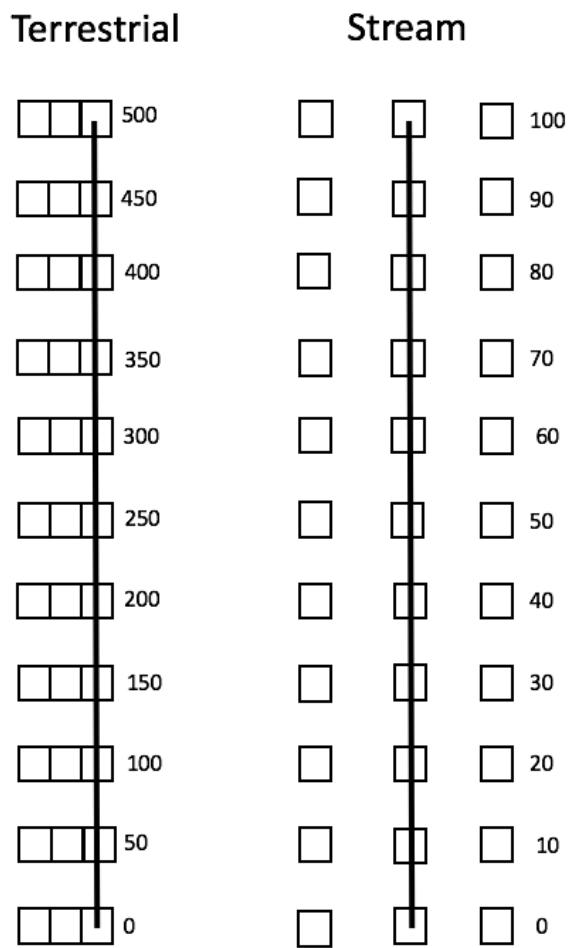


Figure 3.2. Diagram showing transect layout with terrestrial transect on the left and stream transect on the right. Black vertical lines indicate the mid-line of each transect. Black boxes indicate the three individual 1 m² quadrats where habitat variables were measured. Distance in meters from the start of each transect is given as a number on the right of each set of black boxes

In 2016 visual and auditory encounter surveys (VES) were conducted at night between 18:30 and 01:00 hrs. At each terrestrial site, a survey was conducted along a 500 m transect, covering 2 m to either side of the centre line, leading to a minimum area of 2000 m². Surveys were conducted for an active search time of 60 minutes, meaning that time for handling of individuals was accounted for to ensure standardisation between sites. At the start and end of each terrestrial survey, relative air humidity and air temperature were recorded.

The VES in each stream was conducted along a 100 m transect, covering the whole stream bed and 1 m up on the stream bank on either side and up to 2 m height, leading to a minimum area of 400 m³. At the start and end of each stream survey relative air humidity, air temperature, water temperature, and water pH were recorded. Both visually encountered and acoustically encountered amphibians were recorded. Since the AST per unit square meter is the same between streams, search effort can be standardized between streams by specifying it as a fraction of the stream with the highest search time, times the number of occasions the site was surveyed.

Additional occurrence records were sought for in the literature using “*Ansonia platysoma*” as a search term, resulting in two records. After correction to ensure only one record per grid cell, six records remained. I used R (R Core Team, 2017) for data preparation and analysis. Maps displaying environmental variables were created using the “Global Administrative Areas” from the GADM database (www.gadm.org). A total of 19 bioclimatic layers (www.worldclim.org), a soil layer (www.soilgrids.org), an NDVI vegetation layer (NOAA: <https://earthexplorer.usgs.gov>), and elevation (SRTM: <https://earthexplorer.usgs.gov>) were original used for a total of 22 environmental data layers at a resolution of 30 arc seconds. A variance inflation factor (VIF) analysis was used to detect multicollinearity between variables. A VIF works on un-centred data and identifies how multicollinearity affects the precision of a regression coefficient (C. Robinson & Schumacker, 2009). A stepwise backwards deletion of variables using a threshold of 10 for the VIF was performed. Only five layers were retained for further analysis: bioclimatic variable 15 (precipitation seasonality) and 19 (precipitation of coldest quarter), soil, vegetation, and elevation. The species distribution analysis was conducted using MaxEnt (Elith et al., 2011; Steven J. Phillips, Anderson, Dudík, Schapire, & Blair, 2017). MaxEnt finds the maximum entropy of the probability distribution by using environmental variables

and the most uniform distribution of these relative to the presence points (Elith et al., 2011; Keith et al., 2008; Steven J. Phillips et al., 2006). MaxEnt was run using linear features due to the low sample size, and it was set to produce a complementary log-log result (cloglog). MaxEnt uses an inhomogeneous Poisson process, meaning that the occurrence records it models are proportional to the abundance of the species since a higher proportion of occurrence records are expected in an area where the density of the species is higher (Steven J. Phillips et al., 2017). The cloglog produces a result specifically for data in a unit interval. MaxEnt was replicated 15 times and had a burn-in period of 5000 iterations. A 150 km buffer zone was used for training with 10,000 background samples. This was for increasing the likelihood of obtaining true absences, as the region covered by a buffer of this size includes areas that have been part of previous amphibian fieldwork. Since five of the occurrence points were from locations very close to each other the sixth point from Gunung Mulu National Park was used as a testing point to assess how well the model performed. Some recent studies have advocated the use of a stepwise backwards deletion process to attain a better fitting model (Zeng et al., 2016). However, since MaxEnt equates to a multinomial logistic regression and together with technical concerns about stepwise backwards deletion (Thompson, 1989, 2001; Whittingham, Stephens, Bradbury, & Freckleton, 2006), only VIF was used for environmental variable selection. MaxEnt was evaluated using area under the curve (AUC) and true skill statistic (TSS). AUC values are judged as fair in the range 0.7-0.8, good in the range 0.8-0.9, and over -fitted if greater than 0.95. Values below 0.7 are indicative of a poorly fitting model. The AUC measures the discrimination and the ability of the model to correctly assess where the species should be present (L. Zhang, Liu, Sun, Wang, & Wang, 2015). The TSS evaluates both omission and commission errors and corrects for dependence on prevalence (Allouche, Tsoar, & Kadmon, 2006). Values of TSS less 0.4 are considered poor; values in the range 0.4-0.8 are fair, values in the range 0.8-0.95 are good;

and values above 0.95 indicate potentially over-fitted models (L. Zhang et al., 2015). Ensemble models using both presence and absence points were also performed. Ensemble modelling combines different models that perform well giving an average prediction. The ensemble was performed with surface range envelope, classification tree analysis, random forest, multiple regression splines and flexible discriminant analysis. The ensemble delivered only TSS values above 0.95, indicating over-fitting, so were excluded from any further analysis or discussion.

Density of Individuals

The impact of habitat variables on the number of individuals of *A. platysoma* being detected was assessed. As there were no records of *A. platysoma* in any of the terrestrial sites, only the stream data were analysed giving a total sample size of 35 records across 12 different sites with 2-4 surveys per stream. The habitat variables collected from the streams were assessed for collinearity using Spearman's test of correlation, leaving seven variables in the analyses (Altitude, Slope, Depth of Water, Width of Stream, Water pH, Bank Width and Canopy Cover). The data were fitted with a generalised linear mixed model (GLMM), first with a Poisson and then a quasi-Poisson family. The GLMM model showed issues with spatial auto-correlation so the data were analysed using a general least square (GLS) model that accounted for spatial autocorrelation. A total of 17 ecologically meaningful models were selected through an information theoretic approach. The models were compared with Akaike information criteria (AIC) to select the best one.

Occupancy

The presence and absence of *A. platysoma* and the effect of habitat variables was assessed. Out of 12 streams that were surveyed, 5 were occupied. The occupancy data was

analysed using a generalised linear mixed model (GLMM), but this model would not converge with any of the link functions suitable for binomial data, and thus it was excluded from any further discussion.

Results

Species Distribution Model

The MaxEnt results indicate numerous places where the environment is potentially suitable for *A. platysoma* (Figure 3.3). The model had an AUC value of 0.77 and a TSS of 0.74. The model accurately depicts the presence of *A. platysoma* in Gunung Mulu National Park (purple square, Figure 2.3) and indicates areas that could also be suitable for *A. platysoma*. The white squares indicate sites where *A. platysoma* was detected, but the model also indicate other areas with a very high probability of presence of the species on the other side of Mount Kinabalu (Figure 3.3, arrow 5), an area that is rarely visited. In addition, Mount Taman Negara (Figure 3.3, arrow 4) is also predicted to have a high probability of presence of the species. Crocker Range National Park (Figure 3.3, arrow 1) potentially has the correct conditions for the species to be present (the species has been reported from the park without specific locality information). The model failed to predict species presence at Ula Temburong National Park in Brunei (Figure 3.3, arrow 3), despite the species being reported there. This error could be due to locality data available for training only being present from higher elevation areas above 1000 m asl. Another interesting site of note is Truss Madi (Figure 3.3, arrow 2) – there are no official reports of the species from this park, but the model shows areas with very high probability of presence. Several areas between the Crocker Ranges and Gunung Mulu are also indicated as having a high probability of suitable conditions for the species to be present.

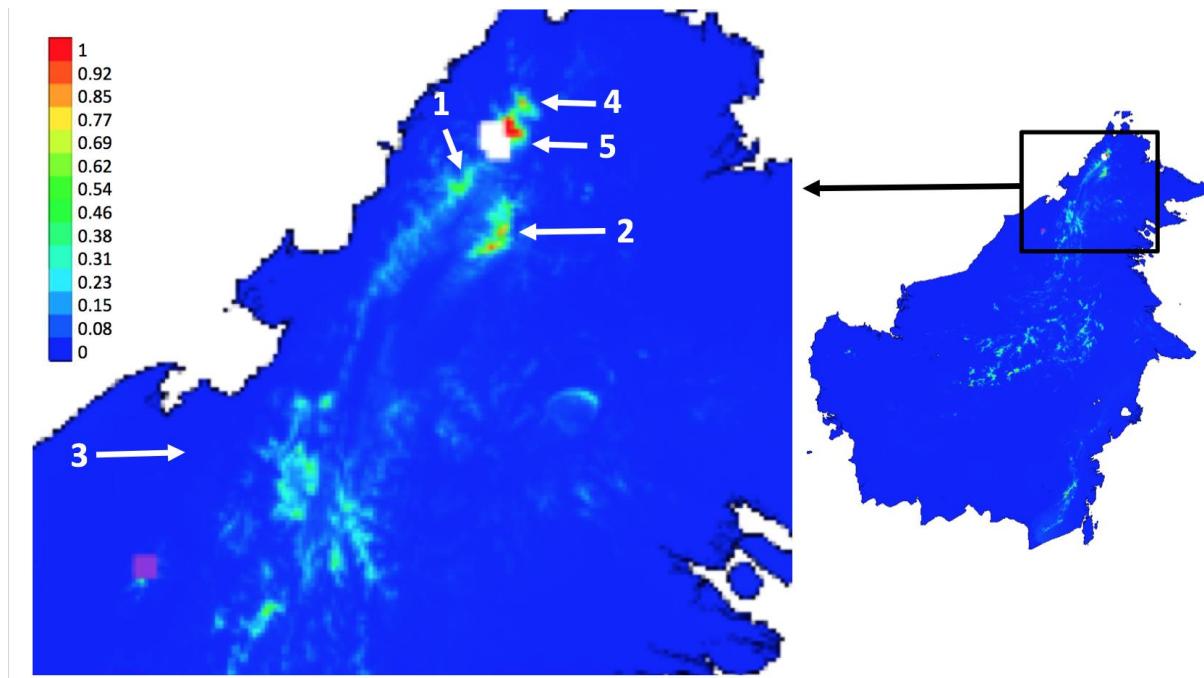


Figure 3.3. MAXENT model for *A. platysoma*: warmer colours show areas where conditions are predicted to be more suitable. White squares indicate points used for training and the purple square indicates the point used for testing. Colours range from dark blue (0; zero is a low probability of presence) to red (1; 100% and a very high chance of presence). White arrows indicate areas of note: arrow number 1 indicates Crocker Range National Park; arrow 2 indicates Trus Madi Nature Park; arrow 3 indicates approximate area where Ula Temburong National Park is located; arrow 4 indicates Mount Taman Negara; and arrow 5 indicates Mount Kinabalu.

Soil has the highest gain on model performance during training with altitude having the second highest contribution. Soil also decreases the gain the most when omitted (Figure 3.4). For testing of the model, the highest gain is achieved with altitude, and omitting soil increases the gain the most (Figure 3.5).

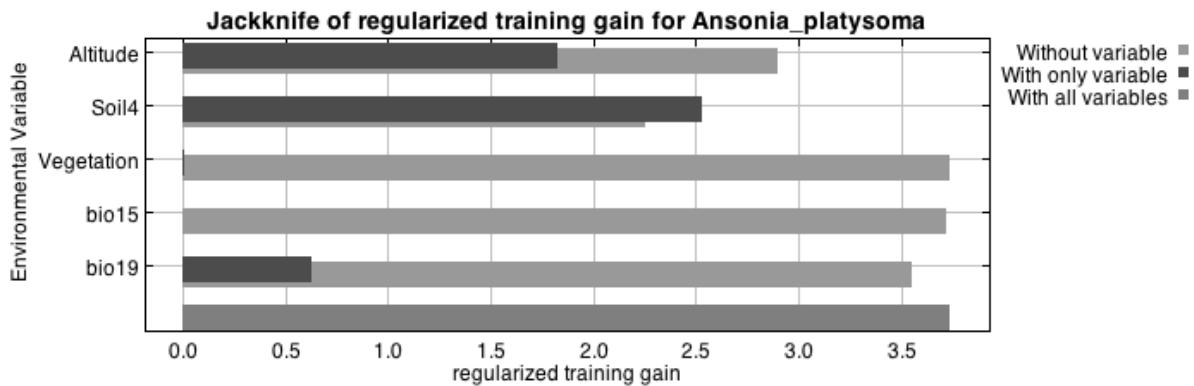


Figure 3.4. Jack-knife results of training data.

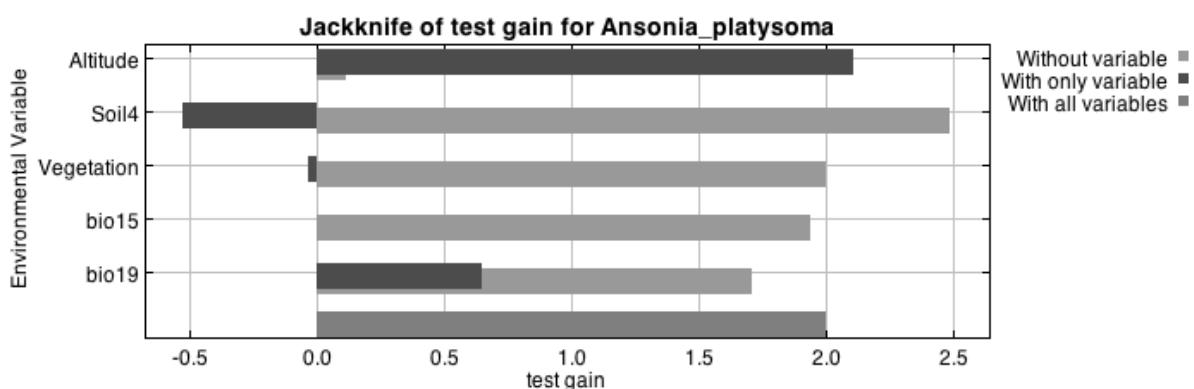


Figure 3.5. Jack-knife results of test data.

Soil is the variable with the highest percent contribution to the MaxEnt model, followed by altitude. There was a very low contribution of bio19 (precipitation of the coldest quarter) and bio15 (precipitation seasonality), and no contribution by vegetation. Altitude also has the highest permutation importance, followed by bio19 (precipitation of the coldest quarter), with the remaining variables showing no importance (Table 3.1).

Table 3.1. Estimates of relative contribution to the MaxEnt model.

Variable	Percent	Permutation
	contribution	importance
Soil	49.2	0
Altitude	43.5	88.9
bio19	5.7	11
bio15	1.6	0
Vegetation	0	0

Density of Individuals

The number of individuals detected against habitat variables was assessed. A total of 33 individuals were detected in five of the 12 streams, mostly (91%) on leaves above the ground (mean = 63.6 cm; range =[1 cm, 100 cm]); the other three occurrences were on the ground. The final GLS model had an AIC score of 55.7 (Table 3.2) and contained only two variables, water pH and water depth, without statistical significance. The number of individuals found in a stream is potentially affected by water pH and the depth of the water: more individuals tend to be found in streams with a lower pH.

Table 3.2. Results for generalized least squares model for number of individuals detected.

Variable	Value	Standard error	t value	P value
Water pH	-0.805	0.968	-0.832	0.427
Water depth	-0.055	0.071	0.778	0.457

Occupancy

Habitat variables were collected during surveys and the effect of these on the occupancy of *A. platysoma* was evaluated. The effect of habitat variables on the presence and absence (rather than density) of *A. platysoma* was assessed using GLMMs. However, the models would not converge due to the low sample size so only qualitative results are reported. *A. platysoma* was not found in streams above 1400 m asl (Figure 3.6a), and most toads were in streams around 1000 m asl. Slope (Figure 3.6b) apparently does not influence species presence (mean: 29.85 m, range: [7 m, -125 mm]). Water pH has some effect on presence, as the species was only recorded in streams with a pH above 7 (Figure 3.6c and Figure 3.7). It appears that the presence of the species is more likely in wider streams (Figure 3.6d), and despite the water depth not having a significant effect there appears to be an increased presence in streams with an average depth over 15 cm (Figure 3.6e). Canopy cover does not appear to influence species presence (Figure 3.6f)

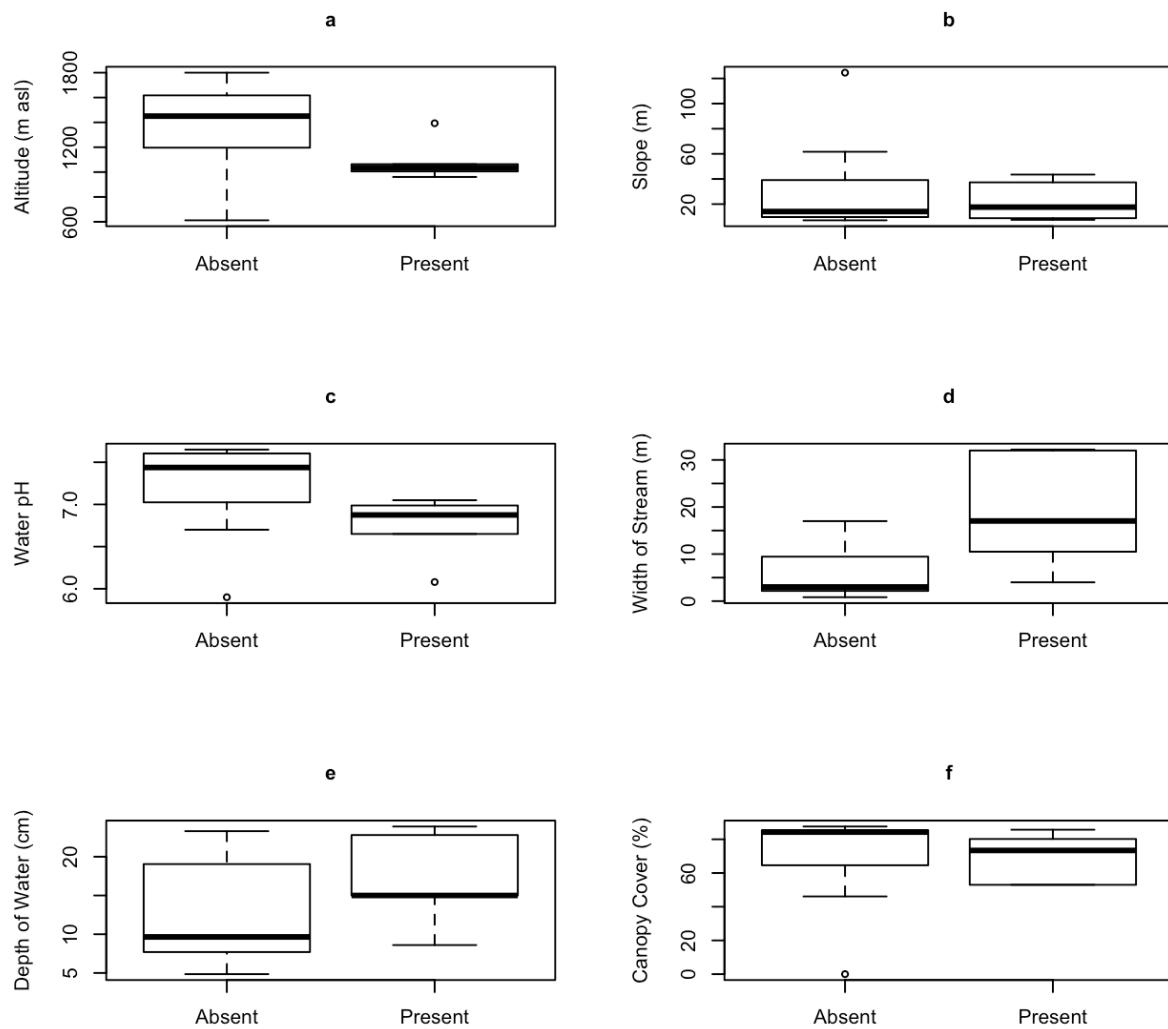


Figure 3.6. Habitat variables of altitude (a), slope (b), water pH (c), width of the stream (d), depth of the water (e), canopy cover (f) versus presence/absence of *Ansonia platysoma* in streams.

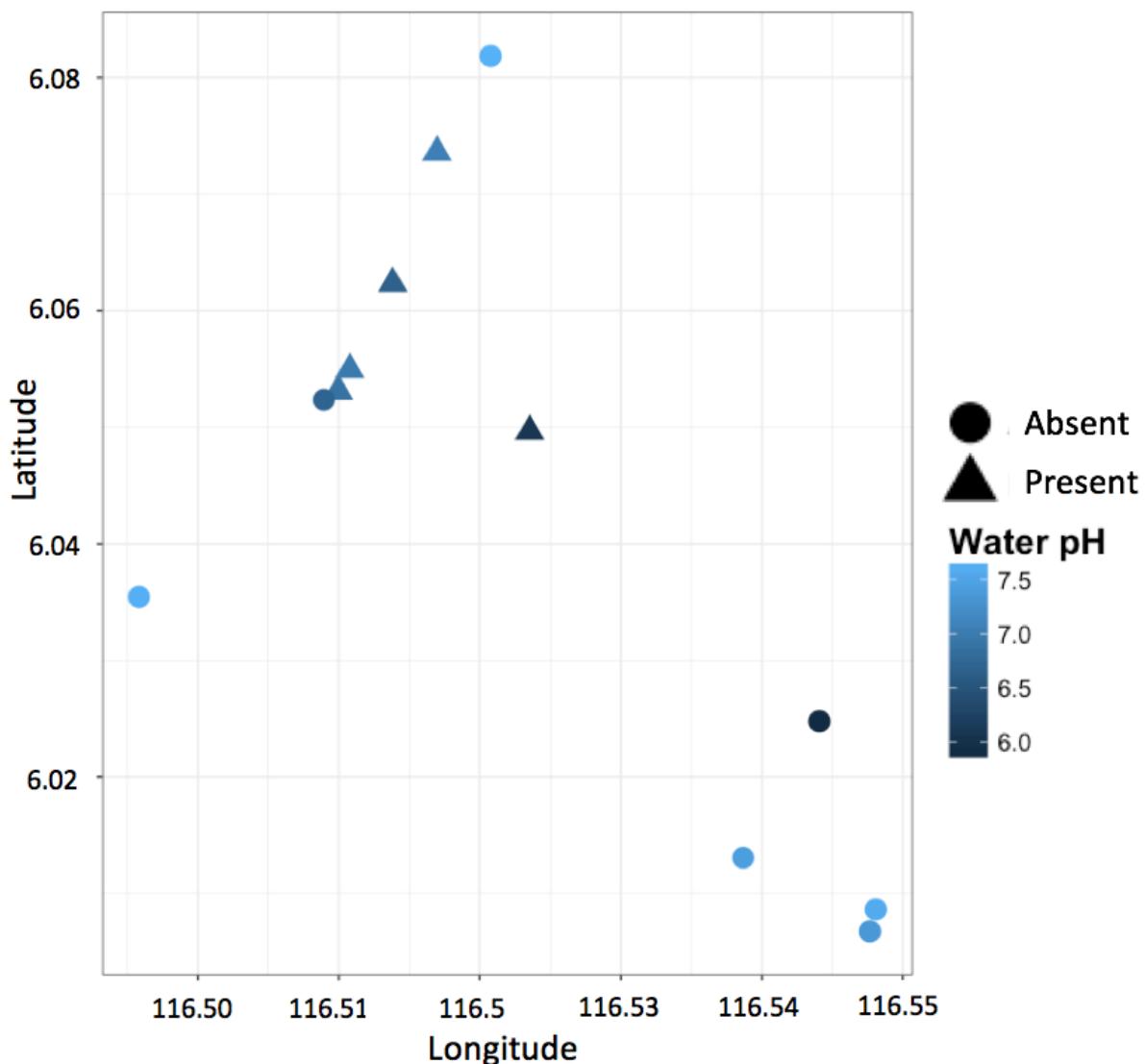


Figure 3.7. Distribution of the streams surveyed, indicating which have *A. platysoma* present (triangles) or absent (circles), and the average pH recorded for the stream (colours).

The presence of *A. platysoma* in several streams severely disturbed by flash floods in 2015 is also confirmed. Three out of the five sites where the species was present were severely disturbed in the floods and are still completely devoid of vegetation a year after the original flash flood events (Figure 3.8).



Figure 3.8. Pictures from four of the streams where *A. platysoma* is present. The top two panels indicate streams that have not been disturbed in flash floods and have vegetation in the stream-bed and moss and algae on the rocks. The bottom two panels show two streams where disturbance by flash floods occurred in 2015 and where there has still not been a full recovery of vegetation, moss or algae cover on the rocks in the stream-bed. The white number in left corner of each panel indicates the average width of the stream-bed in metres.

Discussion

Several environmental variables appear to affect the distribution of *A. platysoma*. The results show that there is a high chance of the species having a wider distribution than is currently known. The predicted distribution maps indicate several locations where environmental conditions are suitable for the species. Altitude appears to be the best predictor and soil might have some effect. Biologically, this is potentially because soil impacts the conditions in the streams as well as the surrounding habitat. Soil conditions affect the type of plants that grow in an area (John et al., 2007; Palmiotto et al., 2004), which have downstream effects on canopy cover as well as temperature and humidity conditions. Due to the low number of location points currently available for analysis, the results should be viewed with caution, specifically since the species is known to occur in areas of lower elevation than the model predicts (e.g., Ula Temburong National Park in Brunei). These results do not show that the species is conclusively present in predicted areas, but that it has a higher potential of being present there. Due to the low number of locations from which this species is currently known, it is important to search for any other locations where it is present. The species distribution map provides locations that should be targeted for surveys as they have a higher probability of presence and surveys targeted there are likely to be more successful than randomly targeted surveys.

The density of individuals detected appears not to be affected significantly by any of the habitat variables – even by those variables that successfully predict presence – absence . However, water depth and water pH were both retained in the final model. Considering that only 12 streams were available to sample and only five streams had the species present, the lack of statistical significance is not surprising. The species appears to prefer slightly deeper water (over 15 cm) and slightly acidic streams (below a pH of 7). Acidity has been shown to have a large impact on amphibian larval development as well as altering the conditions of the

stream itself. Low pH changes the interaction with chemical compounds found in the substrate of streams, for example by increasing the release of toxic compounds, as well as negatively affecting algal growth and therefore altering food conditions in the streams (Glos, Grafe, & Linsenmair, 2003). The acidity values of the streams in this study are not extreme but it would be interesting to investigate the range of pH values the species can persist in, since low pH has been shown to induce rapid adaptation, including selection for larger body size, in other species (Glos et al., 2003; Räsänen, Laurila, & Merilä, 2003).

The probability of stream occupancy by *A. platysoma* increases with width of the stream. However, among the streams in which the species is present, the density of individuals is greater in the narrower streams. This is expected because the streams with very wide banks tend to have very low levels of vegetation and the species is found on the leaves of plants most the time. This leads to an overall non-linear effect of trend stream width, but this result is tentative due to the low statistical power in the model. Stream width was collinear with bank width although it was not collinear with the width of the water, meaning that the dry areas of the stream bed tend to increase with the width of the stream from bank to bank, whereas the width of the water does not necessarily increase in the same ratio. The slope of the stream (collinear with stream flow) does not appear to affect the number of individuals detected, which is surprising given how water speed would normally be expected to have a substantial impact on tadpoles (Keller et al., 2009; Konopik, Steffan-Dewenter, & Grafe, 2015). However, it is possible that due to low sample size it is simply not possible to detect an effect of slope. Altitude does not appear to affect the number of individuals detected, indicating that despite altitude being correlated with variables such as air and water temperature, there are other more important factors that affect the number of individuals. Altitude also did not appear to strongly affect presence or absence, although the species does not occur in streams above 1400 m asl. The lack of a significant effect of altitude on

occupancy and density is in stark contrast to the MaxEnt model. MaxEnt picked this out as a factor that had the most effect on where this species occurs and where it can be predicted to occur. MaxEnt is however, known to perform relatively well with a low sample size which potentially accounts for the difference in results between the two modelling approaches.

A. platysoma is absent in streams above 1450 m asl. At these altitudes, the environmental conditions are potentially not suitable for the species, even though the associated variables were not identified by the models. Alternatively, *A. platysoma* may be outcompeted by other species occupying a similar niche (e.g., stream breeding) above this altitude. *A. platysoma* prefers streams over 15 m wide (up to 32 m). However, the density of individuals found in the streams does not increase linearly with stream width but appears to be highest in the streams closest to the lower end of the range. The results also indicate a preference for streams with slightly acidic water: the species was not present in streams with a pH over 7. The streams where *A. platysoma* is absent, despite suitable pH, functionally look very different from the streams where it is absent as they are much narrower and have high canopy cover and high density of understory plants. Despite the species tending to be present more often in streams that have no vegetation in the stream bed, the highest densities of individuals were found in streams with some vegetation in them.

A. platysoma was also observed in three streams that were disturbed by flash floods after the 2015 earthquake. This indicates that although the species has previously been reported only from primary forest, it can tolerate some degree of disturbance to its breeding habitat. Streams affected by flash floods still have a very low level of visible siltation, which might be why the species is present despite the effect on the streams from the flash floods being severe. That siltation has a generally negative effect on amphibians has been shown previously: in particular the establishment of plantations has a major impact on the sediment levels in stream water (Malmer, 1996) and high levels of sediments in the water can have a

detrimental impact on tadpoles (Gillespie et al., 2012; Wood & Richardson, 2009). If logging or other types of damage occur upstream it can cause siltation in the waterways, which in the case of *A. platysoma* might be the reason that the species is normally only found in primary forest habitat.

I am aware that this species might be part of a species complex. Verification photos of all individuals are available upon request in case this species is split into multiple ones in the future and identification can be performed upon morphological features.

This Chapter is complimentary to the community-level data Chapters 1 and 2, in the sense that looking at an individual species rather than the community can yield considerable insight into the specific requirements of that species. Multi-species surveys are still advocated if general surveys are performed to maximise the use of the ensuing data, since the species-level data can be extracted from it. *A. platysoma* is endangered with very little information available and if it is also shifting its distribution, as several other species appear to be doing (see Chapters 1 and 2), it is important for more in-depth species-specific analysis to assess whether there is a need for species-specific management rather than community-level management.

Conclusions

A. platysoma is still present in Kinabalu Park: the species persists despite recent flash floods affecting many of the streams where it is found. There are many other locations not yet surveyed where the species could occur, especially in Kalimantan. Because habitat variables such as water pH predict presence/absence of this species, its distribution is potentially better explained by environmental variables than competition with other species.

Considering the lack of information available for this species (e.g., on life history, ecology, behaviour, etc.), further work on this species is recommended to enable better conservation management strategies.

CHAPTER 4. Co-occurrence and connectivity in a tropical mountain amphibian community

Introduction

Despite several studies predicting that climate change, specifically changes to the temperature, will have major impacts on the distribution of species, there are now numerous reports indicating that changes in the interactions between species might be more influential than changes to abiotic factors such as temperature (Lathlean, McWilliam, Pankhurst, & Minchinton, 2017b; Valiente-Banuet et al., 2015), rainfall (Laurindo et al., 2017) or spatial heterogeneity (Swift, Rodewald, & Senner, 2017). Changes in abiotic variables are thought to affect the biotic interaction network. Changes to the biotic network could potentially exacerbate the effects of changes to abiotic variables such as temperature. The effect of changes to abiotic variables and their effect on taxa is therefore potentially beyond what single-species models can predict (Laurindo et al., 2017; Loughnan & Gilbert, 2017; Swift et al., 2017). Individual species generally have a very small effect on other species, leading to the conclusion that any major impacts on the community comes from a few species with high impact (Berlow, Navarrete, Briggs, Power, & Menge, 1999). These conclusions are often drawn from studies that look at the relationships between trophic levels since these are easier to study than competition within a trophic level (Desjardins-Proulx, Laigle, Poisot, & Gravel, 2017; Leach et al., 2017; A. Robinson, Inouye, Ogilvie, & Mooney, 2017).

Biotic interactions are highly likely to change in nature with changing temperature (Davis, Lawton, Shorrocks, & Jenkinson, 1998; Gilman, Urban, Tewksbury, Gilchrist, & Holt, 2010; Tylianakis, Didham, Bascompte, & Wardle, 2008; Wittman et al., 2014). If the main reason for a species to shift its range is change in temperature, then the response by the community can be predicted from an individual-species level. However, if the impact of the

temperature change are shifts in interactions then community responses cannot be predicted from individual species' responses (Lathlean et al., 2017b). It is therefore imperative that monitoring studies follow not only individual species, but also the community and assess the community-level response. Models predicting changes in species distributions have been shown to be more accurate when biotic interactions are considered (Gavish et al., 2017; Guisan & Thuiller, 2005; Heikkinen et al., 2006). Studies integrating biotic interactions in their models also report larger differences in predicted future distributions, than studies using only abiotic variables (Gavish et al., 2017; Leach et al., 2017; Pollock et al., 2014; Song et al., 2017; Vitale, Lorenzetti, Francesconi, Attorre, & Di Traglia, 2017).

The number of interactions in a community is not directly linearly related to the number of species present (Valiente-Banuet et al., 2015). The implications are that the loss of one species can lead to losses of interactions that significantly impact the function of the ecosystem (de Ruiter, Wolters, & Moore, 2005; Fortin et al., 2005; Ripple & Beschta, 2004; Ripple, Larsen, Renkin, & Smith, 2001; Valiente-Banuet et al., 2015). It also means that changes in interactions might also destabilise the functioning of the ecosystem, even if only a few species are directly involved (Ripple et al., 2001). Calculating the direct effect of loss of species in an ecosystem if the number of and the strengths of the different interactions in the community has not been quantified becomes difficult (Valiente-Banuet et al., 2015). Biotic interactions are also thought to disappear faster than species themselves, i.e., interactions can disappear without the species involved going extinct, if, for example, a change occurs in the environment that reduces the number of individuals in the landscape below a minimum required threshold for the interaction to be maintained (Evans, Pocock, & Memmott, 2013; Valiente-Banuet et al., 2015).

Interaction loss has the potential to accelerate or delay the extinction of species making it a key area in need of research for both conservation and understanding ecosystem

function (Valiente-Banuet et al., 2015). Within a taxonomic group (e.g., amphibians), it can be presumed that the most common interaction between species is competition. If two species are positively associated with each other (they tend to be present at the same site), it can be presumed that they have evolved niches that are sufficiently different to minimise competition for resources available; whereas a negative association between two species indicates the opposite (Liow et al., 2017). This balance can shift if there are changes to the environment. Discussions regarding the stability of communities often focus on the diversity of these communities, whereas it is the composition and the interaction network that impacts these and gives insight into the mechanisms behind this diversity (Ives et al., 2003). The changes of interactions in ecosystems are therefore of high importance. It is possible for the function of an ecosystem to degrade at a faster rate than the loss of interactions (Hillerislambers et al., 2013; Valiente-Banuet et al., 2015).

The predominant type of interaction in ecological communities are competition and predation. In amphibian communities, both are expected to occur between species. For instance, there is competition between tadpoles for food (algae growth) in streams, and there is potential predation of smaller tadpoles by larger tadpoles (P. J. Morin & Johnson, 1988; Wilbur, 1970). Resource competition between adults is also expected for breeding areas. For species such as *Kalophryalus baluensis* and *Nyctixalus pictus*, availability of tree holes is a limiting resource (Grosjean, Delorme, Dubois, & Ohler, 2008; Karlsson, Mitchell, Imbun, & Bickford, 2018). Changes to the structure of the interaction network can have major impacts as they have an impact on the balance in the competitive networks. If changes to the temperature allows one species to grow faster than another, the nature of the two species' relationship might change from a competition-for-food interaction to a predator-prey interaction (Wilbur, 1970). In some cases, changes affecting amphibian populations might be due to shifts in species from other taxonomic groups (i.e., snails). This might have a positive

or negative effect on the success rate of amphibians. Within groups such as snail, some species suppress harmful algae growth which might have a positive effect (Brönmark & Rundle, 1991), whereas species that negatively affect the availability of necessary algae resources for tadpoles could have a detrimental effect (Shine, 2003). Changing environmental conditions could also have a direct effect on adult amphibians and their competitive ability by affecting the availability of food resources; insects have already been shown to shift their distribution on the mountain and since insect are a major food resource for amphibians this could have detrimental effects if the wrong species of insect shift their distribution (I.-C. Chen et al., 2009; I. Chen et al., 2010).

Currently the knowledge of species-interactions and how these vary temporally in different types of ecosystems is poorly known, with most studies performed on plant-pollinator networks (Laurindo et al., 2017), insects (Robinson *et al* 2017), and aquatic systems (Alexandridis, Dambacher, Jean, Desroy, & Bacher, 2017; J. E. Kemp, Linder, & Ellis, 2017; Rodrigues-Filho, Gurgel-Lourenço, Lima, de Oliveira, & Sánchez-Botero, 2017; Wulff, 2006). Amphibians are one of the most threatened vertebrate groups, with IUCN reporting that around 30% of currently known species are threatened with extinction (IUCN, 2009). Because amphibians are ectothermic, the ability of species to cope with changes in ambient temperature will greatly influence their adaptive response and in turn, affect their geographic distribution in the face of climate change. Amphibians and their interaction networks are relatively understudied in comparison to other taxonomic groups, even though amphibians are one of the vertebrate groups threatened with the highest levels of extinction. In this study, the co-occurrence between amphibian species on Mount Kinabalu in Borneo was investigated, with the aim of 1) establishing if there is a co-occurrence network pattern between species; 2) establishing whether negative co-occurrence patterns between species exist; 3) assessing co-occurrence between species together with abiotic factors; and 4)

assessing which of several co-occurrence analysis methods work the best for this amphibian community.

Methods

This study was conducted on Mount Kinabalu in Kinabalu Park between February and June 2016. This study used the visual encounter survey and habitat survey data from 2016. The data used were the number of individuals detected for each species in each site. For specifics on study site and visual and auditory encounter survey protocol, see the methods section of Chapter 1. In this chapter, references to “interactions” or “connection” means that the species was occurring in a site or in a survey, and the interaction and connection is due to co-occurrence with other amphibian species. The data used for the analysis are discrete counts, and survey effort differs depending on how many times a site was visited; in addition, there was variation in site size among stream sites and between stream sites and terrestrial sites (terrestrial sites were all the same size). Search time was standardised per unit square metre and survey effort is thus defined as a function of search area relative to terrestrial sites multiplied by the number of occasions a site was surveyed. Survey effort for terrestrial sites was simply the number of times it was visited. For environmental data, average site altitude in the site (m), average humidity (%) between the survey visits and average temperature ($^{\circ}\text{C}$) between survey visits were used.

Network Analysis

Networks of species co-occurrence can yield information on changes and variation in a community and the interactions in the community (Poisot, Canard, Mouillot, Mouquet, & Gravel, 2012). The packages iGraph (Csardi, 2006) and metacom (Dallas, 2017) in R were used to assess networks of co-occurrence between sites, species and density of individuals.

Probabilistic Co-occurrence Analysis

Co-occurrence between species was assessed to establish whether any species impacts the probability of presence of another, either negatively or positively. A probabilistic analysis of co-occurrence was used to assess pairwise interactions because this method produces lower rates of Type I and Type II errors (Griffith, Veech, & Marsh, 2016; Veech, 2013). This model does not use randomisations or simulations to assess what the frequency of co-occurrence is in relation to the observed value. It achieves this by assuming that the co-occurrence at a site is the same as the frequency of occurrence across all of the sites (Veech, 2013).

Joint Species Distribution Modelling

Joint species distribution modelling (JSDM) allows the incorporation of environmental data and accounts for the effects of other species and their co-occurrence. A multivariate distribution model, generalised joint attribute model (GJAM) using the gjam package (Clark et al., 2017) was used. The GJAM is probabilistic but is also able to incorporate survey effort, account for different data types, and incorporate environmental data. The GJAM can handle a high number of zeroes in the data and uses a zero-inflation matrix to account for both detection rate as well as a zero being a true absence.

Environmental variables were scaled. They were also centred to allow inclusion of interaction terms in the model. Without centring on a mean of zero some degree of collinearity would be included. Squared terms were included, without centring the squared predictor would be related to the unsquared term making the results difficult to interpret. The MCMC repeat for the GJAM was set to 500,000 iterations and a burn-in of 10,000. The data were trimmed for species occurring in at least two sites, resulting in a total of 25 sites with 24 species.

To the best of my knowledge, the probabilistic co-occurrence model and GJAM do not produce statistics for evaluation of model performance (such as AIC, AUC or TSS), and so for GJAM model performance was assessed from plots that evaluate convergence as well as prediction performance against observed data. Performance was also assessed according to the following pre-defined criteria: i) negative interactions were expected between certain species that rarely occur with other species (e.g., *Philautus amoenus*, which only ever occurs together with *P. saueri*); ii) many random interactions were expected to be present; and iii) no species were expected to have all positive co-occurrences, this would be a cause for concern over model performance as no species is present in all sites. All statistical analyses were performed using R (R Core Team, 2017).

Results

Network Analysis

For the network analysis, the term “connected” refers to a species presence at a site and therefore sharing connections between sites and the other species present at that site. The network analysis indicates that the number of species connected through occurrence to a single site decreases with increasing altitude (Figure 4.1). This is in concurrence with the results from Chapter 1. At higher altitudes, there are fewer species, but each is connected to several sites. The site at 3300 m asl and the one at 3100 m asl had no species recorded. The sites between 2200 to 2900 m asl had only one species recorded. The sites at an elevation of around 1000 m also display species that are not connected to other sites or connected to few other sites. Mid-elevation sites from 1300-1600 m have a high number of species connected to a high number of sites.

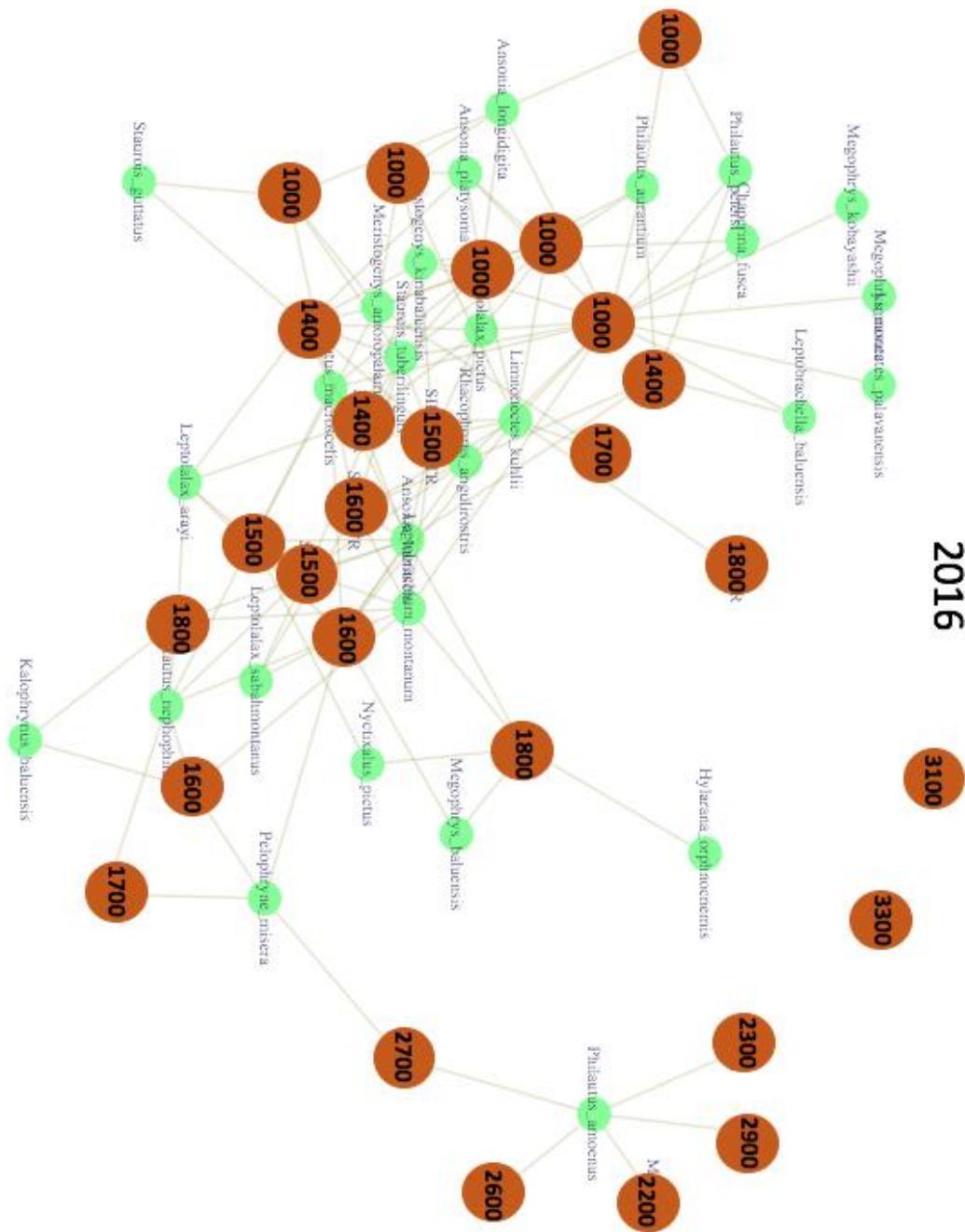


Figure 4.1. Network analysis with a Fruchterman-Weingold layout. Species nodes are in green and site nodes are in orange circles with the site altitude written inside. A connection from a species to a site (yellow line) indicate that a species was present at that site.

Co-occurrence interactions between species appear to be highly complex (Figure 4.2).

In Figure 4.2, nodes, indicated by yellow circles, are species, whereas grey lines are the connections between species. The layout of the graph places nodes that share more connections closer to each other and the size of the nodes is relative to the total number of individuals that were found of that species. The main network has a few species in the middle which appear to have a higher density of individuals and are well connected. Species with low density mostly appear at the edges of the network. One outlier species (*P. amoenus*) has the highest density of individuals and is connected to only one other species. This In the high-altitude sites, this is almost exclusively the only species present (apart from sporadic occurrences of *Pelophryne misera*). The high density of *P. amoenus* is driven largely by just one survey in one site in which 126 individuals were found.

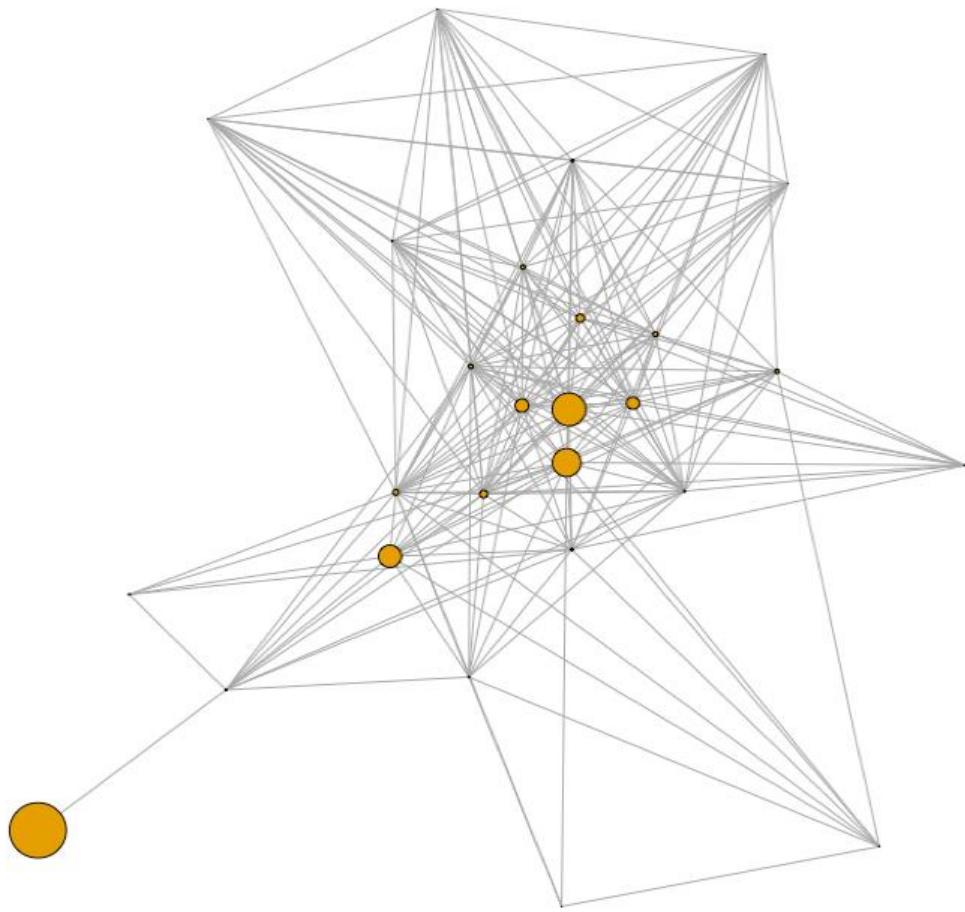


Figure 4.2. Species occurrence interaction network using the Fruchterman-Weingold layout, where nodes that share more connections are closer to each other. Species nodes are shown as yellow circles and links are grey lines. Sizes of nodes are proportional to densities (number of individuals) of species found during surveys. Links are weighted depending on the number of links (sites they co-occur in) to a specific species.

Probabilistic Co-occurrence

Along the altitudinal gradient there is a pattern of few species, and hence very simple community structure, at higher altitudes, and more complex species community structure below 1600 m asl (Figure 4.3).

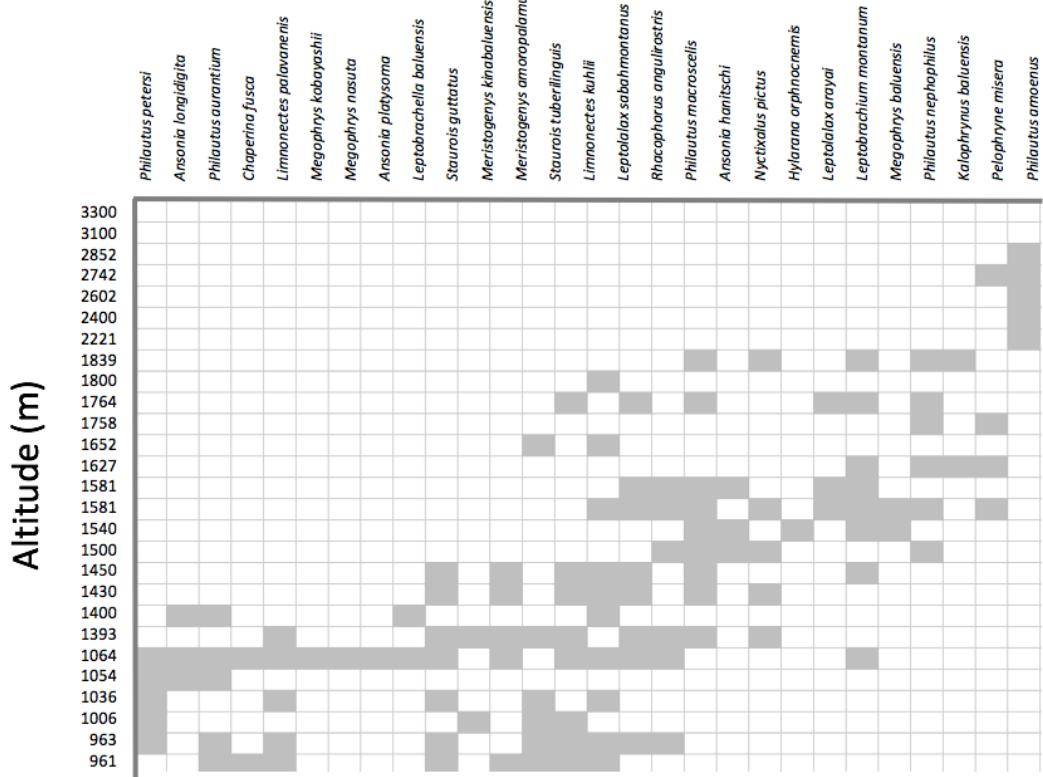


Figure 4.3. Species presence matrix across different sites (species names are given on the x-axis and site altitude are given on the y-axis). Each grey box represents presence, and white represents absence.

The probabilistic co-occurrence model shows no statistically significant negative relationships between species, but several positive ones (Figure 4.4). Species such as *Staurois tuberilinguis*, *Rhacophorus angulirostris*, *Meristogenys amorphalamus* and *Ansonia hanitschi* show four or more positive occurrence interactions with other species, indicating that one species' presence at a site entails a higher chance of certain other species being present than there would be by chance.

