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Amphibian diversity in Amazonian flooded forests of Peru

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Abstract

Global biodiversity is currently facing the sixth mass extinction, with extinction rates at least 100 times higher than background levels. The Amazon Basin has the richest amphibian fauna in South America, but there remain significant gaps in our knowledge of the drivers of diversity in this region and how amphibian assemblages are responding to environmental change.

Surveys were conducted in the Pacaya-Samiria National Reserve (PSNR) in Amazonian Peru, with a view to (1) comparing assemblage structure on floating meadows and adjacent terrestrial habitats; (2) determining the predictors of diversity in these habitats; and (3) exploring the effects of disturbance and seasonal flooding on diversity measures. Eighty-one species of amphibians have been recorded in these habitats since 1996 representing 11 families and three orders. In 2012-2013 22 anuran species used the floating meadow habitat, of which 10 were floating meadow specialists. These specialists were predominantly hylids which breed on floating meadows all the year round. Floating meadows therefore host an assemblage of species which is different to that found in adjacent terrestrial areas which are subject to seasonal flooding. Floating meadows enhance the amphibian diversity of the region, and rafts of vegetation that break away and disperse frogs downstream may explain the wide distribution of hylids within the Amazon Basin.

Fourteen different reproductive modes were represented within the 54 anuran species observed. The number of reproductive modes present was influenced by localised disturbance and seasonal flooding. Diversity increased in the low water period, with hylids breeding in temporary pools. When the forest is inundated most species disperse away from the flood waters.

Disturbance, habitat change, emerging diseases and climate change would likely lead to changes in species composition and assemblage structure rather than wholescale extinctions. However, further studies are needed to evaluate long-term consequences of synergistic environmental change.

KEY WORDS: Amazonian amphibians; floating meadows; climate change; flooding; reproductive modes

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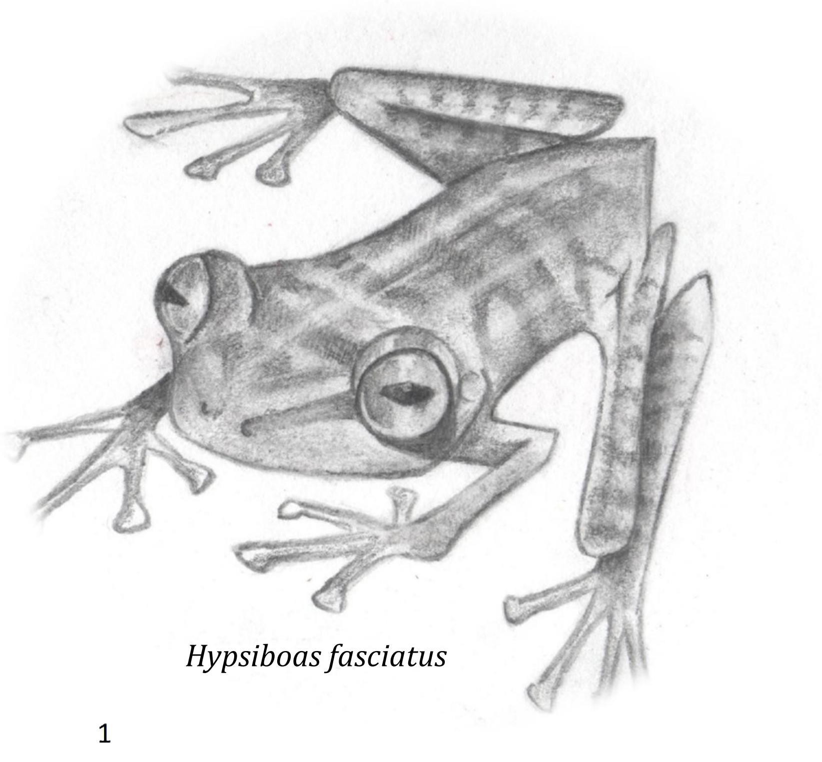
Introduction