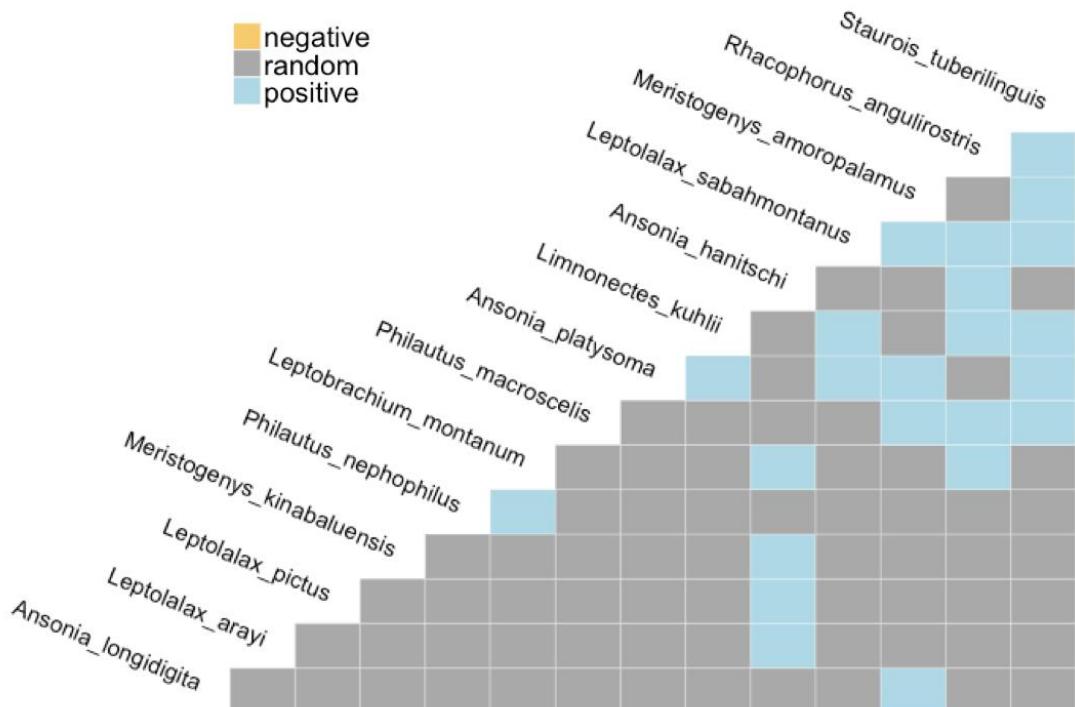


Figure 4.4. Co-occurrence results from the probabilistic analysis. Blue indicates positive relationships, yellow negative relationships (none found) and grey random relationships. As an example, the bottom species *Ansonia longidigita* has only one statistically significant co-occurrence interaction (blue box) with *Meristogenys amoropalamus*.

Joint Species Distribution Modelling

The GJAM evaluates the interactions between species against environmental variables and groups species that have similar responses to environmental variables. The model exhibits reasonable predictive ability against observed data for temperature and humidity for the GJAM model (Figure 4.5). The predictive ability for altitude is high (see Appendix 4.1 for MCMC convergence graphs).

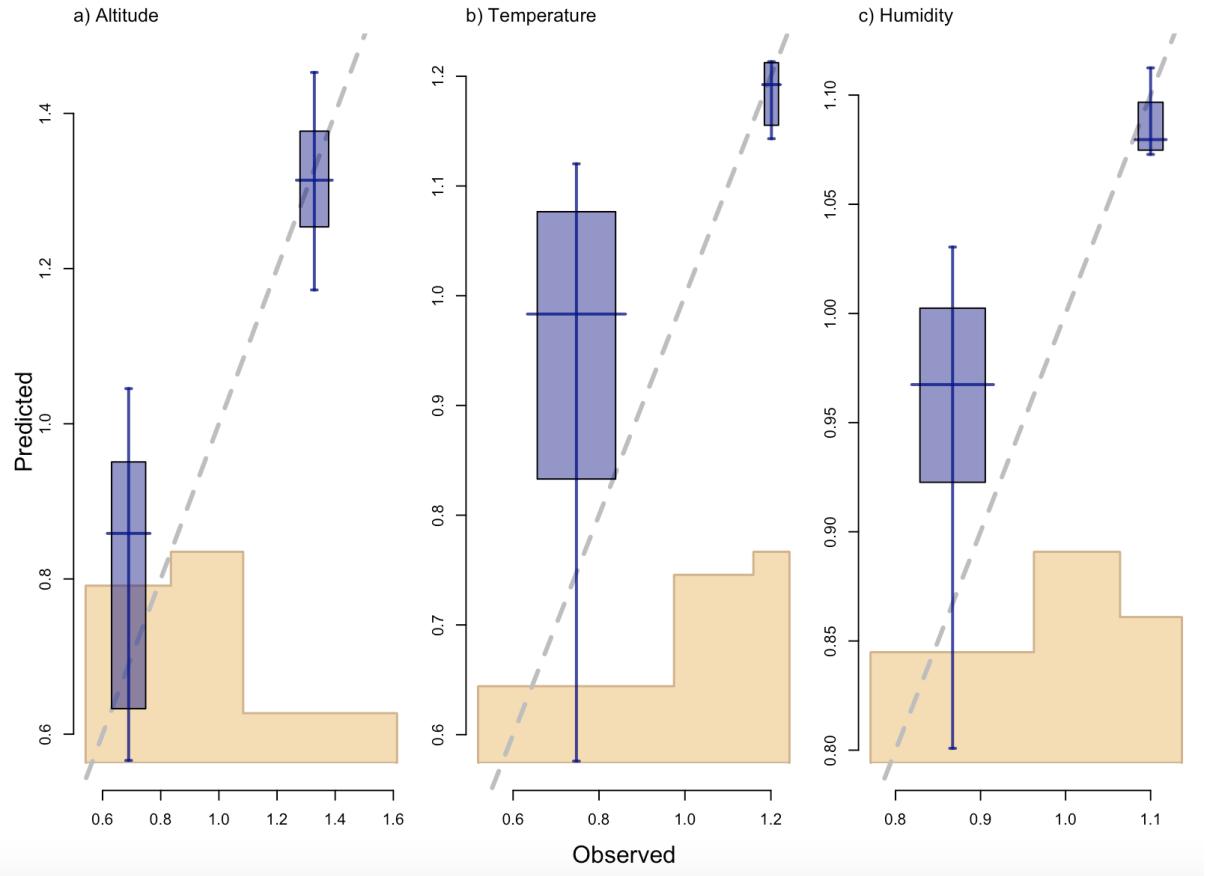


Figure 4.5. GJAM results. Inverse prediction of a) altitude, b) temperature and c) humidity against observed values. Blue boxes indicate 68% predictive interval and whiskers a 95% predictive interval. All values are scaled and centred. Frequency distributions of observations are shown in yellow. Grey dashed lines show lines of best fit. If the model performs well the boxplots should be clustered close to the dashed line. These graphs indicate how well the model performs in predicting the response of species to these variables compared to actual, observed values. Y indicates the environmental variables.

The results also indicate that the model is better at predicting species richness than species diversity, as measured by the Shannon index H (Figure 4.6), because the predicted values deviate substantially from the observed values for the latter (Figure 4.6b).

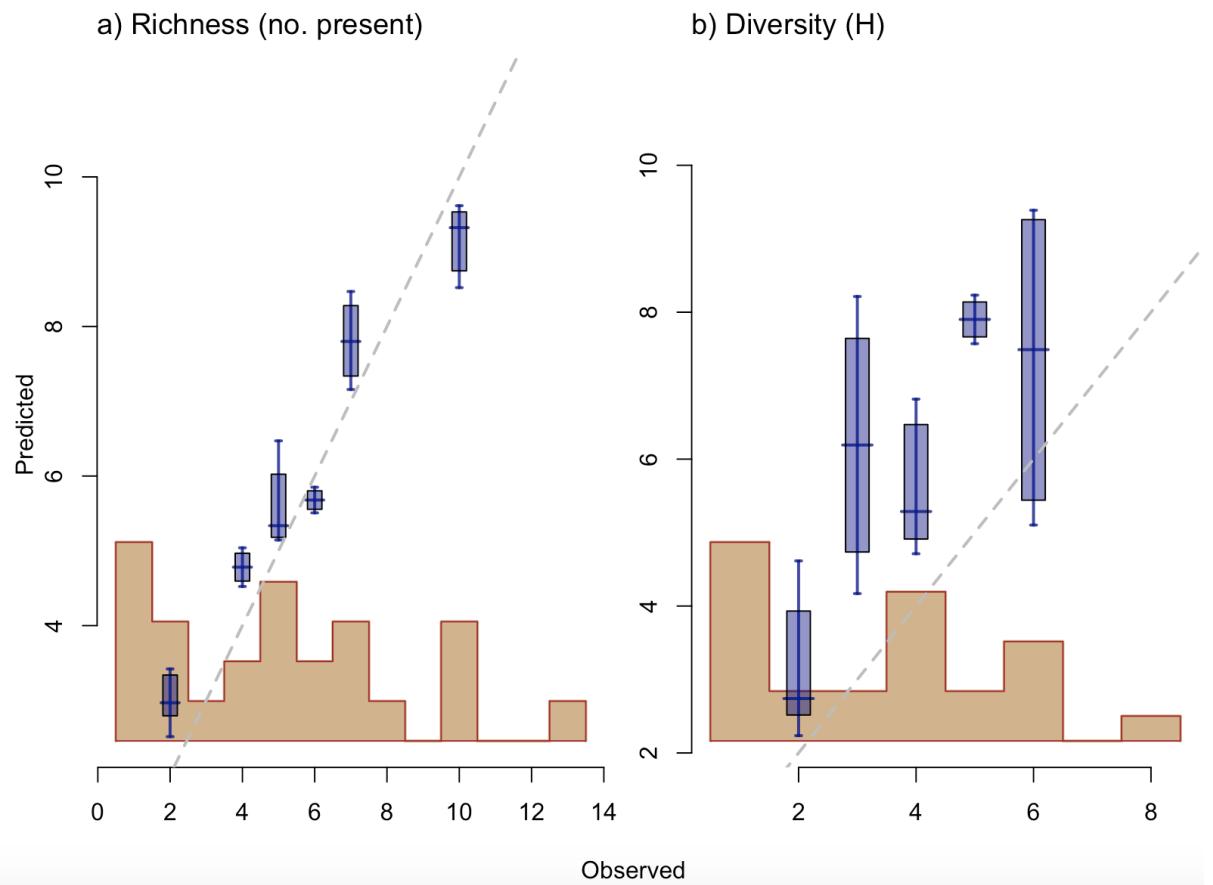


Figure 4.6. Predicted relationship to observed data for a) species richness and b) species diversity.

Temperature and altitude have the largest positive effect on species (Figure 4.7). A high number of species, starting from *Nyctixalus pictus*, show little to no response to many of the predictors. Several species show a negative response relative to altitude, such as *Rhacophorus angulirostris*.

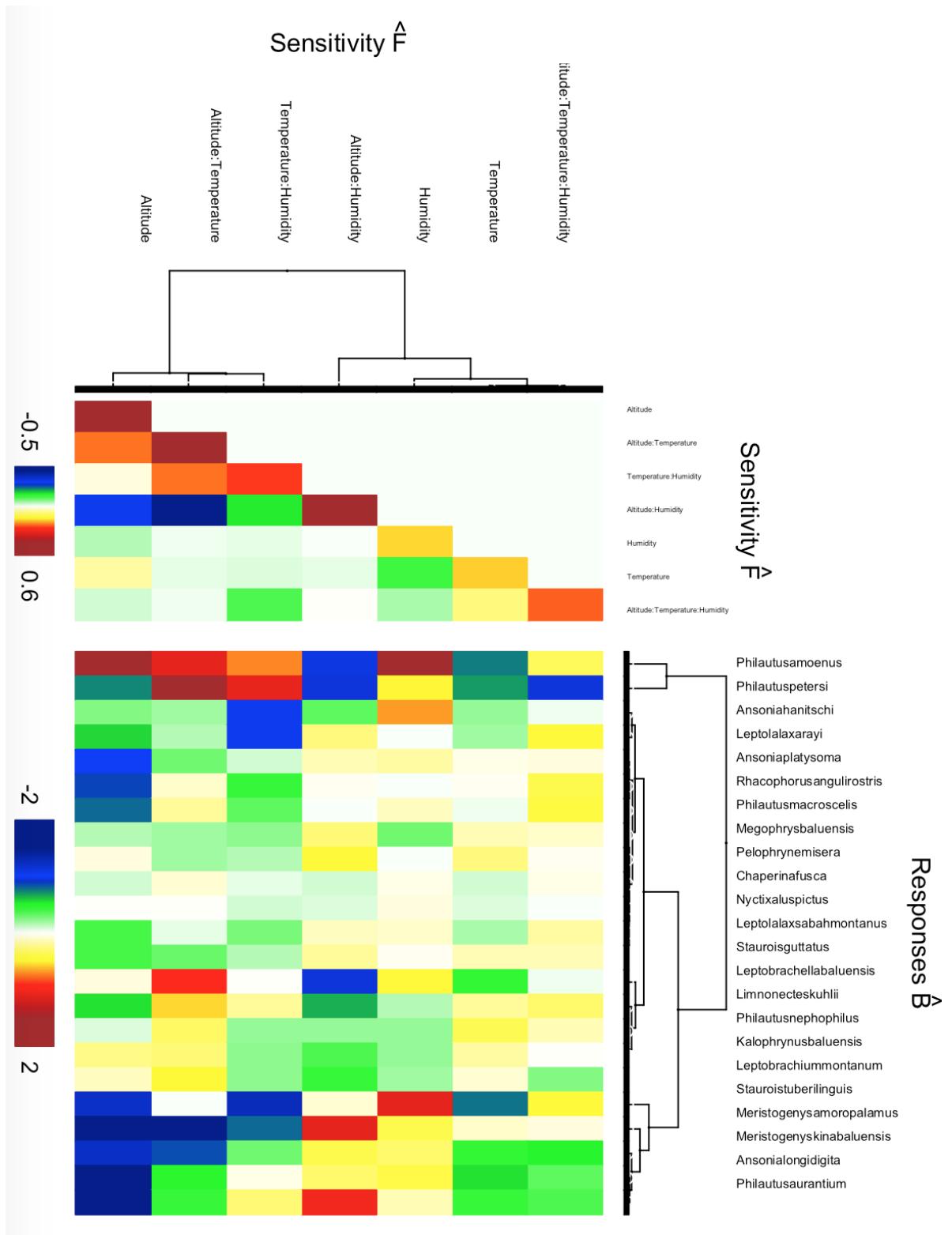


Figure 4.7. Results from GJAM analysis. The top matrix is the sensitivity matrix (\hat{F}); the bottom matrix is the response matrix (\hat{B}). The colour scale runs from strongly negative (blue) to strongly positive (red). Dendograms indicate the sensitivity of predictor variables \hat{F} . Large values in the sensitive vector \hat{f} (the diagonal of \hat{F}) indicate a strong effect from matrix

\hat{B} . F corresponds to eqn. 8 in Clark., *et al* 2017: $F = B\Sigma - 1B'm$ where B is the sensitivity coefficients.

The GJAM analysis split the species into four distinct groups. Group 1 consists of stream species, the majority with strong positive relationships with each other (Figure 4.8). The group appears to respond negatively to altitude and positively to humidity. Group 2 also consists of almost exclusively stream species with many strong positive co-occurrence values between them. This group is different from Group 1 as the constituent species exist at lower elevations; several species also show a negative response to altitude. The high-altitude species *Philautus amoenus* has strong to weak negative co-occurrence relationships with most other species and forms its own group, Group 3. Group 3 has a strong response to most environmental variables: the response to altitude is positive and the response to temperature is negative. Group 4 is a mixed group with some stream species and some species that occupy either only terrestrial sites or a mixture of stream and terrestrial sites. Many of these species have very weak responses to each other and a mixed but mainly weak response to environmental variables.

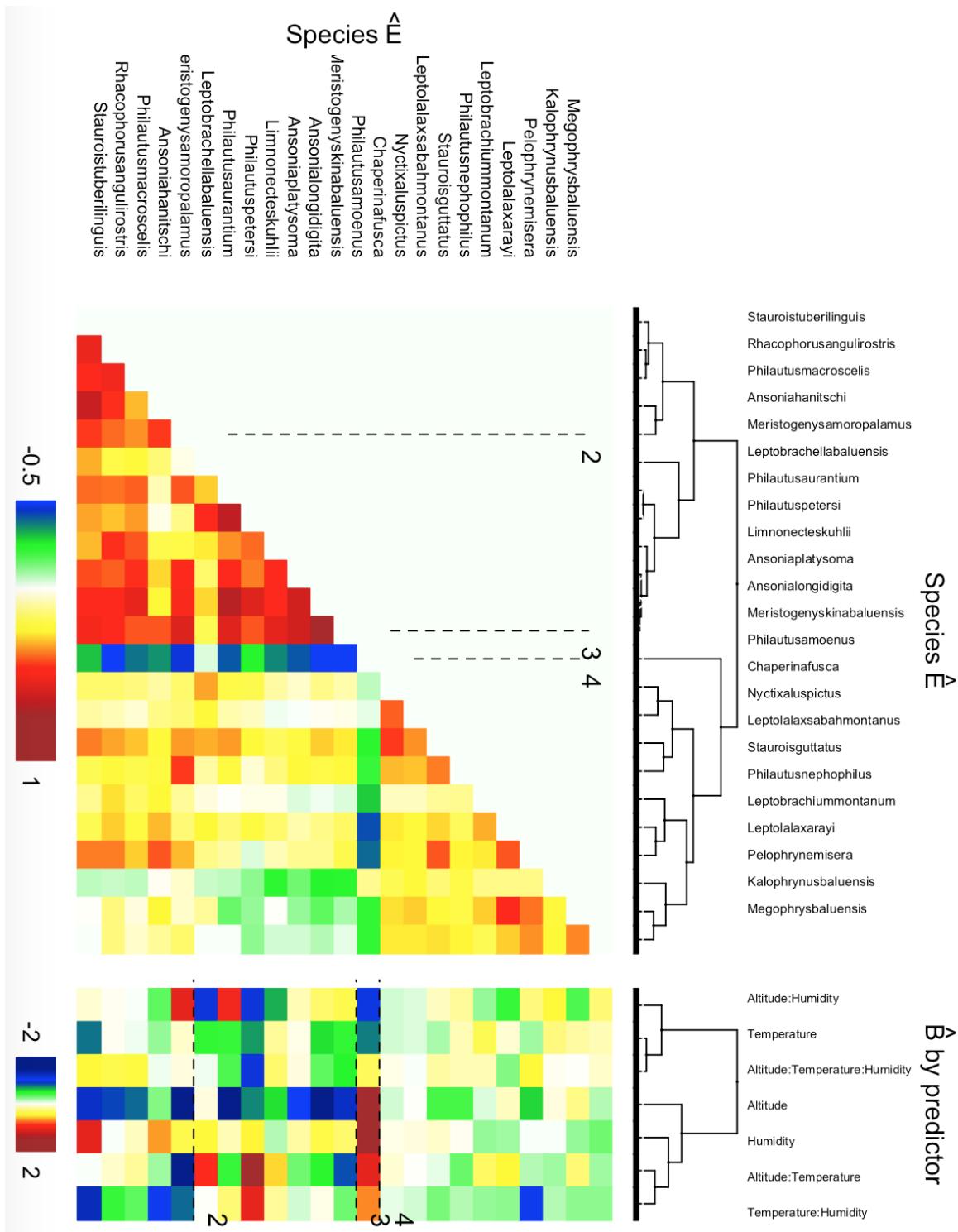


Figure 4.8. GJAM results. Matrix \hat{E} shows groups of species that have a similar response to environmental variables. Matrix \hat{B} shows the environmental predictor variables underlying the grouping in \hat{E} . Dendograms indicate clustering of species and predictors. Predictor blocks are related to species and should be read as the predictor having that level of effect on the corresponding species. Species responses to each should be read as the species in one row and the species matching that block having the indicated level of co-occurrence.

Discussion

The network analysis of the amphibian communities on Mount Kinabalu indicate that connections are high between just a few species and a few sites. This is not necessarily related to number of individuals. Specialist species such as *P. amoenus* have a high number of individuals but very low connectivity to other species and exist in a high number of sites. However, this is a high-altitude species which can persist in a landscape where the environment is challenging for other species. The lower altitude sites, in contrast, indicate high connectivity between sites and between species.

As the effect of biotic interactions on a species should be most apparent at the limit of its range (Hillerislambers et al., 2013) a synergistic effect of climate change is expected at these points. A very strong effect on amphibians is expected from an increase in temperature since they are ectothermic and should exhibit a direct physiological response to changing temperatures. However, if two species are occupying realised niches, rather than fundamental niches, one or both might not be at their physiological extremes and even if a competitive species is starting to shift its range it might lead to increased interaction at these meeting points. As seen in Chapter 1 of this thesis, not all the species on Mount Kinabalu are shifting upwards. It is plausible that the species that are shifting downwards have lost their competitive edge at the higher altitudes because other species are starting to shift their range and change the interaction network. I attempted to identify negative species interactions as these species pairs would be especially important for future studies on the effects of environmental change. The probabilistic co-occurrence model (Veech, 2013) indicated no negative co-occurrence patterns. Considering that from field observations alone, high-altitude species such as *P. amoenus* have very limited co-occurrence interaction with most other species in our study this threw serious doubt over the reliability of the results. *P. amoenus* was included in this analysis, however, was not displayed by the results of the model as this

model predicted that this species only had random interactions with all other species. It indicates that caution should be employed with the interpretation of the positive co-occurrence interaction results this model indicated.

One limitation of the simple probabilistic co-occurrence model is that it considers only presence-absence data and is unable to account for unequal survey effort and incorporation of environmental data. The GJAM analysis offers a result that, despite the low number of sites and low number of species, appears to be highly reliable and ecologically plausible. In comparison to the probabilistic co-occurrence model, the GJAM discards data and makes use of individual density values, and environmental variables. It also takes into account unequal survey effort and solves the problem of zero inflation (Clark et al., 2014, 2017). As such, the GJAM overcomes some of the limitations of previous joint species distribution models (D. J. Harris, 2015; Pollock et al., 2014), whilst still accounting for biotic interactions. The GJAM sensitivity response for these data indicates that many of the amphibian species have weak to no response to the different predictor variables. Despite this, the species with weak responses to environmental variables were not placed together by the GJAM's grouping analysis. The grouping analysis tends to group species that do not show the same response to predictor variables, indicating that the model is inferring a community response rather than a response purely at the individual species level. This is important because predicted species-level responses to climate change might not transfer to real scenarios involving communities of species, in accordance with the conclusions of several other studies (Clark et al., 2014, 2017; Gilman et al., 2010; D. J. Harris, 2015; Suding et al., 2008). Limitations of this model are that it does not account for detection probability (Waddle et al., 2010), and it may not always clearly separate species co-occurrence interactions and habitat responses (Ovaskainen, Hottola, & Shtonen, 2010). *P. amoenus* shows a negative response to a higher number of species, including *K. baluensis*, which is

consistent with field observations because *K. baluensis* occurs very close to the range limit of *P. amoenus* but does not actually overlap with it. This indicates that these species might be suitable for future studies on the effects of environmental change of species in contact zones of range limits.

The results for the species *A. platysoma*, for which a species-level analysis was performed in Chapter 2, also need to be highlighted. This species shows high levels of positive co-occurrence with *A. longidigita*, *L. kuhlii*, *P. macroscelis* and *R. angulirostris* within its species group as well as *C. fusca*, *M. kinabaluensis* and *M. amorphopalamus* in another group containing high numbers of stream species. There does not appear to be any negative co-occurrence of note for this amphibian species. It furthermore indicates a very negative response to increasing altitude and a positive response to increasing temperature. On the other hand, humidity had very low effects on this species. The GJAM provides information on *A. platysoma* additional to the species-level model in Chapter 3. It raises the possibility of inferring potential locations of *A. platysoma* by attempting to model the species in conjunction with other species with which it tends to co-occur.

In general, it is difficult to determine whether it is the effects of the environment or the interactions and competition between species that structure and ecological community. In the case of amphibians, because they are themselves ectothermic and mainly reliant on ectothermic insects for food, one could predict that they would follow their main food sources. Amphibians are normally generalists though, so if another food source takes the place of the existing one they should be able to persist in the same sites. Changes in the distribution attributed to temperature are thus likely to be due to physiological responses rather than shifting food resources. However, several of the amphibian species on Mount Kinabalu are also reliant on water for breeding, suggesting that another factor dictating their distribution is the availability of breeding habitats. Overall, mountains present extreme

challenges for many of the animals that inhabit them. Persistence of these species in the face of climate change is reliant on accurate assessment of abiotic and biotic effects on the amphibian communities.

Conclusions

The results of the network analysis are difficult to interpret, suggesting that more data are needed, both in terms of number of species but also number of sites sampled to be able to get a clear picture of competitive co-occurrence interaction networks in amphibian communities. The information it does yield is that there are a few species with several directional co-occurrence interactions between them. The probabilistic co-occurrence model gave inconclusive results, which I attribute largely to its lack of incorporation of environmental variables. The GJAM analysis, which can incorporate both environmental variables as well as survey effort directly into the analysis, appears to give results that are both reliable and ecologically intuitive. Considering the small number of sites and species that were available, the conclusion is that future analysis of co-occurrence of amphibian datasets is highly feasible and could greatly improve management recommendations for this taxonomic group. Continued monitoring of the amphibian community in Kinabalu Park on a regular basis is recommended, and community-level analysis is recommended for assessing the long-term effects of climate change and other drivers on the community.

CHAPTER 5. Acoustic and environmental monitoring using open-source hardware and software

Introduction

An impediment to development of effective conservation plans is lack of baseline information such as population estimates, dynamics, and ecology of species at risk of extinction (Rodríguez, 2003). Collection of these data, and development of effective conservation plans, requires persistent and extensive inventory-based studies (Collins et al., 2005; Ferrier et al., 2004; B. H. Kueh & Maryati, 1998; BH Kueh & Mohamed, 2005; Matsui, 2006). However, lack of funds and trained personnel means that new methods for rapid monitoring are needed.

Passive acoustic monitoring has led to recent advancement in species survey techniques (Aide et al., 2013; Amezquita, Flechas, Lima, Gasser, & Hodl, 2011; Bedoya et al., 2014; Towsey et al., 2013, 2014). Benefits of passive acoustic monitoring are (i) ability to easily and cheaply collect large data sets; (ii) ability to monitor a site continuously without ongoing disturbance of the environment; and (iii) use of a consistent methodology that is not influenced by surveyor expertise or other human-related factors.

Several commercial systems for acoustic monitoring are available, including the Song Meter (Wildlife Acoustics), Froglogger (Frogloggers) and Arbimon (Aide et al., 2013). A low-cost alternative is open-source systems, which have been used increasingly in recent years with open-source microprocessors, such as the Raspberry Pi (Hill et al., 2018; Whytock & Christie, 2017). These open-source systems are advantageous for multiple reasons: for large-scale studies, they are cheaper and modular, allowing easy replacement of parts and customisation; the power source is flexible, allowing for combinations of different types of

batteries and/or solar panels; choice of data relay (e.g., Bluetooth, Wi-Fi, 3G or LoRa) is also flexible (Aide et al., 2013; Whytock & Christie, 2017).

Some of these systems also include environmental sensors, but stations with combined acoustic monitoring sensors and environmental sensors have not yet been built or tested. It is important to monitor environmental factors because they affect both the acoustic signals and the behaviour of the species being monitored. Sound is affected by changes to environmental variables such as air temperature and humidity (Bohn, 1988; Cramer, 1993; C. M. Harris, 1966). In turn, environmental variables, including light, rain, and temperature, affect animal activity and behaviour (including frequencies of vocalisation) (Han & Gatehouse, 1991; Lengagne & Slater, 2002; Royer, Mcneil, De, Laval, & Gik, 1991; Saenz, Fitzgerald, & Baum, 2006). To interpret time series of acoustic data correctly, it is essential to collect data on environmental variables and acoustic data simultaneously. In this study, I tested an acoustic monitoring system that is based on a Raspberry Pi microprocessor combined with environmental sensors in Southeast Asia, using Kinabalu Park in northern Borneo (Sabah, Malaysia) as well as four nature parks in Singapore as trial sites. A short trial was also performed in tropical lowland forest using LoRa as a data relay system.

Methods

This study was conducted in Kinabalu Park, Sabah, Malaysia on the island of Borneo (Lat: 6.157, Long: 116.638) between 1300 to 1800 m asl. The habitat on the site consists of cloud Fagaceae forest. A trial was also conducted in four nature parks in Singapore. Singapore consists of isolated fragments of lowland tropical rainforest in an urban matrix, where the nature parks range from primary and secondary rainforest to highly managed parks.

In Kinabalu Park, monitoring stations consisted of a Raspberry Pi 3 (model B+), a hygrometer (AM2302), a barometric pressure sensor (Bosch BMP180) a lux sensor (Adafruit TSL2561), and a Real Time Clock (RTC, Adafruit PCF8523). A waterproof omnidirectional condenser microphone (Kingstate KEEG1538WB-100LB) with a frequency response of 20 Hz – 20 kHz and a sensitivity of -42 dB (see Figure 5.1A) was tested. The stations were attached to a tree 1.5 m above ground level and supplied with electricity through two connected 24 Ah 12 V Camdenboss deep cycle batteries. The stations were housed in a waterproof 248 mm \times 197 mm \times 71 mm MTM survivor box.



Figure 5.1. Passive automated monitoring stations used in the present study. A) Station used in Kinabalu Park with a Master Python lock (model no. 8419DPF). B) Inside view of a station in Singapore powered with a 20,000 mAh Pineng power bank. C) As in B but powered with a solar panel. D) External view of a station in Singapore powered using a solar panel.

Field tests were also performed with Raspberry Pi 3 (model A+) computers in Singapore across four sites with 38 stations. The same sensor array as in Kinabalu Park was used but the microphone was changed to a primo EM172 omni-directional microphone with a signal: noise ratio of 80 dB and sensitivity of 28 db. The microphones were mounted using a rugged mount from FEL communications LTD (www.micbooster.com) which has an O-ring. The waterproof box was changed to a 200 mm \times 131.5 mm Tackachi Electronics Enclosure (www.monatoro.sg). The stations were powered using 20,000 mAh Pineng power banks

(Figure 5.1B) and, in a few cases 30,000 mAh Proda power banks. A set-up of two serial-connected deep cycle Camdenboss 7.5Ah 12v car batteries was also tested. A field-test of solar panel set ups was performed in two suitable locations using a polycrystalline HX-P30W 535 mm x 515 mm x 25 mm panel weighing 2.9 kg and a 7.5 Ah 12 V Camdenboss deep cycle car battery with an Anself CMP12 10 A 12 V/24 V charge controller (Figure 5.1C-D). Since the solar panel was mounted 1.5 m above ground rather than in the canopy, a larger sized one than would be required under high-light conditions was used to offset lower light conditions. To reduce humidity inside the box, each station had a bag of silica gel inside (a 100 g bag in the Singapore boxes and a 50 g bag in Kinabalu Park). The stations in Singapore also had ant bait of the brand Combat (three-month supply) to avoid ant and termite infestations. For full details on coding and assembly see Appendix 5.1.

Field tests of LoRa using a Raspberry B+ fitted with an 868 MHz Dragino LoRa/GPS hat with an 868 MHz glue stick antenna as a server (receiver) and a Raspberry Pi A+ with an Adafruit RFM9X 868 or 915 MHz and an Adafruit 900 MHz antenna as a client (transceiver) were conducted in June 2018. The server was thus only receiving data from the client, whereas the client was recording the acoustic and environmental data and sending on information to the server. Because LoRa is not capable of sending data files of the size of one-minute acoustic wav files (10 Mb), it was set up to send the just last row of the CSV file containing environmental data instead (see below).

The stations in both Kinabalu park and Singapore were configured to record acoustic data for one minute in every ten, and to record temperature, humidity, lux and barometric pressure every ten minutes. The environmental data were saved in a CSV file and the acoustic data were saved as WAV files. Calls were identified to species using call descriptions and reference pictures of call spectrograms, as well as sound libraries reported in the literature (Inger et al., 2018; Malkmus et al., 2002). In addition, many calls were

identified either to species or higher taxonomic level (cricket, cicada and bird) during the field season in 2016 meaning that auditory identification could be performed for these groups and then cross-referenced with the spectrograms from the sound recordings (see Figure 5.2 for representative calls for species). The free audio software Audacity® was used for manual inspection of sound files (see Figure 5.2 for examples of screenshots from Audacity®).

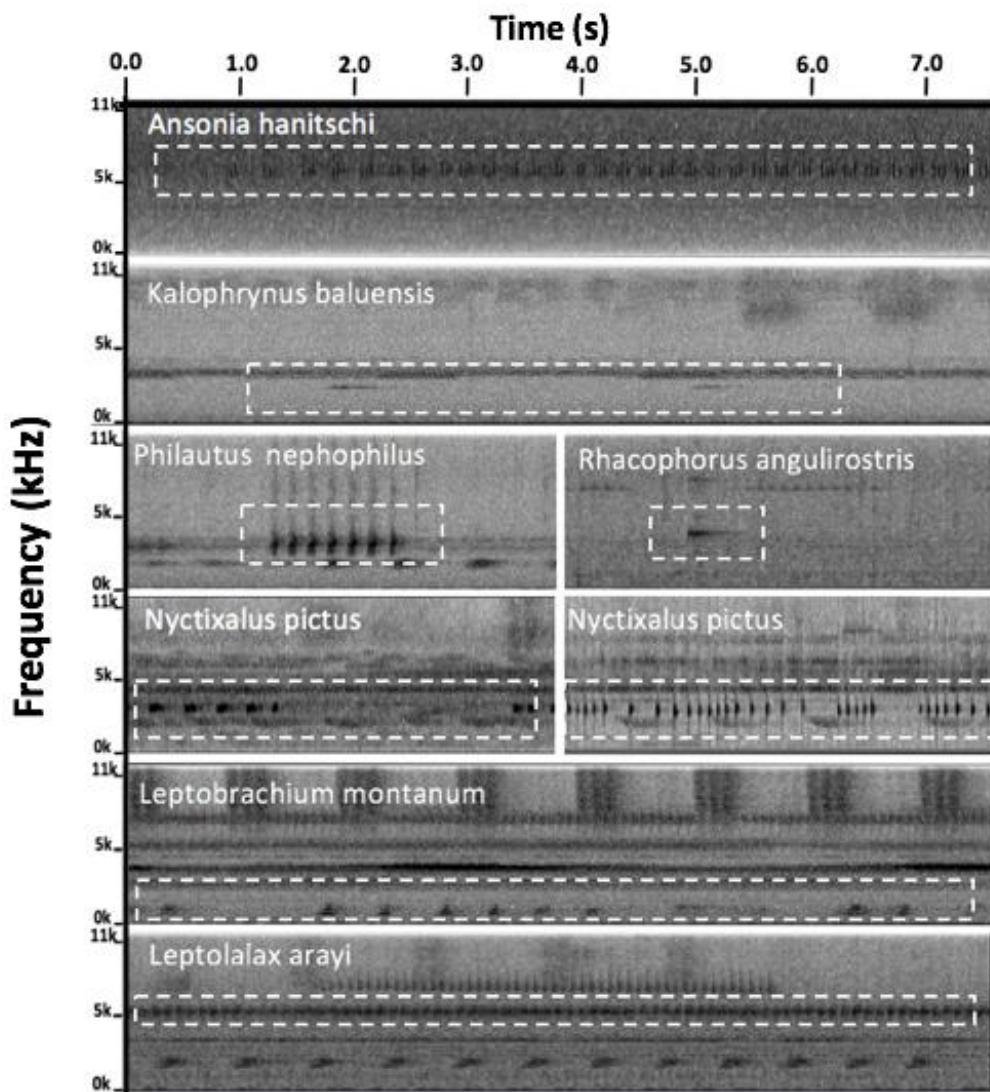


Figure 5.2. Spectrograms of the calls of the identified amphibian species, visualized in Audacity ®. On each spectrogram the species name is given in white and region of the spectrogram corresponding to the call is highlighted with a white dashed box for the window in question. The *M. orphnognemis* call is from the sound library accompanying Inger *et al* 2017. The white dashed boxes denote the main characteristics used for identification, usually indicating the dominant frequencies. Most species have harmonics reaching other frequencies as well (e.g., *P. nephophilus*), but most of the time the harmonics are not captured by the monitoring stations. The call of *Rhacophorus baluensis* is also shown despite this species never being recording with the acoustic monitoring stations (the spectrogram comes from a manual recording with a directional Sennheiser microphone conducted in October 2016).

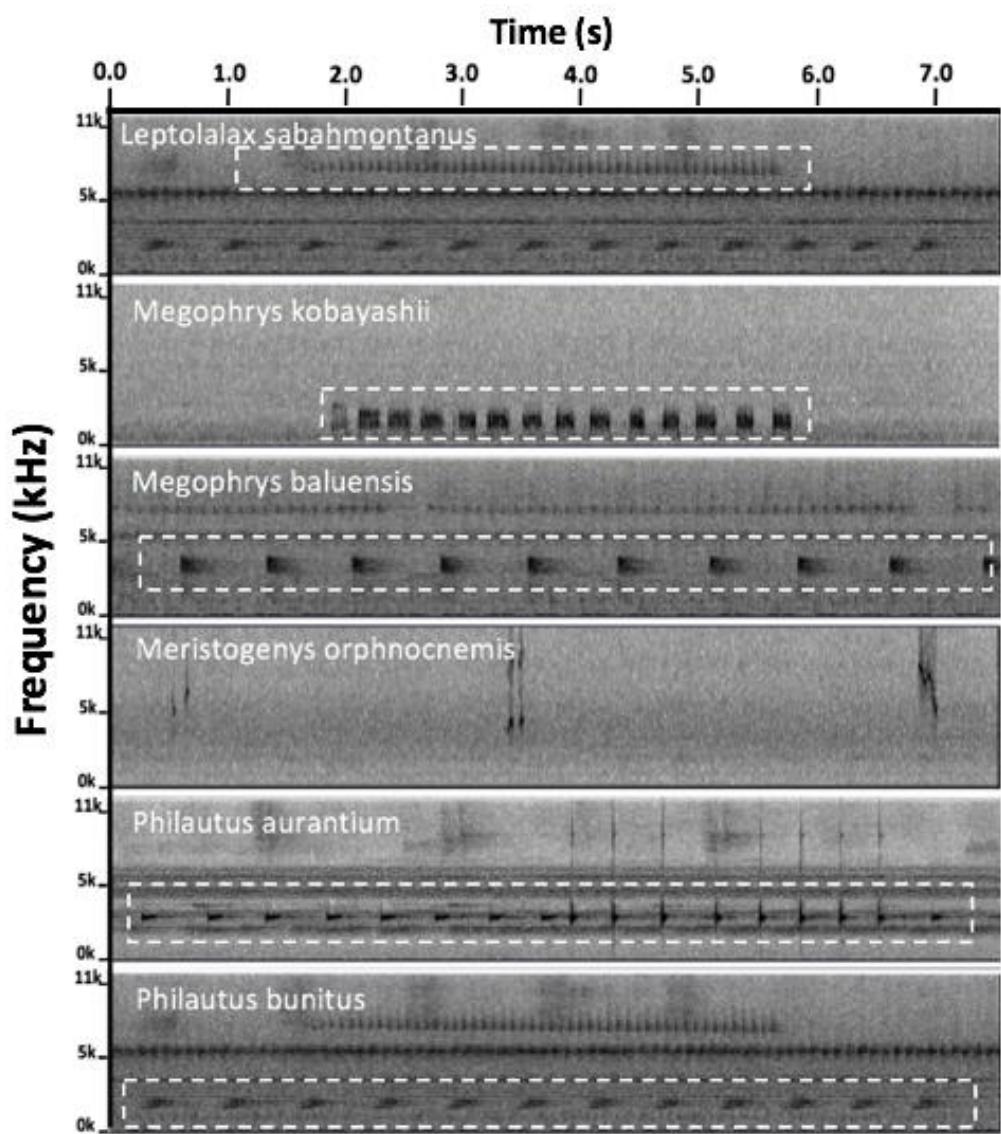


Figure 5.2. Contd.

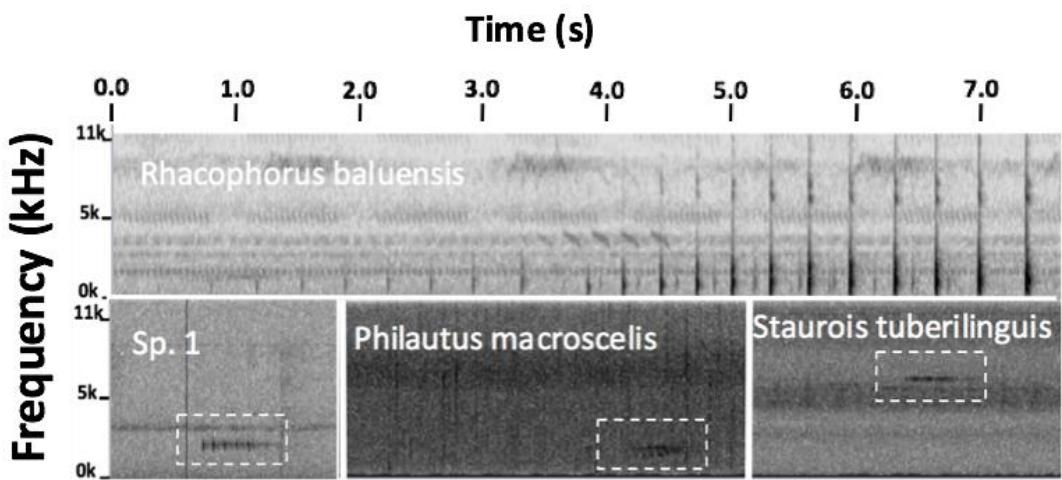


Figure 5.2. Contd.

Data on call occurrences or intensity can either be extracted manually by inspection in Audacity® or by using automated classification algorithms. R (R Core Team, 2017) has acoustic analysis packages (J Sueur, 2018) available including the package warbleR (Araya-Salas & Smith-Vidaurre, 2017) for species identification and extraction (see Appendix 5.1 for code using the contact call of *Kalophrynxus baluensis* (Karlsson et al., 2018)). The software Aureas (Bedoya et al., 2014) is also freely available for automated species identification, with the benefit that this software is capable of handling and identifying species it was not trained on and of dealing with dialect variations. Aureas require a training library whereas warbleR can be tuned on samples. Training libraries and instructions for Aureas are available on the software's website (<http://sistemic.udea.edu.co/es/investigacion/proyectos/analisis-bioacustico/>). To get an overview of the process of using these two types of software packages, see the flowchart in Figure 5.3. Call occurrences were extracted from one site over five days using manual extraction in Audacity®, the data were used to produce a radar plot in R. Call intensity, the number of call series per minute, were manually extracted and linear models were produced and visualised in R. Continuous acoustic data were also recorded in Kinabalu Park in June 2016 for soundscape analysis. The soundscape data were analysed

using false-colour spectrograms (Towsey & Zhang, 2014) produced in Python (https://github.com/sarabsethi/false_colour_index_spectroram). The indices used were: acoustic power (POW), acoustic complexity index (ACI) and temporal entropy (FENT). These indices were chosen as they are largely independent of each other: POW assesses the signal amplitude and averages it; ACI measures the complexity of the acoustic environment by measuring the change in signal amplitude between frames (Pieretti, Farina, & Morri, 2011; Towsey et al., 2014); and FENT measures the acoustic energy dispersed temporally (Towsey & Zhang, 2014).

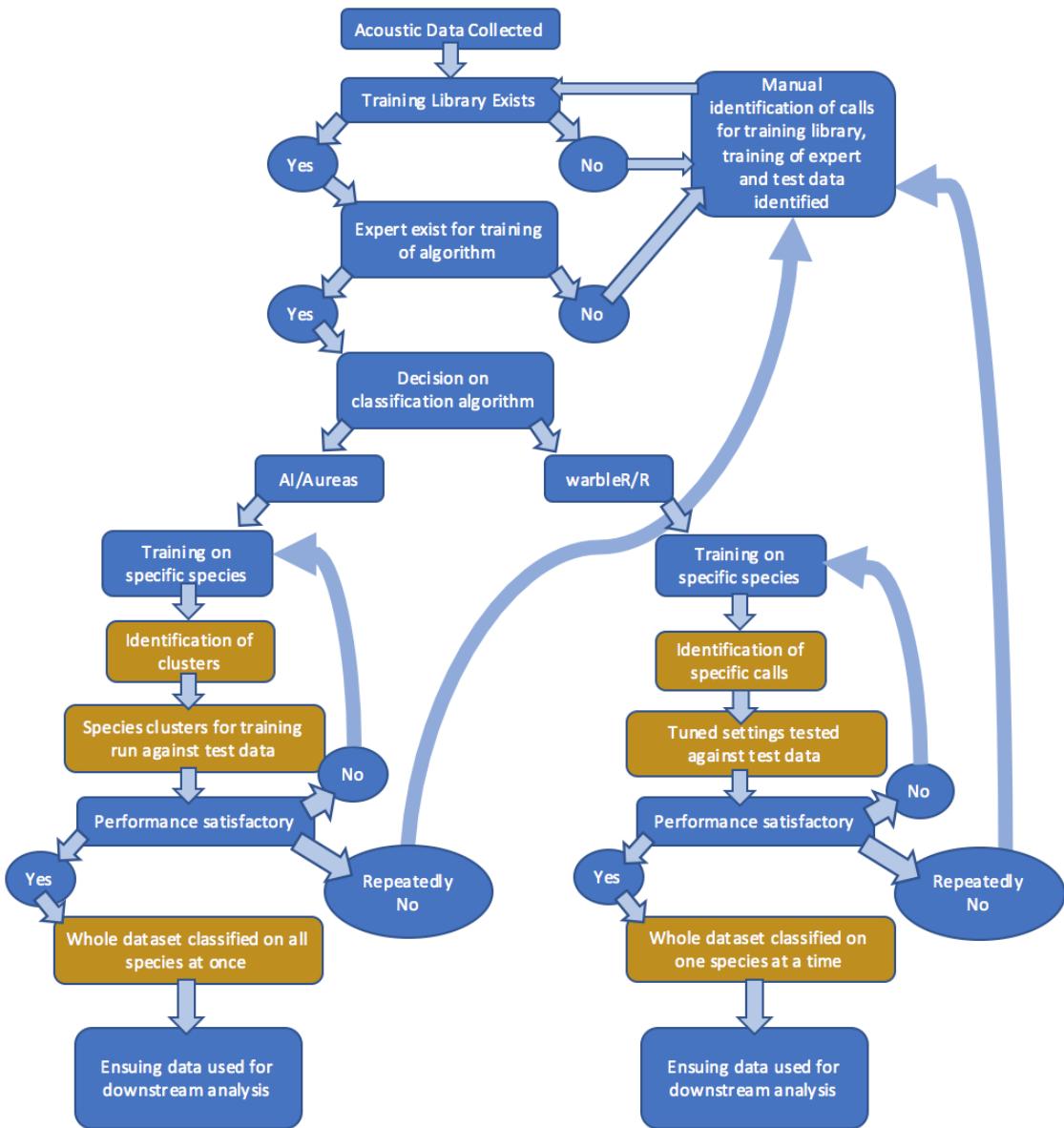


Figure 5.3. Flowchart detailing the steps from data acquisition to the creation of training libraries to using two open-source software packages for automatically classifying the acoustic data: Aureas at the lower-left and warbleR at lower-right. Orange blocks show stages that differ between Aureas and warbleR. Note that, for either software package, repeated performance failures indicate that manual identification of suitable calls for training need to be repeated.

Results

Only five of 10 sites initially tested in Kinabalu Park collected data successfully for the entire test period. One station failed due to RTC corruption after six weeks, two had the SD card fail, one suffered immediate RTC failure, and one had microphone failure. From March–June 2017, over 50,400 minutes of acoustic data were collected in Kinabalu Park, with the associated environmental data totalling 158 GB. The data consist of temperature, barometric pressure, lux (light levels), relative air humidity as well as the acoustic recordings. A total of 123 GB of soundscape data were also collected across ten sites (Figure 5.4D) in Kinabalu Park. The study in Singapore collected over 34,667 minutes of acoustic data with the associated environmental data from November 2017 – January 2018 totalling 128 GB. The Singapore project had an initial failure rate of six out of 38 stations, all failures being due to the RTC. Two additional stations suffered RTC corruption after a few weeks of running which was subsequently corrected: if these are included as failures, the Singapore failure rate is 21%, compared to 50% in Kinabalu Park.

Almost all wind covers for the EM172 microphones in Singapore were destroyed in the field with only two of 38 undamaged after three months. Weather conditions in tropical areas deteriorate recording quality very quickly because of electronic and mechanical failure, requiring regular replacement of even supposedly weather-proof microphones. Clear declines in recording quality were observed over the three-month study period despite Kingstate microphones being marketed as waterproof.

In Kinabalu Park, power consumption was ~300 mA per hour with the B+ stations. In Singapore, power consumption of the stations were ~100 mA per hour using a Raspberry A+. Power banks drain power faster in hot and humid conditions. In Singapore the 20,000 mAh Pineng power banks last on average and the 30,000 mAh Proda power banks lasted, on average, only three days. Car batteries, like the Camdenboss deep cycle model, were more

stable in power delivery and the set up with two 7.5 Ah batteries lasted on average eight days in Singapore, despite the high temperature. The Kinabalu Park stations also used car batteries as a power source, but due to lower temperature (average 20 °C) they lasted much longer with two serial-connected 12 V batteries of 24 Ah delivering enough power for 44 days with the A+ stations and 30 days with the B+ stations. On average, the solar panel set ups powered stations for four weeks before batteries were drained, despite being mounted under the canopy. One station with a solar panel in Singapore collected over 5000 minutes of acoustic data and 5000 environmental data points over a period of two months.

The silica gel packs needed to be replaced approximately every month in Singapore, indicating that despite the box being waterproof and any access holes being sealed with silicone, there was still considerable humidity in the box. The Kinabalu park boxes were much smaller in size, and they were also not opened for three months since batteries were mounted externally, reducing need for replacement of silica gel. Five boxes had ant infestations in Singapore. Two boxes had major infestations with the ant bait consumed and needing to be replaced before the end of the three-month test. The Kinabalu Park boxes did not suffer any infestations of ants or termites.

The LoRa system was functioning, with the maximum distance of data relay being 50 m with a server being stationed indoors and without directional antennae.

Data were extracted manually from the acoustic files. Five randomly selected days from the Silau Silau Stream site in Kinabalu Park were used for demonstration purposes. The environmental data indicate major fluctuations in a 24 h period of barometric pressure and temperature (Figure 5.4A-B). The call occurrences of all the species over the five days were displayed in a radar plot (Figure 5.4C), which shows that there is high activity from most species at night time. However, one species, *Xenophrys baluensis*, was instead active during the day; interestingly this species used a dominant frequency like one of the night-calling

species (2.8 kHz for *X. baluensis* and 2.8 kHz for *P. nephophilus*). The soundscape data from the 4th of June (Figure 5.4D), show that this site has very low acoustic activity during the day. During the night, activity levels increase and species such as *Leptolalax sabahmontanus* and *L. arayi* create a band of ACI just above 6 kHz and a band of FENT just below 7.5 kHz, respectively, between 18:00 – 24:00 hrs. The presence of the bands indicating these species was verified by manually inspecting the sounds files in Audacity® and assessing the corresponding indices in the false-colour spectrogram at a given frequency. Analysis of the environmental variables temperature and barometric pressure against calling intensity (calls per minute) with linear models (Figure 5.5) indicates that effects of temperature and barometric pressure do not follow a predictable or general pattern. Species such as *Philautus bunitus*, for example, show an increase in calling intensity with higher barometric pressure and a decrease in calling intensity with increasing temperature, in comparison to *Rhacophorus angulirostris*, for which there were no apparent effects of barometric pressure or temperature on calling intensity.

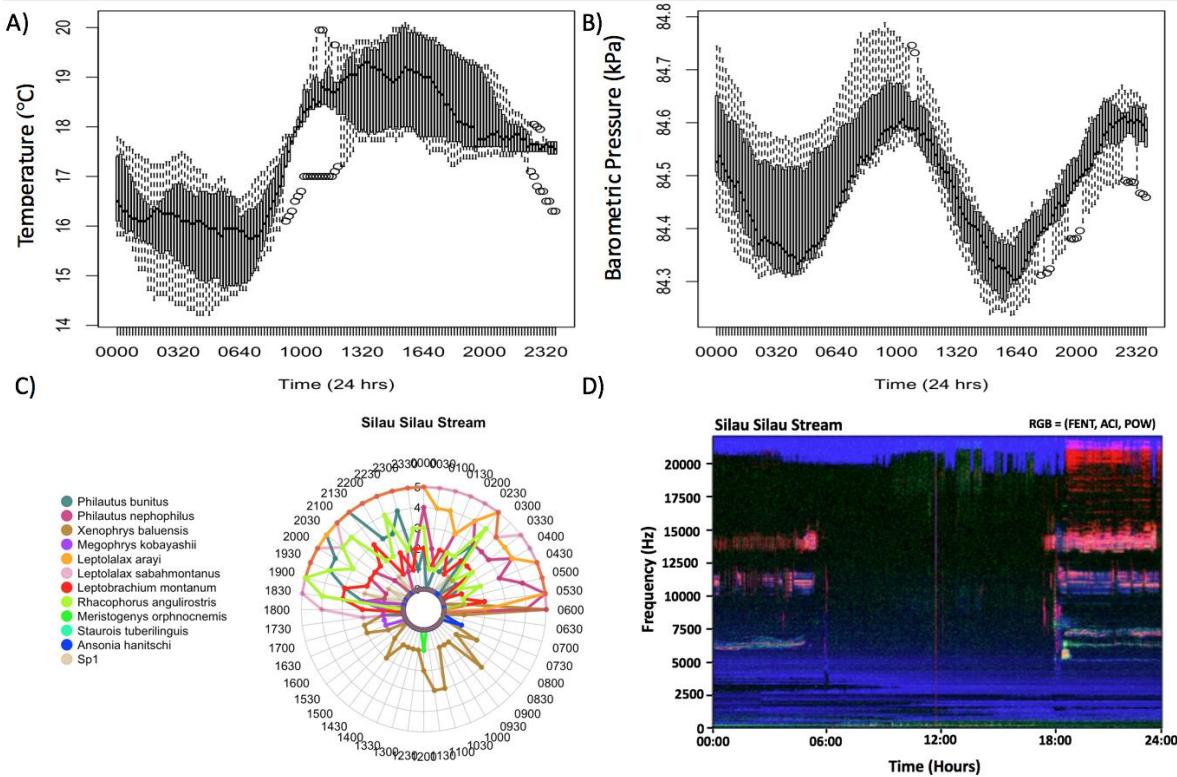


Figure 5.4. Sample data retrieved from one site in Kinabalu Park (Silau Silau Stream): A) Temperature fluctuations over a total of five days; B) barometric pressure data over five days; C) call occurrences for amphibian species over a total of five days; D) an example of soundscape analysis over one 24 hr period, indicating that most acoustic activity in this site occurs during the night.

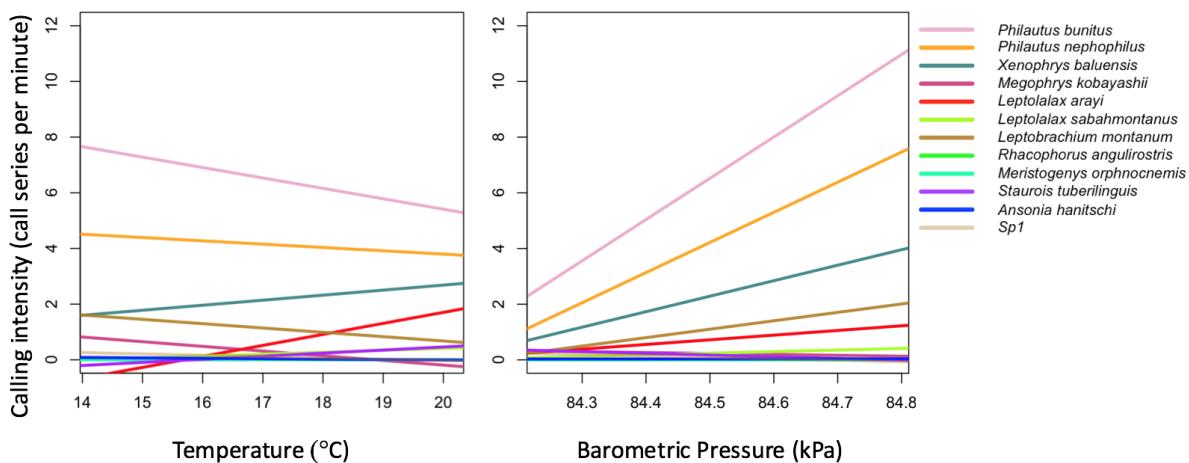


Figure 5.5. Results from linear models of calling intensity (calls per minute) against temperature and barometric pressure for each species present at Silau Silau Stream.

Discussion

This study managed to collect > 400 GB and > 180,000 minutes of data with the Raspberry Pi set up, and I conclude that this system is a cost-effective and viable alternative to more expensive off-the-shelf products. Soundscape data were also successfully collected (> 100,000 minutes). While soundscape data take up more data storage space, they can be analysed faster as they do not require a training library. Training libraries are calls used to tell the classification algorithm what to look for in the data. The creation of a training library takes considerable manual work and requires a certain level of operator expertise to ensure that the algorithm is performing optimally and that mistakes are few. Soundscape analysis gives an overview of the acoustic landscape in a specific site, and though it does not require a training library, it still requires a certain level of manual verification of the sound files to establish accuracy. The public release of Python scripts by Sarab Sethi on GitHub to generate the false-colour spectrograms has substantially increased the accessibility and utility of soundscape data for a broad range of users, including scientist.

While soundscape analysis gives a useful overview of the acoustic landscape, identification of species calls using classification algorithms can potentially yield a much richer and detailed data set. Several open-source software packages are now available for management, analysis and automated species identification of acoustic data. Training a classification algorithm is an arduous process that requires the trainer to be familiar with the call characteristics of specific species and inter-specific differences in multi-species environments. Several R packages are available for various types of sound analysis.

For automated classification, the R package warbleR is available. This package does not require a training library and is instead based on time-to-frequency information and gets tuned by the trainer to a specific call. The limitation of this package is that in multi-species environments with a high number of species calling at the same time, it is difficult to extract

data from all the species. The analysis must be set up and extracted for each individual species. Because this package (and other like it) relies on manual identification of call characteristics, it cannot tell us anything about species unknown to the operator, which is potentially a disadvantage because passive acoustic monitoring would have its greatest utility in areas that are under-surveyed, where there is corresponding lack of information available on which species are present and their calls characteristics.

Another software available for sound analysis, Aureas, can be trained to identify multiple species if enough training data are available. The trainer thus must go through and train the software on each individual species and tell it which clusters are correctly identified from which species. A limitation is that training is sometimes difficult with omni-directional microphone data, such as those from passive acoustic monitoring, because of the multitude of sounds heard concurrently, multiple species and background noises. As an example, the acoustic data used in this study contain a lot of bird noises, in addition to the amphibians, and for optimum performance the algorithm must be trained to identify these as well. This places a considerable strain on the expertise level of the trainer. Aureas is artificial-intelligence based and uses algorithms based on speech recognition. This increases the utility of the software greater, because it can deal not only with dialect variation but also highlight unknown species in the dataset. The Aureas algorithm operates with fuzzy logic (where values are on a scale from 0 to 1 instead of just binary) and if it determines that any sound it hears is far enough away from any call in the training library but still likely to be a call, it will flag the sound as an unknown species. This is immensely useful in applications, because the training libraries can be updated with tagged calls from unknown species, and areas with unknown species can be monitored before formal descriptions of the species and their calls have been performed. Experts can then be directed to the area's most in need of taxonomic work or call identification.

Other free software available for sound analysis, such as Audacity® is useful for manual call identification and inspection of acoustic files, and because of its intuitive interface is particularly appropriate for training of students. It is recommended that any human operator who needs to increase his or her familiarity with species' calls spend a significant time using software such as Audacity. This ensures later ability to train classification algorithms to a high accuracy.

I conclude that open-source hardware offers flexibility in customisation of sensor arrays and the ability to modify relay of data. Initial results also indicate that environmental data should be collected simultaneously when recording acoustic data due to species responding in different ways to environmental conditions. In terms of power sources, car batteries were more reliable than power banks, and when combined with solar panels they worked relatively well even under conditions of low light and high-power consumption. Despite their use in other studies, the high mass of car batteries presents logistical constraints for large-scale studies or studies in isolated areas. The power banks had severe issues, lasting in Singapore (at 30°C) only three days, and up to five days in cooler Mount Kinabalu (at 20°C). Solar panels worked despite less-than-ideal mounting conditions under the canopy. During deployment of solar panels in Singapore, weather was very poor, being overcast and rainy with very few hours of sun, indicating that with more sun, the system should be able to deliver more consistent power.

The durability of acoustic monitoring stations and electronics is considerably shorter in tropical settings compared to more temperate areas since both extreme heat and humidity strain the materials. Even though the units in this study were housed in waterproof boxes, internal humidity in such boxes is a persistent issue that reduces longevity of electronic instruments. As shown here, this can be partially solved by including either a 50 g or a 100 g bag of indicator type silica inside, but it requires space in the station. Five stations in this

study were also infested by ants, despite the use of ant repellent. The covers of solar panels also became infested by ants. This indicates a need to allow space for insect repellents and perhaps back-up deterrents. Issues with destruction of wind covers also appear to be large. Considering the need for wind covers to ensure standardisation among recording stations, it would be of interest to find out what is destroying the wind covers so the destruction can be mitigated. Another approach is to remove all external wind covers on all microphones before deployment. I advocate further testing of durability of off-the-shelf acoustic monitoring products in both temperate and tropical regions. Unfortunately, few data are available on failure rates of off-the-shelf or open-source products for acoustic monitoring, making it difficult for users to judge which is the best investment for their project.

The 3G set-ups used in some studies is not necessarily the best solution for large-scale studies where a high number of stations are involved due to the current cost of the data plans and the high-power requirements. The LoRa system (Hornbuckle 2010; LoRa Alliance Technical Marketing Workgroup 2015), being a low power wide area network, offers a potential low-power and low-cost solution that warrants further testing in tropical forests. In this study the dense understorey posed an obstacle to LoRa because it limits data-transfer distance. In principle, with a clear line of sight and directional antennae LoRa should be able to send data over kilometres. Another limitation of LoRa is the limit of the size of the data packages it can transfer: WAV files, for example, are too big for transfer and must be saved locally on the system and retrieved later. Another possibility is to program the Raspberry Pi client to calculate acoustic indices on the WAV file that can then be added appended to the CSV file that is sent over LoRa. This allows basic information from the stations to be retrieved continuously and provides information on whether the station is still functioning making maintenance visits more efficient.

Conclusion

The environmental sensors available for the Raspberry Pi are relatively inexpensive to replace. The system is modular and can be customised for specific requirements. The data that are extracted from acoustic and environmental monitoring stations provide considerable insight into acoustic ecology in sites where they are deployed. An additional benefit is that training of local scientists encourages development of new technological solutions and applications in their own countries or region.

CHAPTER 6. Bioacoustics of the amphibian community on Mount Kinabalu

Introduction

Amphibians display a wide array of physiological adaptations and have colonised a diversity of terrestrial and aquatic environments. They rely on multiple habitats, generally exhibit low vagility and have permeable skin that makes them sensitive to changes in their direct environment (Bickford et al., 2010; Howard & Bickford, 2014; Navjot S. Sodhi et al., 2008; Wake & Vredenburg, 2008). Due to their sensitivity to changes in both aquatic and terrestrial environments, amphibians are often used as indicators of overall environmental health (Wake & Vredenburg, 2008). Devising effective strategies for monitoring amphibian diversity is therefore imperative in the broader framework of understanding global environmental change.

Southeast Asia incorporates three of the world's 25 biodiversity hotspots and display very high amphibian biodiversity. The remaining tropical forests in the region are being rapidly cleared and many species are threatened with extinction (Bickford et al., 2010; J. Rowley et al., 2010; Navjot S. Sodhi et al., 2010). There is a recognized need for conservation actions related to the amphibians of Borneo, where 23% of the amphibian species are listed in one of the IUCN threatened categories (Inger, 2017). The rates of deforestation due to logging for hardwood and land-conversion for oil-palm plantations and urbanisation are high. This high rate of landscape modifications causes population fragmentation as well as extinction and is severely impacting the viability of many species (Bickford et al., 2010; J. Rowley et al., 2010; Navjot S. Sodhi et al., 2008). Therefore, it is crucial that conservation actions be implemented for specific species. Despite this urgent need, conservation plans remain scarce. An impediment to the development of effective

conservation plans is the lack of baseline information such as population numbers, population dynamics and ecology (Rodríguez, 2003). The development of effective conservation plans, requires persistent and extensive inventory-based studies (Collins et al., 2005; Ferrier et al., 2004; B. H. Kueh & Maryati, 1998; BH Kueh & Mohamed, 2005; Matsui, 2006). Southeast Asia need new technologies and methods implemented to combat a shortfall of funds and trained personnel in the field of biodiversity monitoring.

Passive acoustic monitoring is a rapidly developing technology for monitoring amphibians (Aide et al., 2013; Amezquita et al., 2011; Bedoya et al., 2014; Towsey et al., 2013, 2014) without interfering with the behaviour of the individuals that are being recorded. The volume of data generated could give great insights into species occupancy and population fluctuations over time whilst providing a permanent data record. Recent advances in classification algorithms reduces the need for previously labour intensive manual species identification of the sound files (Araya-Salas & Smith-Vidaurre, 2017; Bedoya et al., 2014; Crump & Houlahan, 2017). Indices for quantifying sound space have also been developed that allow rapid assessment and comparison between sites (Gasc et al., 2015; Jérôme Sueur et al., 2014, 2008; Towsey, 2013; Towsey et al., 2013, 2014; Towsey & Zhang, 2014; Truskinger, Towsey, & Roe, 2015; Wimmer, Towsey, Planitz, Williamson, & Roe, 2013).

Anurans use calls for mate attraction and territory advertisement, making these auditory cues highly important for reproductive success (Grafe et al., 2012; Hödl & Amézquita, 2001; Ringler, Ursprung, & Hödl, 2009; Starnberger, Preininger, & Hödl, 2014). The effect of other species on the frequency bands and the temporal partitioning of a species' calling activity can be strong (Both & Grant, 2012; Mossbridge & Thomas, 1999), and in species-saturated communities the acoustic niche breadth of individual species is expected to be low (Preininger et al., 2007; Sinsch, Lümkemann, Rosar, &, & Dehling, 2012).

Hutchinson's niche theory predicts that the fundamental niche will limit the number of

species in a community dependent on the resources that are available (Hutchinson, 1957). The acoustic niche for anurans is predicted to be one of the most important resources available and should put specific constraints on species' temporal (time-of-day), acoustic (physical structure of the call) and spatial (microhabitat used for calling) characteristics (Amezquita et al., 2011; Sinsch et al., 2012; Starnberger et al., 2014). Despite this most studies on anuran calling communities ignore the temporal and spatial aspects (Ospina et al., 2013), making inferences about the community, and also the effects of potential changes to the acoustic structure difficult.

Multiple studies already indicate that changes to the noise signals in the environment cause adjustment of the calls of individual species (Gibbs & Stanton, 2017; Parris, Velik-lord, & North, 2009; Patricelli & Blickley, 2006). In addition, the expected changes to the occurrence of these species are likely to cause shifts also in the acoustic community which are difficult to infer without thorough data. Animals evolve specific acoustic codes to ensure accurate transfer of information and, as this method of information transfer is costly, it is under strong evolutionary pressure. When the environment changes and species shift their potential location, the acoustic niche they previously operated in might be already occupied, creating a masking effect. This should ensure that species quickly adjust their acoustic codes to compensate for the masking (Almo Farina & Pieretti, 2014; Authors Farina, Farina, & James, 2016). In any case, competition between species in acoustic spaces is expected to be very high. Species undergoing range shifts may thus exert high pressure on resident species in their new ranges if their existing acoustic niche is already occupied.

Acoustic monitoring data can be collected in a variety of ways. Often only a few minutes of every hour are collected (Aide et al., 2013; Whytock & Christie, 2017). However, there is also the option of collecting acoustic data continuously. This allows for soundscape analysis, where "soundscape" is defined as all the sounds that are present in a location

(Farina, 2014). The joining of the fields of acoustic ecology and landscape ecology has yielded the new field of soundscape ecology (Truax & Barrett, 2011). In this field, there is a need to assess the acoustic composition of the environment, the temporal and spatial variation of the acoustics, and the interactions between acoustic events and the rest of the landscape (Almo Farina & Pieretti, 2014; Authors Farina et al., 2016; Jérôme Sueur & Farina, 2015; Truax & Barrett, 2011). Analysis of soundscape data in a fashion that ensures that changes to the acoustic landscape are easy to assess is paramount. Recent advances have seen the development of multiple acoustic indices and methods for portraying this information (Pieretti et al., 2011; Towsey & Zhang, 2014; Towsey et al., 2014).

This study sought to answer the following research questions: 1) Is there a difference in the species list produced by visual encounter surveys and from passive monitoring surveys? 2) Is there evidence of acoustic and/or temporal niche partitioning amongst amphibian communities? 3) Does the acoustic community structure differ between the sites sampled? 4) Do the soundscape indices capture the amphibian species in the sound files? and 5) How do the soundscape results differ between the sites?

Methods

This study was conducted in Kinabalu Park, Sabah, Malaysia on the island of Borneo (Lat: 6.157, Long: 116.638) between 900 and 3300 m asl. The habitat on the site consists of cloud rainforest which is divided into clear vegetation zones: lowland dipterocarp forest (0 – 900 m asl); mixed dipterocarp and Fagaceae forest (900 – 1300 m asl); Fagaceae forest (1300 – 1800 m asl); cloudy moss forest (1800 – 3000 m asl); and sub-alpine hard-leaved plants (3000 – 3700 m asl). The acoustic monitoring stations were placed only in the habitat band between 1300 – 1800 m asl to investigate the degree of overlap in the acoustic community in an area with uniform environmental conditions.

Between January and March 2016, two stream sites and three terrestrial sites were established in Kinabalu Park. In each terrestrial site a 500 m transect was set up and in each stream site a 100 m transect was set up. The shorter length of stream versus terrestrial sites is due to the higher complexity and issues with accessibility of the streams. visual and auditory encounter surveys (VES) were conducted at each site between February and May 2016 (refer to methods in chapter 1 for details). Species heard but not seen were also recorded. Searches were conducted using Princeton Tech Apex head torch at full strength single beam (275 lumens).

Acoustic Monitoring Stations

In 2017, five stations were placed in the previously established transects (see Figure 6.1). The monitoring stations were placed in the mid-point of each transect (at point 50 in each stream transect and point 250 in each terrestrial transect relative to VES; see Figure 2.2 in Chapter 2 for layout). The specifics of the station configurations are reported in Chapter 5 under the sections reporting the specifics for Kinabalu Park. Apart from recording acoustic

data the stations also collected data on temperature, humidity, lux and barometric pressure.

The units were attached to a tree at 1.5 m height off the ground.

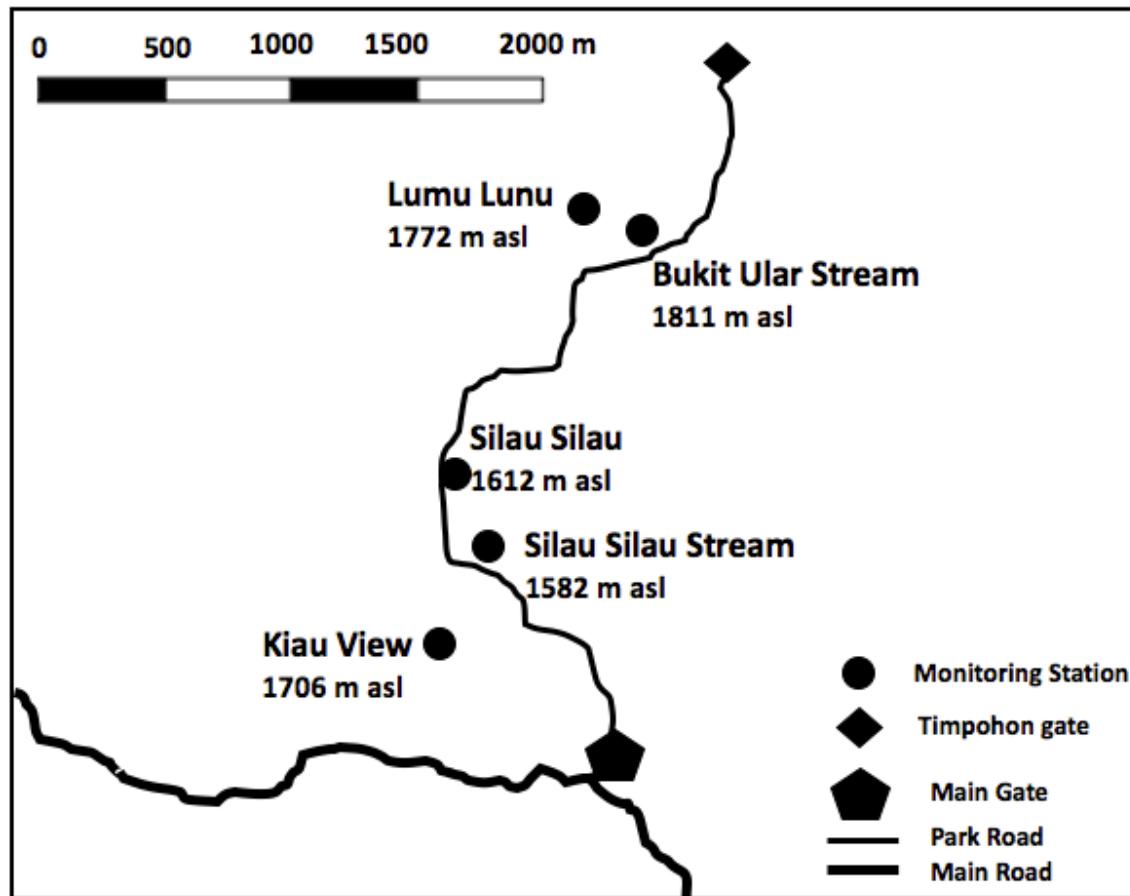


Figure 6.1. Map showing the location of the five sites where data were successfully collected. Map indicates main road, the road in the park, the main entrance gate to the park as well as Timpohon gate where the hiking trail to the peak begins.

Passive Acoustic Monitoring

In February and March of 2017, one automated acoustic monitoring station was deployed in each of the five sites, with a minimum distance of 250 m between stations. The sampling interval was for one minute out of every ten for 24 h per day, with the data stored on a 64 GB micro SD card. The data were downloaded from the units manually after three

months. Sound tests were performed to assess the area a unit can cover using sinusoidal tone signals at 0.5, 2, 4 and 8 kHz at 2, 4, 8, 16, 32 and 64 m away from the monitoring station and at a height of 10, 200 and 500 cm at each interval (Darras et al., 2016). Each signal was repeated three times and an average taken.

Species calls were identified manually from the five sites in Kinabalu Park from five days for each site meaning that 720 minutes were extracted per site and a total of 3600 minutes for all five sites. Calls were identified to species using call descriptions and reference pictures of call spectrograms, as well as sound libraries reported in the literature (Inger et al., 2018; Malkmus et al., 2002). In addition, many calls were identified either to species or higher taxonomic level (cricket, cicada and bird) during the field season in 2016 meaning that auditory identification could be performed for these groups and then cross-referenced back with the spectrograms in the sound recordings (see Figure 5.2 in data Chapter 5 for call identification of the amphibians). The dates used for manual extraction were selected randomly. The acoustic files were visually inspected using Audacity (2.2.0). Presence of species and number of times a call occurred were recorded. The manually extracted dataset will be used for verification of the accuracy of species classification algorithms for later studies. The results were displayed using radar plots to assess levels of calling activity. The plots were generated in R (R Core Team, 2017).

Soundscape

Soundscape data were collected from the five sites over four days, in the period 2 – 7 June 2017. This involved collecting continuous audio data for four days. The 4th of June was used for analysis. The soundscape data were analysed using false-colour spectrograms (Towsey & Zhang, 2014) produced using Python (Sethi, 2017). The indices used were: acoustic power (POW), acoustic complexity index (ACI) and temporal entropy (FENT).

These indices are largely independent of each other: POW assesses the signal amplitude and averages it; ACI measures the complexity of the acoustic environment by measuring the change in signal amplitude between frames (Pieretti et al., 2011; Towsey et al., 2014); FENT measures the acoustic energy dispersed temporally (Towsey & Zhang, 2014).

Results

The signal strength attenuated faster with higher frequencies (Figure 6.3). Higher height off the ground appears to maintain signal strength over longer distances. The furthest distance a unit is calculated to reach was 250 m at a frequency of 2 kHz. Sound at 0.5, 2 and 4 kHz attenuate the furthest in the two stream sites. At 8 kHz the sound attenuates most rapidly with distance and the maximum detectable distance is close to or below 150 m for all stations at all heights. The signal attenuates slightly faster when it is closer to the ground.

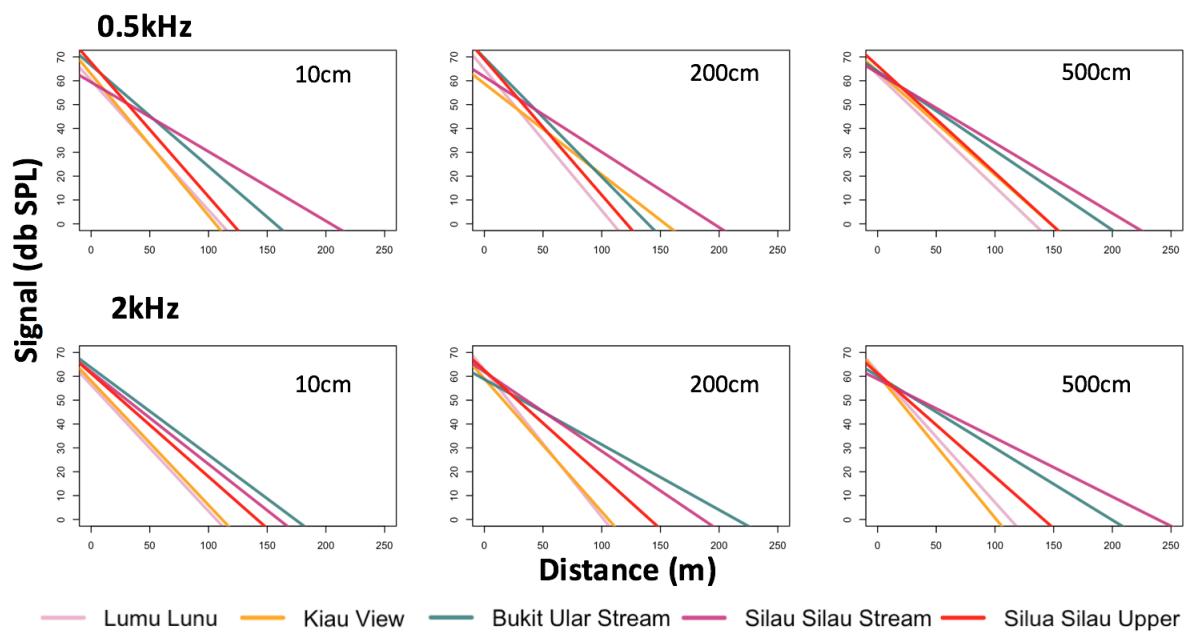


Figure 6.3. Signal strength attenuation at the frequencies: 0.5, 2, 4 and 8kHz. The signal strength was measured at three heights of 10, 200 and 500cm off the ground.

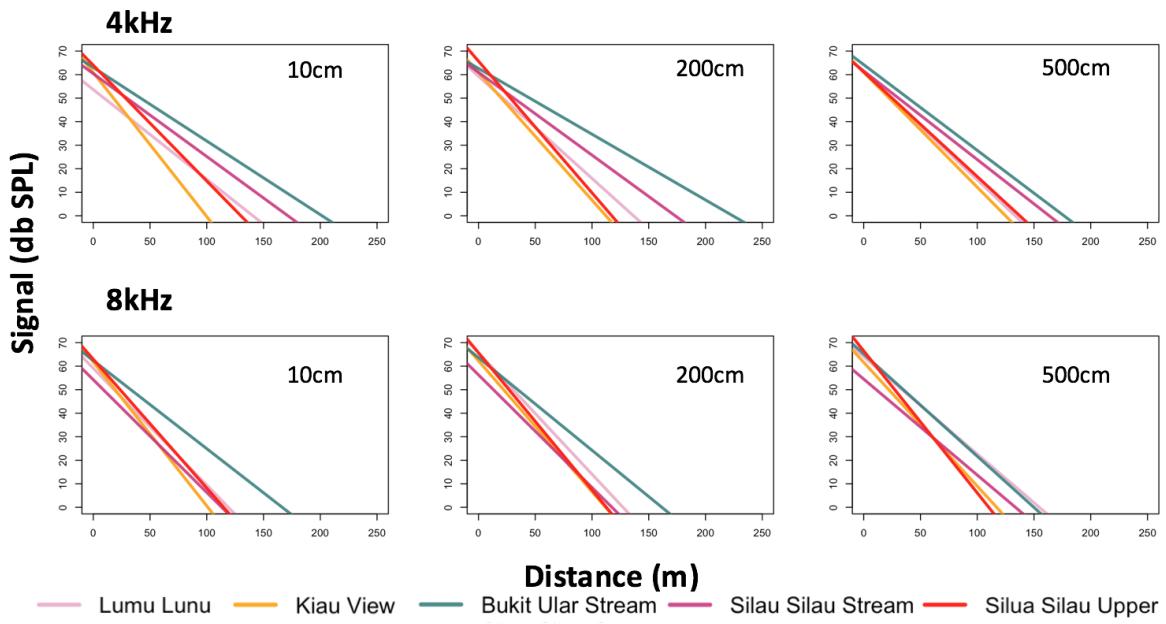


Figure 6.3. contd.

Passive Acoustic Monitoring

In total, 16 amphibian species across the five sites were identified in a total of 3600 minutes of data (7% of our total acoustic dataset of 50,400 minutes) from five random days. The sites differ substantially in the number and identities of species present acoustically. Activity levels also vary substantially, with some sites having many species with high activity through the night (Figures 6.5, 6.6 and 6.8). Two sites have fewer species but show high activity through the daytime as well (Figure s6.4 and 6.7). The overlap in species detected in both VES and acoustic species varies considerably between sites (Table 6.1, see Appendix 6.1 for Venn diagrams). Kiau and Silau Silau Upper had a similar number of independent species found and considerable overlap. Bukit Ular Stream had no overlap between survey methods and both Bukit Ular Stream and Lumu Lunu had more species detected with the acoustic monitoring than with VES. Silau Silau Stream had a high number of species detected by both methods, but there was still a high number of species detected only through acoustic monitoring.

Table 6.1 Number of species detected by survey method (VES stands for visual encounter survey).

Method/overlap	Kiau	Silau Upper	Silau Stream	Bukit Ular Stream	Lumu Lunu
VES	2	3	2	1	1
Acoustic	3	4	6	3	7
Overlap	2	7	5	0	1

Kiau View had five species recorded using acoustic monitoring (Figure 6.4) with the majority being active between 1800 and 0600 hrs. *Philautus bunitus* also showed some day activity. This varied from the VES data recorded in 2016 (see Appendix 6.2 for full tabulation) which recorded four species, two of which were not recorded in the acoustic monitoring study in 2017: *Leptobrachium montanum* and *Pelophryne misera*. The acoustic monitoring study recorded three species that were not seen in the VES study: *P. bunitus*, *Philautus aurantium* and *Rhacophorus angulirostris*. Despite the low number of species, the ones that are present showed very high activity levels with *P. nephophilus* and *P. bunitus* calling almost continuously from 1800 until 0600 hrs. *Kalophrynus baluensis* showed acoustic activity almost around the clock with a break between 0600 and 0830 hrs.

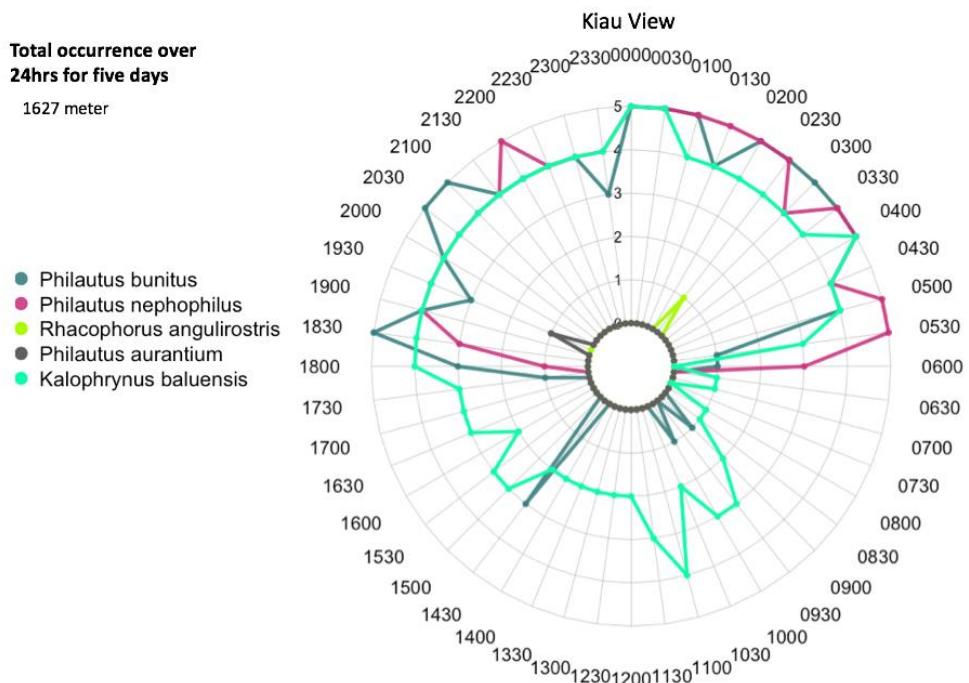


Figure 6.4. Occurrence of calling of amphibian species in the Kiau View site over 24 h for a total of five days. Concentric circles indicate the number of days (as shown by numerals 1-5) a species was detected at each hour.

The Silau Silau Upper site showed high species diversity with 11 amphibian species recorded using acoustic monitoring (Figure 6.5), one more species than the ten recorded from VES in 2016 (Table 6.1). The species list differs in three species recorded from VES in 2016: *Limnonectes kuhlii*, *Pelophryne misera* and *Philautus macroscelis*. Four species were recorded in the acoustic monitoring study that were not seen in the VES study in 2016: *Megophrys kobayashii*, *Meristogenys orphnognemis*, *P. bunitus* and *Staurois tuberilinguis*. This site has considerably high activity levels during the night with multiple species calling consistently from 1800 until 0600 hrs. However, they all occupy different frequency bands. *M. baluensis* again showed high levels of day activity and especially dawn activity at 0600 hrs. There is some activity from known day-calling species such as *A. hanitschi*, *S. tuberilinguis* and *M. orphnognemis*. *P. bunitus* also shows some activity during the day in addition to the high activity levels seen during the night.

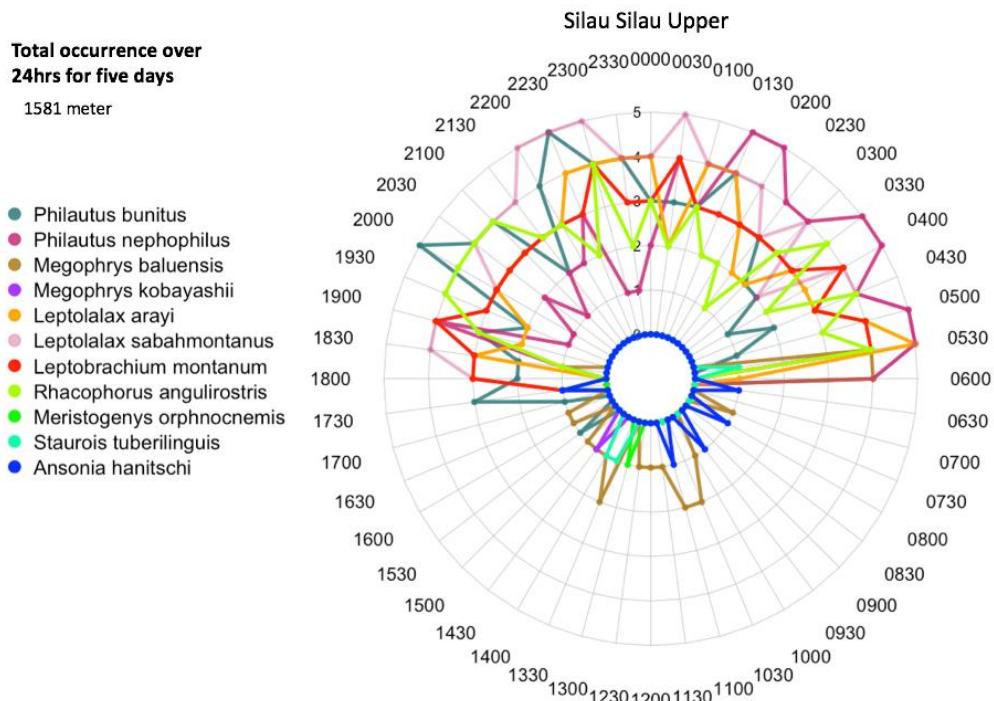


Figure 6.5. Occurrence of calling amphibian species in the Silau Silau Upper site over 24 h for five days. Concentric circles indicate the number of days (as shown by numerals 1 – 5) a species was detected at each hour.

Eleven species were recorded in Silau Silau stream from the acoustic dataset (Figure 6.6), in comparison the VES dataset from 2016 recorded only seven species. With six species: *K. baluensis*, *M. baluensis*, *M. kobayashii*, *M. orphnognemis*, *P. bunitus* and *P. nephophilus* recorded in the acoustic survey but not in the VES and two species (*L. kuhlii* and *Philautus macroscelis*) recorded in the VES but not the acoustic surveys. However, *Leptolalax sabahmontanus* was always missed from auditory identification in the VES even if it was visually identified, due to similarity in call to *Leptolalax arayi*. *M. baluensis* appear to call almost exclusively during the day; the frequency level it calls at is the same as *P. nephophilus*, which calls frequently through the night time.

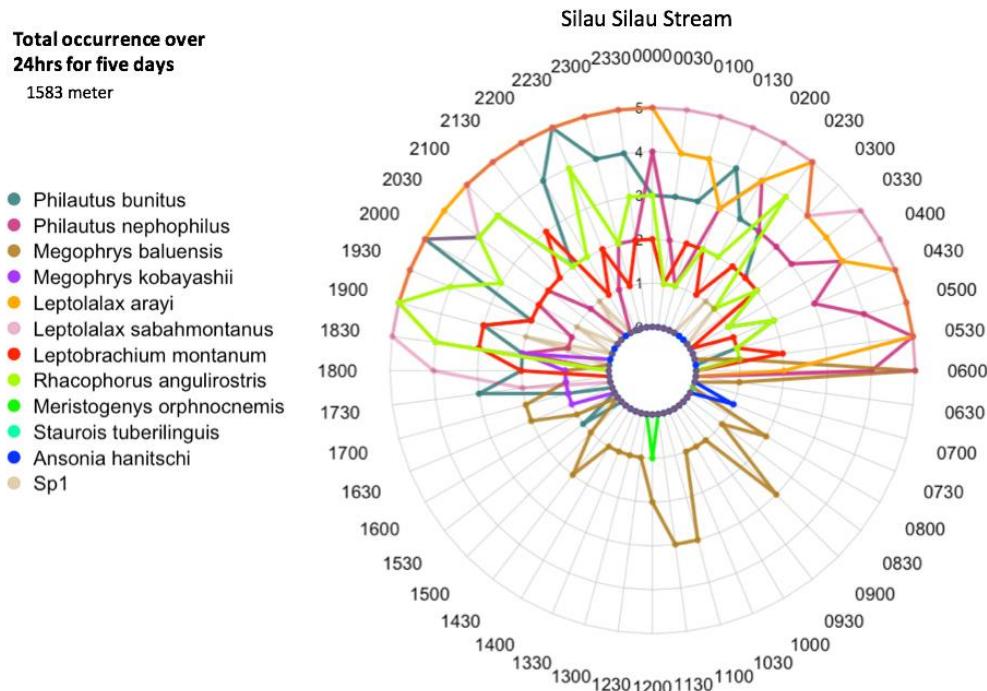


Figure 6.6. Occurrence of calling of amphibian species in the Silau Silau Stream site over 24 h for five days. Concentric circles indicate the number of days (as shown by numerals 1 – 5) a species was detected at each hour.

Bukit Ular Stream had four species detected in the acoustic surveys (Figure 6.7), one of them unknown but believed to be an amphibian (the sound was not characteristic of a bird or insect call). The VES in this site in 2016 recorded only one species, *L. kuhlii*, which was not recorded during the acoustic surveys. But since this species does not call (Malkmus, 2002) lack of presence in the acoustic surveys does not imply absence. Activity is very low during the day with only one amphibian species ever recorded. At night, *K. baluensis* appears to be the most active species.

**Total occurrence over
24hrs for five days**

1800 meter

- Philautus bunitus
- Leptobrachium montanum
- Sp1
- Kalophrynx baluensis

Bukit Ular Stream

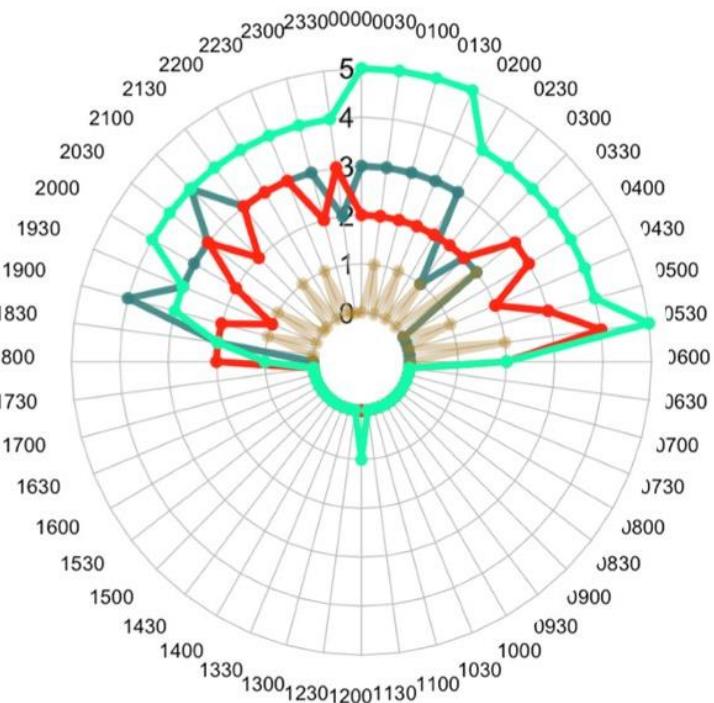


Figure 6.7. Occurrence of calling of amphibian species in the Bukit Ular Stream site over 24 h for five days. Concentric circles indicate the number of days (as shown by numerals 1 – 5) a species was detected at each hour.

Lumu Lunu had eight amphibian species active throughout the acoustic recordings (Figure 6.8), whereas the VES in 2016 recorded two species. Only *P. nephophilus* was recorded by both VES and acoustic monitoring. There is some slight day activity recorded for *K. baluensis*, *M. baluensis* and *M. kobayashii*. The remaining species show consistent activity from around dusk at 1800 hrs until 0530 hrs. *N. pictus* was heard calling once, as was *P. macrosceles*.

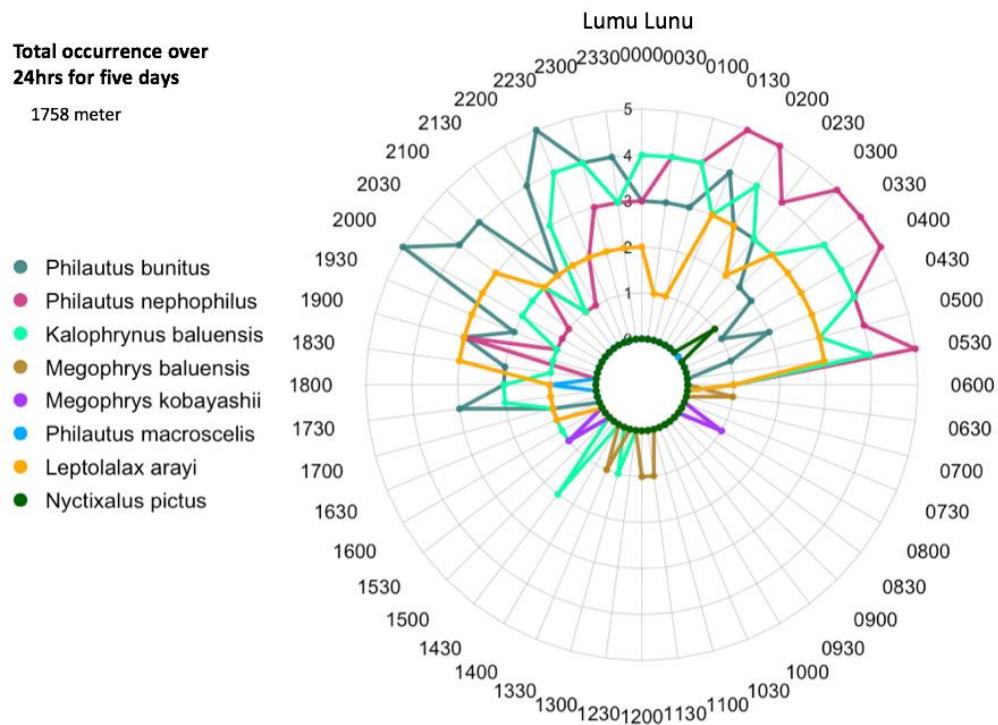


Figure 6.8. Occurrence of calling of amphibian species in the Lumu Lunu site over 24 h for five days. Concentric circles indicate the number of days (as shown by numerals 1 – 5) a species was detected at each hour.

Sites with many species present tend to have a range of frequency bands up to around 7 kHz being used (Figure 6.9A for example). Sites with a low number of species did not show species in frequency bands above 5 kHz (Figure 6.9C for example) and generally there was low activity from amphibians during the day when taxa such as birds are calling and cover a range of frequencies (Figure 6.9B).

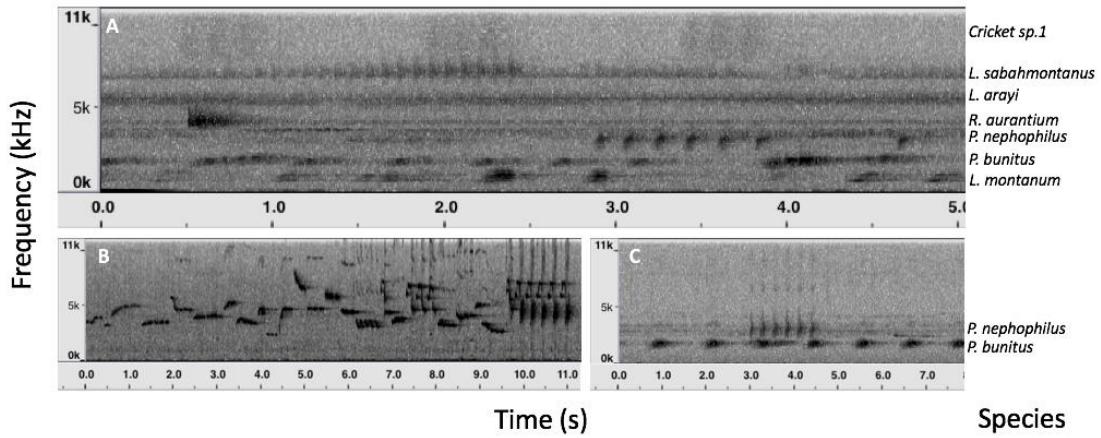


Figure 6.9. Spectrograms of A) high diversity site (Silau Silau) with six amphibian species and one cricket, B) unknown bird species from Silau Silau and C) a low species-diversity site (Kiau View) with two amphibians calling.

Soundscape

The Kiau View soundscape (Figure 6.10) shows a more activity than some of the other sites. There are slight FENT dashes at 3000 Hz with unknown origin. There appeared to be rain present from 0600 hrs, which might explain the lack of a dawn chorus. Different species of cricket showed up above 5000 Hz as bands of FENT, ACI and POW between 0000 and 0600 hrs and 1800 and 2400 hrs. The vertically stacked ACI bars at 0730 and 0930hrs are an unknown species of bird. The big swaths of POW from 1100 until 1800 hrs are a species of cicada. The vertical streak of FENT at 1800 hrs is a species of bird. *K. baluensis* show up as dots of FENT and ACI at just above 2500Hz between 0000 and 0600 hrs and 1800 and 2400 hrs. Manual inspection of the sound file showed that *P. nephophilus* calls frequently between 0000 and 0600 hrs and 1800-2400hrs but does not appear to be picked up by any of the indices.

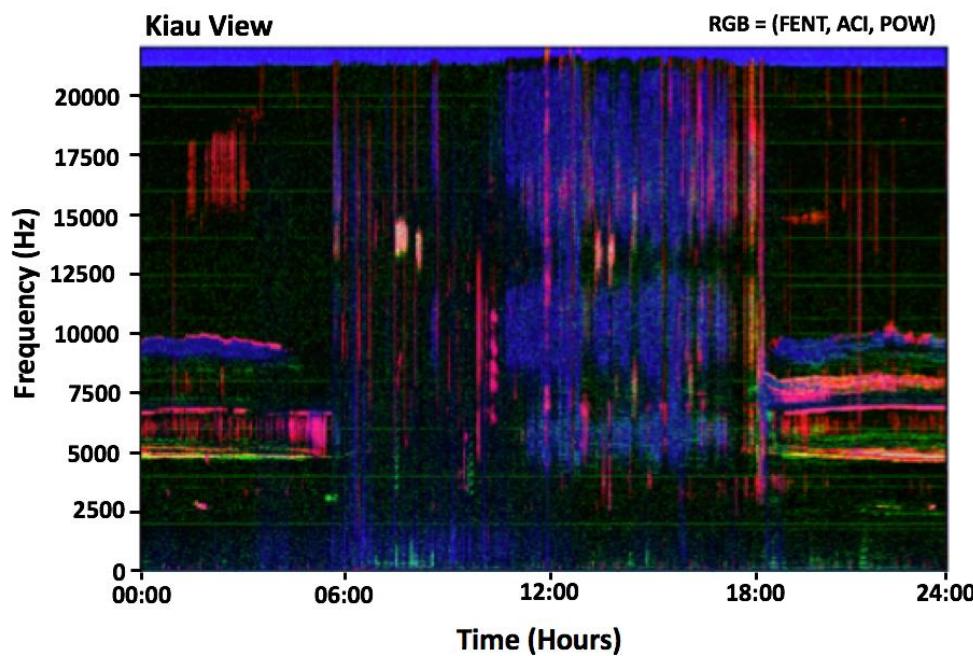


Figure 6.10. False colour spectrogram for Kiau View for three acoustic indices (FENT, ACI and POW) over a 24 h period.

The soundscape results for Silau Silau (Figure 6.11) show that ACI picks up crickets between 0000hrs and 0600hrs. The dawn chorus of birds was picked up by FENT shortly before 0600hrs. Birds were again picked up but by POW around 0800 hrs and 1000 hrs as stacked green vertical bars (species unknown). Day activity appeared to mostly be constrained to sporadic bird calls and some high frequency insect calls starting around 0900 hrs and ending around 1730 hrs. A cricket called at dusk just below 5000 Hz at 1800 hrs, whereas *L. arayi* showed up as a band of ACI from approximately 1800 hrs until midnight just above 5000 Hz. *L. sabahmontanus* showed up as a band of ACI from 1800 hrs until midnight at around 6000 Hz. Above this, crickets show up both as FENT and ACI. At 1800 hrs. A streak of FENT at around 1250 Hz is *L. montanum*. Visual inspection of the sound files shows that *P. nephophilus*, *P. bunitus*, *R. angulirostris* were frequently calling between 0000 hrs and 0600 hrs as well as 1800 to 2400 hrs but were not picked up by any of the indices. *L. montanum* were also calling frequently between 0000 hrs and 0600 hrs but was not picked by any of the indices.

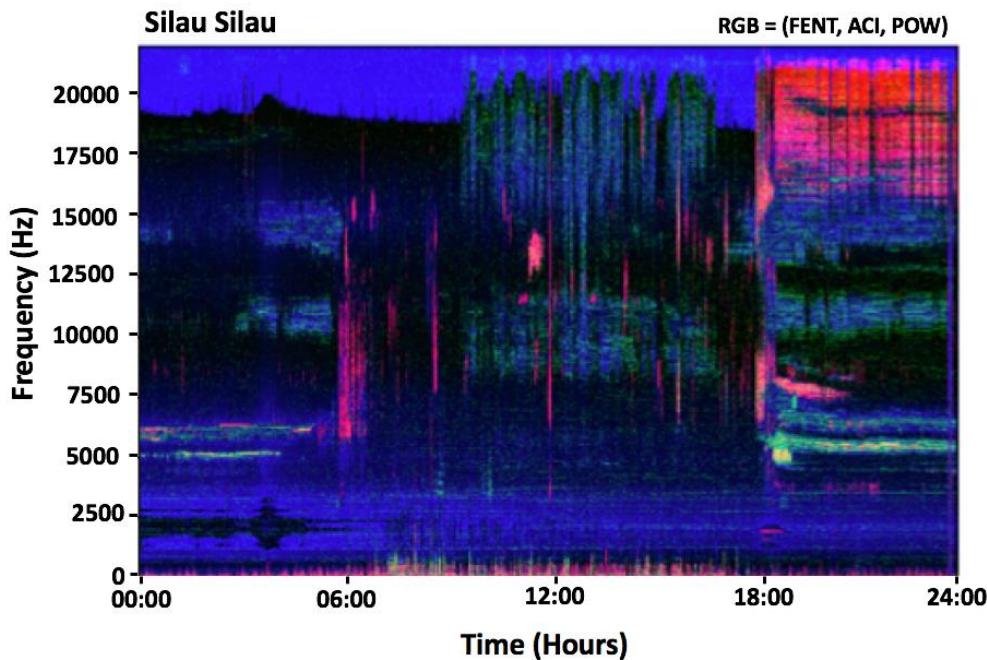


Figure 6.11. False colour spectrogram for Silau Silau for three acoustic indices (FENT, ACI and POWER) over a 24 h period.

Silau Silau Stream displays a soundscape (Figure 6.12) that appears very similar to Silau Silau. The main difference is very little activity during day (0600 – 180 hrs) and a minimal dawn chorus from birds at 0600 hrs. The period from 0000 until 0600 hrs mainly showed activity by *L. sabahmontanus*. *L. sabahmontanus* is again active from 1800 – 2400 hrs as a streak of ACI at around 6000 Hz. *L. arayi* was present as a faint POW streak at just above 5000 Hz. A cricket also showed up as a brief POW band at around 1830 hrs just above 5000 Hz with another species of cricket that called continuously from 1800 to 2400hrs as a streak of FENT at 7500 Hz. Upon inspection of the sound file *R. angulirostris* sporadically called between 0000 and 0600 hrs without any of the indices picking up the calls. *L. montanum* called regularly from 1800 to 2400 hrs but did not appear to be picked up by any of the indices. *P. nephophilus* also called occasionally between 1800 and 2400 hrs without getting picked up by any of the indices.