Hypsiboas fasciatus

1.1 Introduction

1.2 Global biodiversity conservation

Global biodiversity is currently facing the sixth mass extinction; with current extinction rates 100 times higher than the background rate (Ceballos et al. 2015). Whilst the previous five events were likely caused by natural climate change, volcanic eruptions and asteroids, the current event is caused by anthropogenic climate change and habitat destruction (Ceballos et al. 2015). Global biodiversity is distributed unevenly, as are the threats they face. Many high biodiversity areas are located within developing, economically poor countries. Yet, the majority of funding for conservation is raised and spent within economically rich countries. One of the major challenges of biodiversity conservation is designating priority areas in which to allocate funds and resources (Jenkins et al. 2013; Brooks et al. 2006). Many studies have addressed this, highlighting priority areas, defined using a variety of criteria and taxa, including: threatened mammals, birds and amphibians (Brooks et al. 2004); threatened and endemic birds (Orme et al. 2005); mammal endemism and endangerment (Ceballos and Ehrlich 2006); species richness and endemism of amphibians, reptiles, birds and mammals (Lamoreux et al. 2006); and threat risk of birds mammals and amphibians (Grenyer et al. 2006). The majority noted that priority areas for different taxa rarely overlapped and protected areas do not cover a large enough area to conserve biodiversity (Rodrigues et al. 2004).

Most studies use the IUCN Red List as a measure of species extinction risk. Using mammals, birds and amphibians, Hoffmann et al. (2011), assessed worldwide conservation success in terms of changes in these threat categories. This highlighted that while effective conservation measures are increasing populations of some species, the current levels of conservation are insufficient (Hoffmann et al. 2011). Verissimo et al. (2014) showed that in recent years climate change has received a vast quantity of media and publication attention, and runs the risk of overshadowing biodiversity loss. It is vital that climate change issues are therefore incorporated into biodiversity conservation.

1.3 Global amphibian declines

Amphibians are important consumers of invertebrates, whilst also being a prey species (Wake 1991). By using both the aquatic and terrestrial environment they play a key role in energy flow and nutrient cycling (Collins and Crump 2009). Amphibians are highly threatened; recent research has calculated that 41% are at risk of extinction (IUCN 2014; Hoffmann et al. 2011; Stuart et al. 2004). These amphibian declines may have cascade effects in the ecosystems they inhabit.

Amphibians are believed to be good biological indicators, due to their use of both aquatic and terrestrial habitats and their permeable skin, making them vulnerable to environmental contamination (Lips 1998; Blaustein and Wake 1990). However, rigorous tests proving the suitability of amphibians as indicator species are lacking (Kerby et al. 2009; Sewell and Griffiths 2009). Amphibians do not meet all the criteria required to be considered as suitable indicator species (Collins and Crump 2009; Sewell and Griffiths 2009; Beebee and Griffiths 2005). The declines amphibians currently face are attributed to several threats, which are not necessarily threats that will affect the entire ecosystem and the species within; making them less suitable as an indicator species.

Amphibians face a multitude of threats. These threats include: 1) Habitat destruction / fragmentation / alteration caused by increasing human populations which results in habitats being converted for construction and agriculture (Jongsma et al. 2014; Hamer and McDonnell 2008; Pearman 1997); 2) Diseases which causes dramatic mass mortalities, the most notable is chytridiomycosis which has caused population declines and extinctions worldwide (Garner et al. 2009); 3) Climate change which is increasing global mean temperature, potentially disrupting breeding cycles and changing habitat suitability (Blaustein et al 2001; Beebee 1995) in the Amazon extreme weather patterns are already more common as a result of climate change, over the past 5 years a greater intensity of flooding and droughts has been observed (Bodmer et al. 2014); 4) Overexploitation of amphibians which are used as a food resource, for education, medicine, research and even as pets (Warkentin et al. 2009; Pepper et al. 2007; Andreone et al. 2006); 5) Introduced species which compete with and predate upon native species (Kats and Ferrer 2003); 6) UVB radiation caused by anthropogenic ozone depletion (Middleton et al. 2001); and finally 7) Synergism which involves interactions between several threats, working together to cause population declines (Burrowes et al. 2004).

Amphibian declines are well documented in the literature, with worldwide initiatives being implemented to help address the amphibian decline crisis. Yet prior to 1989 this was not the case. It is acknowledged that at the First World Congress of Herpetology in September 1989 herpetologists were able, for the first time, to compare notes on what they believed to be localised small scale declines (Stuart 2012; Stuart 2004; Jutterbrook 1992). However they soon realised that this was a worldwide problem which needed addressing. After the congress the number of publications on amphibian declines rapidly increased (Stuart 2012).