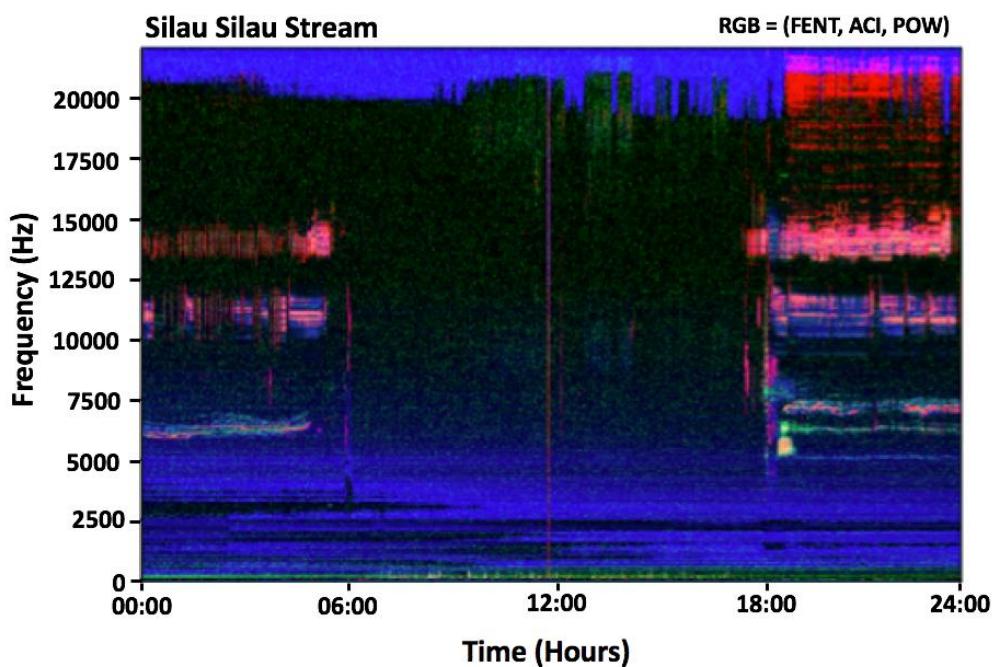


Figure 6.12. False colour spectrogram for Silau Silau Stream for three acoustic indices (FENT, ACI and POW) over a 24 h period.

Bukit Ular showed very low acoustic activity in the soundscape (Figure 6.13). *K. baluensis* showed up as little dots of ACI at around 0400, 1000 and 1930 hrs. There are some faint streaks of cricket activity at around 6000 Hz between 0000 and 0600 hrs. No bird dawn chorus was evident at 0600 hrs or thereafter. However, cicada activity was present from around 1300 hrs as vertical strips of FENT and appeared briefly at around 0430 hrs and 1000 hrs. No amphibian species appear to have been missed at this site in the false colour spectrogram, judged by comparison with manual inspection of the sound file.

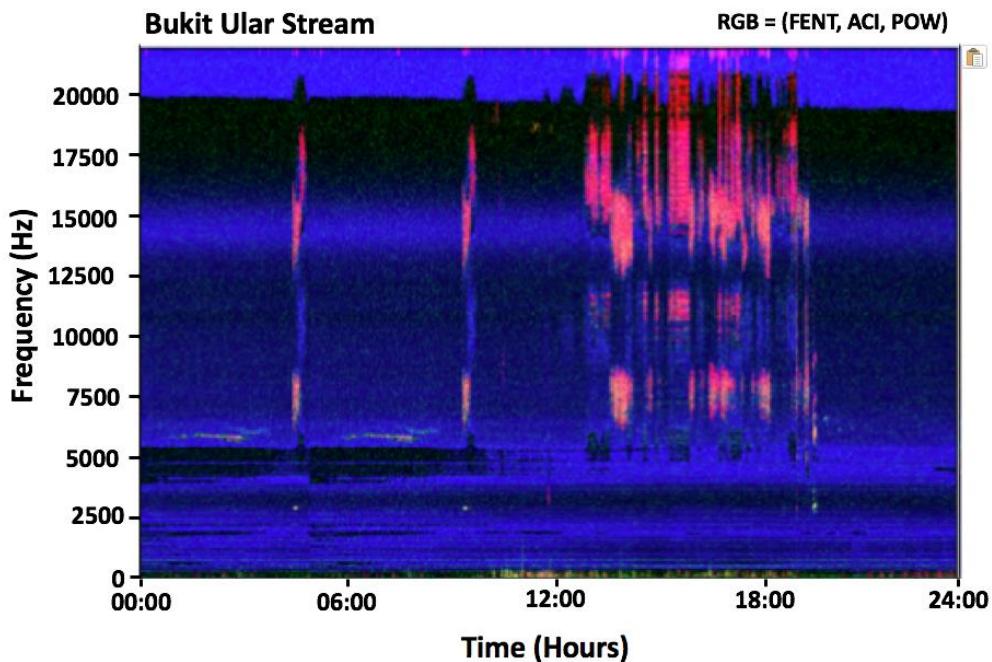
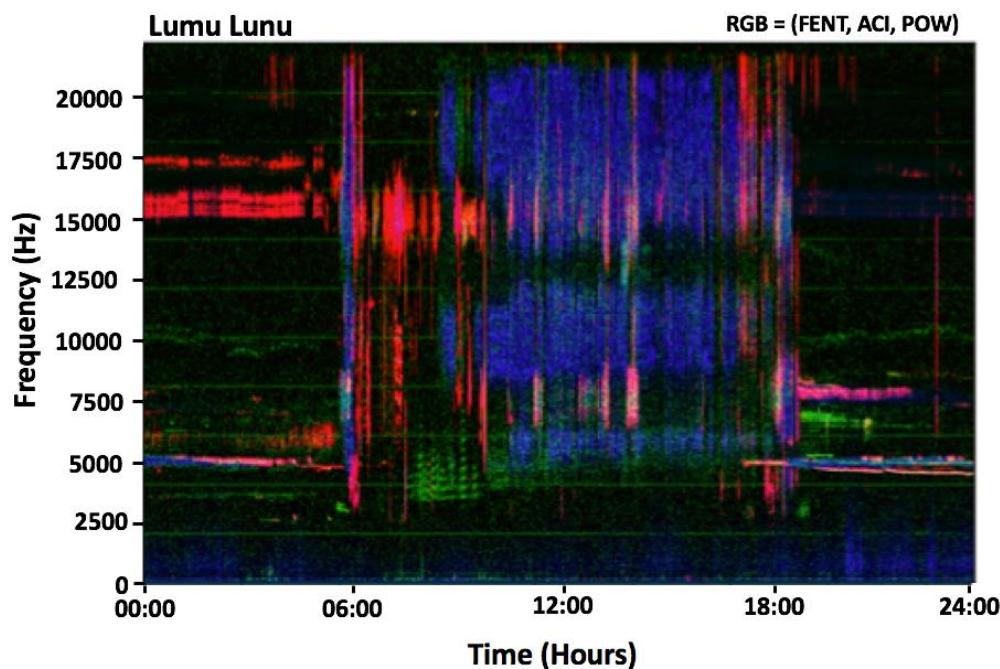


Figure 6.13. False colour spectrogram for Bukit Ular Stream for three acoustic indices (FENT, ACI and POWER) over a 24 h period.

The Lumu Lunu soundscape (Figure 6.14) indicated high acoustic activity, especially during the day. Some faint FENT dots at 2500 Hz close to 0500 hrs is *K. baluensis*: this species called frequently between 0000 and 0600 hrs and between 1800 and 2400 hrs but appeared to largely be missed by the indices. The dawn chorus of birds was evident at 0600

hrs and cicada activity started after this as vertical FENT bands. A bird showed up as stacked ACI between 3000 and 5000 Hz starting at 0700 hrs. From around 0900 until 1800 hrs above 4500 Hz two species of cicada were active, often at the same time. One species of cicada shows up as bands of POW and the other one as FENT. At 1800 hrs, there are FENT bands indicative of one of the birds calling. Crickets call at around 5000 Hz and show up as either bands of or POW or FENT. Two other species of cricket show up as a POW band at 6500 Hz and another one as FENT at 7500 Hz from 1830 hrs onwards. *P. nephophilus* called frequently between 0000 and 0600 hrs when the sound file was inspected manually and was apparently missed by the false colour spectrogram indices.



6.14. False colour spectrogram for Lumu Lunu for three acoustic indices (FENT, ACI and POWER) over a 24 h period.

Discussion

Passive Acoustic Monitoring

Sounds at higher frequencies attenuate faster with distance (Marten & Marler, 1977).

The signal deterioration test in this study shows that there is a difference in attenuation between the sites, and between the different frequencies. This means that for species-specific analysis, the difference between sites in terms of detection space, must be calculated. This is to ensure that any effects detected (e.g., variability in species presence across sites) are not due to variations in the detection area of the monitoring station themselves.

The frequency window where attenuation over distance is the least is between 1 and 3 kHz (Marten & Marler, 1977). If higher audibility distance is an adaptive trait for amphibians (because calls attract mates and identify territories), one would expect evolution to have selected amphibians that call in this frequency band. There is a trade-off however, as calls can also advertise the location of the individual predators and parasites (Higham & Heberts, 2014; Ryan & Kime, 2011). Previous studies have shown that in complex calling communities the recognition space is relatively narrow (Amezquita et al., 2011; Hödl & Amézquita, 2001). It is interesting also to note, that the species calling at higher frequencies, such as *Leptolalax spp.*, appear to call almost continuously between 1800 and 0600 hrs, whereas species calling at lower frequency bands do not. Potentially this is due to a need to invest more in ensuring that a calling signal reaches the intended recipients should they be hard to reach, or maybe the species calling in the frequency bands with lower attenuation call irregularly to minimize the exposure to unintended recipients. Calling is energy demanding (Ryan & Kime, 2011), meaning it should only be performed when the chance of reaching intended recipients is high and the chance of reaching un-intended recipients is the low. The frequency of calls are also modulated by body size: larger species are normally constrained to

lower frequencies (Gingras, Boeckle, Herbst, & Fitch, 2013; Higham & Hebets, 2014; Ryan & Kime, 2011). The environment should also act as a constraint on attenuation of calls (Goutte et al., 2016). Streams are perceived to be highly complex environments, both acoustically and in terms of vegetation density. However, it has also been shown that the stream itself does not actually mask the communication attempts of amphibians (Grafe et al., 2012; Preininger et al., 2013). It is the calls by conspecifics or other taxa that make the communications attempts difficult allowing for the evolution of visual communication signals such as the foot flagging in torrent frogs or the use of the vocal sac in other species (Grafe et al. 2012; Boeckle et al. 2009; Preininger et al. 2013; Starnberger et ahl. 2014). This would explain the absence of the calls of some of the species known to be present in our streams, e.g., the genus *Staurois* is known to resort to foot flagging as an alternative communication mode.

The VES in 2016 were performed between 1900 and 2400 hrs and not throughout the night or the day. There was some concern that the activity of species differs depending on which hour of the night a site is surveyed, and that species might be missed in VES performed at the wrong time. The acoustic data collected in 2017 indicate that the species that are night-active have a similar level of acoustic activity throughout the night. The detection of these species with VES at night is likely to be effective if auditory detection is combined with visual detection. However, as previous studies have noted (Bridges & Dorcas 2000), some species are not acoustically active during the night. Ground-dwelling and well camouflaged species, such as *M. baluensis* and *M. kobayashii*, were consistently missed during the VES, yet they were detected in fewer than five days of passive acoustic monitoring. Species such as *S. tuberilinguis* and *A. hanitschi* that vocalise during the day were not missed during the night surveys, but their behaviour is different to the *Megophrys*

spp. as they are not ground dwellers and instead are located quite openly on leaves in streambeds during the night, at 0 – 40 cm off the ground or water body.

It is also believed that during the VES, *L. sabahmontanus* was consistently missed using auditory identification. This species is identified auditorily as well as visually in VES. *L sabahmontanus* and *L. arayi* sound very similar in the field and the calls are often hard to tell apart when they both call simultaneously as it is difficult to identify the shorter call series of *L sabahmontanus*. The acoustic data indicate that they call concurrently almost all the time, making it difficult to estimate presence from both species in VES from auditory identification alone. When the spectrograms are produced, the two species are easy to differentiate due to the use of different frequency bands and call series.

Acoustic surveys had difficulties with identification of *A. hanitschi*, despite the species being locally abundant in the site Silau Silau Stream. This species emits a high frequency call, above 5 kHz, so it is possible that the monitoring station placed in this site was too far away from any active individuals for them to be recorded. The originating SPL dB is not known for this species and the detection area of the monitoring station relative to this species cannot be calculated. Locally abundant species such as *L. kuhlii*, were also consistently missed, this species is not known to vocalise in lowland areas. In Kinabalu Park the males are thought to make low growling and barking sounds, which were not picked up by our monitoring stations. It is possible that these sounds are contact calls, meant for close contact and not meant to be heard over large distances, as opposed to advertisement calls meant to be projected over far distances. It is noted that the difference in the acoustic community between sites is high, even over small distances and even when sites are in the same type of habitat band. Monitoring of amphibian communities using acoustic techniques thus requires adequate cover of different micro-habitats within larger scale habitat bands to ensure that results are reliable.

Thorough knowledge of the calling community at a site is necessary before automatic classification algorithms can be trained, and gathering such knowledge is encouraged by authors of classification algorithm software (Bedoya et al., 2014). The training stage of classification algorithms from complex environments, in terms of the community of species, takes a considerable amount of time to ensure accuracy. As time consuming as manual extraction is, it ensures knowledge of the structure of calls from different species, general knowledge of the relationship between species and activity levels, which serves to reduce issues that are common during training stage such as failing to identify the correct calls or not identifying calls at all (Bedoya et al., 2014; Crump & Houlahan, 2017). In addition, it is necessary to assess the level of accuracy in the classification algorithms, which can be done by comparing results with a manually extracted dataset. Especially as different studies might use different classification algorithms, comparison between these requires knowledge of how accuracy and sensitivity vary across algorithms.

Soundscape

The false colour spectrograms generated in this study highlight the soundscape differences between the five sites in question, despite their relative proximity to each other. The complexity of the acoustic environment often appears higher at night time, despite the indices often failing to pick up several species that were present in the sound files. I believe that some of the other indices available warrant further testing to assess whether they are better able to pick up amphibian species. The POW index used here is supposed to be suitable for detecting night time activity, but other indices, not used here, such as the acoustic diversity and normalized difference soundscape index, are also reportedly suitable (Fuller, Axel, Tucker, & Gage, 2015). Comparison of how these three indices perform for species such as *L. montanum*, *P. nephophilus*, *P. bunitus* and *R. angulirostris*, which do not call

continuously through the night but have high presence in our recordings would be beneficial to ensure that analysis of soundscape data from Kinabalu Park in the future provides results that are as accurate as possible. Soundscape data, however, provide an easy way to visually assess the relationship between insects, birds, amphibians and other biotic factors such as rain and wind. In this study, the soundscape data showed that there are relationships between the different taxonomic groups. The night-calling cricket species show partition in acoustic space and the cicadas appear to temporally partition to avoid these crickets by calling during day time. Birds that occupy a wide number of frequency bands also temporal partition in relation to the other taxonomic groups with their dawn chorus. The bird species that call during the night, when most of the other taxonomic groups are acoustically active, appear to have fewer complex calls that do not span a wide frequency band. Considering the high activity by other species at night time, it is not likely that even the birds that call at night get acoustically limited to a narrow frequency band. It is proposed that soundscape data collection be incorporated into regular acoustic monitoring programs to facilitate a higher level of understanding of the acoustic landscape, especially to assess the relationship between specific species and their relationship with a potentially complex acoustic environment and how this affects calling behaviour (Preininger et al., 2013; Jérôme Sueur, 2002). These data could be particularly useful in assessing the soundscape properties over time and how this relates to the environment (Almo Farina & Pieretti, 2014; Towsey & Zhang, 2014). Long-duration recordings also collect data on background noise and can give important information on how changes to noise impact the communication of different species (Brumm, 2013). Soundscape analysis is valuable because it collects a high volume of data, which can be analysed relatively fast and be used for fast biodiversity appraisals within and between sites (J Sueur, 2018; Jérôme Sueur & Farina, 2015; Jérôme Sueur et al., 2014, 2008) meaning that it can potentially facilitate optimization of management actions.

The day calling species *A. platysoma*, from data Chapter 3, was not detected in this study as it does not occur in the sites that were sampled. Analysis techniques such as GJAM, from data Chapter 4, that can combine different types of data should be usable with both passive acoustic monitoring data as well as VES data in the future. This would yield considerable insight into amphibian community structure, habitat preferences and distributions and how these change over time. To perform analyses such as those in Chapter 1 on the acoustic data is less feasible, because the data collected in manual surveys and acoustic surveys cannot be directly compared. Currently density numbers are not calculated from the acoustic data: doing so will require further assessment of how population estimation equations work with acoustic data. This means that analysis techniques such as iNEXT cannot currently be used. However, occupancy data, including variability over time, is generated from acoustic surveys, so standard species distributions models such as MaxEnt can be used. Coupled with information on general soundscape characteristics, as provided by the false-colour spectrograms, acoustic datasets such as those collected here can indicate occupancy and reveal any major changes to the acoustic environment that might require investigation.

Conclusions

Humans make mistakes during field surveys, both with visual and auditory species identification. With field surveys conducted by humans it is also difficult to assess in hindsight whether identification was correct, if there is contention regarding the accuracy. Acoustic monitoring provides a tool that is not reliant on interest and skill of the data collector and provides a permanent record that can be used for verification or re-analyses far beyond the conclusion of the initial study. As shown in this study, the species recorded can differ between classical survey and acoustic survey techniques, making it important to verify

and reconcile differences to ensure that a full and accurate species list is created for a site. Initial results in this study indicate that species separate out into their own acoustic niches, with very minimal acoustic and temporal overlap, indicating that these niches are well established in Kinabalu Park. The acoustic community differs substantially across sites, even for sites that are relatively close to each other and in the same type of habitat. The number of acoustic monitoring stations needed per unit area is potentially quite high and warrants further investigation to ensure that the recommended number of stations is adequate. Future studies should also work on developing classification libraries of species in Southeast Asia and focus on multi-taxa studies to get a thorough understanding of the multiple vocalising species temporal, acoustic and spatial resource partition.

CONCLUSION

The aim of this thesis was to use amphibian monitoring data to answer questions relating to shifts in diversity and in distribution of species, interaction effects on community structure, and the use of passive acoustic monitoring. To this end a historical dataset and recently collected datasets were used to assess whether there had been a shift in the elevational distribution of amphibians on Mount Kinabalu. The beta diversity of this amphibian community was also investigated to address whether this had changed over time and why. Ecological data were also collected on an endangered species for which limited information was previously available, and potential new areas that might contain populations of this species were predicted. At a community level, the co-occurrence patterns and the network relationships of amphibians on Mount Kinabalu were also investigated, specifically to assess the effect of the environment at the community level rather than the species level. Lastly, the use of open source hardware to collect acoustic data was tested. The acoustic data collected were assessed and compared to classical survey techniques and were used to draw general conclusions about acoustic niches.

The research in this thesis also relates to issues arising from anthropogenic influences, such as habitat fragmentation and environmental change. Climate change is predicted to have major impacts on the distributions of species (Parmesan et al., 2003; Peterson, 2003; Walther et al., 2002). Anurans present a specifically vulnerable group as they are ectothermic and are thus often unable to adapt to temperature change locally and instead need to migrate (Bickford et al., 2010; Navjot S. Sodhi et al., 2008). Despite this, few datasets are available to test the extent to which anurans have shifted in recent decades, and investigating the mechanisms behind these changes are difficult (J. Rowley et al., 2010; Navjot S. Sodhi et al., 2008, 2010). In Chapter 1 I used a historical dataset from 1929 on the distribution of anurans from a tropical mountain, Mount Kinabalu in Borneo, and compared this dataset to recent-

day surveys. Of 19 species recorded in both datasets, 14 moved upwards and 5 moved downwards over 80 years, with the average shift being 48 m upwards. Site species richness at low altitudes remained roughly constant over time, whereas at mid latitudes it increased and at high latitudes it decreased. I found that the size of the species is a predictor of elevation shift. This latter result might be an artefact of ability to detect species, but on the other hand, why should this detectability vary with altitude? Future studies using models of amphibians in different habitats could yield important insights into detection rates and how these vary between body size and habitat types. If the elevation shift is truly size-dependent, then answering why becomes important. Amphibians are ectothermic, and the group does not follow any general pattern of body size with temperature, such as Bergmann's rule, and instead shows a mixed response. No studies have been performed on any of the species in Kinabalu Park with respect to physiology and temperature. Research studies assessing the effect of temperature on physiology, and how it relates to body size between species would be of considerable interest due to the expected impact of a changing climate on this community. It is also recommended that the amphibians of Mount Kinabalu be continuously monitored through surveys. This to ensure that the response of the amphibian community to any environmental change be detected in time for any necessary management actions. To further understand the effects of individual species shifting their ranges, the diversity of the community and how this change over time also needs to be investigated.

In Chapter 2, I analysed the beta diversity of the amphibian community and how this has changed over a period of 87 years. The beta diversity was influenced by year of survey and altitude. The drivers of beta diversity appear to have changed over time with a mixture of nestedness and turnover driving beta diversity in 1929, but mainly turnover in the recent years. The current community structure shows a mismatch with the environment, indicating a lag effect in response to shifts in the environmental conditions. These results exhibited

substantial variation between years. Hence, just as the results from Chapter 1 indicated, it would be of considerable interest to continuously monitor this community. This would enable research into the level of natural variation in beta diversity over time. It would allow assessment of whether at any point in the future beta diversity stabilises and the community moves towards equilibrium with the environment. It would be of interest to track the community and determine if it generally moves towards equilibrium with the environment or whether the disequilibrium increases. Ensuring the community level response is recorded is of high importance as it will improve the predictions of impacts on individual species.

Amphibians in Borneo are under serious threat, with 23% of reported species currently listed in one of the threatened categories by IUCN (Bickford et al., 2010; Howard & Bickford, 2014; Inger et al., 2018; Navjot S. Sodhi et al., 2008, 2010). The number of species that are listed as threatened is expected to rise, as the rate of new species descriptions is still high. Comparatively little is known about each species, with almost no data on ecology, population estimates, life history, behaviour, and distribution (Gillespie et al., 2012; Howard & Bickford, 2014). This makes baseline data collection urgent for many Bornean amphibian species. *Ansonia platysoma* is a small stream toad listed as endangered with very few known locations, disjunct populations, low gene flow, and dependency on intact primary forests. In Chapter 3, the ecological habitat requirements of this species were assessed. Areas that should be targeted for future surveys to determine if more populations of this species exist were also predicted. Several new sites that fit the habitat requirements of this species were indicated by our species distribution model. *A. platysoma* occurs up to 1400 m asl and seems to prefer neutral or slightly acidic streams (pH6–7 pH) with no indication of whether stream flow was important for determining occupancy. There was no conclusive result on what affects the density of this species in streams. However, the species is mostly detected on plants growing in the streambed and the level of vegetation might be important. This would

suggest a need for more sampling in streams and over a larger altitude range, with replicates, to yield conclusive results. Despite reports of this species being intolerant to disturbance, it persists on Mount Kinabalu in streams severely damaged by flash floods. I recommend continuous population monitoring to track persistence and further studies into reproductive biology and population numbers. In view of the results from Chapter 4 that indicate multiple species with a positive co-occurrence with *A. platysoma*, I suggest that predictions of the distribution of this species that take account of distributional data from other species may yield more accurate distribution maps. This species also needs considerable work on the currently known populations to assess population numbers, life-history of the species (including for tadpoles) and behavioural ecology; this is in addition to work required to locate more populations. The research in this chapter was performed on just one species with very few known occurrence points. However, with the advancement of modelling techniques new methods are arising that can use co-occurrence of rare species with more well-known species to inform predicted distributions.

Most studies on the impact of environmental change on the distribution of species are performed at the species level (Desjardins-Proulx et al., 2017; Leach et al., 2017). However, a species' response to abiotic changes is believed to be strongly influenced by the response of other species (Cam, Nichols, Hines, & Sauer, 2000; Gavish et al., 2017; Leach et al., 2017; Pollock et al., 2014; Vitale et al., 2017). Accounting for interaction effects and the co-occurrence of other species is therefore important to ensure that prediction of responses and monitoring of species are done at the community level (Lathlean et al., 2017b; Laurindo et al., 2017; Swift et al., 2017; Valiente-Banuet et al., 2015). In Chapter 4, the network and co-occurrence patterns of the amphibian community on Mount Kinabalu were investigated. The network and probabilistic co-occurrence analyses both indicated that a few species have high connectivity to others. The GJAM analysis can fully utilize the information available from

field data, including the number of individuals found, survey effort and environmental information. Negative co-occurrence values between species were found where they were expected based on biological knowledge, and strong positive co-occurrence values were found between pairs of species that occur in similar environments such as streams.

The GJAM model assessed species-level responses to environmental predictors while accounting for species interactions and assessing the group-level response. In comparison to the probabilistic co-occurrence analysis, the GJAM provides an important extension of the JSMDs previously available. The GJAM appears to perform well despite the relatively small dataset used here, in terms of number of species and number of sites, promising high applicability for future community studies and prediction of the distribution of species. The amphibian community in Kinabalu Park appears to separate into four distinct groups: one high altitude group, two separate stream community groups, and one group with a mixed response comprising most of the terrestrial species. The responses to environmental variables varies across species, indicating that results from one species cannot easily be generalised.

Philautus amoenus showed a negative co-occurrence with several other species. This high-altitude species is therefore a good model species for in-depth studies on the effect of environmental change on competition. The lower altitude limit of this species appear to be an upper boundary zone for other species, and changes to interaction strengths are expected to be the strongest in boundary zones between species that negatively co-occur. This is particularly relevant on Mount Kinabalu as Chapter 1 shows, species are moving upwards on the mountain, putting pressure on this boundary zone. Kinabalu Park has a relatively well described amphibian community, compared to the rest of Borneo, and presents a good system for future studies into community structure and changes over time relative to environmental variables. It would be instructive to assess the accuracy of prediction maps of the distribution of species created by GJAM.

GJAM has not been used for future predictions of distribution, even though it has been used for predictions of soil conditions based on plant assemblage (Clark et al., 2014). This tool might provide valuable insights into the future structure of amphibian communities under global change scenarios and be especially useful for making multi-species predictions. It would also be of interest to launch a study investigating the interaction strength between trophic levels. I suggest that future studies focus on the prey items of the different amphibian species to explore the relationship not only between different amphibian species and the prey for which they compete, but also individual species' relationships with their prey. Studies could also be performed to assess the species that might predate adults using clay models. The tadpoles have also largely been left out of this thesis as there was no opportunity to collect data on these, but competitive foraging studies between tadpole species in streams would be of interest as would studies exploring the kinds of predators to which tadpoles are exposed. One limitation is that collecting the survey data required for GJAM is very time consuming and costly. Considering the fast rate of change in the landscapes of Southeast Asia, new methods are required to improve the speed at which data can be collected, and the quality and volume of the data.

Southeast Asia is facing a high level of land clearance and modification whilst being one of the most bio-diverse regions in the world. Despite this, many species are still undescribed, and monitoring of the effects of habitat and environmental changes on species in the region is scarce in proportion to the magnitude of the issues (J. Rowley et al., 2010; Navjot S. Sodhi et al., 2008, 2010). Large-scale acoustic monitoring and soundscape projects provide a possible avenue to overcome the shortfall in funds and trained staff that currently plague the field (Digby et al., 2013; Towsey et al., 2013, 2014; Towsey & Zhang, 2014; Whytock & Christie, 2017). In Chapter 5, an acoustic and environmental monitoring station based on open source hardware and software was tested. Acoustic data were collected at set

intervals amounting to six minutes every hour for each station, over a period of three months. Continuously recorded data were also collected over 24 h to use in soundscape analysis. The acoustic monitoring stations provide a cost-effective solution that can be viable for large-scale acoustic monitoring projects in tropical countries given the correct configuration and circumstances. One limitation is that the initial set-up of these stations requires a certain level of expertise because this is not an off-the-shelf product there is no professional support if issues should arise. I successfully collected data both in Borneo and in Singapore and the initial failure rate of stations reduced from 50% to 20%, most likely due to an increase in my experience. Future studies using both self-built and off-the-shelf products should report failure rates accurately, instead of just reporting data from successful stations, to ensure that project managers can make the most cost-effective decision for their project. The method of data relay also needs considerable investment to reduce both power consumption and cost. Power consumption can be reduced by both reducing the power requirements and improving the reliability and efficiency of batteries. The acoustic dataset collected using these stations for this thesis amounted to over 50,000 minutes; a corresponding environmental dataset was collected concurrently.

In Chapter 6 the acoustic dataset, whose collection was reported in Chapter 5, was analysed. The results suggest that the amphibian species detected in a site varies considerably, despite all the sites being in the same type of habitat. At least two species were frequently picked up by the acoustic stations despite not being reported in previous classical human-based surveys. In addition, certain species detected in the classical surveys were not detected by the acoustic monitoring stations. This shows that passive acoustic monitoring cannot replace classical surveys. Conversely, classical surveys cannot replace passive acoustic monitoring. Instead the two techniques need to be used as complements to each other to ensure that accurate species lists are created for study sites.

The results from the soundscape analysis show that there are considerable differences in the acoustic environments between these sites and that several amphibian species were missed by the acoustic indices used. However, the results provided insights into the relationship between different taxonomic groups. Further testing is advocated to ensure that the acoustic indices used are suitable for detecting species in sites studies. In addition, for this thesis, there was not enough time available to explore different acoustic indices for the soundscape dataset, but this is suggested as a future avenue to explore using soundscape data. For a specific taxonomic group, such as amphibians, monitoring data provides a more thorough understanding of the acoustic ecology of the community in comparison to soundscape data.

The acoustic dataset revealed some interesting results relating to acoustic niche, with specific frequency bands used by each species. I suggest that future studies focus on the energy requirements of calling behaviour in this community, and how this relates to the body size of the species. Especially for species in the genus *Leptolalax*, which appear to call consistently at a very high frequency and have medium body size. The acoustic dataset collected here needs a more thorough analysis involving specific data on frequency information from individual calls, coupled with environmental data, body size information and information on energy expenditure during calling. There has also been very little work done on the amphibians in Kinabalu Park on the sensory ecology of receivers (e.g., nearby conspecific amphibians) and how receivers respond to calls and the information they contain. This is an interesting area for future research, because both the signalling and the receiving individuals exert evolutionary pressures on each other, resulting in coevolution of signalling and receiving traits. The six data chapters presented all have potential for future avenues of research, but they also suffered from limitations.

Chapter 1 is limited by the unknown survey effort in 1929: if it were known, the reliability of the results would be higher. The observed shift in mean altitude therefore needs to be viewed with some caution. Chapter 2 is limited by the available datasets: it would be considerably strengthened by more between-year data in addition to more thorough environmental sampling. Chapter 3 is limited by the lack of streams that are logically available to survey, firstly in pure numbers giving us relatively low statistical power. Secondly, there are no paired streams available, limiting the comparisons of effects on occupancy and density numbers of the species. Chapter 4 would be strengthened substantially if the competitive interaction strengths between the amphibian species and between trophic levels with prey and predators could be quantified. Chapter 5 is limited by the time and funding available to explore data relay. Especially in the Southeast Asian region where funds are scarce and there is a need for increased biodiversity monitoring at a lower cost. With areas that are often inaccessible but still need to be monitored, a reliable data relay method is of high importance. Chapter 6 is limited by the time required to train classification algorithms to automate the identification of species from sound files. The expertise level required when it comes to identifying species calls is high and takes a considerable amount of training and familiarity with a specific site. Manual extraction of data are thus often required to ensure adequate training of an expert, who will later train the algorithm. The initial setting up of an acoustic training library is a process that is expected to take at least six months for Kinabalu Park, given that the acoustic community there is already well described, but even longer for sites where acoustic descriptions of species are few. As part of this thesis, a soundscape dataset was also collected—a limitation here was the large size of the files, which restricts the duration over which the soundscape can be monitored. Despite acoustic indices and false-colour spectrograms speeding up the analysis process considerably, these techniques appear to miss a considerable number of species. This indicates that the combination of indices used might

need to be tuned for specific sites. The results both from the soundscape dataset and the acoustic monitoring dataset are promising when it comes to improving the data collection and the biodiversity monitoring of amphibians in Southeast Asia.

The six chapters all indicate that the implementation of long-term monitoring of amphibians in the Southeast Asia region is necessary. The fast pace of land conversion in the region, coupled with the high biodiversity and continued high rates of species descriptions, makes it paramount to increase data collection before species disappear. This would also yield opportunities for investigating important ecological questions regarding contact zones for competition, biotic interaction effects due to responses to climate change, and for exploring acoustic niche theory from a multi-taxon setting rather than single-species or single-taxon avenue.

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APPENDICES

Appendix 1.1. Site justification between sites reported from 1929 and the sites used in recent surveys.

Site name in 1929	Elevation in 1929	Site name in 07/10	Elevation	Site name in 2016	Elevation
Kiau	917	Kiau	1000	Kiau	1000
Kenokok	1005	Kenokok	1060	Kenokok (Tinokok?)	1006
Lobang	1220	Lobong	1413	Lobong (?)	1036
Tenompok	1432	Tinompok	1500	Tinompok (Kinataki?)	1393
Marei Parei	1524	Marei Parei	1600	Marei Parei	1652
Lumu Lunu	1676	Lumu Lunu	1780	Lumu Lunu	1758
Kamborangah	2194	Kamborangah	2220	Kamborangah	2221
Pakka	3100	Pakka	3000	Pakka	2966

Appendix 1.2. Justification for changing species names to modern equivalent for 1929 survey.

Table 1. Indicating which names, we believe some of the species from Smith's 1929 survey have changed to. Some have just changed their names, column three is for the ones where we believe the identification in 1929 was mistaken or the species had not been described yet. We also indicate the reason we think a complete species change is justified. Species where the identification is uncertain whether it was correct or not in 1929 is also addressed (such as *Leptobrachella baluensis* that appear to have been found at a very high altitude in 1929).

From Smith	To	Thought to be species:	Reason	ID mistake
Megophrys_gracilis	Leptolalax_gracilis		Change of name	Unlikely
Simomantis_leptopalmata	Staurois_latopalmatus		Change of name	Not likely it is very different from the other Staurois in the area
Rana_macrodon	Limnonectes_macrodon		Change of name	Unlikely
Rana_kuhlii	Limnonectes_kuhlii		Change of name	Unlikely
Rana_palavanensis	Limnonectes_palavanensis		Change of name	Unlikely
Rana_luctuosa	Abovarana_luctuosa		Change of name	Unlikely
Rana_nicobariensis	Amnirana_nicobariensis	Meristogenys_amoropalamus	amoropalamus not described until 1986, nicobariensis only known from type locality in India	Unlikely
Rana_cholconota	Cholcorana_megalonesa		Change of name	Unlikely
Rana_jerboa	Meristogenys_jerboa	Meristogenys_amoropalamus	jerboa not present in Kinabalu (but in Sarawak)	Unlikely
Rana_whiteheadi	Meristogenys_whiteheadi		Change of name	Unlikely
Rhacophorus_acutirostris	Philautus_acutirostris	Philautus_aurantium?	Change of name	Unlikely
Philautus_spiculatus	Philautus_macroscelis		Change of name	Unlikely
Megophrys_monticola	Megophrys_montana	Xenophryns_baluensis	montana is not present in Borneo but looks similar and have a similar size to kinabaluensis	Unlikely

Megophrys_hasseltii	Leptobrachium_hasseltii	Leptobrachium_montanum	hasseltii not present in Borneo but montnaum is and seem abundant	
Bufo_leptopus	Ansonia_leptopus		Change of name	Unlikely
Nectophryne altitudinis	Ansonia_fuliginea		Change of name	No
Leptobrachella_baluensis	-			Hard to mistake, very distinct species
Nectophryne_misera	Pelophryne_misera		Change of name	Unlikely

Appendix 1.3. Checklist of species recorded from Kinabalu Park.

Table 1. Checklist of currently known species from the park (including species with distribution known from across Borneo with high likelihood of presence in the park).

Number	Family	Genus	Species
1	Bufonidae	<i>Ansonia</i>	<i>fuliginea</i>
2	Bufonidae	<i>Ansonia</i>	<i>guibei</i>
3	Bufonidae	<i>Ansonia</i>	<i>hanitschi</i>
4	Bufonidae	<i>Ansonia</i>	<i>longidigita</i>
5	Bufonidae	<i>Ansonia</i>	<i>platysoma</i>
6	Bufonidae	<i>Ansonia</i>	<i>spinulifer</i>
7	Bufonidae	<i>Ingerophrynus</i>	<i>divergens</i>
8	Bufonidae	<i>Phrynobatrachus</i>	<i>juxtasper</i>
9	Bufonidae	<i>Leptoyphryne</i>	<i>borbonica</i>
10	Bufonidae	<i>Rentapia</i>	<i>everetti</i>
11	Bufonidae	<i>Sabahphryalus</i>	<i>maculatus</i>
12	Bufonidae	<i>Pelophryne</i>	<i>misera</i>
13	Bufonidae	<i>Pelophryne</i>	<i>signata</i>
14	Ceratobatrachidae	<i>Alcalus</i>	<i>baluensis</i>
15	Megophryidae	<i>Leptobrachella</i>	<i>baluensis</i>
16	Megophryidae	<i>Leptobrachella</i>	<i>parva</i>
17	Megophryidae	<i>Leptobrachium</i>	<i>abbotti</i>
18	Megophryidae	<i>Leptobrachium</i>	<i>gunungense</i>
19	Megophryidae	<i>Leptobrachium</i>	<i>montanum</i>
20	Megophryidae	<i>Leptolalax</i>	<i>arayi</i>
21	Megophryidae	<i>Leptolalax</i>	<i>sabahmontanus</i>
22	Megophryidae	<i>Leptolalax</i>	<i>maurus</i>
23	Megophryidae	<i>Leptolalax</i>	<i>pictus</i>
24	Megophryidae	<i>Xenophrys</i>	<i>baluensis</i>
25	Megophryidae	<i>Borneophrys</i>	<i>edwardinae</i>
26	Megophryidae	<i>Megophrys</i>	<i>kobayashii</i>
27	Megophryidae	<i>Megophrys</i>	<i>nasuta</i>
28	Microhylidae	<i>Chaperina</i>	<i>fusca</i>
29	Microhylidae	<i>Kalophrynus</i>	<i>baluensis</i>
30	Microhylidae	<i>Kalophrynus</i>	<i>meizon</i>
31	Microhylidae	<i>Kaloula</i>	<i>baleata</i>
32	Microhylidae	<i>Kaloula</i>	<i>pulchra</i>
33	Microhylidae	<i>Metaphrynella</i>	<i>sundana</i>
34	Microhylidae	<i>Microhyla</i>	<i>borneensis</i>
35	Microhylidae	<i>Microhyla</i>	<i>petrigena</i>

36	Dicoglossidae	<i>Fejervarya</i>	<i>limnocharis</i>
37	Dicoglossidae	<i>Limnonectes</i>	<i>finchi</i>
38	Dicoglossidae	<i>Limnonectes</i>	"kuhlpii"
39	Dicoglossidae	<i>Limnonectes</i>	<i>leporinus</i>
40	Dicoglossidae	<i>Limnonectes</i>	<i>palavanensis</i>
41	Dicoglossidae	<i>Occidozyga</i>	<i>baluensis</i>
42	Dicoglossidae	<i>Occidozyga</i>	<i>laevis</i>
43	Ranidae	<i>Huia</i>	<i>cavitypanum</i>
44	Ranidae	<i>Ingerana</i>	<i>baluensis</i>
45	Ranidae	<i>Meristogenys</i>	<i>amoropalamus</i>
46	Ranidae	<i>Meristogenys</i>	<i>dyscritus</i>
47	Ranidae	<i>Meristogenys</i>	<i>kinabaluensis</i>
48	Ranidae	<i>Meristogenys</i>	<i>orphnognathus</i>
49	Ranidae	<i>Meristogenys</i>	<i>whiteheadi</i>
50	Ranidae	<i>Chalcorana</i>	<i>megalonesa</i>
51	Ranidae	<i>Hylarana</i>	<i>erythrea</i>
52	Ranidae	<i>Odorrana</i>	<i>hosii</i>
53	Ranidae	<i>Abovarana</i>	<i>luctuosa</i>
54	Ranidae	<i>Amnirana</i>	<i>nicobariensis</i>
55	Ranidae	<i>Pulchrana</i>	<i>glandulosa</i>
56	Ranidae	<i>Pulchrana</i>	<i>picturata</i>
57	Ranidae	<i>Pulchrana</i>	<i>signata</i>
58	Ranidae	<i>Staurois</i>	<i>guttatus</i>
59	Ranidae	<i>Staurois</i>	<i>latopalmatus</i>
60	Ranidae	<i>Staurois</i>	<i>natator</i>
61	Ranidae	<i>Staurois</i>	<i>tuberilinguis</i>
62	Rhacophoridae	<i>Nyctixalus</i>	<i>pictus</i>
63	Rhacophoridae	<i>Philautus</i>	<i>amoenus</i>
64	Rhacophoridae	<i>Philautus</i>	<i>aurantium</i>
65	Rhacophoridae	<i>Philautus</i>	<i>bunitus</i>
66	Rhacophoridae	<i>Philautus</i>	<i>ingeri</i>
67	Rhacophoridae	<i>Philautus</i>	<i>macrosceles</i>
68	Rhacophoridae	<i>Philautus</i>	<i>mjobergi</i>
69	Rhacophoridae	<i>Philautus</i>	<i>neophophilus</i>
70	Rhacophoridae	<i>Philautus</i>	<i>petersi</i>
71	Rhacophoridae	<i>Philautus</i>	<i>sauvagei</i>
72	Rhacophoridae	<i>Polypedates</i>	<i>leucomystax</i>
73	Rhacophoridae	<i>Polypedates</i>	<i>macrotis</i>
74	Rhacophoridae	<i>Polypedates</i>	<i>otilophus</i>
75	Rhacophoridae	<i>Rhacophorus</i>	<i>angulirostris</i>
76	Rhacophoridae	<i>Kurixalus</i>	<i>appendiculatus</i>
77	Rhacophoridae	<i>Rhacophorus</i>	<i>baluensis</i>

78	Rhacophoridae	<i>Rhacophorus</i>	<i>cyanopunctatus</i>
79	Rhacophoridae	<i>Rhacophorus</i>	<i>dulitensis</i>
80	Rhacophoridae	<i>Rhacophorus</i>	<i>gauni</i>
81	Rhacophoridae	<i>Rhacophorus</i>	<i>harrissoni</i>
82	Rhacophoridae	<i>Rhacophorus</i>	<i>nigropalmatus</i>
83	Rhacophoridae	<i>Rhacophorus</i>	<i>pardalis</i>

Appendix 1.4. Weighted altitude results table.

Table 1. Weighted altitude results.

Species	1929	2007	2010	2016	Shift in altitude	Habit at band 1929	Modern habitat band	Reproduction
Ansonia_fuliginea	3096.47	NA	3325.00	NA	228.53	High	High	Stream
Chaperina_fusca	953.44	1100.00	1037.08	972.12	82.95	Low	Low	Tree hole
Chalcorana_chalconota	917.00	1044.23	NA	NA	127.23	Low	Low	Stream
Leptobrachella_baluensis	2194.00	1155.58	1046.40	1192.59	-1062.47	High	Low*	Stream**
Leptobrachium_montanum	1676.00	1818.21	1752.51	1662.25	68.32	Mid	Mid	Stream
Limnonectes_kuhlii	1068.00	1413.61	1177.47	1654.07	347.06	Low	Mid	Stream/puddle
Limnonectes_paramacrodon	917.00	1500.00	NA	NA	583.00	Low	Mid	Stream
Limnonectes_palavanensis	917.00	NA	NA	1064.00	147.00	Low	Low	Ground/Stream
Xenophrys_baluensis	1559.08	1780.00	1590.09	1692.15	128.34	Mid	Mid	Stream**
Megophrys_nasuta	917.00	1665.50	1000.00	1064.00	326.17	Low	Low	Stream
Meristogenys_amoropalamus	1676.00	NA	1060.00	1252.49	-519.75	Mid	Low*	Stream
Meristogenys_whiteheadi	1016.52	1462.87	1500.00	NA	464.92	Low	Mid	Stream
Nyctixalus_pictus	1558.63	1430.00	1096.60	1561.13	-196.05	Mid	Mid*	Tree hole
Pelophryne_misera	2417.02	2220.00	1589.72	2065.59	-458.58	High	High*	Small waterbody**
Philautus_amoenus	2194.00	2209.74	2215.50	2505.83	116.36	High	High	Direct developer
Philautus_marcoscelis	1005.00	1430.00	1144.24	1192.17	250.47	Low	Low	Direct developer**
Philautus_mjobergi	1854.70	2210.89	2022.42	1629.20	99.48	High	High	Direct developer
Philautus_petersi	1695.96	1129.78	2096.97	1075.36	-261.92	Mid	Mid*	Direct developer
Staurois_tuberilinguis	918.33	1375.75	1351.85	1398.23	456.94	Low	Mid	Stream

Figure 1. The 19 species that were found in both 1929 and one of the modern years. Altitude shift was averaged over the modern years. Species that shifted habitat bands are indicated in grey, negative shifts are indicated with a *. For reproductive mode/habitat dependency, species where the breeding mode is uncertain and inferred from other species in the genus are indicated with **.

Appendix 3.1. Variogram for GLS model with spatial autocorrelation nugget and normal error structure.

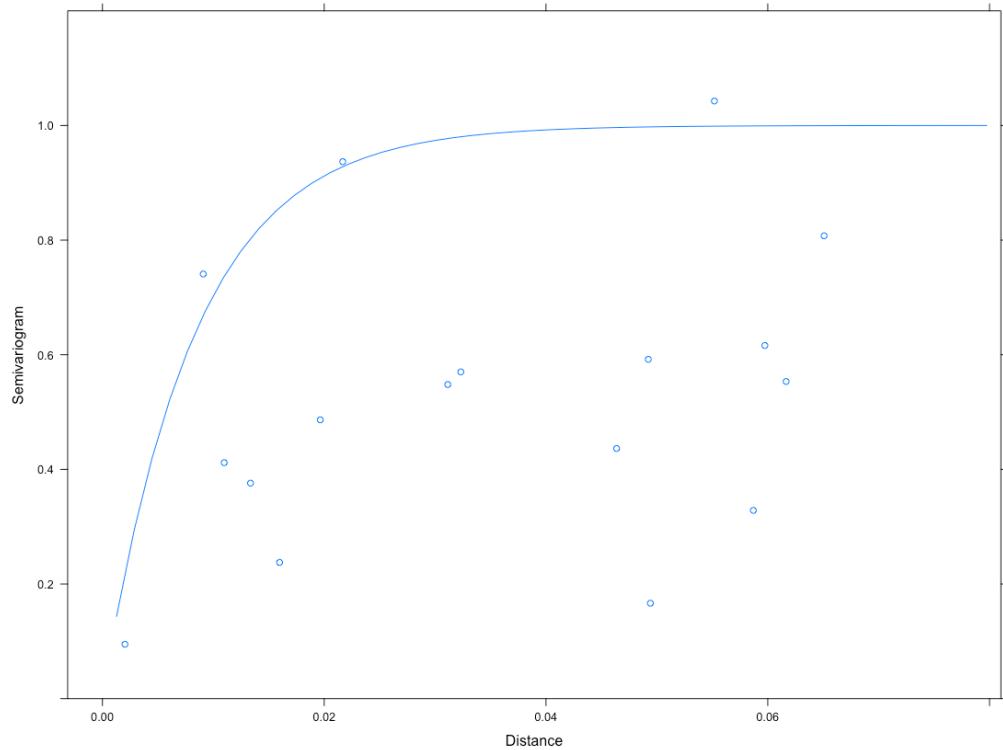
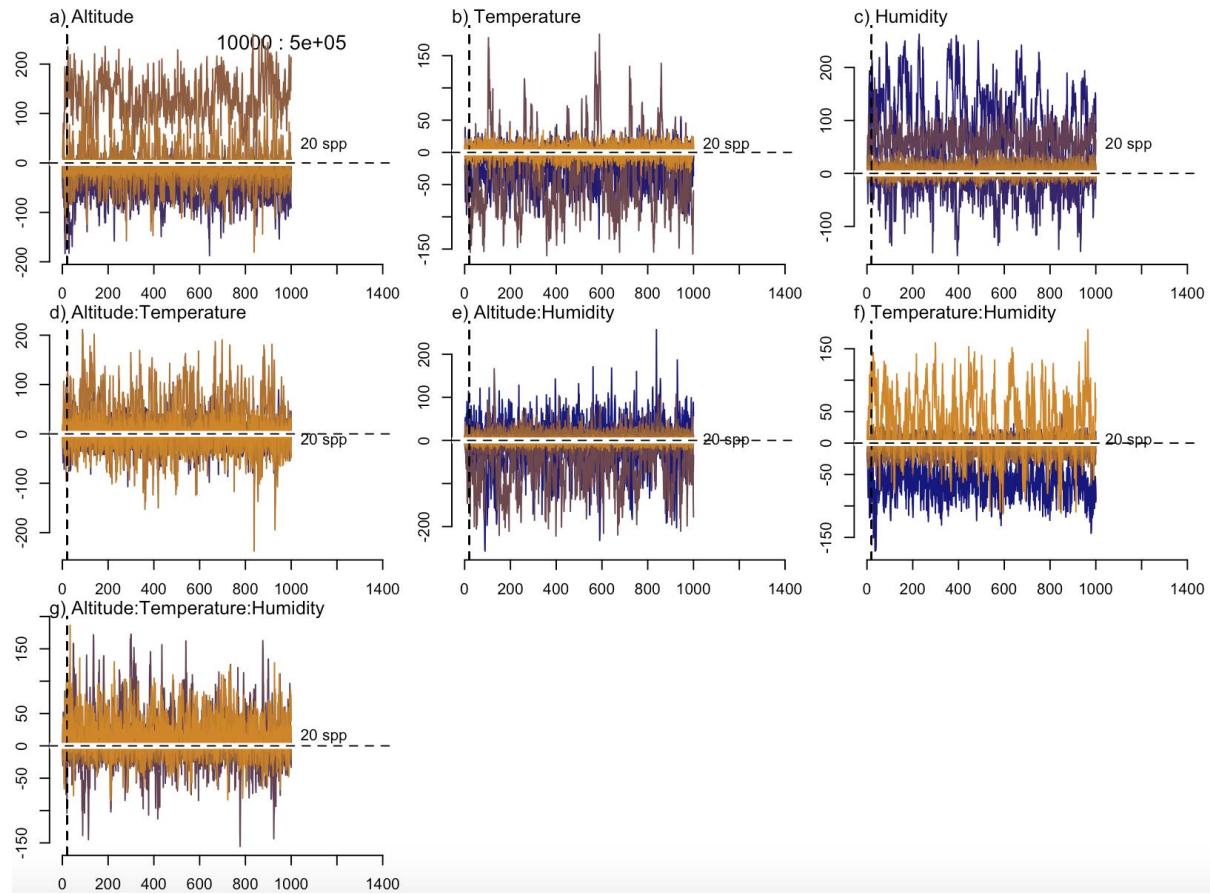


Figure 4. Variogram of normalized residuals of the model with a Normal error structure. For GLS model shown in Figure 2.6.

Appendix 4.1. Convergence plots for the GJAM model.



Appendix 5.1. Manual over assembly and coding for acoustic and environmental monitoring stations.

Automated Acoustic and Environmental Monitoring Stations Assembly and Coding Instructions



NATIONAL
GEOGRAPHIC



Wildlife Reserves Singapore
Conservation Fund

Catharina Karlsson
Version 1.1

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Introduction

Acoustic monitoring is increasingly being used as an alternative to classical survey techniques as the price of the monitoring stations themselves drop as well as availability and price of storage of the data is lowered. With the advancement of classification algorithms for identification of species in the data, collecting large data sets is also becoming increasingly possible.

The material in here stem from two projects. The first one being on automated acoustic monitoring of amphibians in Kinabalu Park in Borneo, a project that was in the field in the beginning of 2017 and was funded by National Geographic Society. The second project was in the field in the last half of 2017 in Singapore on automated acoustic monitoring of the endangered and naturalized amphibians of Singapore, that project was funded by the Wildlife Reserves Singapore Conservation Fund. I would like to offer thanks to the field staff and volunteers as well as park managers that have assisted me in both projects.

These projects were conducted during my PhD I had the fortune to work on assembling acoustic monitoring stations from open source hardware as well as software. When I started it was a bit of a daunting task, I had done a high amount of coding but in R, not Python or in bash. I also had not soldered anything together since 8th grade (over 15 years earlier). I must thank my father from the depth of my heart, because I went home to Sweden and spent some time with him in his workshop, discussing how to put together the hardware and he also ensured that my soldering skills was up to scratch again. The first prototype was working and collecting data after spending a month in Sweden working on it. I have also had considerable help from Dr Peter Todd and his Experimental Marine Ecology Lab at National University of Singapore. Since my own lab is mainly a theoretical one we did not have any workspace for assembly of units and the EMEL lab kindly allowed me usage of their work area. Lastly I would like to offer a thank you to my main supervisor, Dr Ryan Chisholm, and my co-supervisor, Dr David Bickford; the support they have both offered during this process have been tremendous and none of the material in this manual would have been able to be written up without their help and allowance for me to pursue research ideas and areas that at times seem impossible.

The acoustic monitoring projects have been fantastic to work on, mainly because they allowed a regular ecologist to work outside the box on solutions to data collection issues. I believe that students and researchers alike gain strength from realizing and being taught that they can come up with new solutions to some issues and learning to assemble hardware and code opens a whole world of opportunities for inventions. There is a whole universe full of different types of microprocessors, sensors and other components available out there. I hope some people find some use in the manual, not as finite solution, but rather as a stepping stone to new ideas and inventions.

Best regards
Catharina
Kuching 7th of July 2018

Tools needed

- Drill and an assortment of drill bits
- Solder station
- Adjustable spanners
- Pliers
- Metal cutter
- Metal/plastic saw
- Flat headed screw drivers
- Electronics screw driver with assortment of heads
- Power meter
- Nipper
- Solder with rosin core
- Box cutter

Soldering

Introduction

Putting a unit together requires some soldering, mainly for the different types of sensors that will be used but also for things like a button to ensure a safe shut down when the battery is removed or replaced.

It is highly important that the soldering is done properly, bad soldering leads to an increase in units breaking down and thereby data loss and monetary loss for the project. The following instructions are thereby paramount:

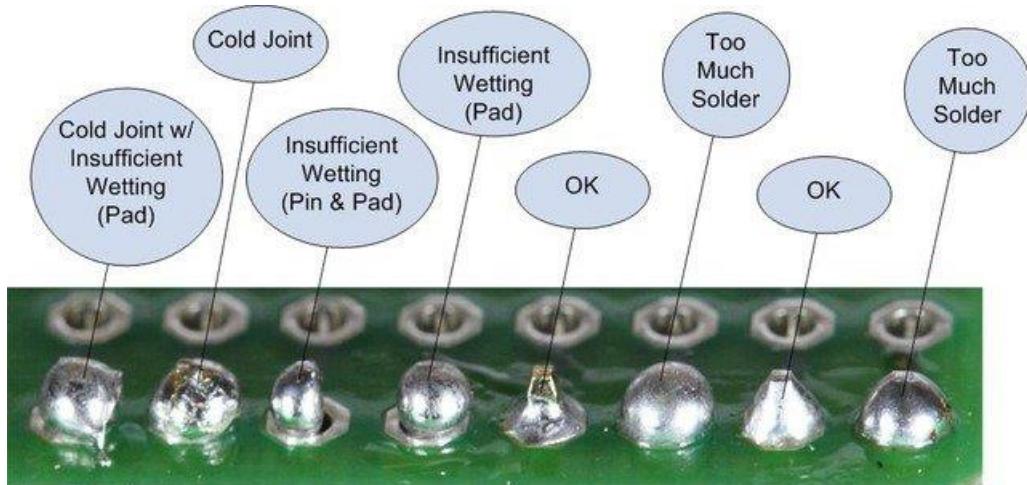
- 1) NEVER use a soldering pen if you can avoid it, use a soldering station.
- 2) Ensure you have the correct temperature depending on what you are soldering.
- 3) Use flux! Ensure it is the correct one for what you are doing.
- 4) Ensure the solder tip is clean.
- 5) Prepare properly.

1. Why not to use a soldering pen.

Soldering pens are highly unreliable, even if they have a couple of temperature settings to choose between the pen often don't reach those. There is a high chance that the pen will either be too hot (especially dangerous for circuit boards) or too cold (leading to cold solder, if the metal has not ligated properly in a solder it will not lead electricity the way it should and will easily break). Instead use a soldering station where you can set the specific temperature we need, like the one in the picture below. There are also portable USB pens as well as butane gas soldering pens that are more portable, they come in different types and versions so also need to be picked carefully to ensure they work as they should in the field.



See the picture below to get an understanding of the different ways incorrect and correct solders look like, the picture below that one shows a diagram of how a good through the hole solder should look like.



Showing some of the issues and how to spot a bad solder (source: adafruit).

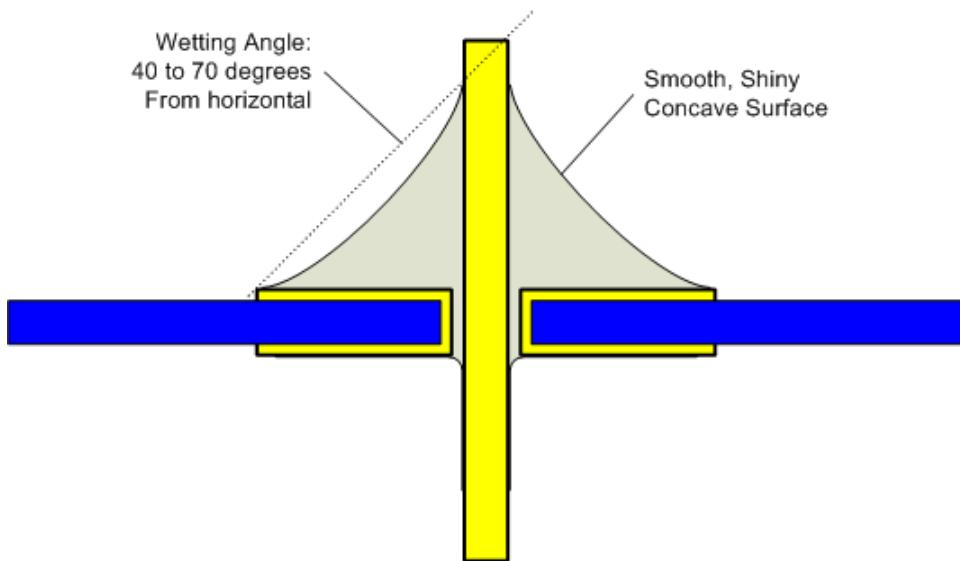


Diagram of what a nice through the hole solder should look like (source: adafruit).

If you want more thorough information, please visit this webpage:
<https://learn.adafruit.com/adafruit-guide-excellent-soldering/common-problems>

2. Temperature.

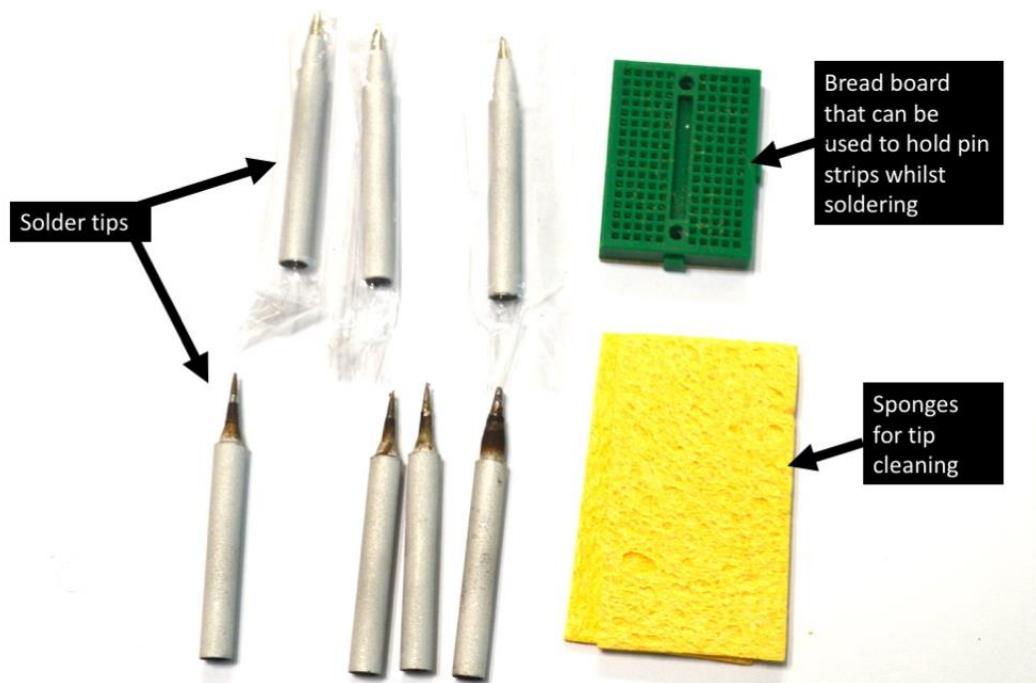
Solder at the incorrect temperature can lead to multiple issues. If the solder iron is too cold there will be issues with melting the solder, it will not wet properly and will not ligate properly where it should. It will thereby easily break and get cracks and will stop working faster than one that is done properly. The iron can also be too hot, causing the flux to burn before the solder has managed to wet properly. The temperature will differ depending on if you solder wires, what kind of wires, the type of solder and whether you are on circuit board. We normally use 60/40 rosin-based solder which should be at a maximum temperature of 370°C.

3. Flux.

Flux is used to clean the surface that you want to solder onto and remove impurities, it creates better solder joints. Wires should always be fluxed! Circuit boards are a bit trickier, they should ideally be fluxed but as they are sensitive you need to use a flux with a low amount of acidity such as rosin.

4. Ensure the solder tip is clean.

If the tip is not clean it will be difficult to get a good solder. Never melt plastic with the solder tip as you get plastic in your solder. Ensure it is cleaned regularly on a wet sponge when you are soldering. There are also different types of solder tips depending on what you solder, thicker tips for wires, thin tips for through the hole and precision soldering and flat head solder tips for circuit board soldering. They are cheap, so it is better to get a few and change regularly than continue with one that has seen better days. To protect the solder tip between use, it should be left with clean solder on it! So, once you are finished, clean off the tip properly, then melt solder onto it before turning it off.

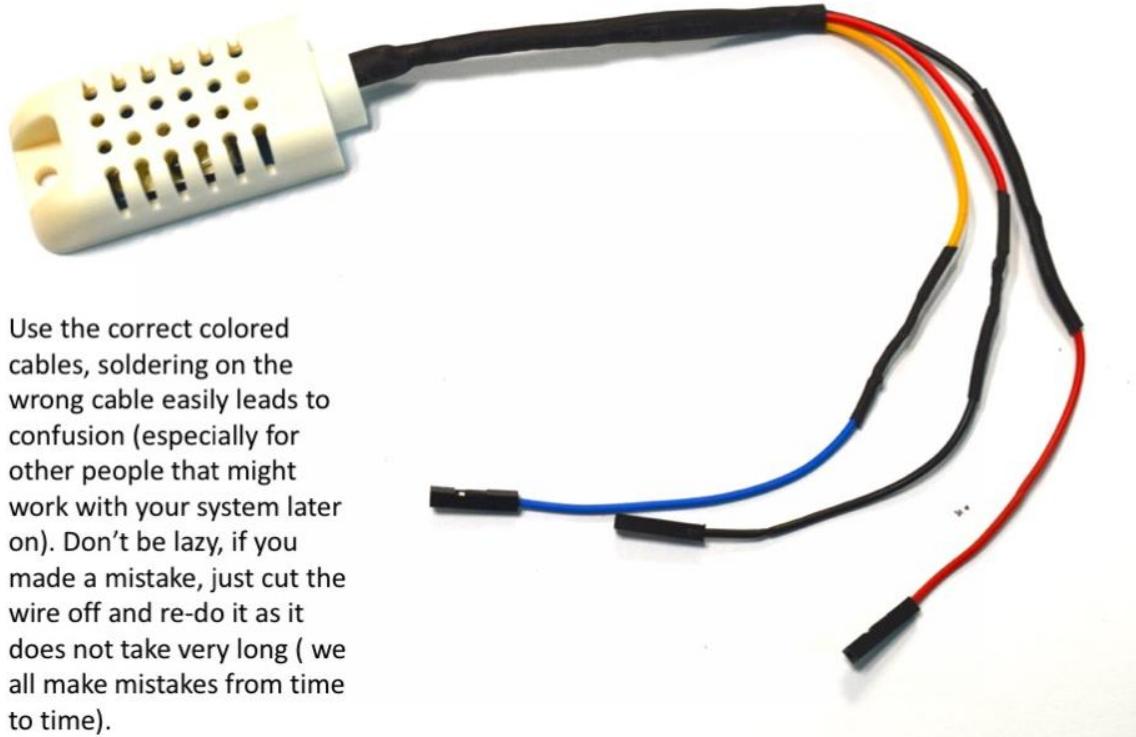


5. Prepare properly.

If you are uncertain, read up before you start, there are plenty of good information on the internet (be critical though and read multiple sources). Ensure your station is set up properly, take it easy and don't stress. If something goes wrong, just re-do it rather than leaving a solder joint or a wire of the incorrect colour attached.

Examples of what not to do

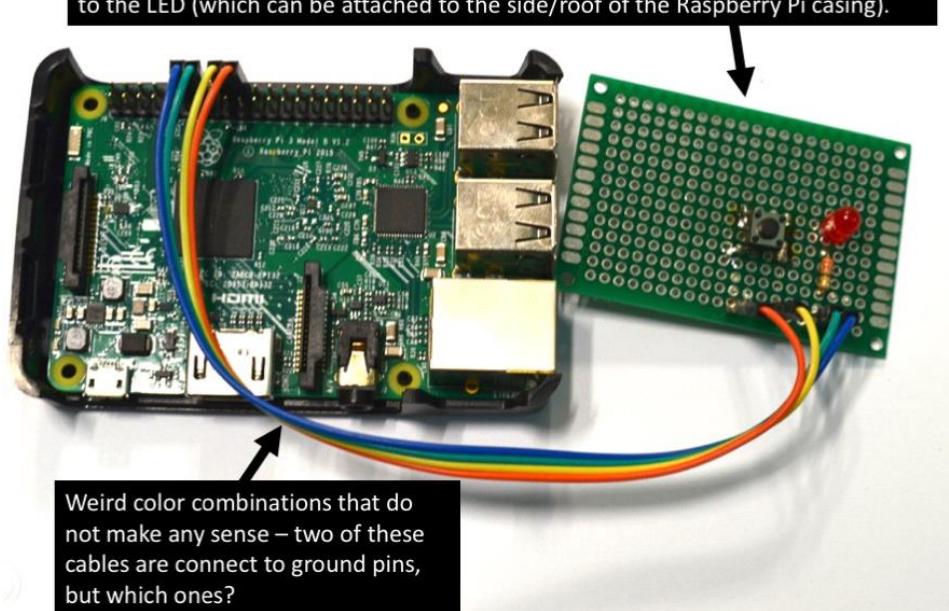
Attach the correct color wires to each other.



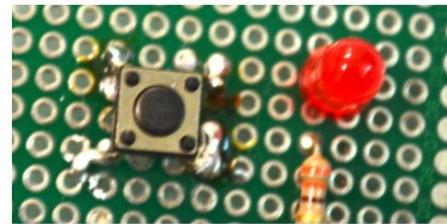
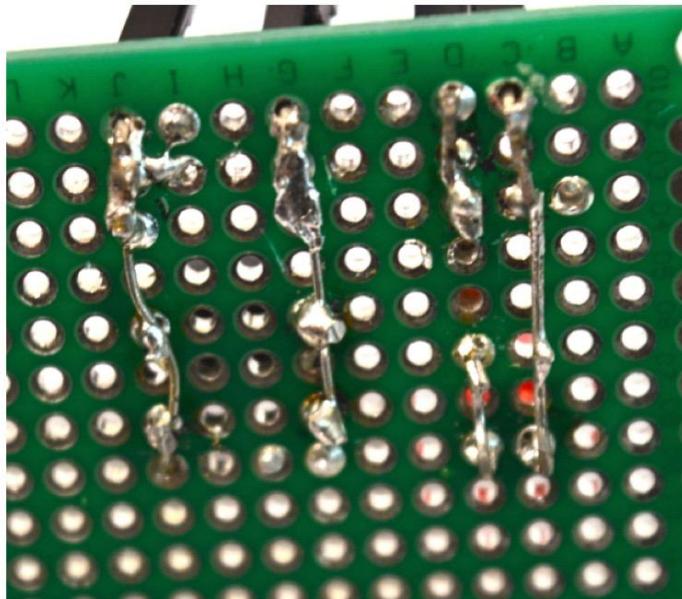
Use the correct colored cables, soldering on the wrong cable easily leads to confusion (especially for other people that might work with your system later on). Don't be lazy, if you made a mistake, just cut the wire off and re-do it as it does not take very long (we all make mistakes from time to time).

Less is more.

Think through your design and make it as easy to use as possible. This is too bulky, and most of all, there is no need for the circuit board at all. It just adds extra surface that is exposed to humidity. A resistor can be attached onto a cable and covered in shrink plastic for protection and the cables can go the whole way to the LED (which can be attached to the side/roof of the Raspberry Pi casing).



Take time to learn how to do things well, it will save time in the future.

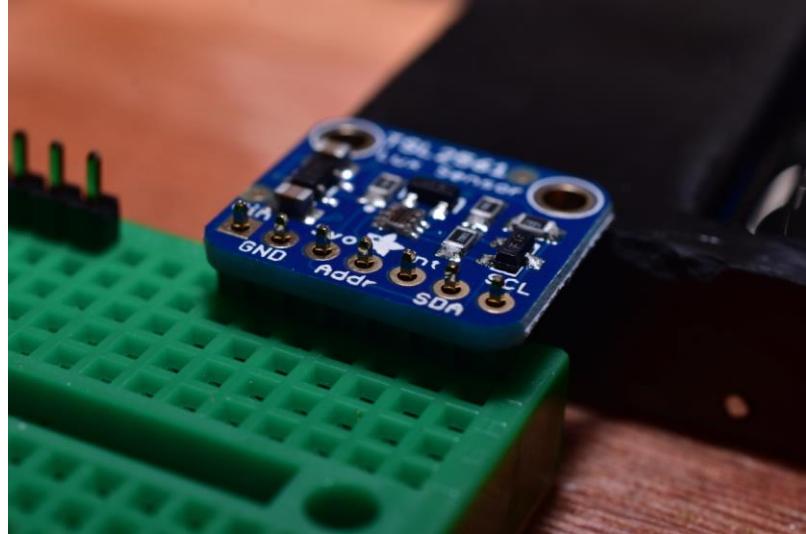


Bad soldering. It is lumpy and in sections not melted properly. Not only are these probably cold soldered, meaning they don't ligate properly. They are more likely to break and stop working. A good solder saves the day in the rainforest!

Sensor soldering

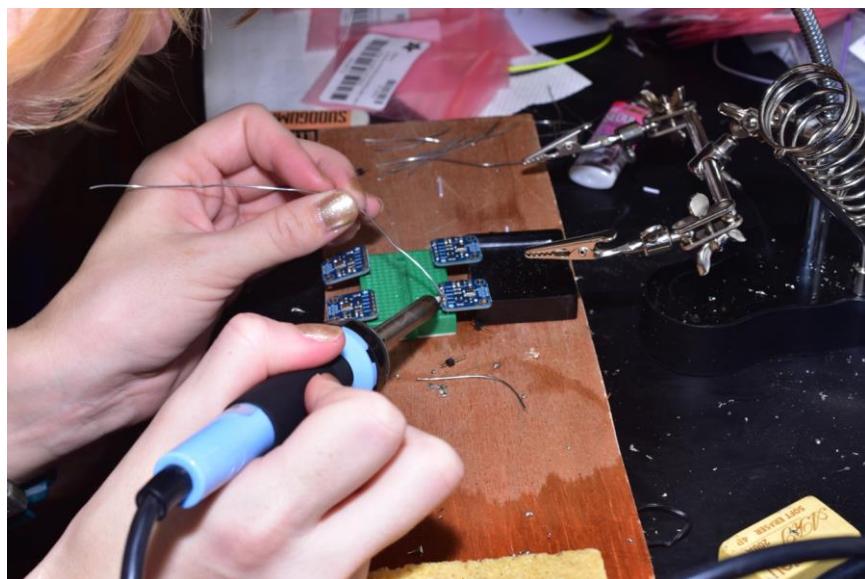
Two of the sensors need through the hole soldering to attach the pins that the jumper cables attach to. The BMP180 (barometric pressure sensor) and the TSL2561 (lux sensor). They come with the pin strip, but the strip might contain more pins than required, cut it to the correct pin length to match the sensor holes. We want to solder the short pin side, and the sensor need to be soldered the correct way around to ensure the actual sensor ends up on the correct side. The long pins should be on the back (where there are loads of text) and the short pins should come out on the side with all the circuitry for the BMP180, for the TSL2561 the sensor is on the other. I use a breadboard to attach the pins to hold it for the soldering, then hold the sensor up so that it is at a 90° angle to the pins. Solder all the pins.

1. Put the pin strip through the pin holes of the sensor.



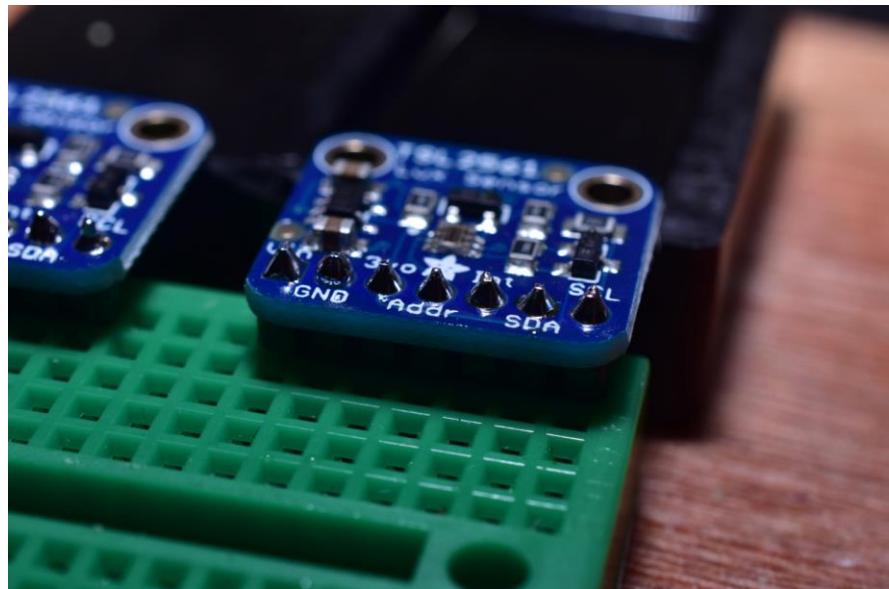
Pin-strips to the left with the short end up and a TSL2561 sensor with the sensor board up and the pins through the holes ready for soldering.

2. Ensure a 90° angle and have the small pin side showing (with the sensor side up).



Through the hole soldering, note that the boards being soldered is held up to ensure a 90° angle to the pins.

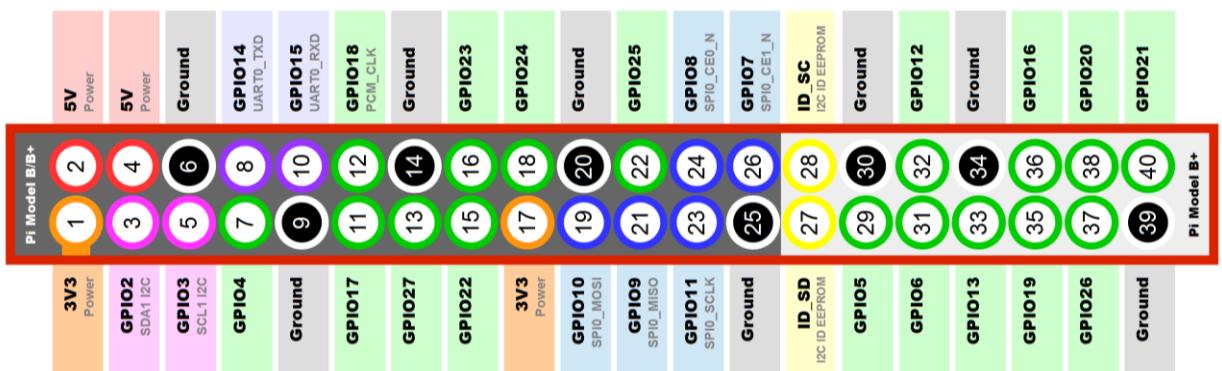
3. Solder each of the pin heads.



Soldered pins.

Wire soldering

We need to solder wires for all three sensors. We are using female/female jumper wires as they can connect directly to the GPIO pins on the raspberry pi. The GPIO pins is what we use to connect all our wires, they provide ground, power and receive/send information to the sensors. Ensure that you use the correct colored wires, someone else might be working on a unit later and having your own color use or mixing the colors used for a specific purpose increases the chance of mess-ups in the future. It is HIGHLY important to ensure that ground and power is always connected correctly! If you connect incorrectly you can destroy the sensor and in worst case, the raspberry pi. The GPIO pin layout is shown below, take note of the ground pins (labelled 6, 9, 14, 20, 25, 30, 34, and 39). There are four power pins, two 3.3 volt (1 and 17) and two 5 volts (2 and 4). Not only do you need to ensure that ground is connected correctly to a sensor, you need to ensure it has the correct power supply! The AM2302 hygrometer sensor needs 5v and the BMP180, TSL2561 and RTC need 3.3v.



Showing GPIO pin layout.

The pins labelled 3 and 5 are the I2C pins, SDA and SCL respectively. They will be used to send information back and forth to the BMP180 and the TSL2561 sensor. If you want more detailed explanations of the I2C bus, please look at the following two websites:

<http://www.robot-electronics.co.uk/i2c-tutorial>

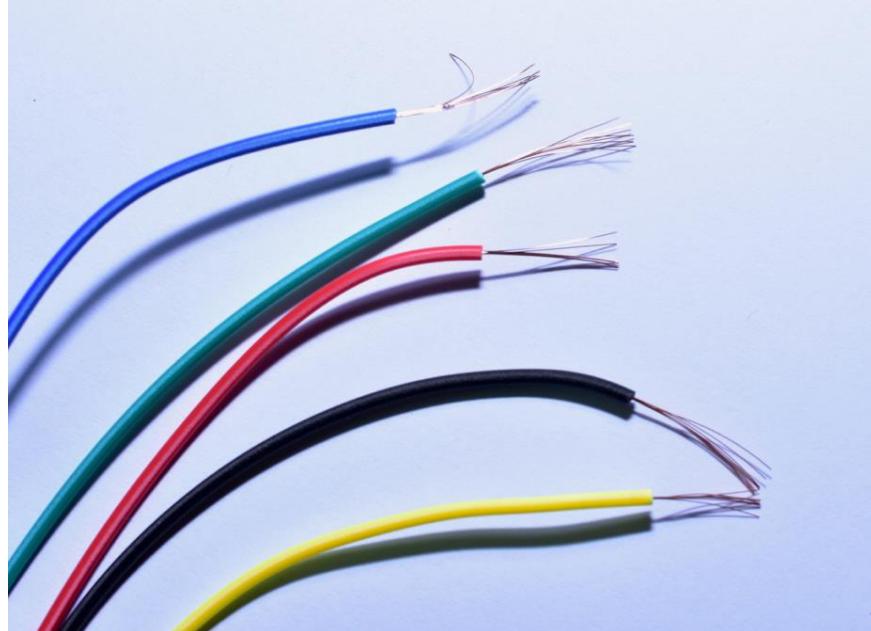
<https://learn.adafruit.com/using-the-bmp085-with-raspberry-pi/overview>

The I2C is useful as we can attach multiple sensors to the same pin, we just solder the wires together. The I2C bus has multiple channels, and each sensor is assigned a specific channel. In our case TSL2561 has channel 39 and BMP180 has channel 77, allowing us to use the same pin for data transfer (we can change the channel as well, if for example we want multiple TSL2561 sensors attached we can assign them different channels).

In our case the sensors will be attached to pin 1 for 3.3v power supply and 9 for ground, pin 3 (GPIO2) for I2C SDA and pin 5 (GPIO3) for I2C SCL.

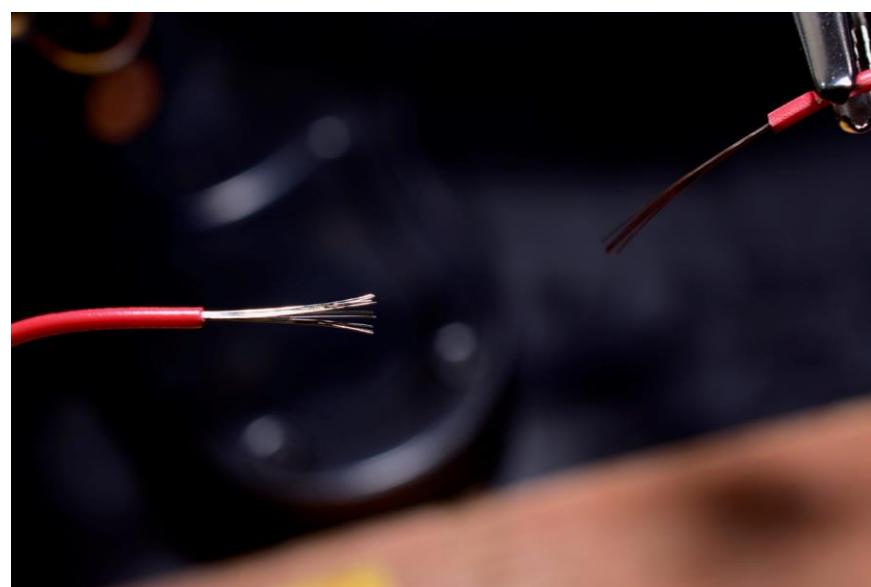
The AM2302 will be attached to pin 4 for 5v power supply, pin 6 for ground and pin 8 (GPIO14 <- important as 14 is the GPIO number we need to tell the raspberry pi to use in our configurations later) for information transfer.

1. To solder wires, you first need to strip the plastic off the wires. Do not use the tip of the soldering pin to remove the plastic regardless of how much easier it is! It is highly important that the tips of the solder iron are kept as clean as possible and do not get residue of plastic on it. Instead use the nippers (there is a specific tool for wire stripping but for some of our smaller wires they do not have small enough stripping holes, so it is worth learning how to do it with the nippers).



Wires with the ends stripped of plastic.

2. Twist the metal wire together in the ends.



Prior to twisting the ends.



Ends of wire twisted to prevent fraying.

3. Cut shrink plastic to size and put over the wire before you solder (away from the stripped wire).
4. Twist the two wires together (you can also wet each wire end with solder first, then attach them together).
5. Solder the two ends together (see figure 10).

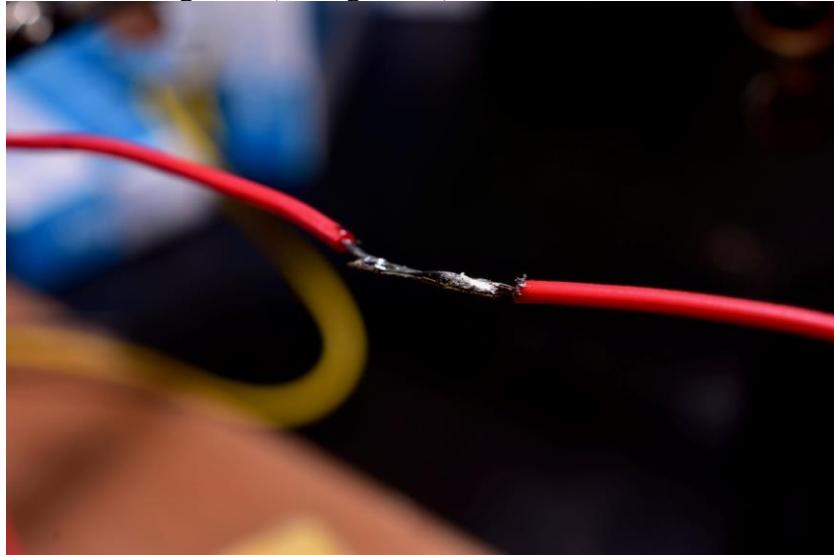


Figure 10. Ends of the wires soldered together.

6. Move the shrink tubing over the soldered area.
7. Shrink the tubing into place using a lighter or heat gun.

If you want more information on how to solder wires you can read more here:

<http://www.instructables.com/id/Soldering-wires-together/>

AM2302

Equipment needed:

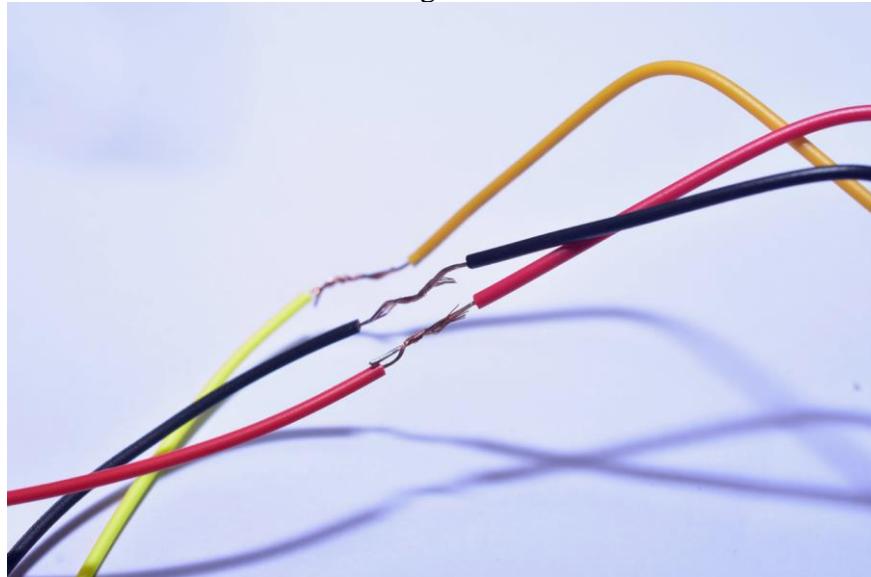
- AM2302 pre-wired sensor
- 40-way 20cm female to female jumper cables (remove a yellow, black and red cable and cut them in half)
- Solder
- Shrink tubing

Tools needed:

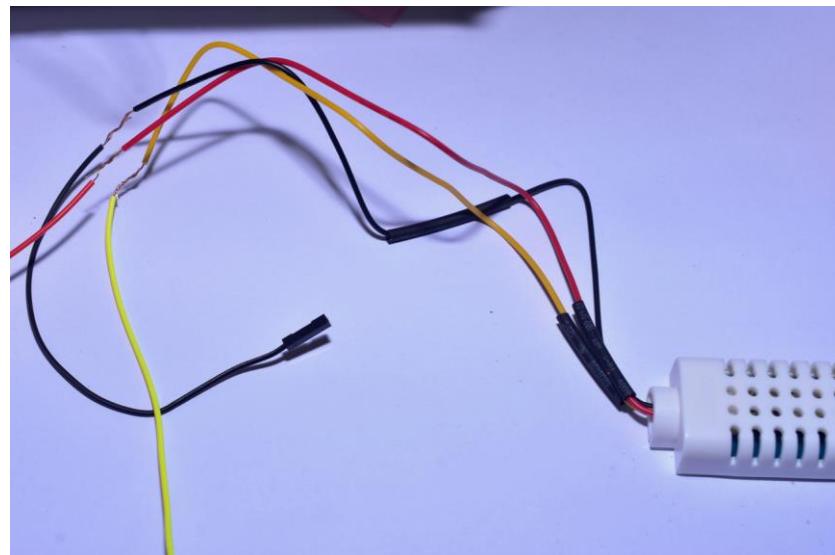
- Solder station
- Nippers
- Lighter or heat gun

This sensor comes with the three wires already attached but without a female jumper cable end. There is a red wire for the 5v power supply, a black one for ground and a yellow one for the GPIO pin for information transfer.

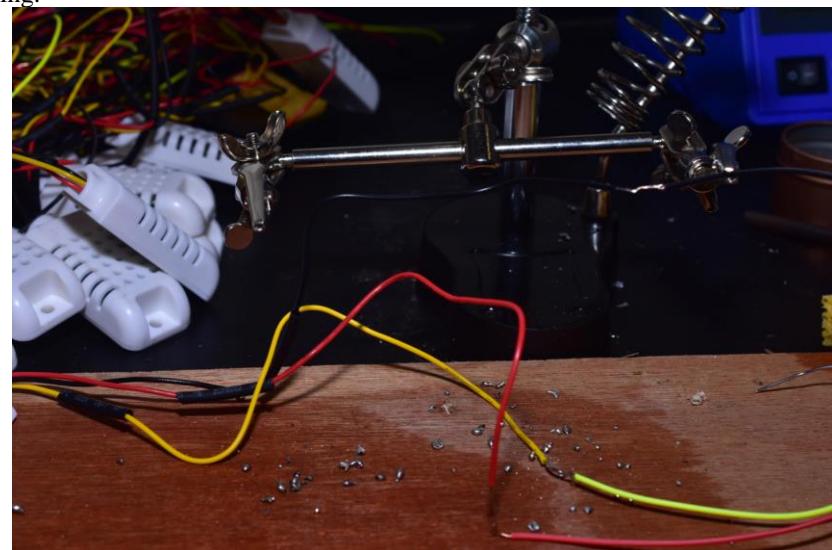
1. Cut a black, yellow and red jumper wire in half and cut three pieces of shrink tubing in the correct size. Strip the ends of the wires and twist together with the wire of the same colour coming off the sensor.



The three wires coming off the sensor twisted together with the correct jumper cable with a female end.

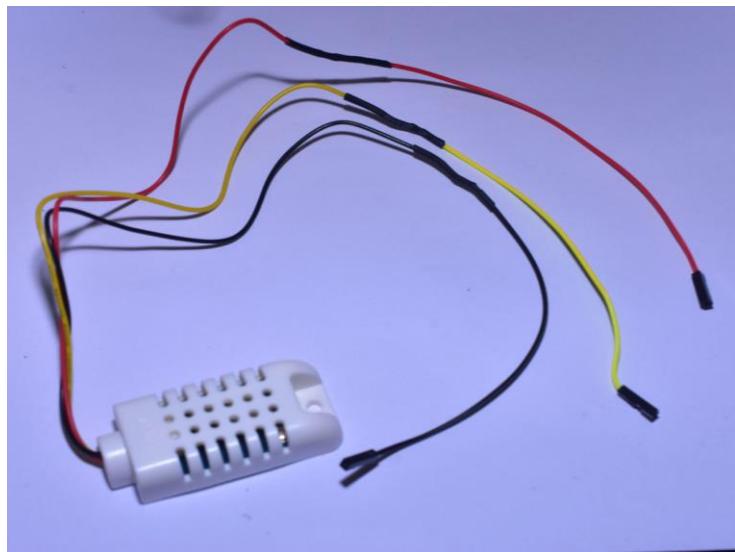


The sensor, unsoldered but twisted together wires from the sensor to jumper cable and shrink tubing.



Wires from the sensor ready for soldering with shrink tubing on the cable.

2. Solder the wires together
3. Move the shrink tubing over the solder area and shrink it using a lighter (not the solder iron!).



Fully assembled sensor with wires soldered and shrink tubing heated over the soldered areas.

BMP180 and TSL2561 (and if needed for any extra sensors or RTC).

Equipment needed:

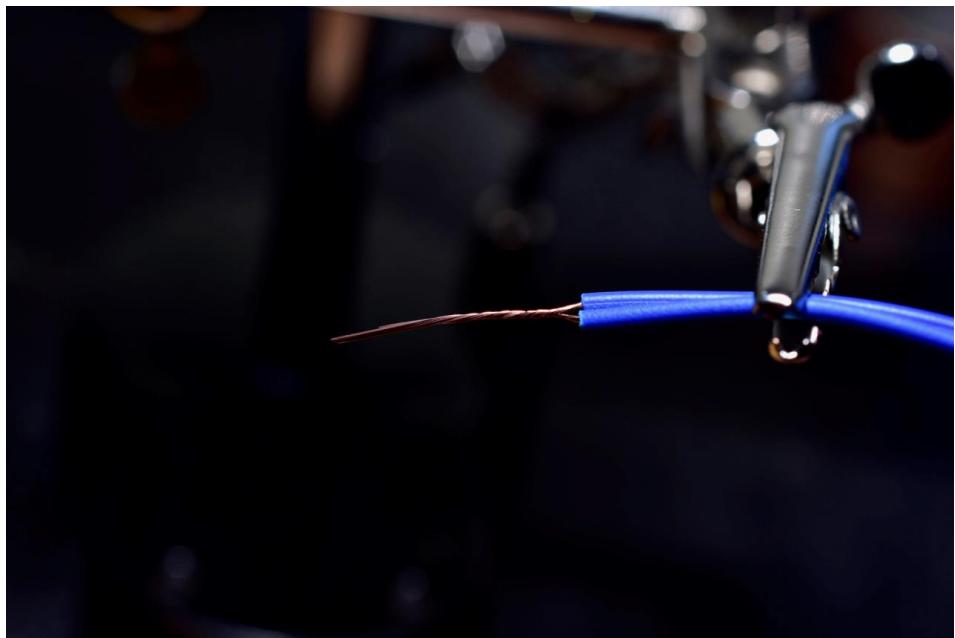
- Sensors (BMP180, TSL2561, RTC) – should come with pin strip.
- Solder
- 40-way 20cm female to female jumper cables. Take out two of each colour: blue, green, orange and black and cut them in half.
- Shrink tubing

Tools needed:

- Solder station
- Nippers
- Lighter or heat gun

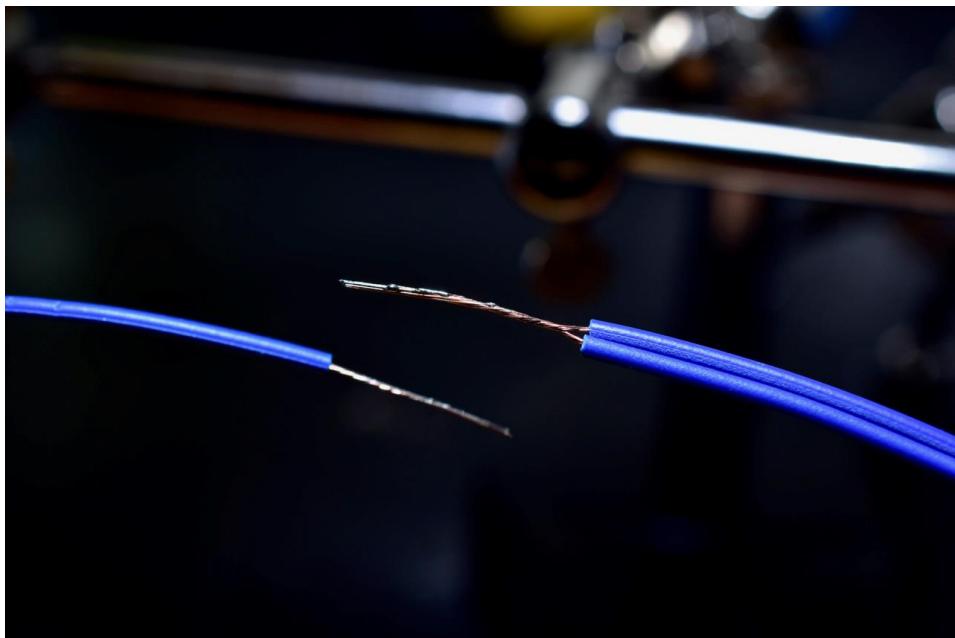
These sensors have no wires attached. They need female/female jumper wires. For power these two both need 3.3v, for this we use orange wires. We want to use green wires for SDA and blue for SCL and we need to solder these. They also need a black wire for ground. If you need to attach any more sensor or a Real Time Clock then just add on an extra wire (i.e. solder three wires against one, or four, or five, depending on how many you need). We typically solder them in 1:3 to get two sensors and an RTC attached.

1. Cut two jumper cables of each colour in half.
2. Strip the ends.
3. Cut one piece of shrink tubing.
4. Put the shrink tubing over one of the cables.
5. Now twist together the ends of the three wires without the shrink tubing lengthwise/side by side/parallel to each other.

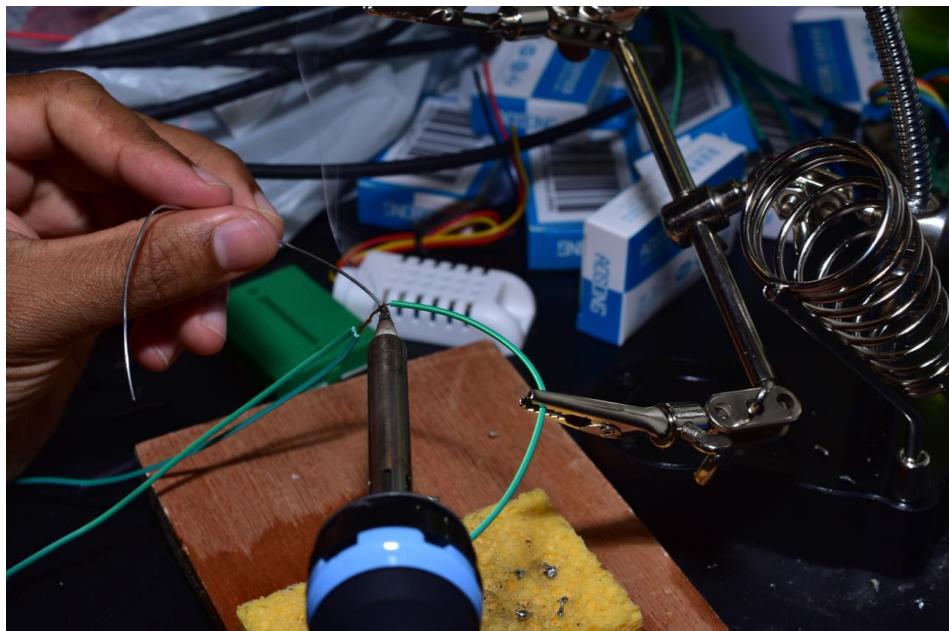


Two wires parallel to each other with the ends twisted together.

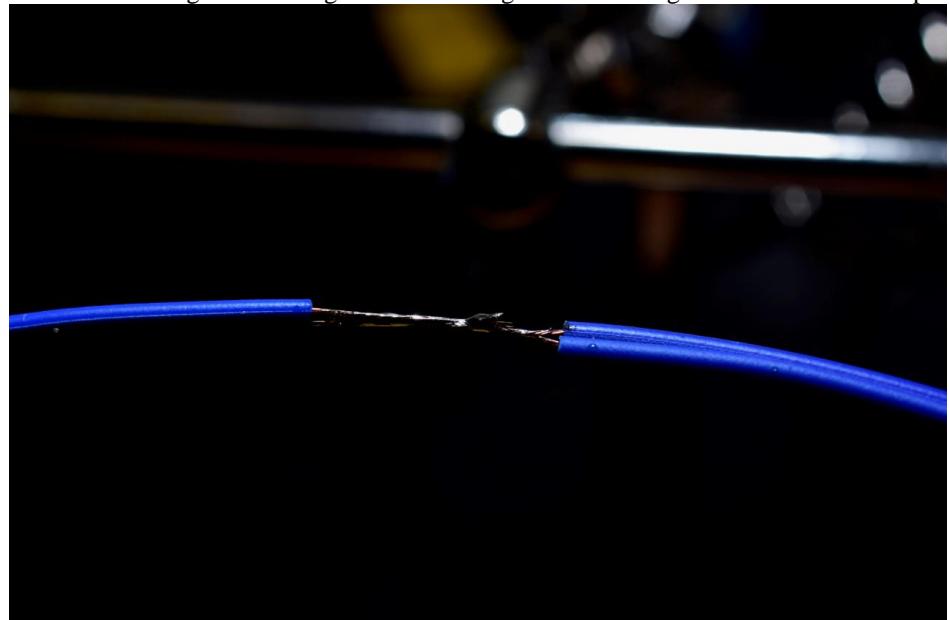
6. Now twist these together with the single one and solder (figure 16-18).



The two side by side wires against the single wire.

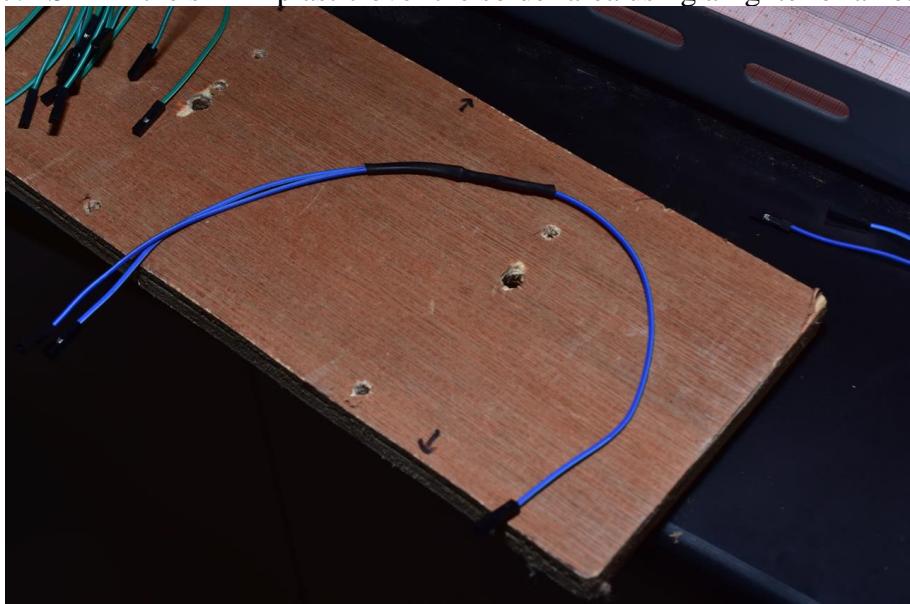


Two wires being soldered together with a single wire creating a Y fashion wire setup.



Single wire soldered to two parallel wires.

7. Shrink the shrink plastic over the solder area using a lighter or a heat gun.



The Y style wire set up with shrink tubing over the soldered area.

Microphones

We have tried two types of microphones. The Kingstate KEEG1538WB-100LB waterproof microphone which comes without the 3.5 mm jack attached and no holder so requires assembly. We also tried the EM172 microphone from FEL Communications Ltd, which is not waterproof but can be ordered with a rugged mount (which has an O-ring making assembly easy). The EM172 can be ordered as just the capsule or as pre-mounted. Below is instruction for assembly of microphone capsules.

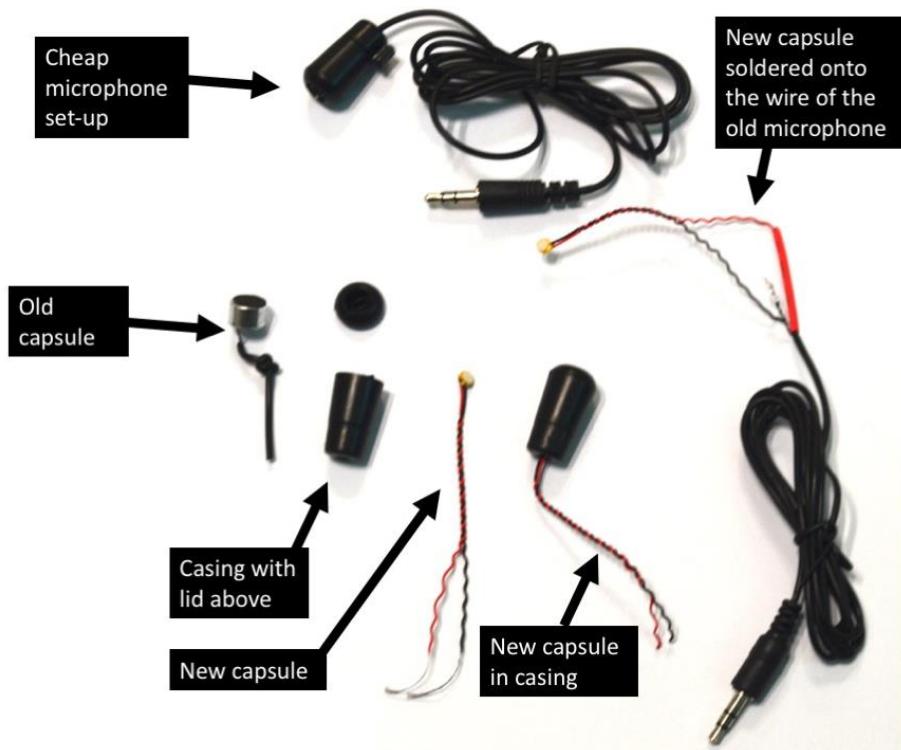
Equipment needed:

- Microphone capsule
- Cheap microphone stripped of the capsule.
- Solder
- Silicon
- Shrink tubing of two sizes

Tools needed:

- Solder station
- Nippers
- Silicon gun

We might want to use waterproof microphones to ensure they last longer if the environment they are in is very humid or have a lot of rain. These come without a 3 mm connection end. We thereby need to solder them onto a cable that has one. There are microphones which are very cheap (1-2 dollars) but of too low quality for us to use, we will use the wire with the 3.5 mm jack and the plastic casing from them.



1. Open the plastic casing of the low-quality microphone.
2. Pull the microphone and the wires through and cut the microphone off.
3. Keep the casing on the wire and strip the ends of the two wires.
4. Strip the wires coming off the microphone.
5. Cut three pieces of shrink tubing, two of the smaller diameters and one of the larger diameters. The smaller ones should be able to fit over a solder on a microphone wire, the larger one should fit both microphone wires.
6. Put the two smaller pieces of wire over the small microphone wires and push it as far as possible over the end.
7. Put the bigger piece of shrink tubing over the main wire.
8. Solder together the black wire from the microphone with the black wire from the cable.
9. Solder together the red wire from the microphone with the white one from the cable.
10. Shrink the smaller pieces of plastic over the soldered wires, it is very important that no pieces of these wires are left exposed! If the solder wires touch each other they short circuit!
11. Move the bigger piece of shrink plastic over the area of soldered and shrink the plastic over both wires.



12. Stick the wires of the microphone through the casing (but do not put in the microphone).
13. Inject some silicon into the base of the microphone holder.
14. Carefully pull/push in the microphone and ensure it does not get silicon on it.