Subsequently a dedicated workshop was arranged to look into the causes of amphibian declines. This workshop resulted in the organisation of the Declining Amphibian Population Task Force (DAPTF) which was allied with the IUCN Species Survival Commission (Wake 1998). However, the status of amphibians globally was still largely unknown, so in 2001 the Global Amphibian Assessment (GAA) was started to assess the conservation status of the world's amphibians. The GAA was first published in 2004 (Stuart 2004) then updated in 2006 and 2008 to include newly described species (Amphibian Specialist Group 2012). Amphibian Red List status was assessed and the threats they faced highlighted, although an action plan was then needed. So in 2007 the Amphibian Conservation Action Plan (ACAP) was published (Gascon et al. 2007; Mendelson et al. 2006). This gave clear guidelines for addressing amphibian declines which has since resulted in numerous field projects as well as ex-situ conservation work (Stuart 2012). More recently many worldwide initiatives such as: the Amphibian Survival Alliance (www.amphibians.org – last accessed 14/11/15); Amphibian Specialist Group (www.amphibians.org – last accessed 14/11/15); Save the Frogs (www.savethefrogs.com – last accessed 14/11/15); and Amphibian Ark (www.amphibianark.org – last accessed 14/11/15) have been created to tackle the global amphibian crisis.

1.4 Amphibian diversity and distribution

There are currently 7424 amphibians species described worldwide (AmphibiaWeb 15/06/2015) however, their distribution is uneven (Fig. 1.1). While they can be found on almost every continent greater abundances are observed in tropical regions (Duellman 1999).

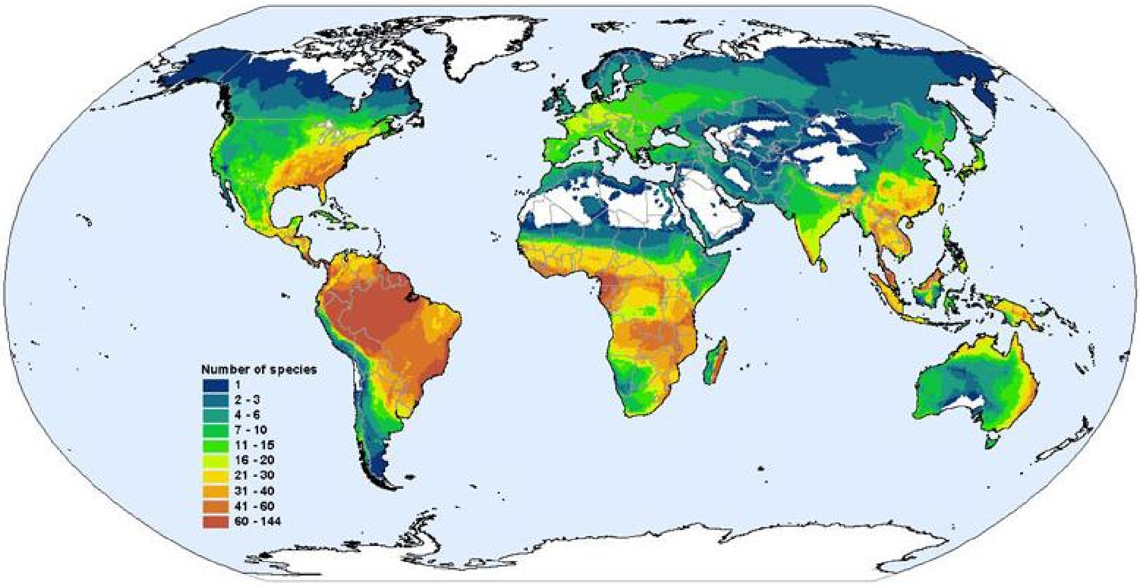


Figure 1.1. Distribution of worldwide amphibian species richness reprinted from the Global Amphibian assessment (IUCN Species Survival Commission 2004).

Amphibians comprise three classes, the largest is Anura (frogs and toads) with 6549 species (AmphibiaWeb 2015), found on almost every continent (Pough et al. 2004). Frogs can be found in all habitat types from below the driest deserts to the high canopies of tropical forests. The second largest amphibian class is Caudata (salamanders) with 676 species (AmphibiaWeb 2015) these have elongated bodies with tails. Salamanders have also adapted too many habitat types; some species can be aquatic, terrestrial, fossorial, arboreal or a combination thereof (Dodd 2010). Salamanders are not as diverse as frogs, and they are most abundant in North America and temperate Eurasia, although some have moved into Central and South America. They are completely absent from sub-Saharan Africa, Australasia, and much of tropical

Asia (Dodd 2010). The third and final class is Gymnophiona (caecilians) with 205 species (AmphibiaWeb 2015) these are the least well known group of amphibians. This is due to their fossorial or aquatic habits which means they are rarely encountered above ground. Caecilians resemble large earthworms and can even be confused with snakes, as they have elongated bodies with no legs. Caecilians can be found in tropical habitats worldwide, however are not found in Madagascar and the Papuan-Australian region (Pough et al. 2004).