Button

Equipment needed:

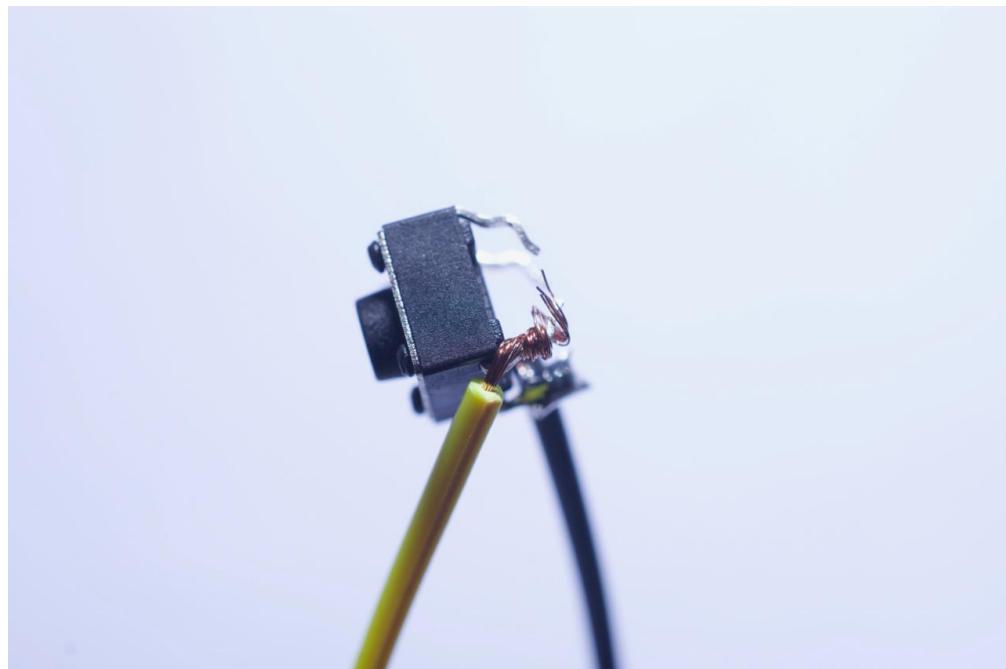
- Tactile push button
- Black and yellow female to female jumper wire cut in half.
- Solder
- Shrink tubing (optional)

Tools needed:

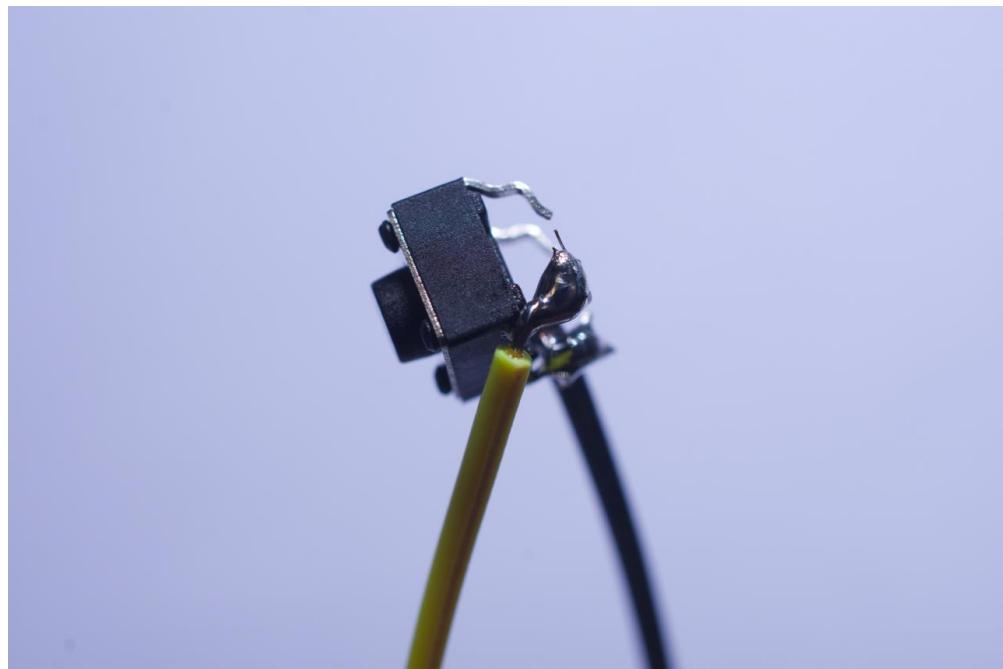
- Solder station
- Nippers

We need to attach a tactile push button for safe shut down of the unit when the battery needs to be removed. Sometimes we also need to waterproof it so that we can put it on the outside of the waterproof box, it removes the need to open the box and let humidity in when the battery needs to be replaced.

1. Take a yellow and a black jumper cable with stripped ends.
2. Solder the black jumper cable to one of the pins on the short side of button.

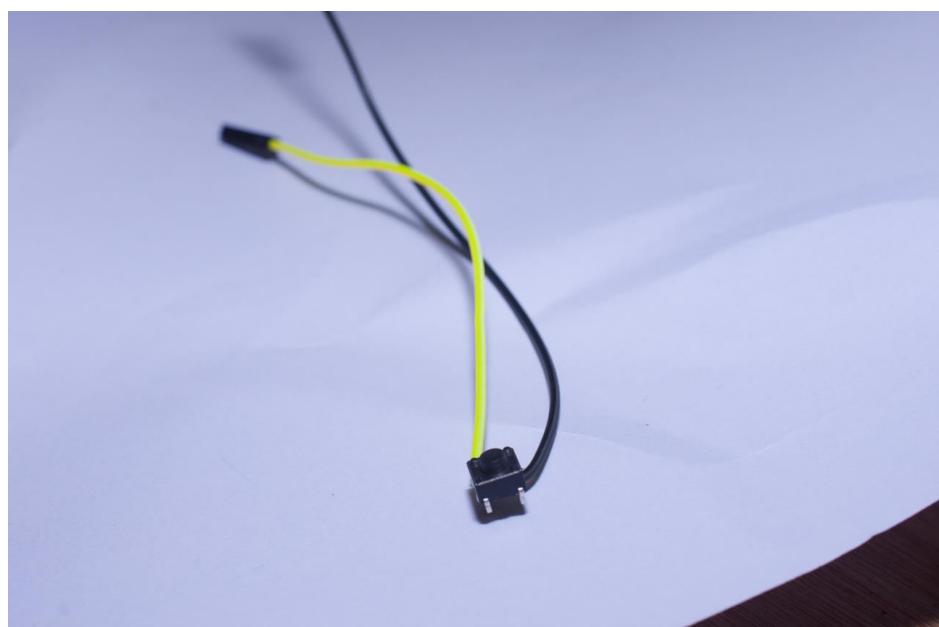


Button with a yellow and a black wire twisted around the pins on one of the short sides.



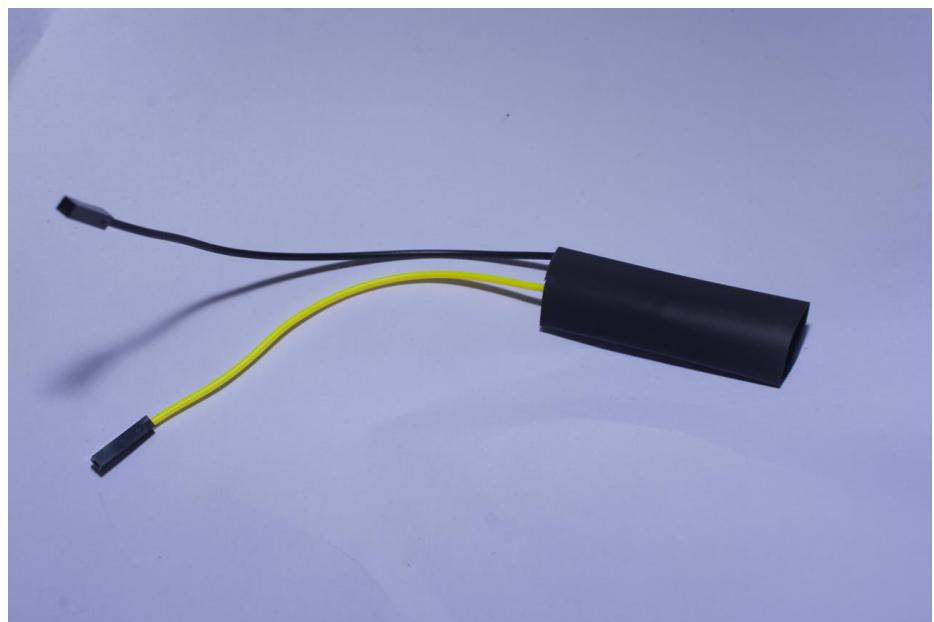
The button after the wires have been soldered to the pins.

3. Solder the yellow one to the opposing pin on the button (still short side).



Button with the two wires soldered to it.

4. OPTIONAL: Get a bit of shrink tubing in the correct size, put it over the button and heat shrink it. Only do this step if you need to keep the push button on the outside of the box! Otherwise it can just be left like it is.



Button set up covered in shrink tubing before heating.



Buttons et up with the shrink tubing heated around for waterproofing.

Coding

OS installation

Download NOOBs from the Raspberry Pi website.

<https://www.raspberrypi.org/downloads/noobs/>

If the SD card is less 32GB or less all the items in the NOOBs folder can be just be copied to the SD card. If it is of a larger size it needs to be formatted for FAT32. If you are on a mac you open the terminal after inserting the SD card into a reader. List all the disks currently on the computer.

```
diskutil list
```

Put in the blank SD card that you want a new OS on and check what disk number it is under and make sure that is the one that is unmounted, so you do not unmount the hard drive of the computer. Then unmount it.

```
diskutil unmountDisk /dev/disk4
```

Format to FAT32.

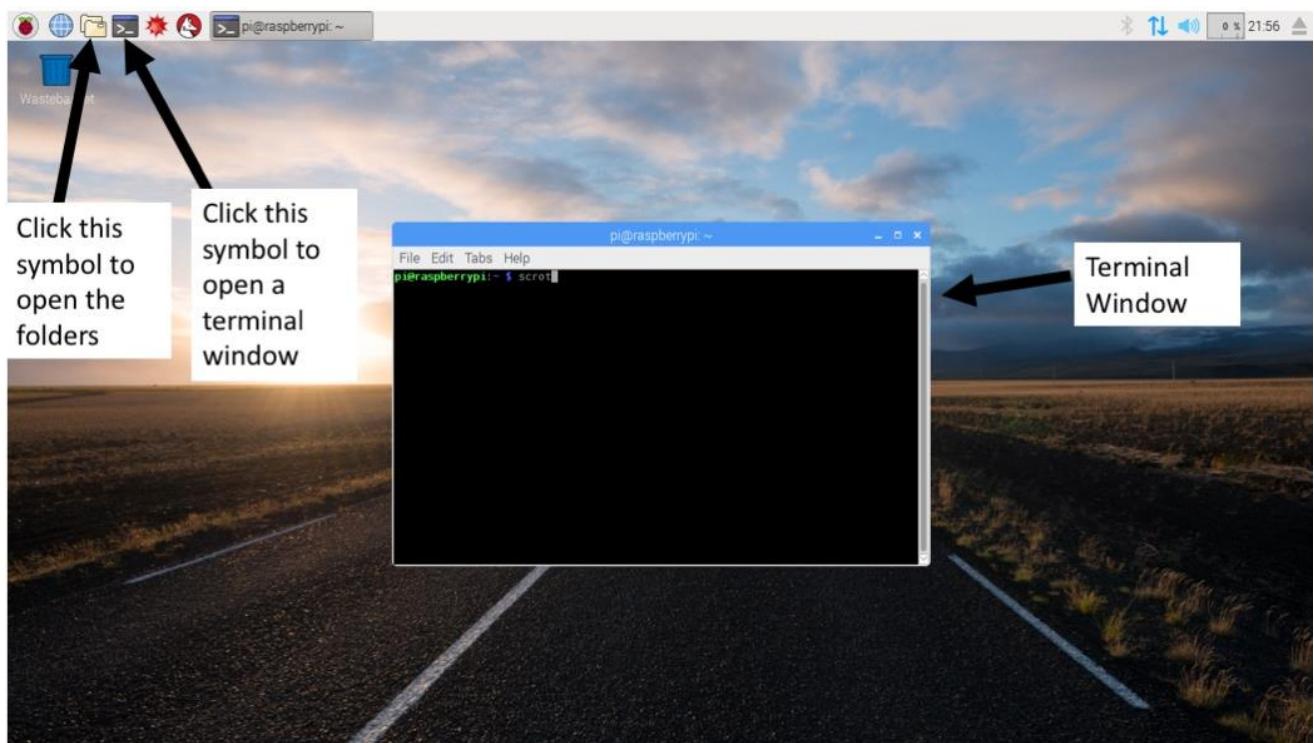
```
sudo newfs_msdos -F 32 /dev/disk4
```

After this is finished you can just copy the files in NOOBs folder and paste them onto the SD card (not the NOOBs folder itself, it needs to be the item inside the folder that gets copied and pasted). When starting the Raspberry Pi the first time, choose Raspbian as an OS system.

Configuration

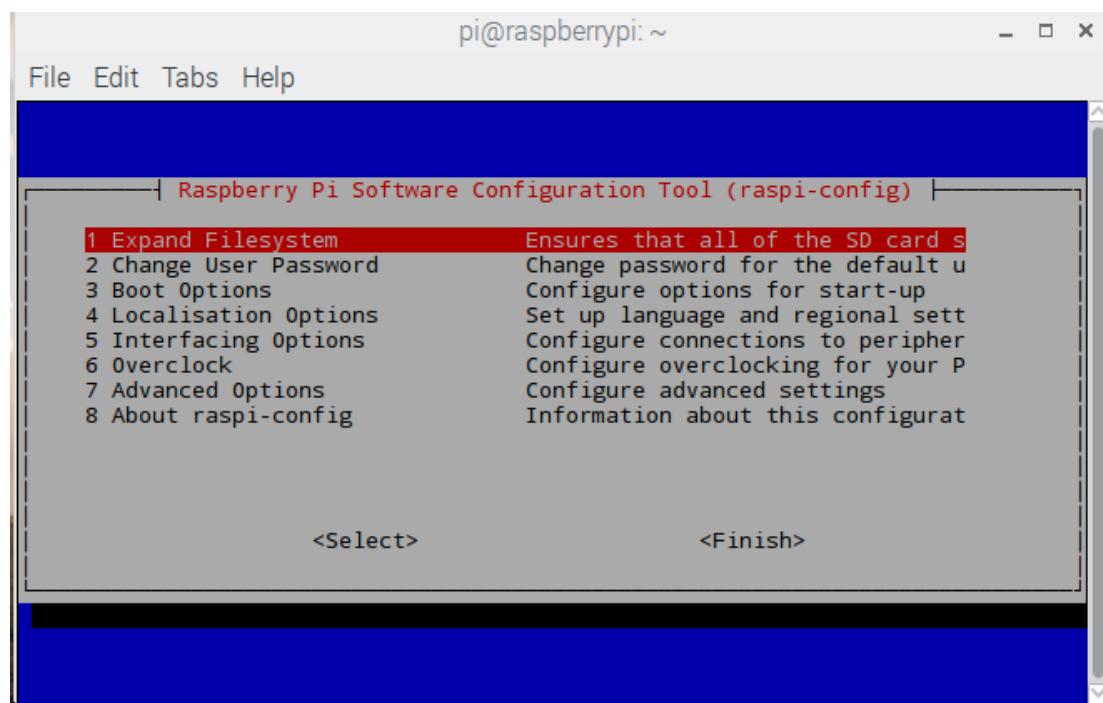
Follow these coding instructions to set up the unit for use as an acoustic environmental recorder. Ensure the Raspberry Pi is connected to Wi-Fi and start it. Code is shown in “Courier New”.

1. Open the terminal window.

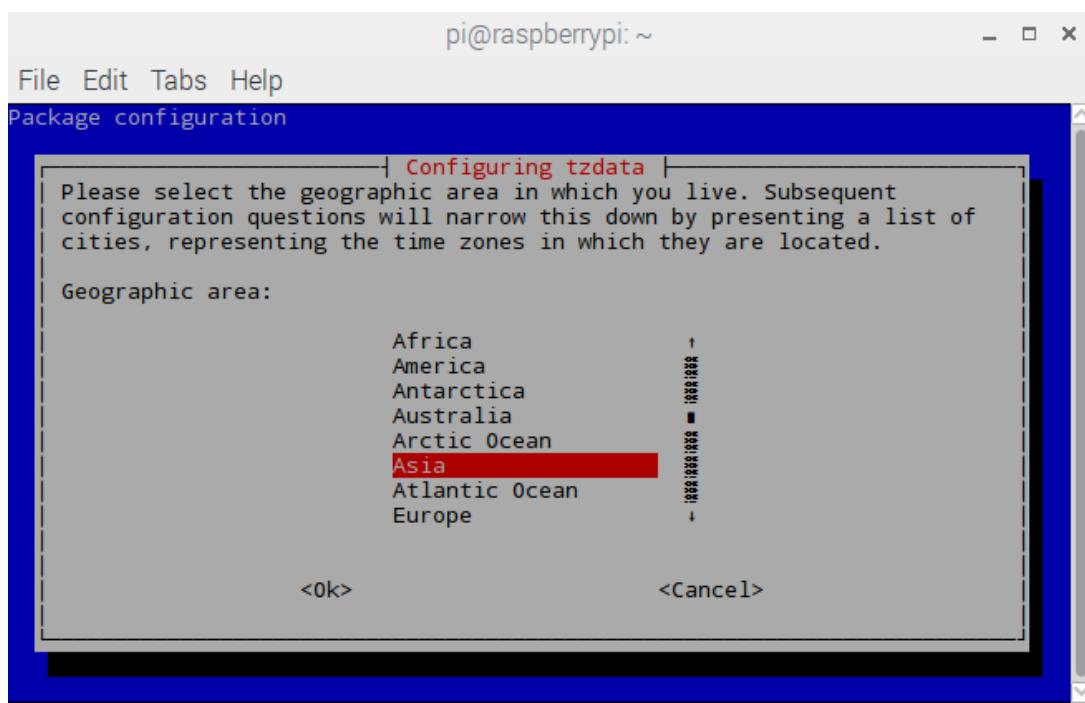
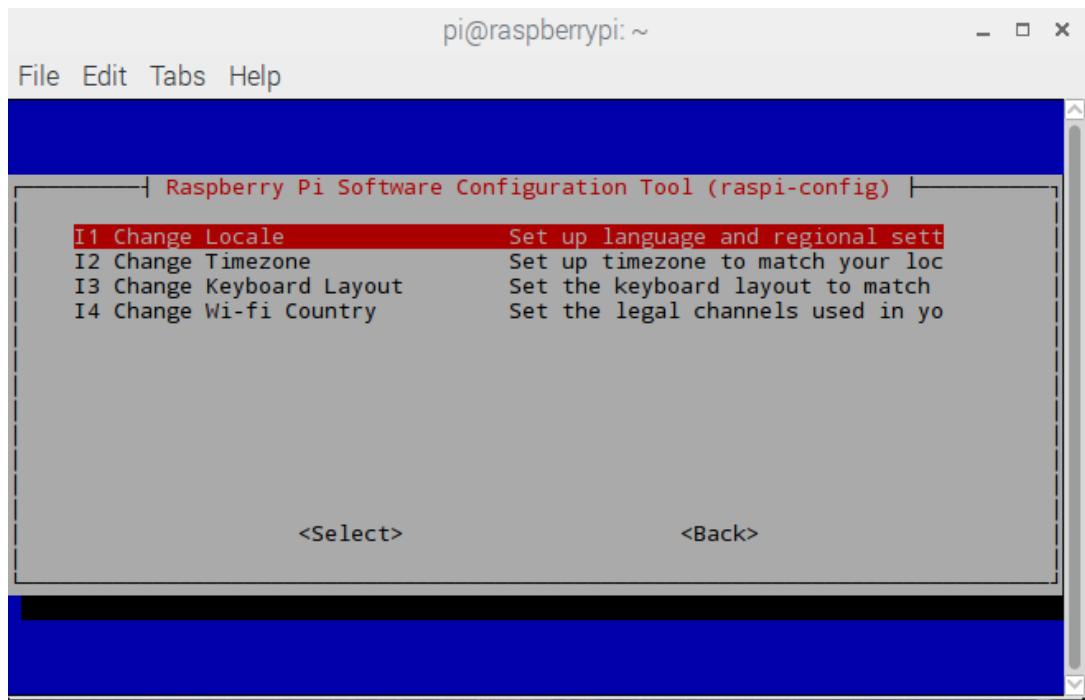


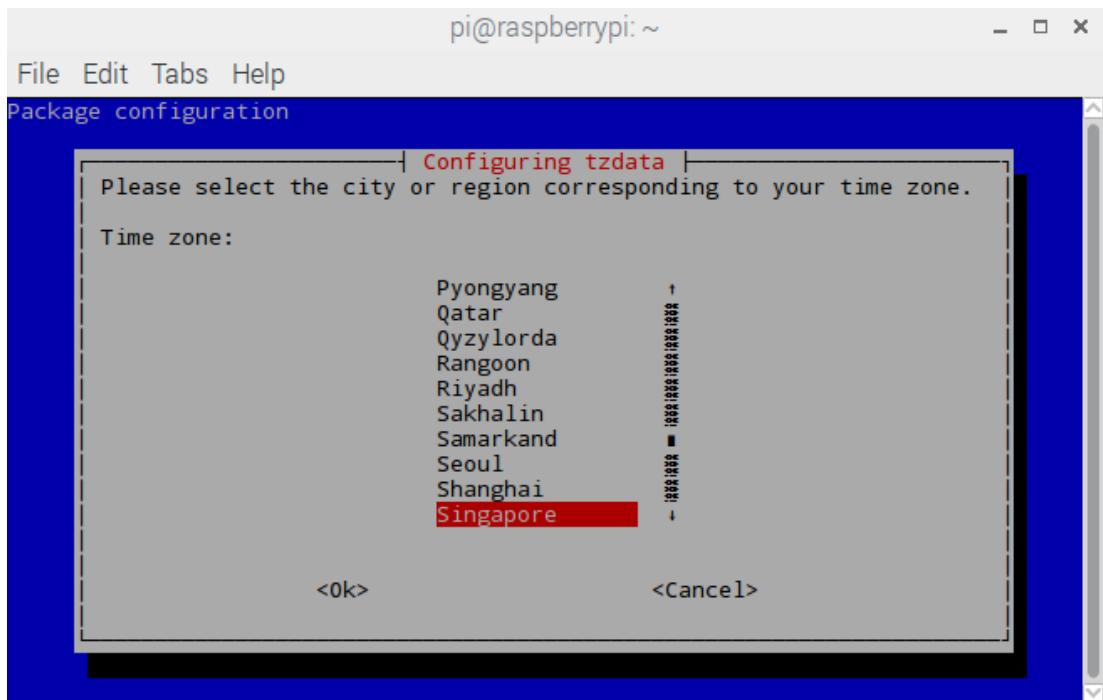
2. We first need to set up the Raspberry Pi and install several packages that we will need. In the terminal, you type out the code then press enter to execute it. Open the configuration window:

```
sudo raspi-config
```



3. Now that you are in the configuration window. Go into localization options and change the time zone (if you are in Singapore set it to Singapore) by first setting the correct region. Save and exit the configuration window.



A screenshot of a terminal window titled "pi@raspberrypi: ~". The window shows the command "sudo raspi-config" being run. The output of the command is displayed, showing the current default time zone as "Asia/Singapore", the local time as "Sun May 13 21:58:06 SGT 2018", and the universal time as "Sun May 13 13:58:06 UTC 2018". The prompt "<pi@raspberrypi:~ \$>" is visible at the bottom.

4. Double check that the time-zone have been changed to the correct one.

```
date
```

A screenshot of a terminal window titled "pi@raspberrypi: ~". The command "date" is run, and the output shows the date and time as "Sun 13 May 21:58:28 SGT 2018". The prompt "<pi@raspberrypi:~ \$>" is visible at the bottom.

5. Install packages that we need by entering the following codes.

```
sudo apt-get install python
sudo apt-get install python-dev
sudo apt-get install libjpeg-dev
sudo apt-get install libfreetype6-dev
sudo apt-get install python-setuptools
sudo apt-get install python-pip
```

```
sudo apt-get install libi2c-dev
```

6. Update Python distribution.

```
sudo easy_install -U distribute
```

7. Install GPIO packages.

```
sudo pip install RPi.GPIO  
sudo pip install pySerial  
sudo pip install nose  
sudo pip install cmd2
```

8. Install I2C library.

```
git clone https://github.com/adafruit/Adafruit_Python_GPIO.git
```

9. Install adafruit pureIO library.

```
git clone  
https://github.com/adafruit/Adafruit_Python_PureIO.git
```

10. Ensure the Python version is upgraded and build it.

```
sudo apt-get upgrade  
sudo apt-get install build-essential python-dev
```

11. Install the I2C configuration.

```
sudo apt-get install -y python-smbus  
sudo apt-get install -y i2c-tools
```

12. We need to make some changes in the configuration file that is opened on boot to activate the I2C.

```
sudo nano /boot/config.txt
```

13. Uncomment the following line by removing the # to activate the I2C.

```
dtparam=i2c_arm=on
```

14. Press Ctrl+X to exit then Y to save. Reboot to start the I2C.

```
sudo reboot
```

15. Update the system.

```
sudo apt-get update
```

16. We need to create some new directories to put scripts and files into which is done with the command mkdir.

```
mkdir Scripts  
mkdir Audio  
mkdir Environ  
mkdir sources
```

17. You can see that they have been creating by clicking the folder on the desktop or simply listing everything that is present in the current directory.

```
ls
```

```
pi@raspberrypi:~ $ ls  
Adafruit_Python_BMP    Desktop    Music      reader    Test  
Adafruit_Python_GPIO   Documents  oldconffiles Screenshots Videos  
Adafruit_Python_PureIO Downloads  Pictures    Scripts  
asoundrc                Environ   Public     sources  
Audio                  Logs      python_games Templates  
pi@raspberrypi:~ $
```

Sensors

1. Move into the directory called sources so that we can start to set up the sensors. The cd command tells the terminal to change directory into whatever location it is being given.

```
cd
```

2. Clone the library for the DHT22 sensor.

```
git clone https://github.com/adafruit/Adafruit_Python_DHT.git  
pi@raspberrypi:~ $ git clone https://github.com/adafruit/Adafruit_Python_DHT.git  
Cloning into 'Adafruit_Python_DHT'...  
remote: Counting objects: 253, done.  
remote: Compressing objects: 100% (4/4), done.  
remote: Total 253 (delta 0), reused 0 (delta 0), pack-reused 249  
Receiving objects: 100% (253/253), 79.11 KiB | 0 bytes/s, done.  
Resolving deltas: 100% (142/142), done.  
Checking connectivity... done.  
pi@raspberrypi:~ $
```

```
sudo apt-get update  
sudo apt-get install build-essential python-dev python-openssl
```

3. Set the sensor up.

```
cd Adafruit_Python_DHT  
sudo python setup.py install
```

4. For the next section the sensors need to be attached to the station. See the assembly section of the manual. Test that the DHT22 is working. Note that the code 14 is telling the script which information pin the sensor is connected to, if you are coding the script for another pin you need to change this.

```
cd examples  
sudo ./AdafruitDHT.py 2302 14
```

```
Finished processing dependencies for Adafruit-DHT==1.3.2  
pi@raspberrypi:~/Adafruit_Python_DHT $ cd examples  
pi@raspberrypi:~/Adafruit_Python_DHT/examples $ sudo ./AdafruitDHT.py 2302 14  
Temp=25.1* Humidity=78.0%  
pi@raspberrypi:~/Adafruit_Python_DHT/examples $ █
```

5. Go back to the initial directory.

```
cd
```

6. Now we need to set up the I2C for the BMP180 and the TSL2561, specifically we need to add modules to their modules to the start-up kernel.

```
sudo nano /etc/modules
```

7. In the kernel add the following lines.

```
i2c-bcm2708  
i2c-dev
```

```
GNU nano 2.2.6          File: /etc/modules

# /etc/modules: kernel modules to load at boot time.  
#  
# This file contains the names of kernel modules that should be loaded  
# at boot time, one per line. Lines beginning with "#" are ignored.  
  
i2c-bcm2708  
i2c-dev

^G Get Help      ^O WriteOut      ^R Read File      ^Y Prev Page      ^K Cut Text      ^C Cur Pos  
^X Exit         ^J Justify       ^W Where Is       ^V Next Page      ^U UnCut Text    ^T To Spell
```

8. Exit and save. The boot file needs to be changed again so open it.

```
sudo nano /boot/config.txt
```

9. Make sure the following lines are uncommented or write them in if they do not exist.

```
dtparam=i2c1=on  
dtparam=i2c_arm=on
```

The screenshot shows a terminal window titled "pi@raspberrypi: ~". The window title bar includes icons for minimize, maximize, and close. Below the title bar is a menu bar with "File", "Edit", "Tabs", and "Help". The main area of the window is a text editor titled "GNU nano 2.2.6" with the file path "File: /boot/config.txt". The text in the editor is as follows:

```
#uncomment to overclock the arm. 700 MHz is the default.  
#arm_freq=800  
  
# Uncomment some or all of these to enable the optional hardware interfaces  
dtparam=i2c_arm=on  
dtparam=i2c1=on  
#dtparam=i2s=on  
#dtparam=spi=on  
  
# Uncomment this to enable the lirc-rpi module  
#dtoverlay=lirc-rpi  
  
# Additional overlays and parameters are documented /boot/overlays/README  
#  
# Enable audio (loads snd_bcm2835)  
dtparam=audio=on  
  
# NOOBS Auto-generated Settings:  
^G Get Help ^O WriteOut ^R Read File ^Y Prev Page ^K Cut Text ^C Cur Pos  
^X Exit ^J Justify ^W Where Is ^V Next Page ^U UnCut Text ^T To Spell
```

10. Connect the TSL2561 and the BMP180 to the GPIO pins. Test to see whether the I2C bus is working, a grid appears and channel 39 and 77 should be active. In the example below the first grid shows the grid with no sensors attached. If the RTC is connected channel 68 should also be active.

```
sudo i2cdetect -y 1
```

```

sudo: i2cdetect: command not found
pi@raspberrypi:~ $ sudo i2cdetect -y 1
      0  1  2  3  4  5  6  7  8  9  a  b  c  d  e  f
00: --- - - - - - - - - - - - - - - - - - - - - - -
10: --- - - - - - - - - - - - - - - - - - - - - - -
20: --- - - - - - - - - - - - - - - - - - - - - - -
30: --- - - - - - - - - - - - - - - - - - - - - - -
40: --- - - - - - - - - - - - - - - - - - - - - - -
50: --- - - - - - - - - - - - - - - - - - - - - - -
60: --- - - - - - - - - - - - - - - - - - - - - - -
70: --- - - - - - - - - - - - - - - - - - - - - - -
pi@raspberrypi:~ $ sudo i2cdetect -y 1
      0  1  2  3  4  5  6  7  8  9  a  b  c  d  e  f
00: --- - - - - - - - - - - - - - - - - - - - - - -
10: --- - - - - - - - - - - - - - - - - - - - - - -
20: --- - - - - - - - - - - - - - - - - - - - - - -
30: --- - - - - - - - - - - - - - - - - - - - - - -
40: --- - - - - - - - - - - - - - - - - - - - - - -
50: --- - - - - - - - - - - - - - - - - - - - - - -
60: --- - - - - - - - - - - - - - - - - - - - - - -
70: --- - - - - - - - - - - - - - - - - - - - - - -
pi@raspberrypi:~ $ 
```

11. Set up the BMP180 sensor.

```

git clone https://github.com/adafruit/Adafruit_Python_BMP.git
cd Adafruit_Python_BMP
sudo python setup.py install

```

12. Test that the BMP180 is working.

```

cd examples
sudo python simpletest.py

```

```

pi@raspberrypi:~/Adafruit_Python_BMP/examples $ sudo python simpletest.py
Temp = 28.00 *C
Pressure = 100645.00 Pa
Altitude = 57.35 m
Sealevel Pressure = 100642.00 Pa
pi@raspberrypi:~/Adafruit_Python_BMP/examples $
pi@raspberrypi:~/Adafruit_Python_BMP/examples $ 
```

13. Now it is time to set up the TSL2561, so we go into the sources folder and copy the configuration files to it.

```

cd
cd sources
wget
https://raw.githubusercontent.com/seanbechhofer/raspberrypi/master/python/TSL2561.py

```

```
wget  
https://raw.githubusercontent.com/born2net/raspberry/master/Adafruit-Raspberry-Pi-Python-  
Code/Adafruit_BMP085/Adafruit_I2C.py
```

```
pi@raspberrypi:~/sources $ wget https://raw.githubusercontent.com/born2net/raspberry/master/Adafruit-Raspberry-Pi-Python-Code/Adafruit_BMP085/Adafruit_I2C.py  
--2018-05-13 18:02:56-- https://raw.githubusercontent.com/born2net/raspberry/master/Adafruit-Raspberry-Pi-Python-Code/Adafruit_BMP085/Adafruit_I2C.py  
Resolving raw.githubusercontent.com (raw.githubusercontent.com)... 151.101.72.133  
Connecting to raw.githubusercontent.com (raw.githubusercontent.com)|151.101.72.133|:443... connected.  
HTTP request sent, awaiting response... 200 OK  
Length: 5501 (5.4K) [text/plain]  
Saving to: 'Adafruit_I2C.py'  
  
Adafruit_I2C.py          100%[=====] 5.37K --.-KB/s   in 0.001s  
2018-05-13 18:02:57 (5.35 MB/s) - 'Adafruit_I2C.py' saved [5501/5501]
```

14. There are some aspects of the code file which have changed so you need to open the python script and manually change these.

```
sudo nano TSL2561.py
```

15. Change the import of Adafruit_GPIO on line 11 to Adafruit_I2C like below.

```
GNU nano 2.2.6                                         File: TSL2561.py  
  
#!/usr/bin/python  
# Code sourced from AdaFruit discussion board: https://www.adafruit.  
  
import sys  
# Not needed here. Thanks to https://github.com/mackstann for highlighting  
#import smbus  
import time  
#Adafruit_I2C from https://github.com/adafruit/Adafruit-Raspberry-Pi  
from Adafruit_I2C import Adafruit_I2C
```

16. On line 27 change the self.i2c from Adafruit GPIO to Adafruit_I2C like below. Exit and save.

```
class TSL2561:  
    i2c = None  
  
    def __init__(self, address=0x39, debug=0, pause=0.8):  
        self.i2c = Adafruit_I2C(address)  
        self.address = address  
        self.pause = pause  
        self.debug = debug  
        self.gain = 0 # no gain preselected  
        self.i2c.write8(0x80, 0x03)      # enable the device
```

17. Check that the scripts are working.

```
sudo python Adafruit_I2C.py  
sudo python TSL2561.py
```

```
pi@raspberrypi:~/sources $ sudo python TSL2561.py  
1095.86625115
```

18. Great! All the sensors are working. Now we need a script that can take readings from all the sensors and then dump it in a CSV file. To do this we will clone a library somebody else already wrote. First go back to the main directory.

```
cd  
git clone  
https://github.com/JeremyMorgan/Raspberry_Pi_Weather_Station.g  
it reader
```

19. Now we need to go into the script called readings and change it for our own purposes.

```
cd reader  
sudo nano readings.py
```

20. This script needs the following changes done to it:

- line 11 comment out line with # (from ds18b20 import ds18b20_read_temp)
- line 18 change the pin to 14 (am2302Pin = 14)
- line35 change to False (useDS18B20 = False)
- line98 change to divide by two and remove temperature 3 (avgTemp = ((temperature1 + temperature2) / 2)
- line99 remove temperature 3 (tempList = [temperature1, temperature2])

21. Perform a dry run with the script.

```
sudo python readings.py dryrun
```

```

pi@raspberrypi:~/reader $ sudo python readings.py dryrun
Sensor Reading Dry Run
*****
AM2302:
Temp Sensor 1 = 26.2 *C
Humidity=70.9%

BMP180:
Temp Sensor 2 = 26.80 *C
Pressure = 100625.00 Pa
Altitude = 58.86 m
Sealevel Pressure = 100621.00 Pa

TSL2561:
Lux: 1434.91371031

Highest: 26.8
Lowest: 26.2000007629
Average: 26.5000003815
Variance: 0.599999237061

*****
pi@raspberrypi:~/reader $ 

```

22. Everything is working. Now we need to move this script into the Scripts folder.

```

cd
cp Reader /home/pi/Scripts

```

23. Change the name of the folder to “Sensors”.

```

Cd Scripts
mv Reader Sensors

```

24. We will now change the readings scripts so that it saves the data into a CSV file instead of onto an online server then change the name of the script.

```

cd Sensors
sudo nano Readings.py

```

25. At the end script after the ## Get average Temperature section delete everything after the section that starts with “if dryrun:” and instead add in the code for creating a csv file and putting data into it. We need to start with assigning date and time as we need this information. As you save it, change the name to “Environmentalreadings.py”.

```

## set current date and time
import datetime
datetime = datetime.datetime.now

```

```

import csv
field=[datetime,humidity,lux,pressure,slpressure,temperture1,t
emperature2,avgTemp,highestTemp,lowestTemp]
with open(r'home/pi/EnvironmentalData.csv','a') as f:
    writer = csv.writer(f)
    writer.writerow(fields)

```

```

GNU nano 2.2.6                               File: Environmentalreadings.py

#sensor = BMP085.BMP085(mode=BMP085.BMP085_STANDARD)
#sensor = BMP085.BMP085(mode=BMP085.BMP085_HIGHRES)
sensor = BMP085.BMP085(mode=BMP085.BMP085_ULTRAHIGHRES)

pressure = sensor.read_pressure()
altitude = sensor.read_altitude()
slpressure = sensor.read_sealevel_pressure()
temperature2 = sensor.read_temperature()

## DS18B20 Waterproof Temperature Probe
if useDS18B20:
    temperature3 = ds18b20_read_temp()

## TSL2561 Lux Sensor
if useTSL2561:
    lux = luxrdr.readLux()      # Auto
    #lux = luxrdr.readLux(1)    # Low Gain
    #lux = luxrdr.readLux(16)   # High Gain

    lux = luxrdr.readLux()

## Get Average Temperature

avgTemp = ((temperature1 + temperature2) / 2)
tempList = [temperature1,temperature2]
highestTemp = max(tempList)
lowestTemp = min(tempList)

## set current date and time
import datetime
datetime = datetime.datetime.now()

import csv
fields=[datetime, altitude,humidity,lux,pressure,slpressure,temperture1,temperature2,avgTemp,highestTemp,lowestTemp]
with open(r'/home/pi/EnvironmentalData.csv', 'a') as f:
    writer = csv.writer(f)
    writer.writerow(fields)

```

Audio Recordings

1. It is time to set up the audio recordings now. First change into the Scripts folder and create a script called MyBoot.sh.

```

cd /home/pi/Scripts
sudo nano Myboot.sh

```

2. Enter the following to kill the triggerhappy and dbus service on boot, then exit and save.

```

#stop the trigger happy service
sudo service triggerhappy stop
#stop the dbus service
sudo service dbus stop

```

```

GNU nano 2.2.6

#stop the triggerhappy service
sudo service triggerhappy stop
#stop the dbus service
sudo service dbus stop

```

^G Get Help ^O WriteOut ^R
^X Exit ^J Justify ^W

Issue the command to run the script on boot. First open rc.local then enter the command.

`sudo nano /etc/rc.local`

3. Before exit 0 enter the following code then exit and save.

`sh /home/pi/Scripts/MyBoot.sh`

4. We need to configure the sound to take input from the USB soundcard and not from the soundcard that is built into the processor. The alsoundrc file does not exists anymore so instead access the alsamixer configuration file.

`sudo nano /usr/share/alsa/alsa.conf`

5. In the following two lines change the 0 to 1.

```
defaults.ctl.card 0
defaults.pcm.card 0
```

6. Save and exit.

7. Go back to the home folder by issuing the cd command.

8. Install sox for audio recording.

```
sudo apt-get install sox lib sox-fat-mp3
sudo reboot
```

9. Start the alsamixer.

`alsamixer`

10. Configure the alsamixer and check recording levels

- Hit Fn F6 (Function Key F6) and select the "USB Audio Device"
- Hit Fn F3 to show playback controls
- Set the speaker up to 100
- Set the mic down to 0 and hit M to mute (yes, this is counter intuitive).
- If necessary, hit M to turn on Auto Gain Control (00 is "on" and MM is "off").

- Hit Fn F4 and set Capture up to 100.
- Hit Esc to exit and save.

11. Test the mono recording.

```
arecord -d 10 -f s16_LE -D plughw:1 ~/rectest.wav
aplay -D plughw:1 ~/rectest.wav
```

12. Test the stereo recording.

```
arecord -d 10 -f S16_LE -c 2 -D plughw:1 ~/rectest2.wav
aplay -D plughw:1 ~/rectest2.wav
```

13. We now want to create a shell script that contains the code that runs the recordings.

```
cd /home/pi/Scripts
sudo nano Record1 minute.sh
```

- 14. Enter the following. The first line establishes that this is a shell script. Line two creates a folder name based on the current day's date. Line 3-5 checks whether there is already a folder created with the current days' date, if there is it continues with the script. If there isn't it creates the folder. Line six creates a filename, first is "site" which should be replaced on each unit with a code for whatever location you are depositing it. Then an underscore is followed with the current date, another underscore gives the current time. This format for the file name is important as it allows the analysis software we use further down the line to automatically extract the correct information (such as site, date and time). Line seven is what executes the actual recording. -r denotes the recording speed which needs to be either 22050 or 44100 (corresponding to Hz). -b is the byte, in this case we want 16. Then the file is named and told where to be deposited as well as what format to record in (wav in this case). Trim establishes the length of the recording, in this case 60 seconds. The last line disables the HDMI and should be commented out when you work with the unit, the # need to be removed before the unit is deployed.**

```
#!/bin/bash
FOLDERNAME=$(date +"%Y%m%d")
if [ ! -e /home/pi/Audio/${FOLDERNAME} ]; then
    mkdir /home/pi/Audio/${FOLDERNAME}
fi
FILENAME=$(date + "%Y%m%d'/Site'_date +"%Y%m%d_%H%M%S")
rec -c 1 -r 22050 -b 16 /home/pi/Audio/${FILENAME}.wav trim 0
60
#Disable HDMI
#/usr/bin/tvservice -o
```

```

GNU nano 2.2.6                               File: Record1Minute.sh

#!/bin/bash
FOLDERNAME=$(date +"%Y%m%d")
if [[ ! -e "/home/pi/Audio/${FOLDERNAME}" ]]; then
    mkdir "/home/pi/Audio/${FOLDERNAME}"
fi
sleep 3
FILENAME=$(date +"%Y%m%d/SITE_%Y%m%d_%H%M%S")
rec -c 1 -r 22050 -b 16 /home/pi/Audio/${FILENAME}.wav gain 28 trim 0 60
#/usr/bin/tvservice -o

```

GNOME Terminal keyboard shortcuts:

- Get Help** (**Alt**+**G**)
- WriteOut** (**Alt**+**O**)
- Read File** (**Alt**+**R**)
- Prev Page** (**Alt**+**Y**)
- Cut Text** (**Alt**+**K**)
- Exit** (**Alt**+**X**)
- Justify** (**Alt**+**J**)
- Where Is** (**Alt**+**W**)
- Next Page** (**Alt**+**V**)
- Uncut Text** (**Alt**+**U**)

15. We are going to use a daemon called crontab to run our scripts. Having one script that runs in a loop continuously increases the risk of it becoming corrupt and data being lost. A daemon is a program that runs in the background anyway so there is no extra power being drawn from using it. Crontab is a task daemon, it means we can schedule tasks to be performed at specific time, date every month or day of the week etc. In this case, we can tell it to run a script at set times, meaning the script is run fresh every time which should reduce corruption and even if one occurs hopefully minimize the events. We will first set it up to run our script that makes the audio recordings as well as the one that takes our environmental readings. We are also going to schedule a daily reboot. A daily reboot again reduces the impact of corruption and ensure the microprocessor runs as healthy as we can. In this case, if something major would go wrong it will hopefully get cleared out in the reboot and minimizing the impact to a maximum of 24hrs. Start by opening crontab.

```
crontab -e
```

16. Now we need to add the following lines. Hopefully you can see the pattern for running the scripts every ten minutes. Repeat it until you have filled the whole 24 hrs.

```

PATH=/usr/local/sbin:/usr/local/bin:/usr/sbin:/usr/bin:/sbin:/bin
SHELL=/bin/bash
55 23 * * * sudo reboot
00 00 * * * /bin/bash /home/pi/Scripts/Record1 minute.sh
10 00 * * * /bin/bash /home/pi/Scripts/Record1 minute.sh
20 00 * * * /bin/bash /home/pi/Scripts/Record1 minute.sh
30 00 * * * /bin/bash /home/pi/Scripts/Record1 minute.sh
40 00 * * * /bin/bash /home/pi/Scripts/Record1 minute.sh
00 01 * * * /bin/bash /home/pi/Scripts/Record1 minute.sh

```

```
10 01 * * * /bin/bash /home/pi/Scripts/Record1 minute.sh
20 01 * * * /bin/bash /home/pi/Scripts/Record1 minute.sh

00 00 * * * /usr/bin/python
/home/pi/Scripts/Sensors/Environmentalreadings.py
10 00 * * * /usr/bin/python
/home/pi/Scripts/Sensors/Environmentalreadings.py
20 00 * * * /usr/bin/python
/home/pi/Scripts/Sensors/Environmentalreadings.py
30 00 * * * /usr/bin/python
/home/pi/Scripts/Sensors/Environmentalreadings.py
40 00 * * * /usr/bin/python
/home/pi/Scripts/Sensors/Environmentalreadings.py
00 01 * * * /usr/bin/python
/home/pi/Scripts/Sensors/Environmentalreadings.py
10 01 * * * /usr/bin/python
/home/pi/Scripts/Sensors/Environmentalreadings.py
20 01 * * * /usr/bin/python
/home/pi/Scripts/Sensors/Environmentalreadings.py
30 01 * * * /usr/bin/python
/home/pi/Scripts/Sensors/Environmentalreadings.py
40 01 * * * /usr/bin/python
/home/pi/Scripts/Sensors/Environmentalreadings.py
50 01 * * * /usr/bin/python
/home/pi/Scripts/Sensors/Environmentalreadings.py
00 02 * * * /usr/bin/python
/home/pi/Scripts/Sensors/Environmentalreadings.py
10 02 * * * /usr/bin/python
/home/pi/Scripts/Sensors/Environmentalreadings.py
20 02 * * * /usr/bin/python
/home/pi/Scripts/Sensors/Environmentalreadings.py
30 02 * * * /usr/bin/python
/home/pi/Scripts/Sensors/Environmentalreadings.py
40 02 * * * /usr/bin/python
/home/pi/Scripts/Sensors/Environmentalreadings.py
50 02 * * * /usr/bin/python
/home/pi/Scripts/Sensors/Environmentalreadings.py
```

```
GNU nano 2.2.6          File: /tmp/crontab.P0Dhvb/crontab

# Edit this file to introduce tasks to be run by cron.
#
# Each task to run has to be defined through a single line
# indicating with different fields when the task will be run
# and what command to run for the task
#
# To define the time you can provide concrete values for
# minute (m), hour (h), day of month (dom), month (mon),
# and day of week (dow) or use '*' in these fields (for 'any').#
# Notice that tasks will be started based on the cron's system
# daemon's notion of time and timezones.
#
# Output of the crontab jobs (including errors) is sent through
# email to the user the crontab file belongs to (unless redirected).
#
# For example, you can run a backup of all your user accounts
# at 5 a.m every week with:
# 0 5 * * 1 tar -zcf /var/backups/home.tgz /home/
#
# For more information see the manual pages of crontab(5) and cron(8)
#
# m h  dom mon dow   command
#
PATH=/usr/local/sbin:/usr/local/bin:/usr/sbin:/usr/bin:/sbin:/bin
SHELL=/bin/bash
55 12 * * * sudo reboot
```

```

00 00 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
10 00 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
20 00 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
30 00 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
40 00 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
50 00 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
00 01 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
10 01 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
20 01 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
30 01 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
40 01 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
50 01 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
00 02 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
10 02 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
20 02 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
30 02 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
40 02 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
50 02 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
00 03 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
30 03 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
40 03 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
50 03 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
00 04 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
10 04 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
20 04 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
30 04 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
40 04 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
50 04 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
00 05 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
10 05 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
20 05 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
30 05 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
40 05 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
50 05 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
00 06 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
10 06 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
20 06 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
30 06 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
40 06 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
50 06 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
00 07 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
10 07 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
20 07 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
30 07 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
40 07 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
50 07 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
00 08 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh
10 08 * * * /bin/bash /home/pi/Scripts/Record1Minute.sh

```

^G Get Help ^O WriteOut ^R Read File ^Y Prev Page
 ^X Exit ^J Justify ^W Where Is ^V Next Page

17. Be sure to end each line with a carriage return as the cron daemon expects this.

18. Let's install a safe shut down button. This will be used when the battery needs to be changed or the unit need to be turned off. Safely shutting the unit off minimizes the risk of corruption that comes with just turning off the power.

```
cd Scripts  
sudo nano shutdown_pi.py
```

19. Power consumption is a major issue! You hopefully noticed that at the end of the audio recording script the last line was commented out. That line turns off the HDMI port which saves around 30 mAh. However, we do not want the HDMI port turned off at boot, this is a safety thing to ensure that we can always access the unit and use it with a screen as we can go in and comment out that line whilst we are working with it. It needs to be uncommented before deployment in the field. First, we need to create the script for it.

```
cd Scripts  
nano shutdown_pi.py
```

20. Copy the following and enter it into the script. The 18 indicates the GPIO pin.

```
#!/bin/python  
#simple script for shutting down the raspberry pi at the press  
of a button  
#by Inderpreet Singh  
  
import RPi.GPIO as GPIO  
import time  
import os  
  
#use the Broadcom SOC pin numbers  
#setup the pin with internal pull-ups enabled and PIN in  
reading mode  
GPIO.setmode(GPIO.BCM)  
GPIO.setup(18, GPIO.IN, pull_up_down = GPIO.PUD_UP)  
  
#our function on what to do when the button is pressed  
def Shutdown(channel):  
    os.system("sudo shutdown -h now")  
    #add our function to execute when the button pressing event  
happens  
GPIO.add_event_detect(18, GPIO.FALLING, callback = Shutdown,  
bouncetime = 2000)  
  
#now wait!  
while 1:  
    time.sleep(1)
```

```
GNU nano 2.2.6                                         File: shutdown_pi.py

#!/bin/python

import RPi.GPIO as GPIO
import time
import os

GPIO.setmode(GPIO.BCM)
GPIO.setup(18,GPIO.IN,pull_up_down=GPIO.PUD_UP)

def Shutdown(channel):
    os.system("sudo shutdown -h now")

GPIO.add_event_detect(18,GPIO.FALLING,callback=Shutdown,bouncetime=2000)

while 1:
    time.sleep(1)

^G Get Help      ^O WriteOut      ^R Read File      ^Y Prev Page      ^K Cut-
^X Exit          ^J Justify       ^W Where Is       ^V Next Page      ^U Undo
```

21. Save the file by Ctrl+Y then X and enter. Test that it is working.

```
sudo python shutdown_pi.py
```

22. We need to add this file to the boot commands to ensure it runs in the background.

```
sudo nano /etc/rc.local
```

23. After fi and before exit 0 add the code below, the & at the end ensures it runs in the background.

```
sudo python /home/pi/Scripts/shutdown_pi.py &
```

24. Reboot again, then test that it is working when you start by pressing the button.

Real-Time Clock

1. We need to add a Real-Time Clock (RTC), unless the unit in the field is connected to a 3g dongle it will lose track of time if it does not have a clock attached. The Raspberry Pi's do not come with an internal clock already attached (your laptop for example would have one somewhere that runs on a little coin battery). First make sure the I2C bus detects it.

```
sudo i2cdetect -y 1
```

2. Disable the fake hwclock as it interferes with the real hwclock.

```
sudo apt-get -y remove fake-hwclock  
sudo update-rc.d -f fake-hwclock remove
```

3. Start the original hardware clock.

```
sudo nano /lib/udev/hwclock-set
```

4. Comment out these lines:

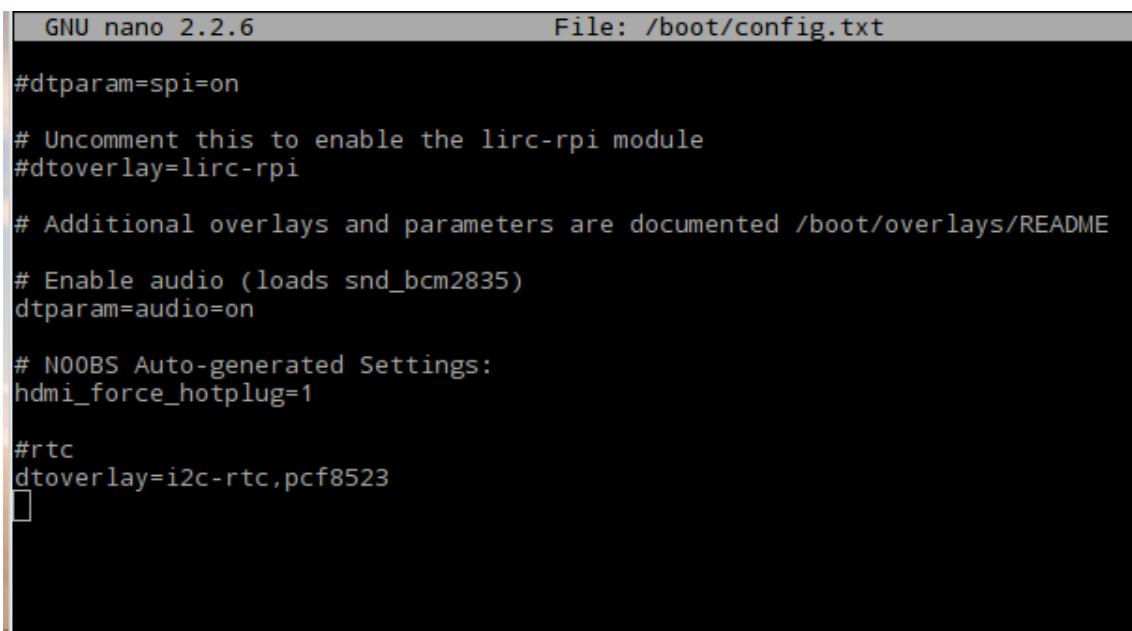
```
#if [ -e /run/systemd/system ] ; then  
#exit 0  
#fi
```

5. Add RTC support by adding device tree overlay.

```
sudo nano /boot/config.txt
```

6. At the end of this file add:

```
dtoverlay=i2c-rtc,pcf8523
```



```
GNU nano 2.2.6          File: /boot/config.txt

#dtparam=spi=on

# Uncomment this to enable the lirc-rpi module
#dtoverlay=lirc-rpi

# Additional overlays and parameters are documented /boot/overlays/README

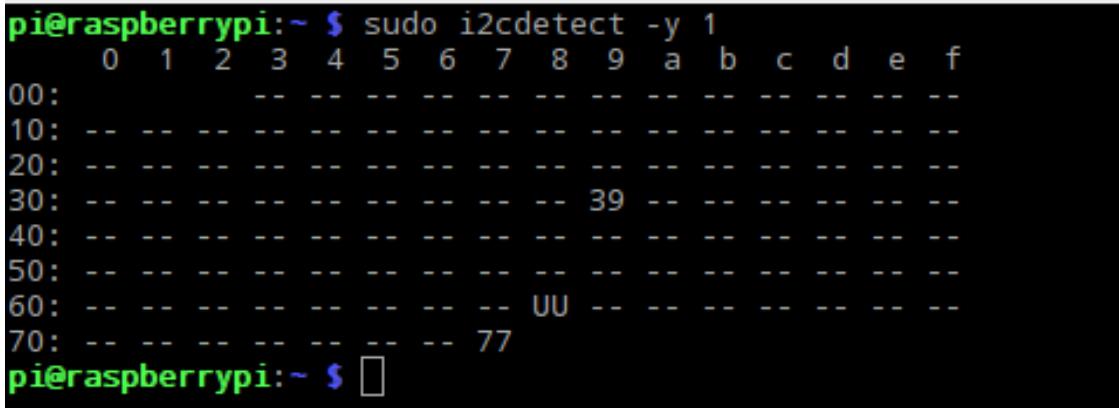
# Enable audio (loads snd_bcm2835)
dtparam=audio=on

# NOOBS Auto-generated Settings:
hdmi_force_hotplug=1

#rtc
dtoverlay=i2c-rtc,pcf8523
```

7. Save the file and reboot. Channel 68 should now say UU.

```
sudo i2cdetect -y 1
```



A terminal window titled "pi@raspberrypi:~ \$". It displays the command "sudo i2cdetect -y 1" followed by a table of I2C addresses from 00 to 7F. The table shows various device IDs, including "39" at address 30, "UU" at address 60, and "77" at address 70. The "f" column indicates the last byte of each row.

	0	1	2	3	4	5	6	7	8	9	a	b	c	d	e	f
00:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30:	-	-	-	-	-	-	-	-	-	-	39	-	-	-	-	-
40:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60:	-	-	-	-	-	-	-	-	-	-	UU	-	-	-	-	-
70:	-	-	-	-	-	-	-	-	-	-	77	-	-	-	-	-

pi@raspberrypi:~ \$

8. Sync the time from the Pi to the RTC. However, the RTC will have the wrong time when it is first plugged in, so it needs to be set. Read the time from the RTC.

```
sudo hwclock -D -r
```

9. Run the date command to check that Pi has the correct time.

```
date
```

10. If the Pi is not connected to the internet we can set the date and time manually (check "man date" for further info).

```
sudo date -s "2017-02-20 20:47:00"
```

11. Now write the date and time, then read it.

```
sudo hwclock -w  
sudo hwclock -r
```

12. Important! Each individual clock needs to get the time set on it! That means that you need to connect each RTC before installation in the field and repeat step 9-11 for each one. Occasionally when you start the clock is not detected as UU on the i2c and step 5-7 need to be repeated as well.

On-screen keyboard

1. We want to install floating screen keyboard; this ensures we can take a small touchscreen with us into the field and check the units for any issues.

```
sudo apt-get install matchbox-keyboard
```

2. Reboot and on start-up go into the menu and go into preferences and into the main menu editor. Press accessories and ensure the keyboard is ticked then press ok, apply and exit. It should now be under accessories.

Power Saving Options

1. Power consumption is a major issue! You hopefully noticed that at the end of the audio recording script the last line was commented out. That line turns off the HDMI port which saves around 30 mAh. However, we do not want the HDMI port turned off at boot, this is a safety thing to ensure that we can always access the unit and use it with a screen as we can go in and comment out that line whilst we are working with it. It needs to be uncommented before deployment in the field. Disable the act LED's.

```
sudo nano /boot/config.txt
```

2. Add the following lines at the end:

```
# Disable the ACT LED.  
dtparam=act_led_trigger=none  
dtparam=act_led_activelow=off  
# Disable the PWR LED.  
dtparam=pwr_led_trigger=none  
dtparam=pwr_led_activelow=off
```

3. To help reduce power consumption.

```
sudo nano /etc/rc.local
```

4. Enter the following command before the “exit 0” at the end.

```
echo powersave >  
/sys/devices/system/cpu/cpu0/cpufreq/scaling_governor
```

Cloning SD cards

Instructions for cloning on a mac computer. Once one SD card is finished, and image of it can be created and cloned onto new SD cards, ensuring that you do not have to sit and code every unit from scratch (just remember to change the site name in the audio recording script for the new ones). Remember that if you are cloning a 64GB card, it will only be able to be cloned onto a new 64GB or larger card. If the new card is larger you need to go into configurations and expand it or it will only make use of the size of the card it was cloned from. Insert the SD card that we want to take an image of into a card reader in the mac. Then open the terminal of the mac. Find the SD card by listing the disks currently read by the computer. Ensure you get the name of the correct one to ensure that you do not clone or imprint on the computer's hard drive! The disk name will change between computers and it will also change if you have other disks attached such as external hard drives or USB sticks, so you should check this every time before you start.

```
diskutil list
```

#:	TYPE	NAME	SIZE	IDENTIFIER
0:	GUID_partition_scheme		*121.3 GB	disk0
1:	EFI	EFI	209.7 MB	disk0s1
2:	Apple_CoreStorage	Macintosh HD	121.0 GB	disk0s2
3:	Apple_Boot	Boot OS X	134.2 MB	disk0s3
/dev/disk1 (internal, physical):				
#:	TYPE	NAME	SIZE	IDENTIFIER
0:	GUID_partition_scheme		*1.0 TB	disk1
1:	EFI	EFI	209.7 MB	disk1s1
2:	Apple_CoreStorage	Macintosh HD	999.3 GB	disk1s2
3:	Apple_Boot	Recovery HD	650.0 MB	disk1s3
/dev/disk2 (internal, virtual):				
#:	TYPE	NAME	SIZE	IDENTIFIER
0:	Apple_HFS	Macintosh HD	+1.1 TB	disk2
Logical Volume on disk0s2, disk1s2 DA9F81FF-2ECE-43AE-85BA-068EC9EE3111				
/dev/disk3 (external, physical):				
#:	TYPE	NAME	SIZE	IDENTIFIER
0:	GUID_partition_scheme		*63.9 GB	disk3
1:	EFI	EFI	209.7 MB	disk3s1
2:	Microsoft Basic Data	LIWS	63.7 GB	disk3s2

Name we need to use for cloning and formatting → disk0s1

Internal disks of the computer → disk0, disk1, disk2

The SD card that we want to format and clone to for this computer → disk3

Create the image and place it on the desktop, the last section “disk4” need to be changed to whatever name the SD card is listed under. This will take some time, potentially a couple of hours so just leave the computer to it.

```
sudo dd if=/dev/disk3 of=~/Desktop/MonitoringStations.dmg
```

Put in the blank SD card that you want to clone onto, check what disk number it is under and make sure that is the one that is unmounted, so you do not unmount the hard drive of the computer. Then unmount it.

```
diskutil unmountDisk /dev/disk3
```

Format to FAT32.

```
sudo newfs_msdos -F 32 /dev/disk3
```

Clone the image onto the new card. This can take a while (~30 minutes for a 64GB one my mac mini).

```
sudo dd if=~/Desktop/MonitoringStations.dmg of=/dev/rdisk3  
bs=1 m
```

Assembly and Installation

How the unit is assembled will depend on what kind of dry box and what kind of sensors are attached. Here is an example of what we did in Singapore. We used water proof plastic boxes from Monotaro of a size that would be able to fit our batteries and power banks. First we need to drill some holes so that we can keep things like the DHT22 on the outside, the BMP180 need a hole to let the air pressure fluctuate, we need a hole for the TSL2561 so we can create a window for it and if we want a solar panel attached we need to drill a small hole for the cables to go through. At least in the tropics keeping the number of holes to a minimum is very important! Humidity is a major issue and any holes increase the risk of letting more of it into the sensitive electronics. Furthermore, ants regularly break into the boxes, despite us plugging them with silicon they will simply send enough ants until they have managed to create a passage in. We do not want ants in the box as they can fry the electronics, chew on the wires and create a mess by making nests and laying eggs. Less holes equates lower number of break ins.

Box modifications

Equipment list:

- Dry box
- Plexi glass window
- Silicon (I suggest Dow corning 791 for the tropics)
- Bent plexi glass shelf
- Ice cream sticks
- Electronics screws

Tool list:

- Drill
- Drill bits of the correct sizes
- Silicon gun
- Electronics screw driver

Plan where things need to go to ensure that the cables are long enough, and things do not get too messy on the inside.

1. take the dry box and drill the holes. In our case, we need at least four holes.
 - I. TSL2561 window at the top.



II. One small or a few micro holes for the BM180 to allow for air pressure changes.



III. A hole that can fit the cables for the DHT22.



IV. A hole for the microphone to fit through in the bottom of the box.



2. Now we need to silicon the things that need it.

- I. We start with attaching a small plexi glass window to the TSL4561 hole.



- II. We attach a small ledge made from bent plexi glass to the bottom of the box, this helps to ensure there is something holding the batteries in place.



- III. We need to be able to attach the BMP180 and the TSL4561 to something, we used ice cream sticks as these were cheap and of the right size.

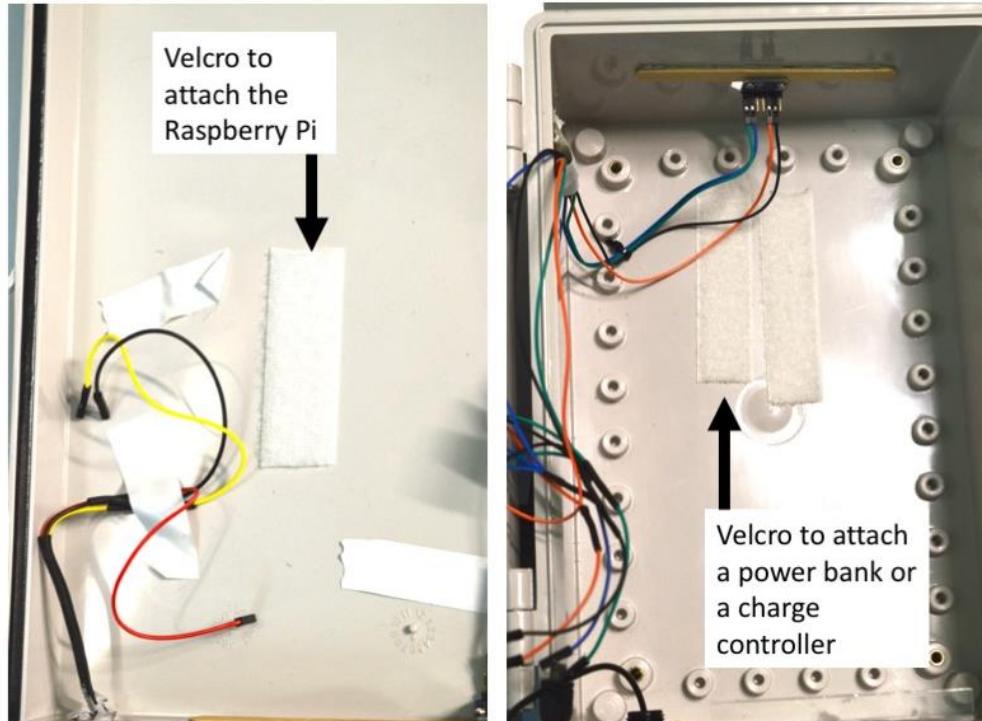
Ice cream stick for light sensor



Ice cream stick for pressure sensor



3. Put some Velcro where the Raspberry Pi will sit and on the wall in the main body of the box. **Let the silicon dry overnight.**



- When the silicon is dry. Take a BMP180 sensor and a TSL4561 and mark the screw holes on the ice cream stick with a pen. Then drill holes there with a drill one size smaller than the electronics screws. Screw the sensors into place.



Assembly of electronics

Equipment needed:

- Configured Raspberry Pi
- Velcro
- USB sound card
- Shut down button
- Four 1:3 female to female jumper cables in colours orange, black, blue and green.
- Four 1:1 male to female jumper cables in colour orange, black, blue and green.
- BMP180 sensor (should already be attached to box)
- TSL2561 sensor (should already be attached to box)
- RTC
- Dry box which has been modified with a DHT22 attached
- Microphone
- Tape
- USB to micro USB cable
- Power source

First, we will go through where the wires should be connected on the Raspberry Pi GPIO strip. We have already assigned the different wires a specific color depending on what it does per the list below:

Red = 5V

Orange = 3V

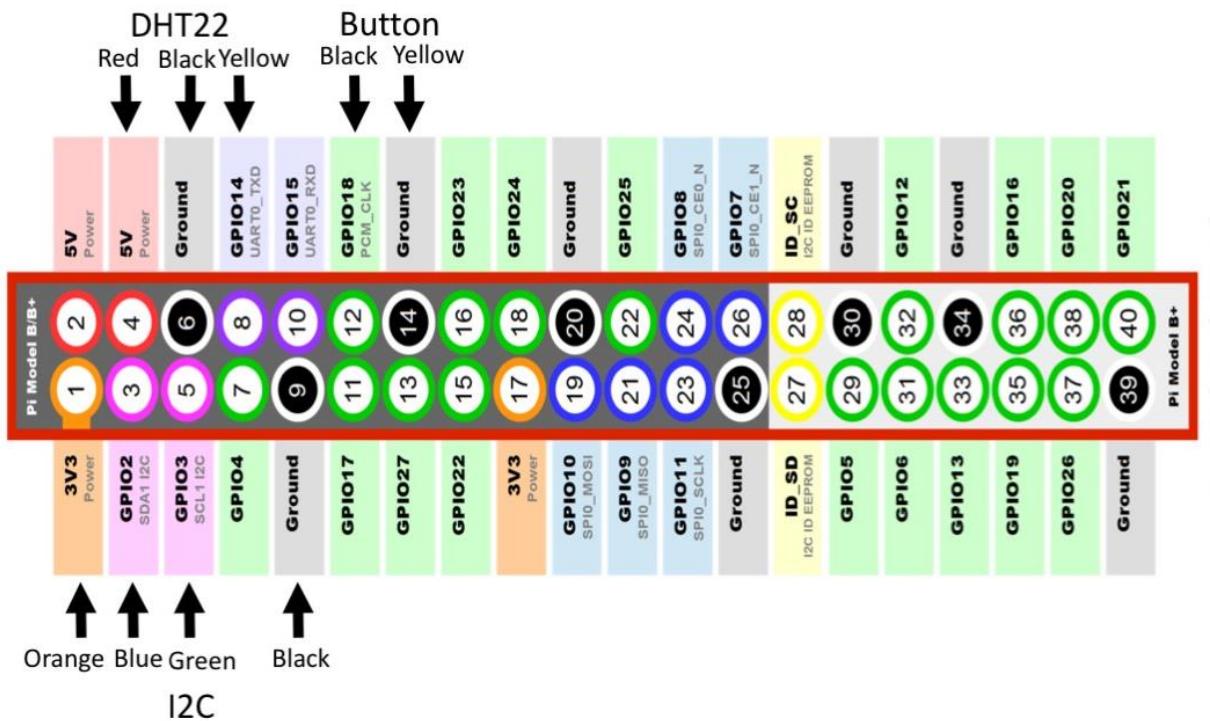
Black = Ground

Yellow = Basic GPIO information Pin

Blue = I2C SDA

Green = I2C SCL

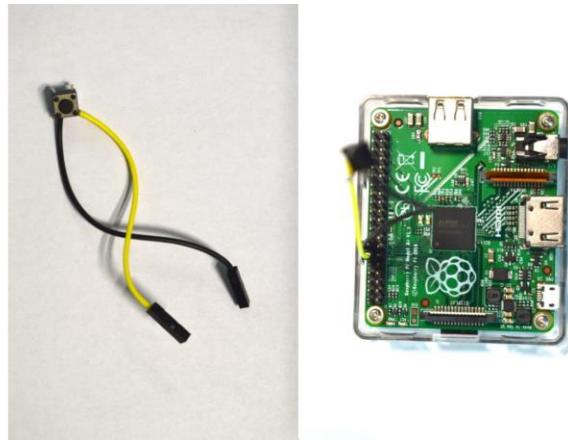
Which means we now need to figure out where to place the cables. Some of them already have specific points as we gave them specific points when we coded. The AM2302 uses GPIO14 for information transfer for example and need a 5V pin as power supply, whereas the ground cable can go to any ground pin (but I normally use the closest one to keep the wires less messy). The shut off button was assigned GPIO pin 18 as its information pin and need a ground pin. The I2C requires four cables, we have one 3V for power and a ground cable. We also need to use the two I2C pins which one this schematic is GPIO pin 2 for SDA and 3 for SCL. See picture below for the layout of where to attach each wire.



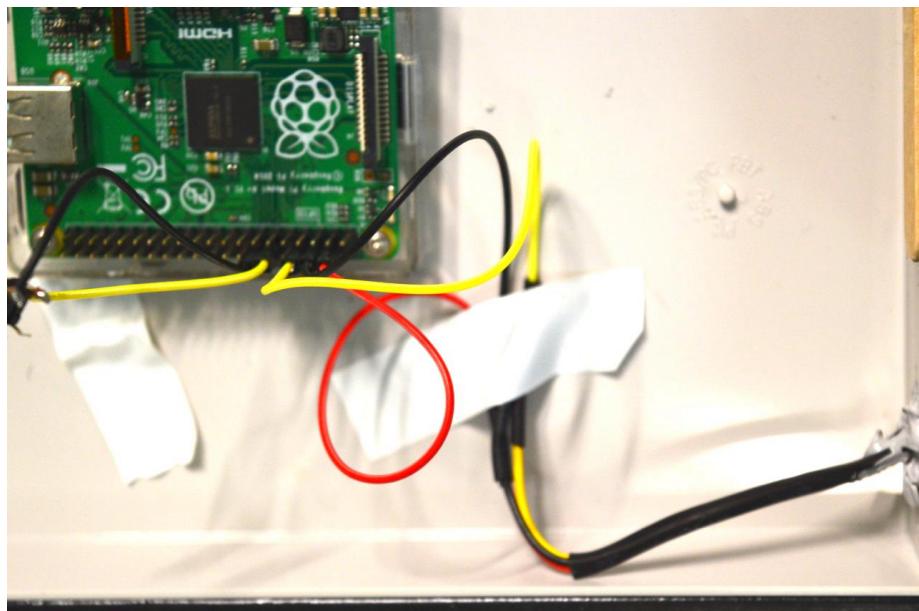
1. Attach the Raspberry Pi to the Velcro and insert the USB soundcard.



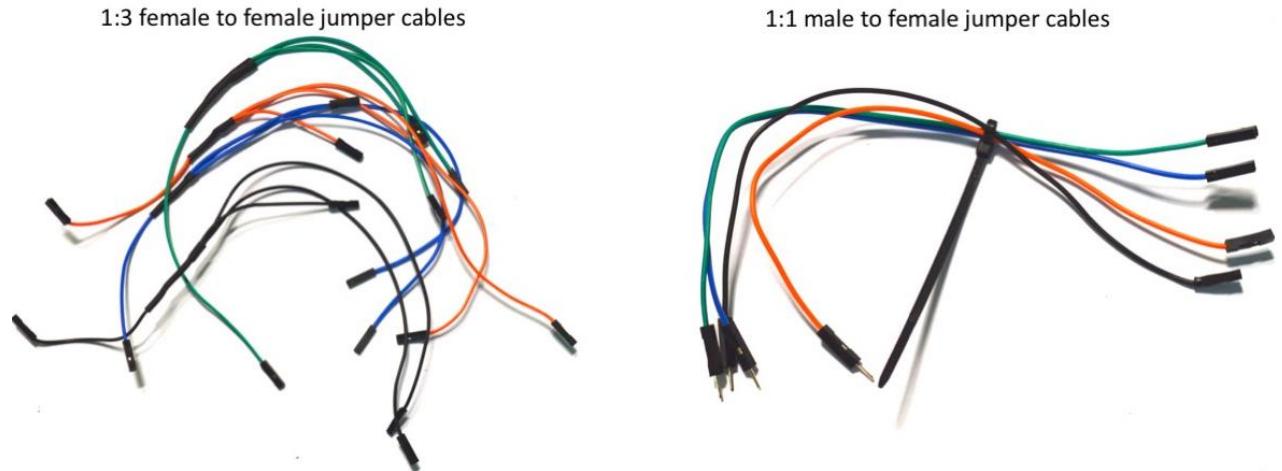
2. Attach the safe shut down button.



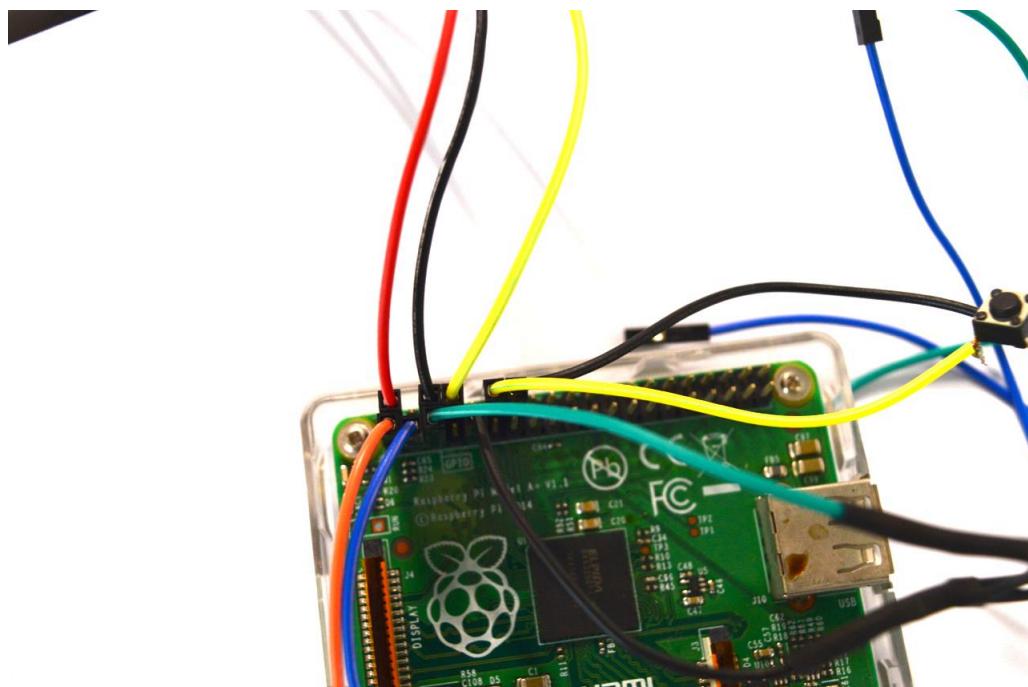
3. Attach the AM2302.



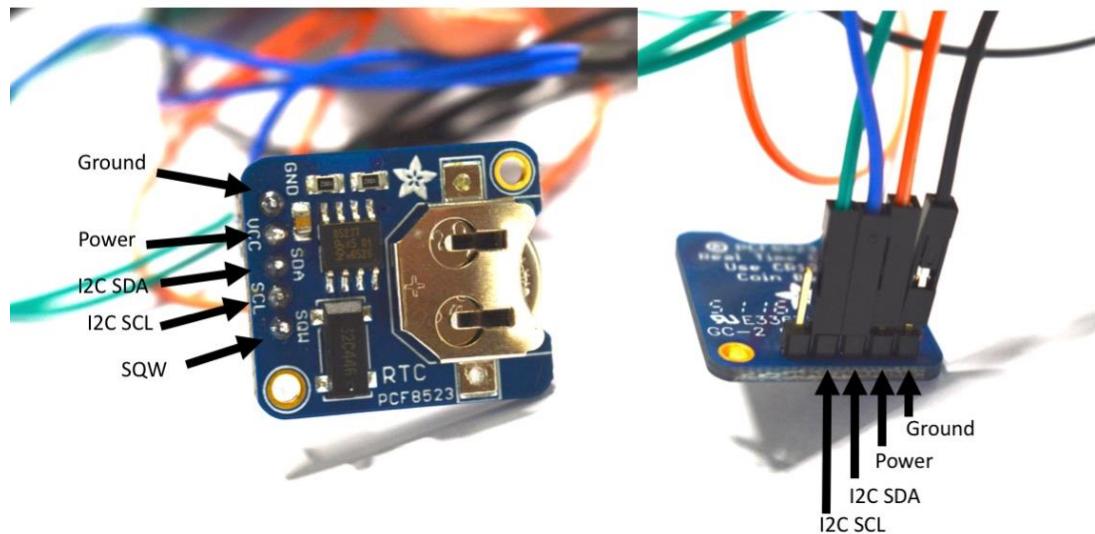
- Take the I2C 1:3 cables, the 1 side needs to be attached to the Raspberry Pi's GPIO pins whereas one of each colour of the 3 side need to be attached to the male side of the 1:1 male to female jumper cables.



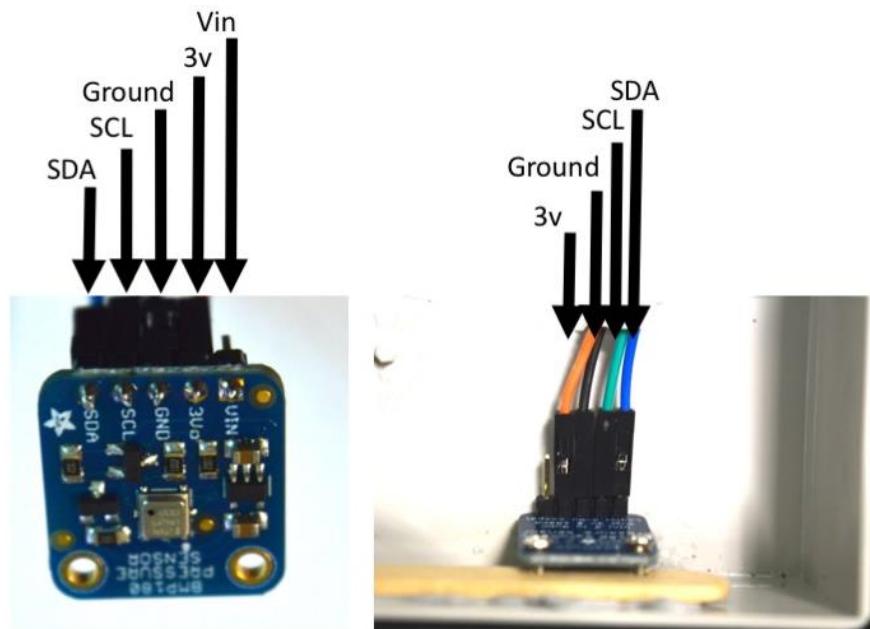
- Attach the I2C cables to the correct pins on the Raspberry Pi. The finished results with all the wires attached should at this point look something like the picture below.



6. Attach one of the I2C wire ends to the RTC. If you look at the RTC one side of it will tell you what each pin is for.

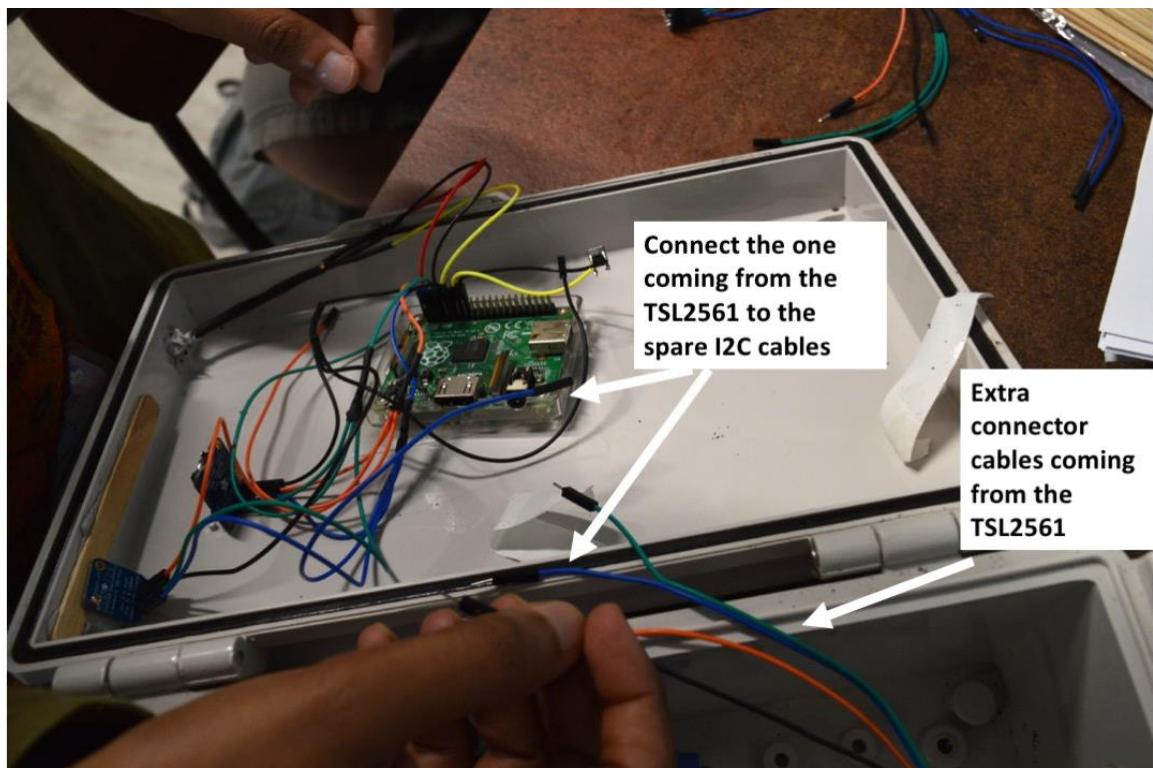
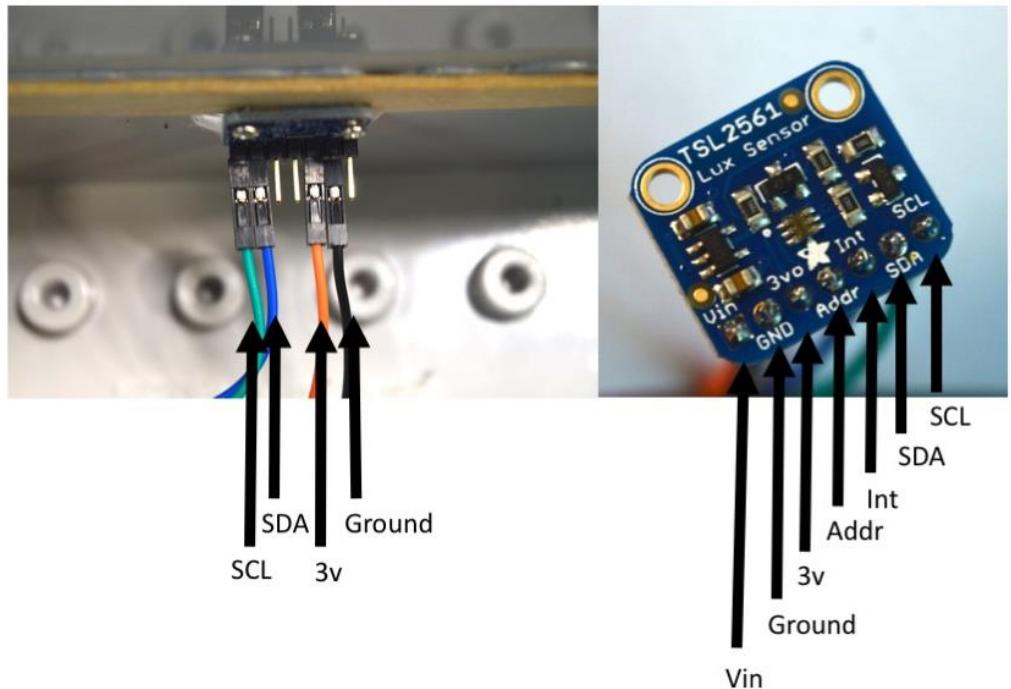


7. Attach one of the I2C wire ends to the BMP180.



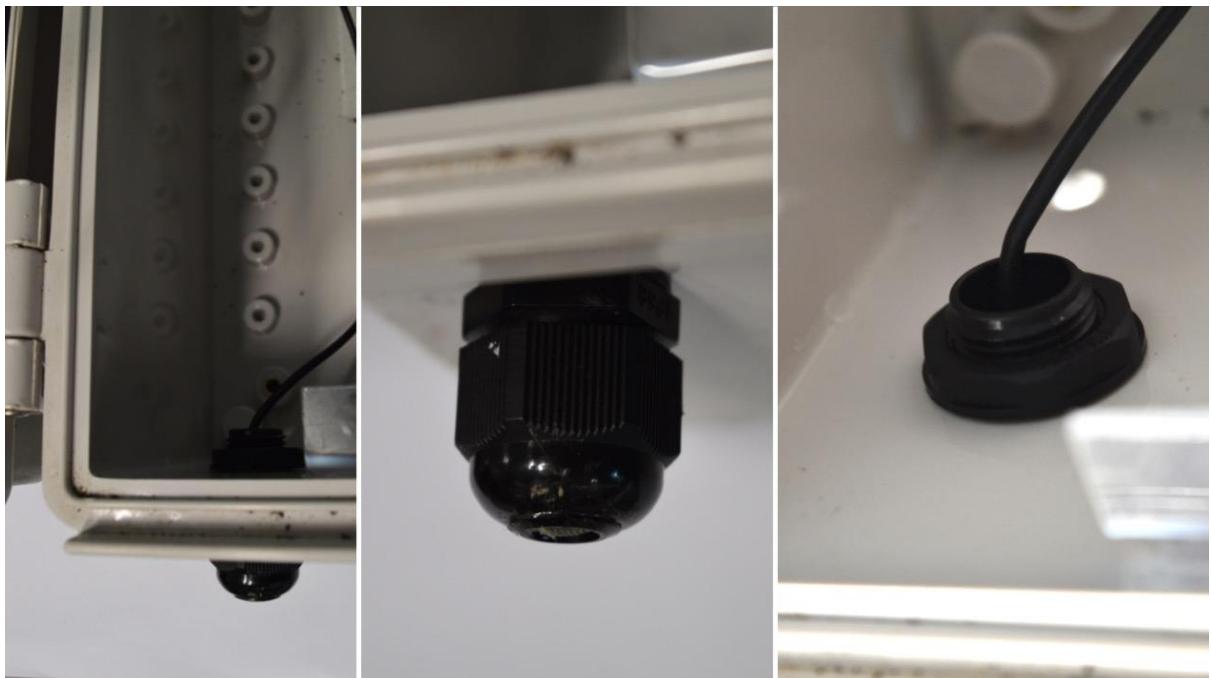
8. Attach the TSL2561. The TSL2561 needs connector cables (male to female jumper wires) in this case to ensure the door can be opened safely as the cables are too short

otherwise (the sensor is the wrong way around here as we accidentally soldered them in wrong, the other side of the sensor should be facing the window).

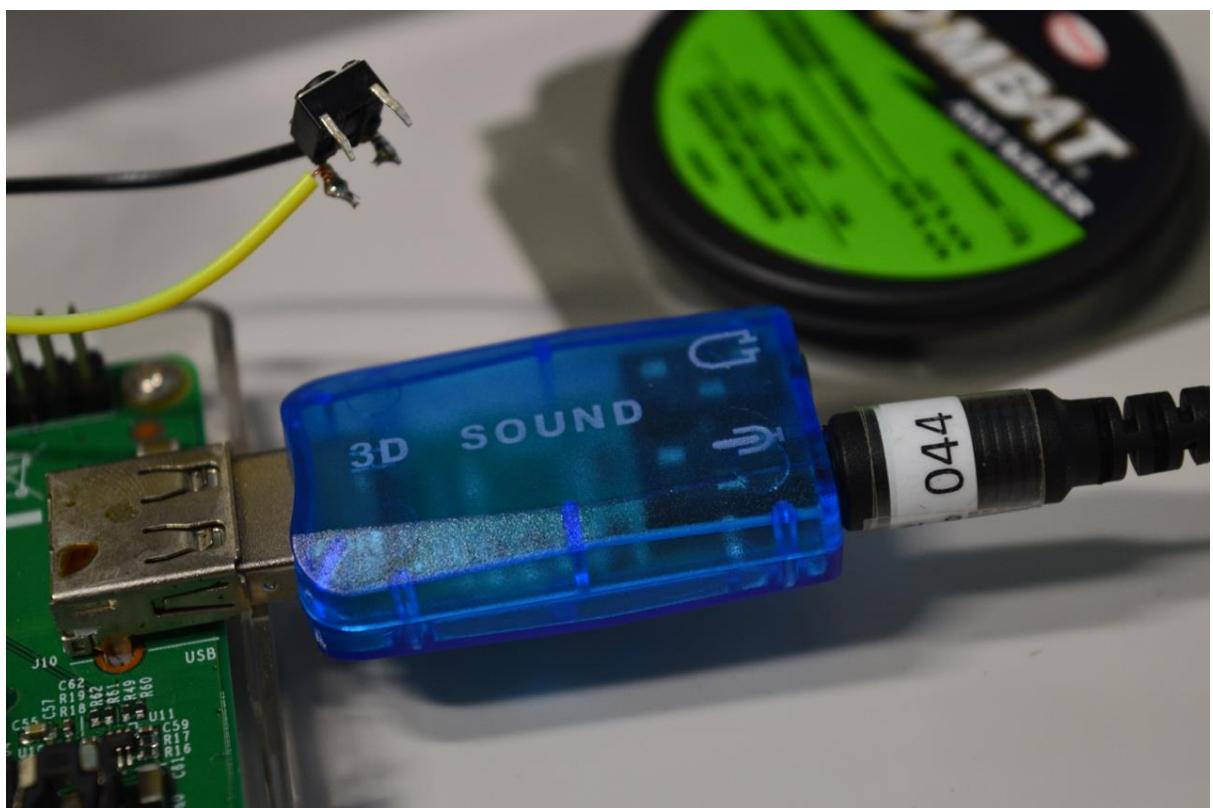


9. Take some tape and attach the wires to the walls where they stick out, so they do not accidentally get caught in the door.

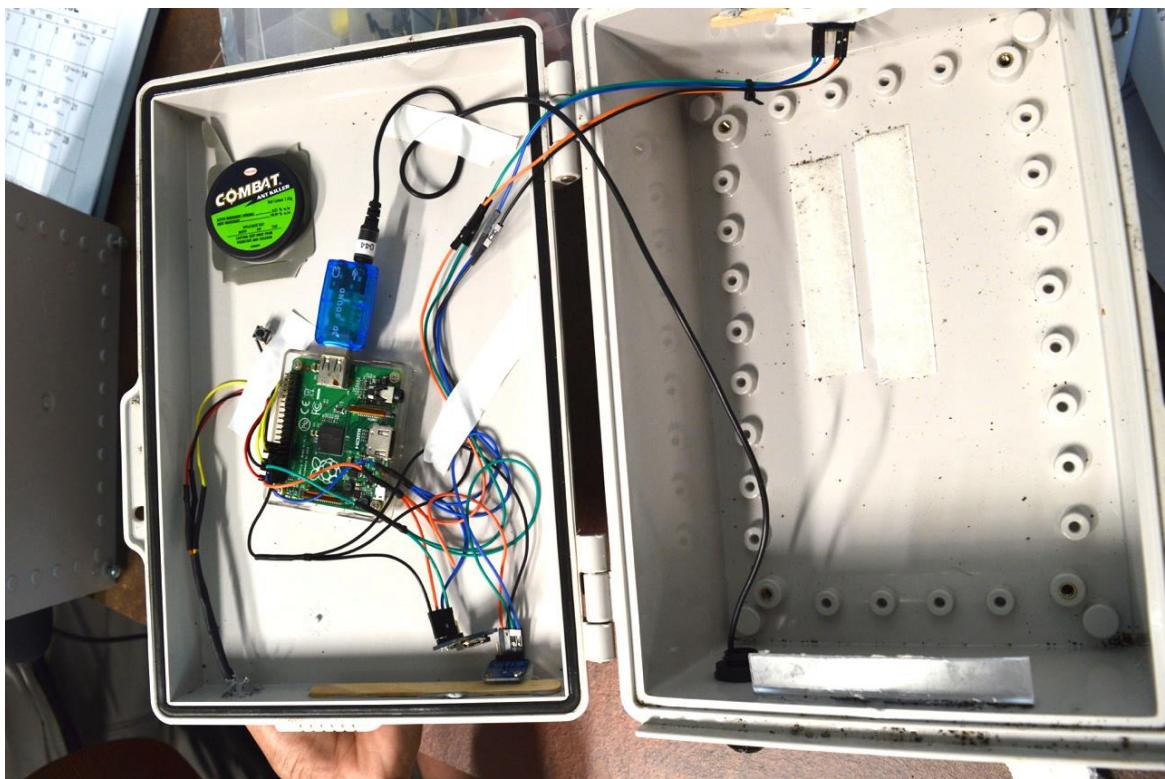
10. Attach the microphone.



11. Connect the microphone.

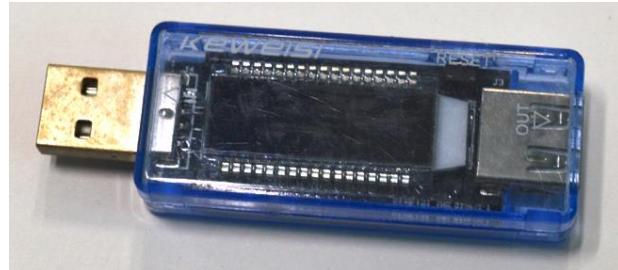


The finished version should look something like this.



Power Supply

We need a power supply in the field! It is very handy to figure out how much power the units take before settling on what to use. Use a USB power meter to assess how much power the unit draws. The current set up on a Raspberry Pi A+ takes around 100 mAh.



Power Bank

If you plan on using a power bank, put some Velcro on the back of it and attach it to the back of the wall. Now the USB cable can be connected between the two and the unit is up and running.



Car Battery

Equipment needed:

- Car battery
- Power cables (black and red)
- Cable clamps
- 12v to 5v converter
- USB to micro USB cable
- Assembled monitoring station

Tools needed:

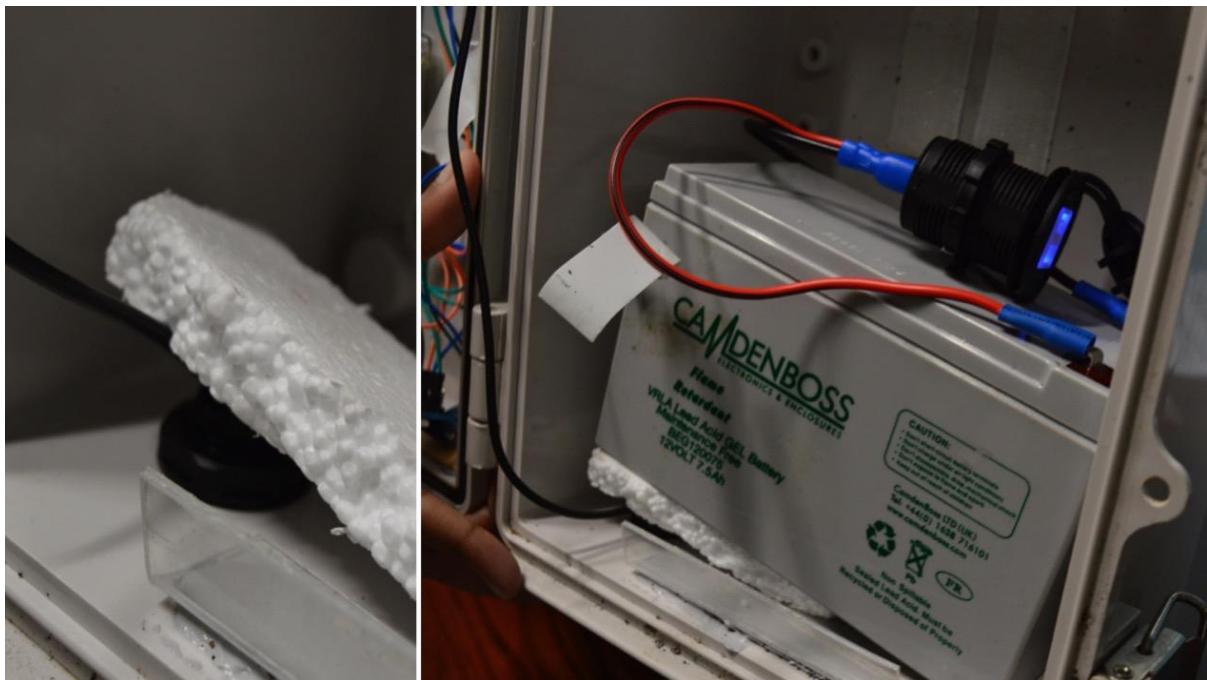
- Pliers
- Nippers (that can cut wire/cable and strip them off plastic)

If you want to use car batteries, then you need to get some cables and a 12v to 5v USB converter. We use batteries that fit inside the box in this example. If you need a larger size and want to keep them outside the box, then drill a hole in a corner of the bottom of the box for the power cables to run through.

1. Cut cables to the correct length using the nippers.
2. Strip the ends of the cables of the cover.
3. Attach and clamp on the cable ends using the pliers.
4. Attach them to the battery and then the corresponding plus and minus to the converter. The converter should light up (if it is the type with a LED light) if there is charge in the battery.



5. Carefully place the battery in the box, in our case unfortunately the microphone attachment sticks up and the battery will squash the microphone cable. To not damage the cable, we put a bit of Styrofoam between the cable and the battery.



6. Connect the USB cable between the converter and the Raspberry Pi.

Solar Panel

Equipment list:

- Solar Panel
- Charge controller
- Power cables (black and red)
- Power cable clamps
- Shrink tubing
- Protective tubing
- Silicon
- Velcro
- 12v to 5v converter
- Assembled monitoring station

Tools needed:

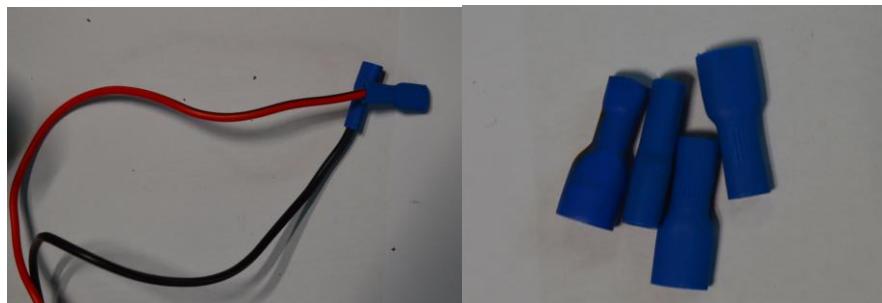
- Flat screwdriver
- Lighter for shrink tubing
- Drill
- Wire cutter

In this example, we are using a 30W solar panel. We also need a charge controller. The power that comes from the solar panel is not even, it will fluctuate a bit since the sun that hits it will not be even all the time. In addition, the battery gets destroyed if it is continuously fed more power even when it is full. The charge controller solves both issues. Firstly, the power does not go directly to the unit, so it is the charge controller that deals with the uneven power and it sends it on to the battery for storage. If the battery is full it does not feed more power to the battery. The power that is sent to the unit is even instead of fluctuating.

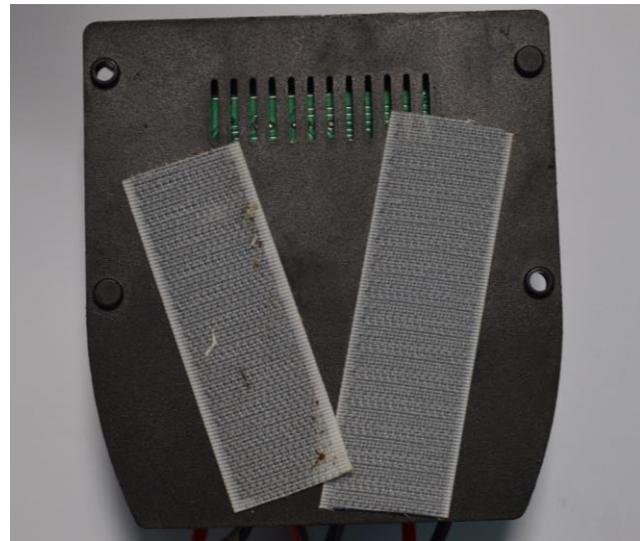
1. Cut three sets of power cables and strip the wire ends. One set from the battery to the charge controller, one set from the charge controller to the converter and the last set from the solar panel to the charge controller. The set that goes from the charge controller to the solar panel should be a lot longer than the others and will vary from installation site to installation site. See picture below for example of a charge controller with the wires attached to it.



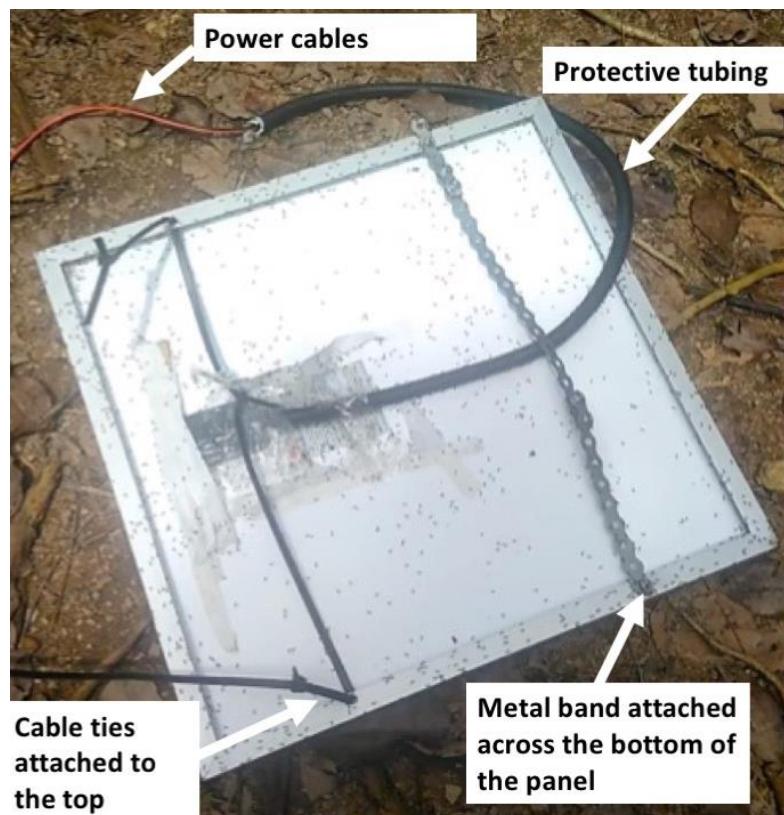
2. Attach and clamp the connection ends to one side of the wires.



3. Put some Velcro on the back of the charge controller. The battery and converter wires can be attached to the correct areas on the charge controller.



4. The solar panel need one end of its wires soldered to the ones coming of the connection box. Once the solder is done cover the area with shrink tubing.
5. Drill a hole in a corner of the bottom of the box that for the power cables coming from the solar panel.
6. Cover the cable in protective tubing and stick the ends in through the hole in the box (yes, all the little black dots on the photo below are ants).



7. Connect the power cables to the charge controller and then connect the battery and then the converter. Once this is done and it looks as if everything is working the USB

cable can be connected to the converter and then to the Raspberry pi. Silicon the hole the solar panel wires come in through.



Attachment to tree

The attachment will depend on the type of box you use. Here we give examples of two types of attachments we have used.

Holed metal band attachment

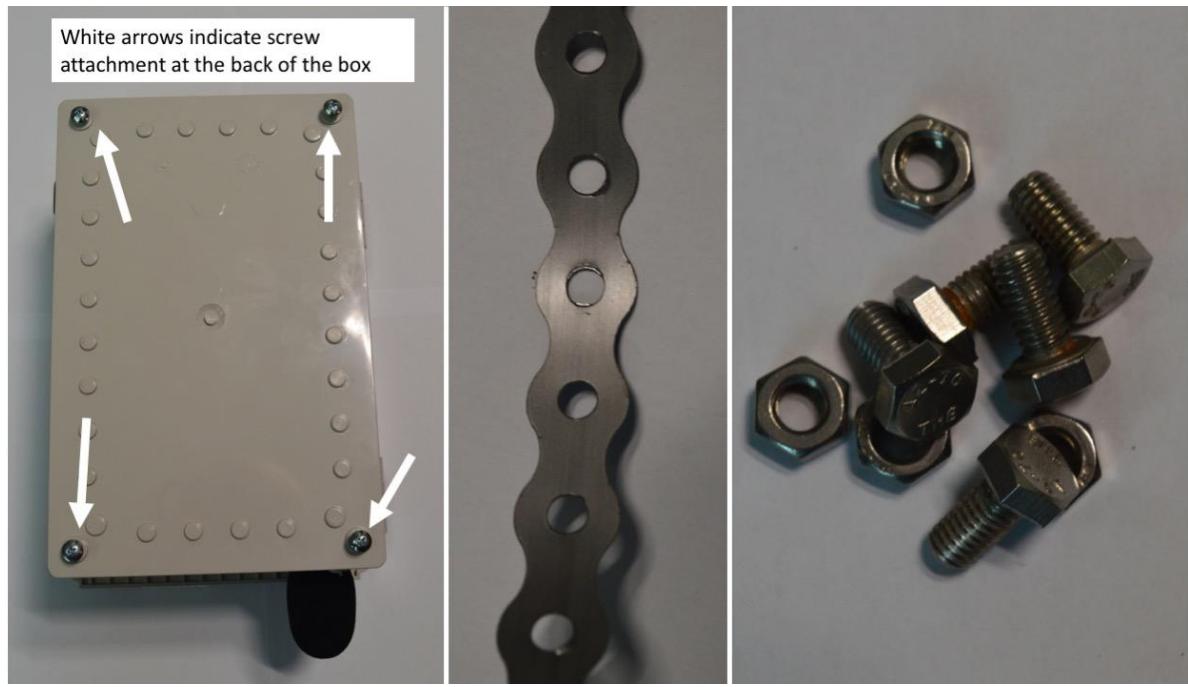
Equipment needed:

- A monitoring station
- Holed metal bands
- Plastic tubing
- Screws and bolts

Tools needed:

- Metal band cutter
- Adjustable spanner or pliers
- Box cutter

1. The bigger type of dry box we use have screws holes on the back for attachment that will fit holed metal bands.



2. Cut some tubing into the desired length with the metal band cutter, then split it length wise with the box cutter. These are going to be used to protect the tree from the metal band.
3. Cut four sections of the metal band to the correct length using the metal band cutter and attach them to the back of the box with the screws.
4. Get somebody to hold the box at around 1.5 meters' height to the tree. Attach the tubing around the tree whilst you close the two top metal bands as tightly as possible with a screw and a bolt. Sometimes it is not possible to get it tight enough for the box to stay up securely, you can either fold some tubing up and push between the tree and the attachment or put in a stick.



Python lock

If you have a box that have attachment holes on the side, a python lock is the best for attachment to the tree.



Solar panel attachment

The attachment of a solar panel will depend on what type of solar panel you buy. We used ones that were already mounted on a frame. This require a solar panel, power cables (from the solar panel to the monitoring station). Protective tubing to cover the power cables with. Plastic pipe to use as support struts between the tree and the solar panel. Holed metal band and three sets of screws and bolts. Cable ties, both a larger size and a smaller size.

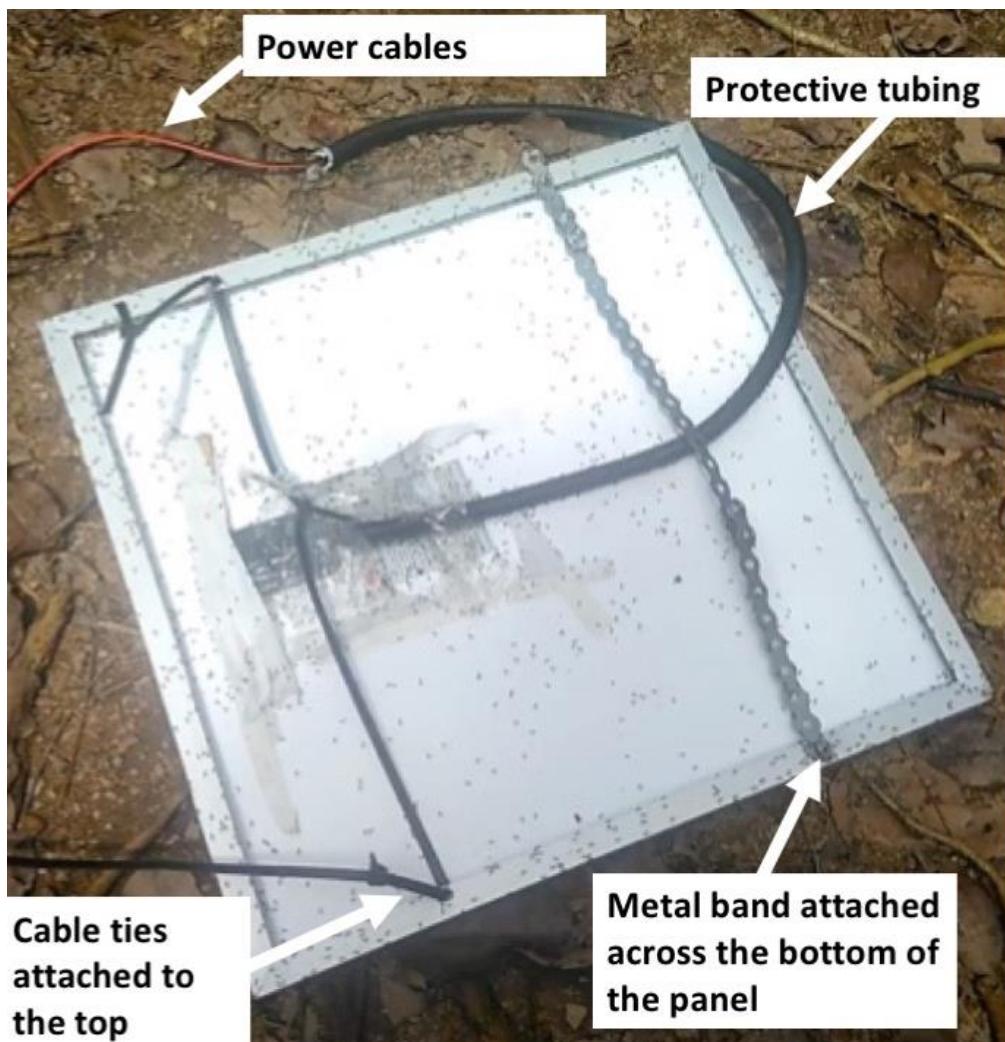
Equipment needed:

- Solar panel (which have been assembled)
- Holed metal band
- Plastic tubing
- Cable ties, large ones and a smaller size.
- Screws and bolts
- Plastic pipe
- Monitoring station that is already attached to a tree/pole

Tools needed:

- Metal cutter
- Plastic/metal saw
- Adjustable spanner or pliers
- Box cutter

1. Cut a holed metal band of the correct length using the metal cutter. Attach it to the bottom holes of the solar panel frame using screws and bolts.



2. We used big cable ties to attach the top of the solar panel to the tree above the monitoring station (but not covering the top of the monitoring station as the TSL2561 window cannot be covered). Do not tighten the cable ties too much at this point as they will be adjusted after the bottom of the panel has been attached to the pipes.



3. Cut a piece of holed metal band that fits the circumference of the tree below the monitoring station. Cut a piece of plastic tubing that also fits the tree with box cutter then split it length wise. Create a ring of holed metal band with tubing as protection around the tree at a suitable height. Ensure that there are two screws on either side of it.



4. Cut the plastic pipe using the saw into two sections of the right length to get a good angle on the solar panel (try to maximize sun exposure).

5. Drill a hole in each end, of a size that the bolts will fit through.
6. The bottom end needs to be cut out with a saw to ensure that that nuts can be tightened.



7. Attach the bottom end to the ring of holed metal band using the nuts and the bolts.
8. Attach the other of the pipes to the metal band on the solar panel using smaller cable ties.



9. Before you tighten the cable ties at the top of the panel, cut a piece of tubing length wise and cover the edge of the top of the panel. This prevents the edge of the panel from damaging the tree. See the picture below.

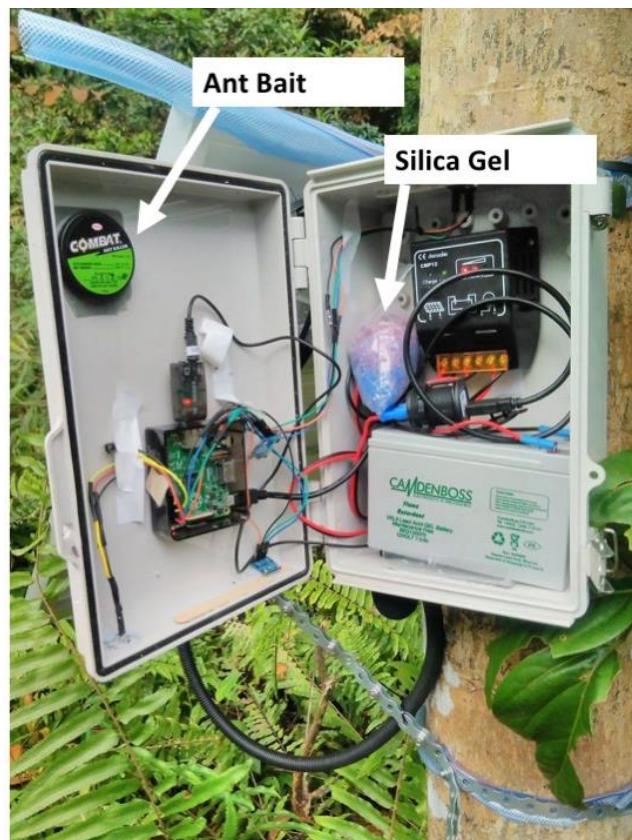


10. Now the solar panel can be connected internally to the monitoring station.



Precautionary Measures

In the tropics, it is almost impossible to not have ants breaking into the box now and then. Similarly, some humidity will always manage to get in when the door is opened. To reduce the impact of this we use a desiccant in the box, such as silica gel that will absorb the moisture. In our case the silica has a color indicator. It means that the individual silica granule changes color when it is full of moisture. It allows us to judge when we need to put in new silica gel and take the silica granules full of moisture back to the lab to dry it out in an oven (to make them ready for use again). If you look at the picture below you will see that the bag of silica gel is half pink and half blue, the blue granules are ready to pick up moisture and the pink ones are full so that specific bag can still be left in the box for a bit longer. We also put in long-term ant bait, so that the ants that manage to break in do not get established.



Detection Space

It is important to assess the area a unit can pick up sound from and calibrate our microphones so that we can account for variation in this in the analysis. This ensures that differences in detection rates or number of species detected are real and not due to variations between the units. If we do not do these tests we cannot compare data between stations or between sites. If you want to know more about this and the technical details behind it, please read the following article:

Darras, K. et al., 2016. Measuring sound detection spaces for acoustic animal sampling and monitoring. *Biological Conservation*, 201, pp.29–37. Available at: <http://dx.doi.org/10.1016/j.biocon.2016.06.021>.

Here is a simplified and slightly modified version of the instructions given by Kevin Darras. To get a thorough understanding of why these tests are necessary I strongly encourage you to read through both the website and the article. For later analysis, he also provides an R script that extracts the sound test data and puts it in a CSV file for later use. You also need to download the clear tone step signals from this website or get them from the appendix of the paper. Remember that it is often necessary to also run this test on the species you are looking for! Download a sound file of the species call and attach it to the end of the clear step tone signal file, then transfer it to your phone.

<http://wwwuser.gwdg.de/~ecosound/index.html>

Speaker

The speaker used in this test is very important! It needs to have the correct frequency range to ensure that the test is accurate. If it only goes to 7KHz for example, then the tests run at 8kHz and 12kHz will not give correct data. Most speakers that you can get off the shelf does not have the correct range, often it does not even say what range they cover. We use the SME-AFS speaker from Mineroff (<http://mineroff-nature.com/nature/smeafs-portable-field-speaker-p-188.html>).

Calibration

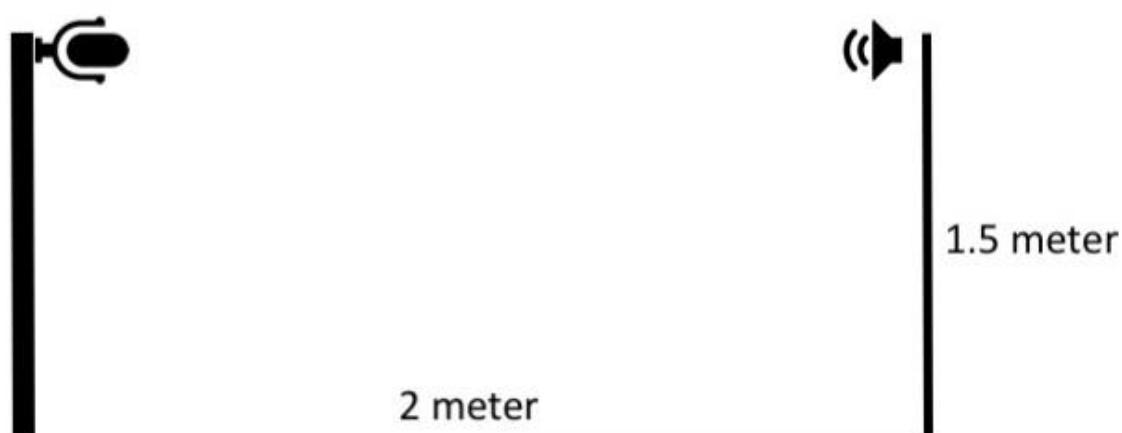
To calibrate, simply configure one of the raspberry pi's to run a script in a loop for an hour or two that records data for 50 seconds, then have a 10 second break before it starts again.

1. Mark all the microphones with individual numbers.



2. Attach the speaker to the extendable cleaning pole at 1.5 meter.

3. Mark out 2 meters away from the test unit.



- Calibrate the sound level from the speaker using a sound meter (this is to standardize the dB every time the speaker is used).



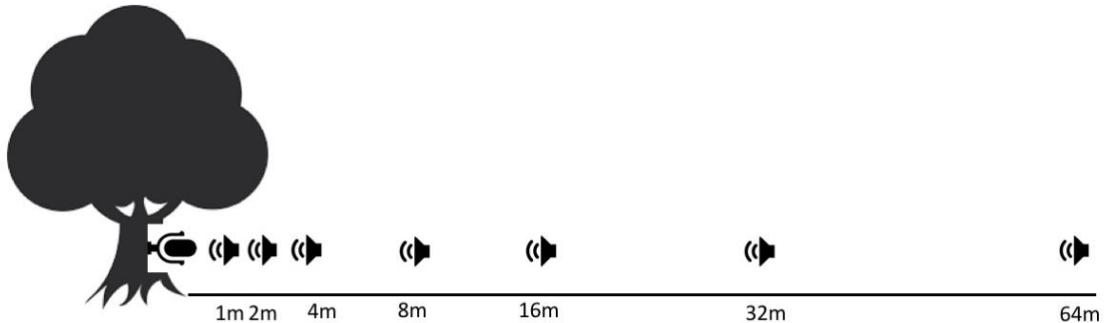
- Attach the Raspberry Pi onto something. A tree or a pole, we used the post of a football goal once – the important thing is that the ground and air between the microphone and the speaker is completely free of obstacles (including humans!!!). Ensure you do not stand in front of the equipment), and that the weather conditions are standardized with not rain and no wind. Mark out 1.5 meter that faces the speaker (this is where the microphone needs to be held during the test).
- Start the Raspberry Pi and wait until the unit is running. Speak the microphone number into the microphone, then hold it at the 1.5-meter mark towards the speaker. Play the test sounds three times in a row.
- When the unit is having a break between recording, quickly swap over to the next microphone and repeat the above step.

Detection Space Test in the field

You need to configure a Raspberry Pi specifically for these tests. Configure it to run soundscape collection constantly. This ensures that it collects data continuously with a break of a few seconds every thirty minutes. First set up the Raspberry Pi that is configured for soundscape recording above the monitoring station, ensure you note down which microphone you are using. We use the same unit with the same microphone at each station because it means that any variation between the tests is not due to microphone, so we do not have to take the sensitivity of the microphone into account when comparing this data. Bring a sound level meter (dB meter) and calibrate the sound from the speaker to ensure it is the same between all stations.

- Either bring along a measuring tape that goes to 64 meters or mark up a piece of string at the required increments. Mark the extendable cleaning rod so that you know

where 2 meters and 5 meters are if it is needed (10cm is normally just measured on its own from the ground). When at the site of the unit create a straight transect from the monitoring station using the measuring tape, remember that this should be level the whole way along – so bring along a clinometer to be able to account for variation in ground height. You need to perform these sound tests at these distances from the monitoring station: 1 m, 2 m, 4 m, 8 m, 16 m, 32 m and 64 m.



2. Calibrate the sound level using a sound meter at 5cm directly in front of the speaker.



3. Go to the 1 m mark. Hold the speaker at 10cm height off the ground and play the sound test file three times. Move the speaker to two meters' height and play the sound test three times. Move the speaker to five meters' height and play the sound test three times again.



- Now move to the 2 m distance mark and repeat the step above, then move to 4 m and after that 8 m and so on.

Habitat Data

We normally collect a standardized set of habitat data. There are a wide range of variables that can be collected but there are two that we take from every site and study. Canopy cover data and under story density. For this you need a camera (we use a Nikon DSLR), a tripod, a measuring tape and our special habitat sheet.

Canopy Cover

- I. Camera
- II. Tripod

How you want to collect this is up to how detailed you want to be. We either take one just where the station is located, or we take one at set intervals 10 m away from the tree (for example, one at North, South, East and West). We usually take flat photos instead of fish eye. With fisheye, you catch under story as well (which we are recording in a better way) which affect the canopy cover data and if you work in mountains (which we do regularly) then the chance is high that you catch the ground as well if it is steep. With regular photos, it is very important that the camera is exactly level and at the same height off the ground. Attach the camera to a tripod that has a spirit level and ensure you have the correct height off the ground. I attach a string to the bottom with a rock which measures out the distance when it is at the ground, 1 meter for example. Ensure that the weather conditions are standardized when these are taken, it can't be sunny, it can't rain, and you can't be in a cloud and so on.

Once the photos are taken the data can be extracted using this Python script.

https://github.com/KarlssonCatharina/Canopy_cover_extraction

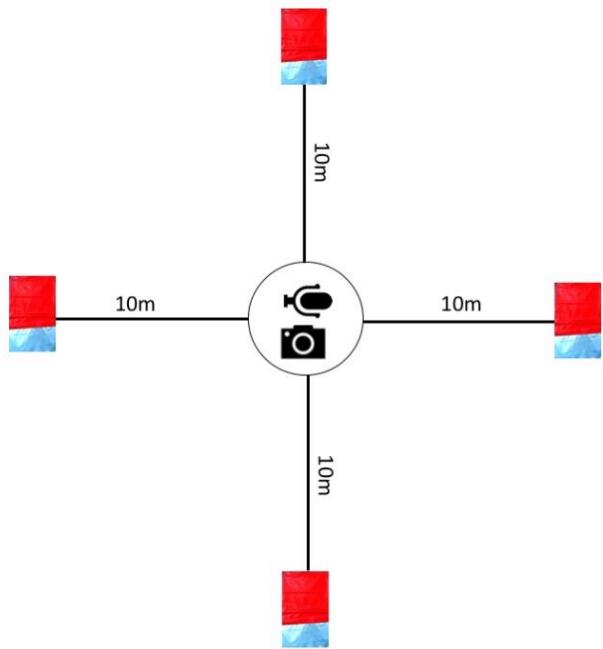
Under Story Density

- Camera
- Habitat sheet
- Measuring tape (10 m)
- Help from another person

The sheet is the important thing here. It needs to be one meter wide a total of two meters high. The bottom 0.5 meter should be in a light blue material and the top 1.5 meter should be darker red/orange color to contrast towards the light bottom. We use a tarp that the bottom blue material is stitched onto, it makes the sheet durable in the rainforest.



When taking, these photos ensure the weather conditions are standardized, it should not have rained recently as it creates a shine in the leaves which will skew the data. The person taking the photos should stand at the monitoring station. A helper needs to hold the sheet up at 10 meters away from the station at set intervals. We took 16 photos in Singapore in a circle around the station. For our set-up that means a photo every fourth meter! We recommend a minimal of four photos at north, south, east and west.



Retrieving Data

To get the data off the units the easiest way is to connect the unit to a screen, keyboard and mouse. The Acoustic files are in the Audio folder and the environmental data is in the folder named Environ. Connect a USB drive and simply copy the Acoustic files and environmental CSV files over to the USB drive (this might take a while! Do not forget to turn off the HDMI port turning off whilst you do this).

You can inspect the files in the free software Audacity:

- <http://www.audacityteam.org>

For species identification, you can use the WarbleR package in R:

- <https://marce10.github.io> (instructions for use)

Or the software Aureas can be used:

- <http://sistemic.udea.edu.co/es/investigacion/proyectos/analisis-bioacustico/> (site for software download and manual)

Mounting SD card

Occasionally a SD card gets corrupted or you just want to retrieve the data without starting the whole Raspberry Pi system up. If you have a mac you can mount the SD card and retrieve the files that way.

Open the terminal and ensure that homebrew is install. Then install the following.

```
brew cask install osxfuse  
brew install ext4fuse
```

Insert the SD card in the card reader. Then retrieve the correct disk number by checking what number it was assigned.

```
diskutil list
```

The list should tell you which the external SD card is under external. We need the non-boot section of the SD card (you can normally tell which one it is as it is the largest size).

```
Catharinas-Mac-mini:~ catharinakarlsson$ diskutil list
/dev/disk0 (internal, physical):
#:          TYPE NAME                SIZE    IDENTIFIER
0: GUID_partition_scheme          *121.3 GB  disk0
1:           EFI EFI                 209.7 MB  disk0s1
2:   Apple_CoreStorage Macintosh HD 121.0 GB  disk0s2
3:   Apple_Boot Boot OS X        134.2 MB  disk0s3
/dev/disk1 (internal, physical):
#:          TYPE NAME                SIZE    IDENTIFIER
0: GUID_partition_scheme          *1.0 TB   disk1
1:           EFI EFI                 209.7 MB  disk1s1
2:   Apple_CoreStorage Macintosh HD 999.3 GB  disk1s2
3:   Apple_Boot Recovery HD       650.0 MB  disk1s3
/dev/disk2 (internal, virtual):
#:          TYPE NAME                SIZE    IDENTIFIER
0:            Apple_HFS Macintosh HD +1.1 TB   disk2
                         Logical Volume on disk0s2, disk1s2
                         DA9F81FF-2ECE-43AE-85BA-068EC9EE3111
                         Unencrypted Fusion Drive
/dev/disk3 (external, physical):
#:          TYPE NAME                SIZE    IDENTIFIER
0: FDisk_partition_scheme          *63.9 GB  disk3
1:   Windows_FAT_16 RECOVERY      1.3 GB   disk3s1
2:           Linux                 33.6 MB  disk3s5
3:   Windows_FAT_32 boot          69.2 MB  disk3s6
4:           Linux                 62.5 GB  disk3s7
Catharinas-Mac-mini:~ catharinakarlsson$
```

Create a mount point for the partitions.

```
sudo mkdir /Volumes/rpi
```

Now mount the drive.

```
sudo ext4fuse /dev/disk3s7 /Volumes/rpi -o allow_other
```

The rpi is now available in the finder and can be accessed just like any other drive. When finished with it safely unmount the disk.

```
sudo diskutil unmount /Volumes/rpi
```

LoRa

Installation and coding instructions for installation of LoRa server and client for relay of basic environmental data and acoustic indices. The LoRa equipment need to have the correct bandwidth which is legal to operate in the country of deployment.

Equipment needed:

- Raspberry Pi A+ set up as an acoustic and environmental recorder
- Raspberry B+
- Dragino LoRa/GPS hat
- Adafruit RFM9X LoRa transceiver
- Antenna attachment
- Antenna
- A LED soldered onto a yellow and black female jumper cables (with a resistor).
- A tactile push button soldered to a yellow and a black female jumper cable.

Client

1. First attach the Dragino hat to the Raspberry B+, it should fit over the GPIO pins and cover all of them. Attach the antenna that came with them.
2. The RFM9X comes without the antenna attachment mounted, which needs to be soldered. Considering the small size of it, this is easiest to do if the antenna itself is attached to the attachment so that it can be held easier. The bottom pad has an inlet pad and two ground pads on the side that needs to be soldered onto the connector area on the radio breakout. First wet the areas of both the antenna connector and on the radio breakout.
3. Hold the antenna connector onto the correct areas on the connector area on the radio breakout, heat them with the solder tip and they should ligate together with the solder that is already on there.
4. Now that the antenna is attached, it is time to attach the wires between the RFM9X and the Raspberry Pi A+. The radio breakout uses the SPIO pins. It should be connected according to the following (note the lack of use of the IRQ pin as this is not enabled on the Raspberry Pi).
 - Orange cable from VIN to pin 17.
 - Black cable from GRND to pin 20.
 - Purple cable from MOSI to pin 19 (SPIO MOSI).
 - Brown cable from MISO to pin 21 (SPIO MISO).
 - White cable from SCK to pin 23 (SPIO SCLK).
 - Blue cable from CS to pin 22.
 - Yellow cable from RESET to pin 11.
5. Now attach the LED with the black cable to pin 39 and the yellow one to pin 40 (GPIO 21).
6. The tactile push button should be attached with the yellow cable to pin 36 (GPIO 16) and the black cable to pin 34.

7. The hardware configurations are now finished so it is time to start up the Raspberry A+ (from now on the client).

8. Download the necessary files.

```
git clone https://github.com/hallard/RadioHead
```

9. The main files for this system run with C++ which uses h files for some of the definitions, one of these defines what pins are used depending on the radio module.

```
cd RadioHead/examples/raspi  
ls
```

10. You should now see a file called “RaspbiBoards.h”, access it.

```
sudo nano RaspbiBoards.h
```

11. There should be a section for the board we are using (Adafruit RFM9W is the correct section for us). We need to undefined the IRQ pin, and we need to define the correct LED pin as per below. Note that since this is C++, the # does not mean that a line of code is commented out anymore. Two // is instead the way of commenting out a section.

```
//#define RF_IRQ_PIN RPI_V2_GPIO_PIN7  
#define RF_LED_PIN RPI_V2_GPIO_PIN40
```

12. Save and exit. Now we need to enter the C++ client file and make some changes.

```
cd rf95  
sudo nano rf95_client.cpp
```

13. We need to tell the script that we are using the RFM9X module so ensure the section for this at the top is uncommented and that all other radio modules are commented out with two //.

14. Now we need to be able to tell how many times data has been sent by the client. This enables us to keep track of any lost packages. Towards the end of this file there is a loop section that tells the client what to send to the server. Before this loop (which starts with a “while”), define that the variable i is an integer.

```
int i =0;
```

15. Now inside the loop before the section where the data is defined tell to add one to each iteration of i.

```
i=i+1;
```

16. The section which tells the loop what to print on the client screen, it starts with “printf”, modify the code with the below code. Note how after the “”, we define what

should be inserted after the % signs. First the variable i, then the variable len (which is defined just before this section as the length of the data) and the number of the gateway which was defined very early on in the code file.

```
Printf("Sending number %d of %02d bytes to node #%d =>", i,  
len, RF_GATEWAY);
```

17. Now we need to do some changes in this section to also send the iteration number to the server somehow. We also want it to send our environmental data, but we just want to check that it can send at all first so just copy and paste the last line of a csv file with environmental data and replace “Hi Raspi” where the data is defined. Before this section we now need to tell the script what kind of data we are sending and how long. So, give it an arbitrarily long number (like 256). Notice how in the second line of these codes, we add in the iteration number to the end of the line of data that we are sending to the server.

```
uint8_t data[256];  
sprint((char*)data,"2018-06-11, 76, 98,33,31, %d", i);  
uint8_t len = uint8_tstrlen((char*)data);
```

18. We are now also using code from the string library, so the script needs to be told to read the h file for this library when it is starting. Go back to the top of this file. You should see several rows of the script calling multiple h libraries. Add in the string one.

```
#include <string.h>
```

19. Now exit and save. Now we need to “make” the files, before we can run it.

```
make  
sudo ./rf95_client
```

20. If everything went as it should, the client should now start sending messages every five seconds and the iteration number should increase by one every time (the last number in the message). If it is running, you should see the LED flashing every time it sends a message. You hopefully noticed that in the C++ file for the client there was a section that defined how many seconds should be between each message should be sent. You can close the running of this script by pressing ctrl+c.

21. Now we want to add a push button so that the client starts sending messages whenever you push a button. We will modify our shutdown button script for this purpose. Copy it and give it a new name.

```
cd  
cd Scripts  
cp Shutdown_pi.py RF95_client_button.py  
sudo nano RF95_client_button.py
```

22. Modify the code to give it the correct GPIO number and tell it what to do when the button is pressed.

```

#!/bin/python
#simple script for executing the RFM95_client script at the
press of a button at the press of a button
#by Catharina Karlsson

import RPi.GPIO as GPIO
import time
import os

#use the Broadcom SOC pin numbers
#setup the pin with internal pull-ups enabled and PIN in
reading mode
GPIO.setmode(GPIO.BCM)
GPIO.setup(16, GPIO.IN, pull_up_down = GPIO.PUD_UP)

#our function on what to do when the button is pressed
def Shutdown(channel):
    os.system("sudo
./home/pi/RadioHead/examples/rf95/rf95_client")
    #add our function to execute when the button pressing event
happens
GPIO.add_event_detect(16, GPIO.FALLING, callback = Shutdown,
bouncetime = 2000)

#now wait!
while 1:
    time.sleep(1)

```

23. Now we need to add this script to the rc.local so that it runs in the background on boot just like the shutdown script.

```
sudo nano /etc/rc.local
```

24. Add the line of code before exit 0 (remember to end with a carriage return).

```
sudo python /home/pi/Scripts/RF95_client_button.py &
```

25. If you reboot the unit now, the LED should start blinking and sending messages whenever you press the button.

Server

1. Time to start up the Raspberry B+ with the Dragino hat (server from now on).
2. We need to download the RadioHead library again.

```
git clone https://github.com/hallard/RadioHead
```

3. We need to again enter the h file that defines the pins. This time we are undefining both the LED and the IRQ (the Dragino hat already has a blue LED that blinks every time the server receives a package from a client).

```
cd RadioHead/examples/raspi  
sudo nano RaspiBoards.h
```

4. In the section where the pins are defined for the dragino, comment out the LED and the IRQ definitions.

```
//#define RF_IRQ_PIN RPI_V2_GPIO_PIN7  
//#define RF_LED_PIN NOT_A_PIN
```

5. It is not time to edit the c++ file for the server.

```
cd rf95  
sudo nano rf95_server.cpp
```

6. First uncomment the section for the Dragino board and ensure any other boards are commented out.
7. For the server to run without crashing the system without an IRQ we need to allow it to create a driver without the IRQ. Where the instant of the driver is created, uncomment the row that allows this to happen (it is relatively early in the script file).

```
//create an instant of a driver  
#ifdef RF_IRQ_PIN  
RH_RF95 rf95(RF_CS_PIN, RF_IRQ_PIN);  
#endif  
RH_RF95 rf95(RF_CS_PIN);
```

8. Exit and save.

9. Make the file and start the server.

```
make  
sudo ./rf95_server
```

10. If it runs without issue you should be able to also start the client (if it is not running already) and the server should be receiving messages from it.

Reading Material

Websites

WarbleR: <https://marce10.github.io>

Aureas: <http://sistemic.udea.edu.co/es/investigacion/proyectos/analisis-bioacustico/>

Sasquatch recorder: <http://sasquatchbioacoustic.blogspot.se/2016/01/long-duration-recorder-ldr-based-on.html>

Weather Station: <https://www.jeremymorgan.com/tutorials/raspberry-pi/how-to-weather-station-raspberry-pi/>

Adafruit: <https://learn.adafruit.com>

Detection space: <http://wwwuser.gwdg.de/~ecosound/index.html>

Raspberry Pi bat project: <http://www.bat-pi.eu/EN/Mikrofone-EN.html>

Audacity: <http://www.audacityteam.org>

I2C: <http://www.robot-electronics.co.uk/i2c-tutorial>
<https://learn.adafruit.com/using-the-bmp085-with-raspberry-pi/overview>

Wire soldering: <http://www.instructables.com/id/Soldering-wires-together/>

Mounting of SD drives: <https://www.jeffgeerling.com/blog/2017/mount-raspberry-pi-sd-card-on-mac-read-only-osxfuse-and-ext4fuse>

Journal Articles:

Araya-Salas, M. & Smith-Vidaurre, G., 2017. warbleR: an R package to streamline analysis of animal acoustic signals. *Methods in Ecology and Evolution*. 8, pp. 184–191. Available at: <https://besjournals.onlinelibrary.wiley.com/doi/epdf/10.1111/2041-210X.12624>

Bedoya, C. et al., 2014. Automatic recognition of anuran species based on syllable identification. *Ecological Informatics*, 24, pp.200–209. Available at: <http://linkinghub.elsevier.com/retrieve/pii/S1574954114001198>.

Darras, K. et al., 2016. Measuring sound detection spaces for acoustic animal sampling and monitoring. *Biological Conservation*, 201, pp.29–37. Available at: <http://dx.doi.org/10.1016/j.biocon.2016.06.021>.

Appendix 5.2. R code to run the package warbleR on contact calls from *Kalophrynus baluensis*. Code adapted from the vignette associated with warbleR

```
#warble R
```

to run this you first need to install the following fftw3, libsndfile and pkg-config through the terminal and enter the following code

```
#ruby -e "$(curl -fsSL
```

```
https://raw.githubusercontent.com/Homebrew/install/master/install)" </dev/null 2> /dev/null
```

```
#brew install libsndfile
```

```
#brew install pkg-config
```

```
#brew install wget --with-libressl
```

```
#brew install fftw
```

#workflow taken from the warbleR online workflow page and adapted for the Kalophrynus audio file

```
#directory: dropbox -> Kinabalu_acoustic_trials -> SilauSilau
```

```
library(fftW)
```

```
library(warbleR)
```

```
library(tuneR)
```

```
#create new directory
```

```
dir.create(file.path(getwd(),"Kalophrynus_audio_analysis"))
```

```
setwd(file.path(getwd(),"Kalophrynus_audio_analysis"))
```

```

# Let's create a list of all the recordings in the directory
wavs <- list.files(pattern="wav$")

# We will use this list to downsample the wav files so the following analyses go a bit
faster

lapply(wavs, function(x) writeWave(downsample(readWave(x), samp.rate = 22050),
                                    filename = x))

# ovlp = 10 speeds up process a bit

# tiff image files are better quality and are faster to produce

# We can zoom in on the frequency axis by changing flim,
# the number of seconds per row, and number of rows
#all files

lspec(flim = c(0, 12), ovlp = 10, sxrow = 3, rows = 10, it = "tiff")

#create new directory for autodetect
dir.create(file.path(getwd(),"autodetect"))

setwd(file.path(getwd(),"autodetect"))

# Run autodetect() on subset of recordings
#threshold controls detection by relative amplitude (%)

#bp serves as a frequency bandpass filter

#mindur & maxdur determine the minimum and maximum duration of the signals to
be detected

```

```
#set controls if settings of the autodetection process are included in the image file  
name. If TRUE, threshold (th), envelope (envt), bandpass (bp), power (pw), smooth (smo),  
maxdur (mxdu), and mindur (midu) are included. This could be very helpful for comparing  
the performance of different detection setttings
```

```
kalo<-autodetec(flist = wavs, bp = c(0, 4), threshold = 5, mindur = 0.001, maxdur  
=0.5, envt="abs",sssmooth = 300, ls = TRUE, res = 100, flim = c(0, 12), wl = 300, set  
=TRUE, sxrow = 3, rows = 10, redo = TRUE, it = "tiff")
```

```
str(kalo)
```

```
#look at the output
```

```
table(kalo$sound.files)
```

```
# A margin that's too large causes other signals to be included in the noise  
measurement
```

```
# Re-initialize X as needed, for either autodetec or manualoc output
```

```
#test on 10% first to esnure the margins are set correctly
```

```
X <- kalo[sample(1:nrow(kalo),(nrow(kalo)*0.1)), ]
```

```
X <-kalo
```

```
snrspecs(X = X, flim = c(2, 110), snrmar = 0.60, mar = 0.7, it = "tiff")
```

```
#Let's look at the number of selections per sound file
```

```
table(kalo$sound.files)
```

```

# A margin that's too large causes other signals to be included in the noise
measurement

# Re-initialize X as needed, for either autodetect or manualoc output

snrspecs(X = kalo, flim = c(2, 110), snrmar = 0.5, mar = 0.7, it = "tiff")

#####
#####this is for the spectrogram

# Run function for all recordings, with final argument settings

specreator(kalo, flim = c(0, 12), osci = TRUE, line = TRUE, wl = 300, ovlp = 90, it =
"tiff", res = 300, title = FALSE)

#####

#create signal to noise ratio data

dir.create(file.path(getwd(),"snr"))

setwd(file.path(getwd(),"snr"))

snrspecs(X = kalo, flim = c(0, 12), snrmar = 0.5, mar = 0.7, it = "tiff")

#We will measure SNR on every other selection to speed up the process

kal.snr <- sig2noise(X = kalo[seq(1, nrow(kalo), 2), ], mar = 0.04)

#As we just need a few songs to characterize each sound file and individual, we can
choose selections with the highest SNR per sound file. In this example, we will choose 5
selections with the highest SNRs.

kal.hisnr <- kal.snr[ave(-kal.snr$SNR, kal.snr$sound.files, FUN = rank) <= 5, ]

```

```
# Double check the number of selection per sound files
```

```
table(kal.hisnr$sound.files)
```

```
#At this point would be a good idea to save the selections as a file
```

```
write.csv(kal.hisnr, "Kalo_balue_hsnr.csv", row.names = FALSE)
```

```
#In some cases manual selection may be preferable, especially if you have shorter recordings or if the automatic detection is not as accurate as you???d like.
```

```
#Note that you can stop the function at any point by clicking twice on the stop button.
```

```
# Run manualoc() with frequency range set for Phaethornis longirostris
```

```
# Recording comments are enabled to mark recording quality
```

```
# Selection comments enabled to include visual classifications
```

```
dir.create(file.path(getwd(),"manuloc"))
```

```
setwd(file.path(getwd(),"manuloc"))
```

```
manualoc(flim = c(0, 12), reccomm = TRUE, selcomm = TRUE, osci = TRUE)
```

```
# Read manualoc() output back into RStudio as an object
```

```
# This data frame object can be used as input for the functions that follow
```

```
manualoc_out <- read.csv("manualoc_output.csv", header = TRUE)
```

```
set.seed(50)
```

```
# Plot selection lines from manualoc() or autodetec()
```

```
specreator(kalo, osci = FALSE, line = TRUE, wl = 300, flim = c(0, 12), it = "tiff")
```

```
# Change frequency limits of y-axis
```

```
specreator(kalo, flim = c(0, 12), osci = TRUE, line = TRUE, wl = 300, it = "tiff")  
# Change width of spectrogram to be proportional to signal duration  
specreator(kalo, flim = c(1, 11), osci = TRUE, line = TRUE, propwidth = TRUE, wl =  
300, it = "tiff")  
# Change spectrogram size  
# Changing inner.mar and outer.mar arguments improves picsize results  
specreator(X, flim = c(1, 11), osci = TRUE, line = TRUE, picsize = 1.5, wl = 300,  
ovlp = 90, inner.mar = c(4, 4.5, 2, 1), outer.mar = c(4, 2, 2, 1), it = "tiff")
```

#Visualize frequency measurements with trackfreqs
#Prior to calculating acoustic measurements, it???'s good practice to visualize the accuracy of some important measurements, namely frequency measurements. The function trackfreqs is the last in the family of spectrogram-creators. It allows you to create spectrograms with dominant frequency and fundamental frequency measurements plotted on top of each selected signal.

#In general, the fundamental frequency measurements are not as reliable as the dominant frequency measurements. Use trackfreqs on all the recordings for which you want to measure acoustic parameters. Scroll through all the spectrograms to get a feeling for how well the frequency measurements will be performed across your recordings.

#Like it???'s sister functions, trackfreqs has many graphical arguments. It has additional graphical arguments to change colors of the plotting symbols, and size and position of legend labels. These arguments will largely depend on the nature of your selections.

```
dir.create(file.path(getwd(),"freq_analysis"))  
setwd(file.path(getwd(),"freq_analysis"))
```

```
#because row three is actually capturing an "echo" it causes an issue in analysis so  
remove it
```

```
kalo2 <- kalo[-3,]
```

```
# Note that the dominant frequency measurements are almost always more accurate  
trackfreqs(kalo2, flim = c(0, 12), bp = c(0, 4), it = "tiff")
```

```
# We can change the lower end of bandpass to make the frequency measurements  
more precise
```

```
trackfreqs(kalo, flim = c(0, 12), bp = c(1, 5), col = c("purple", "orange"),  
          pch = c(17, 3), res = 300, it = "tiff")
```

```
# If the frequency measurements look acceptable with this bandpass setting,
```

```
# that's the setting we should use when running specan()
```

```
#Batch-process acoustic measurements with specan
```

```
# We can now perform acoustic measurements with the function specan. This  
function calculates several acoustic parameters across all the specified recordings. It's a batch  
process that is much faster than calculating measurements one recording at a time. specan  
uses and customizes several functions available in the seewave package.
```

```
#specan uses the time coordinates in the autodetect or manualoc output. It will  
measure acoustic parameters within the start and end times of the selections.
```

```
# Use the bandpass filter to your advantage, to filter out low or high background
```

```
# noise before performing measurements
```

```
# The amplitude threshold will change the amplitude at which noises are
```

```
# detected for measurements
```

```
params <- specan(kalo2, bp = c(1, 4), threshold = 15)
```

```
View(params)
```

```
str(params)
```

```
# write .csv files to your working directory
```

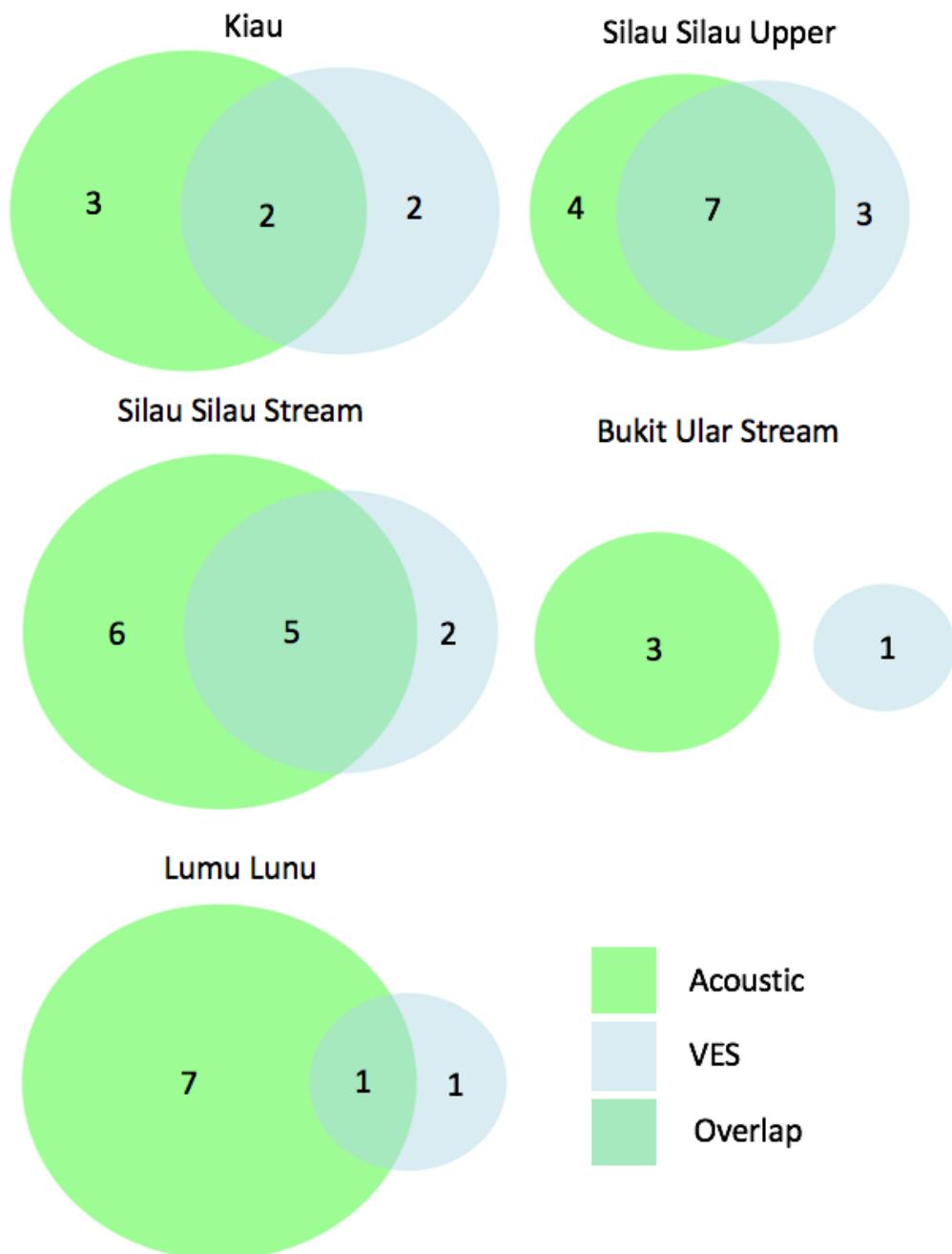
```
write.csv(params,"frequency_parameters.csv")
```

Appendix 6.1. Presence or absence of species in five sites during the VES surveys in 2016.

Presence is indicated with grey block and absence with white.

Species		Silau	Kiau	Bukit	Silau	Lumu
Presence	Absence	Silau	View	Ular	Silau	Lunu
		Upper		Stream	Stream	
<i>Ansonia hanitschi</i>		■			■	
<i>Kalophryalus baluensis</i>		■	■			
<i>Leptobrachium montanum</i>		■	■		■	
<i>Leptolalax arayi</i>						
<i>Leptolalax sabahmontanus</i>					■	
<i>Limnonectes kuhlii</i>				■	■	
<i>Xenophrys baluensis</i>						
<i>Pelophryne misera</i>		■			■	
<i>Philautus nephophilus</i>		■				
<i>Philautus macroscelis</i>		■			■	
<i>Rhacophorus angulirostris</i>					■	
<i>Staurois tuberilinguis</i>						

Appendix 6. 2. Venn diagrams of species detected in acoustic surveys and in VES surveys and overlap in species between the two survey methods.



KALOPHRYNUS BALUENSIS (Kinabalu Sticky Frog). **REPRODUCTION AND VOCAL DESCRIPTION.** The microhylid frog genus *Kalophrynus* currently consists of 25 species (www.research.amnh.org/vz/herpetology/amphibia. 14 June 2017) with 11 of them reported from the island of Borneo. *Kalophrynus baluensis* (Kinabalu sticky frog) has a very restricted range, and is endemic to Mount Kinabalu in Sabah, Malaysian Borneo, ranging from 1000-1800 m elev. *K. baluensis* is often found in leaf litter and has been observed climbing as high as 1.2 m on trunks and shrubs (pers. obs.). Currently, detailed descriptions of *K. baluensis* reproductive ecology and behavior are lacking (Inger & Stuebing. 2005. A field guide to the frogs of Borneo. Natural History Publications. Kota Kinabalu, Malaysia. pp. 97-98). Furthermore, the current known range of vocalizations is limited to a single-noted nocturnal call (Malkmus & Riede 1996. *Herpetozoa*. 9(1/2): 151-155; Malkmus et al. 2002. Amphibians and Reptiles of Mount Kinabalu. A. R. G Gantner Verlag. Königstein, Germany. pp. 115-132). Here, we present the first description of the breeding habits of *K. baluensis*, as well as a novel diurnal vocalization.

K. baluensis was found in a tree buttress hole near the ground during a survey of Kinabalu Park on 22 June 2010 (Bickford, pers. obs.). The buttress hole was approx. 40x20cm wide, and was filled with approx. 150 eggs. In April 2016, several individuals (at one point as many as five) of *K. baluensis* were observed in a tree hole (hollow stump) created from a felled tree fern (*Cyathea spp.*) in Kinabalu Park at approx. 1450 m elev. The ambient air temperature at this altitude averages 20°C (Kitayama 1992; Fig. 5. An altitudinal transect study of the vegetation on Mount Kinabalu, Borneo. *Vegetatio*. 102: 149-171). The tree fern was located near the upper loop road in the park, next to the Padahaan shelter. The top of the tree fern stump ranged between 115 and 120cm from the ground. In April of 2016 the water column was 42cm deep, and 51.5cm deep including the leaf litter at the bottom. The temperature of the water was 20°C and it had a pH of 6. The internal diameter of the tree hole was 9cm. Eggs were observed (see fig. 1a) but disappeared during embryonic development, likely due to predation, preventing measurements of these critical periods.

Upon a subsequent visit to Kinabalu Park, the species was observed in amplexus in the same tree hole at 1356h on 6 June 2017. The pair was filmed using a Nikon AW130 that was left unattended to reduce disturbance to the frogs. They were filmed for 0.5h, from 1415h until 1445, as well as one hour under red light between 1900 and 2100. The male

periodically came up to the surface of the water to emit short calls (see spectrogram, fig. 1b, c) that differed from the typical advertisement call reported in the literature (Malkmus et al. 2002. op. cit.). We analyzed 25 of these contact calls (emitted by the same individual) and they averaged 57 ms in length (range: 43-70 ms) and consisted of a single note with an average mean dominant carrier frequency of 1.4kHz (range: 1.3-1.6kHz; fig 1b, c). In comparison, the previously recognized advertisement call of *K. baluensis* averages 125 ms in length and a dominant carrier frequency at 2.6kHz (Malkmus et al. 2002. op. cit.). The call analysis was performed using the package warbleR in the software R.

After the male vocalized, the female subsequently emerged from the bottom of the water column. The female was seen surfacing just beneath the male on numerous occasions. The male used his forearms to grasp the female on both sides of her head as her snout reached the surface. The back legs were not used to hold onto the female but were left floating at the surface. The female frequently dove down, causing the male to vocalize again, which seemed to cause the female to resurface. These unique behaviors were observed during the day (1415-1445) as well as night (1900-2100). No eggs were observed in the tree hole over the following two days, suggesting that the pair spends several days together prior to oviposition.

Even though egg laying was never directly observed, evidence strongly suggests the eggs photographed in 2016 (fig. 1a) belong to *K. baluensis*. Aside from the eggs appearing in the same location as the amplexed adults, we can rule out most other frogs from this field site as well. Members of the genera *Ansonia*, *Leptobrachium*, *Leptolalax*, *Megophrys*, *Meristogenys*, *Staurois*, and *Rhacophorus* from the same site all rely on streams or water bodies on the forest floor for reproduction. *Pelophryne spp.* use leaves with accumulated water on the forest floor. *Chaperina fusca* and *Nyctixalus pictus* both attach their eggs to the walls of small water bodies or to submerged leaf litter. *Philautus spp.* in this region are all thought to deposit their eggs directly on leaves and are not dependent on water bodies, and their eggs are much larger in diameter than those observed in this tree hole. Furthermore, other *Kalophrynus spp.*, which may have similar breeding habits, do not occur at this elevation (Malkmus et al. 2002, op. cit.). The eggs we observed were approx. 3 mm in diameter (figure 1a) and light grey in colour. The genus is known to use tree holes and other aquatic microhabitats for breeding (Malkmus et al. 2002, op. cit.) and breeding in temporary pools has been speculated for *K. baluensis*. As far as we are aware, this is the first confirmation that *K. baluensis* breeds in tree holes. It also shows that they may climb, in this case to just over one meter in height, to reach available small water bodies.

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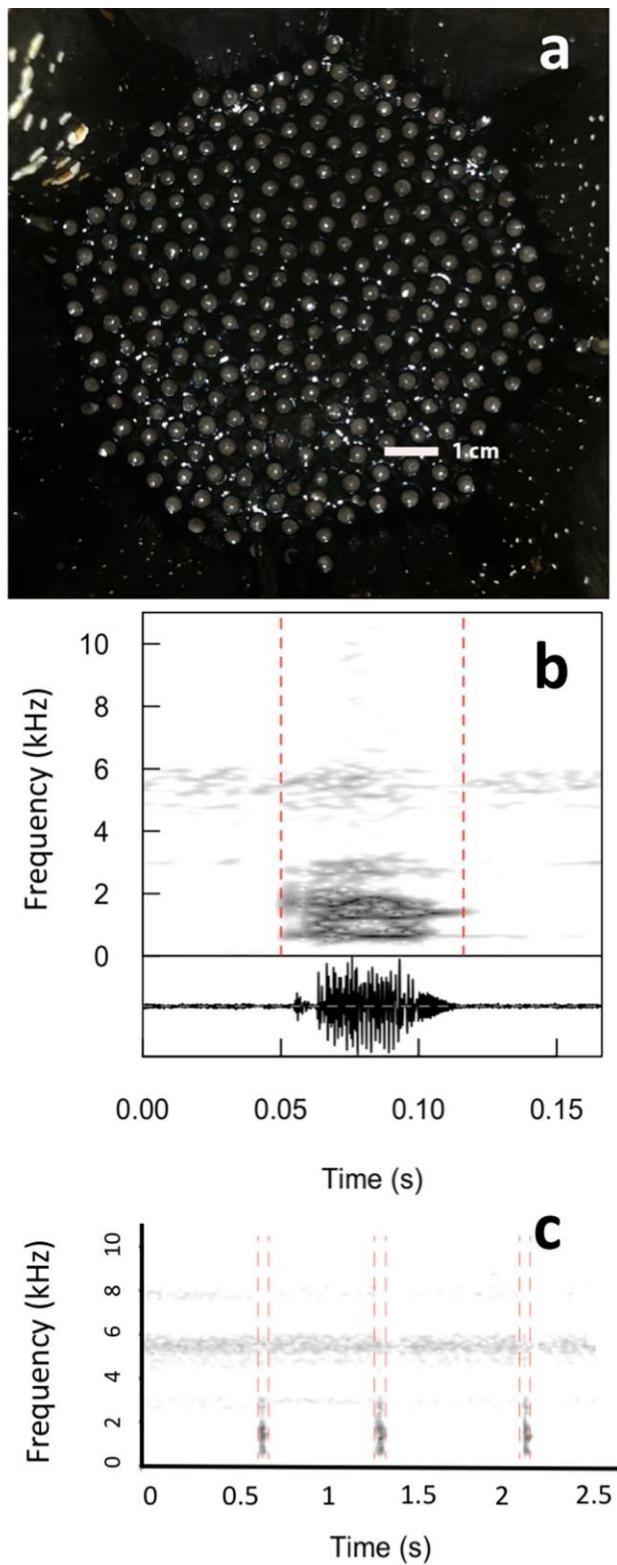


Figure 1. a) *K. baluensis* egg mass (photos ©A. Mitchell 2016), b) spectrogram with oscillogram underneath of *K. baluensis* contact call and c) long spectrogram of *K. baluensis* contact call. Call is identified between dashed lines.