1.5 South American Amphibian Diversity, Distribution and Conservation

South America has the highest diversity and density of amphibians worldwide (Fig. 1.1). Within South America one of the greatest diversities is found in the Amazon Basin (Duellman 1999). Although the exact number of species found here is unknown, the species list has grown in recent decades. For example, Heyer (1976) suggested 100 species in the Amazon Basin, while a few years later Duellman (1988) suggested there were 3533 species in the American tropics. Other more recent studies suggest 1000 species (Butler 2010); 300 species (Duellman 2005); and 500 species (also includes reptiles: Bartlett and Bartlett 2003) all in the Amazon Basin. However, a more recent paper suggests 554 species in the Amazon Basin (Funk et al. 2011). Brazil contains the largest part of the Amazon Basin and studies have recorded 163 amphibian species in the Brazilian Amazon (Azerdo-Ramos and Galatti 2002).

Yet the distribution of amphibian species across South America is uneven; Duellman (1999) highlighted these extreme differences. By splitting South America into both vegetation types and natural regions he identified which areas were the most species diverse and which were the least. The highest species richness was observed in the montane tropical rainforest with 745 species compared with 608 in the lowland tropical rainforest and only 32 species in temperate forests. The natural regions show a similar pattern with 335 species in the Amazonia-Giana region, 753 species in the Andes, 334 in the Atlantic forests and 32 in the Austral temperate forests. Most species are endemic to just one vegetation type or natural region with 95% of all species in the Andes endemic to that region.

The patterns of amphibian evolution and distribution in South America have been correlated with topographical features, climate and vegetation (Duellman 1999). Whilst historical patterns and processes are also believe to play an important role (Wiens et al. 2011; Santos et al. 2009). The uplifting of the Andes is believed to play a very significant role in creating the diversity of habitats found in Peru (Antonelli et al. 2010) which in turn will have influenced the diversification of amphibian species. It is possible that island biogeography theory can help to explain this diversity. Although often related to actual islands surrounded by water bodies, it is also possible to relate these theories to islands of habitats, which are isolated from each other, such as floating meadows (see chapter 2), rotting logs and caves. These three examples represent habitats, in which species have adapted, with less suitable habitat surrounding them and so potentially isolating them. This degree of biological isolation is important in allowing speciation to occur (Osborne 2000). There are two methods in which this isolation can occur the first is a “new” uninhabited habitat, the second is an existing habitat which becomes isolated (be this by flooding / removal of a land bridge etc.). In the first scenario species must migrate from the mainland or surrounding habitat into this new site, dispersal is vital for colonization of this new habitat. In the second method species become trapped with the habitat. The species composition of an island is greatly influenced by colonisation, speciation, extirpation and extinction (Osborne 2000). It will also be influenced by the size of the island and the distance from the mainland, a larger island closer to the mainland is likely to have higher species richness than a small island far from the mainland. The creation of the large wetland system in the Amazon (See Chapter 4) may have created 'Islands' of habitat be this terrestrial forest or floating vegetation in which species were able to evolve and speciate.

In the last decade 216 new amphibian species have been described from the Amazon Basin (Thompson 2009). Twenty-four of these are poison dart frogs, the majority of which were found in the Peruvian Amazon (Thompson 2009). As well as discovering new species, advances in DNA sequencing have allowed the separation of species which were originally clumped together under one clade (Funk et al. 2011). Most new and undiscovered species are likely to be found in the Neotropics, particularly within forests with minimal anthropogenic disturbance (Giam et al. 2012). This may help to explain the high number of newly described species within the Peruvian Amazon. In the Loreto region of Peru, which is just under 370,000 km^2 there are only 891,700 people, over half of whom live within cities: Iquitos; Nauta; Yurimaguas; Requena; Contamana; and Tamshiyacu leaving large tracts of relatively un-researched Neotropical forests (Perez et al. 2010).

Catenazzi and May (2014) assessed the conservation status of Peruvian amphibians and highlighted six main threats: 1) Modification and fragmentation of habitat in the Amazon Basin and eastern slopes of the Andes, with forests being converted through logging, to agriculture, (such as coca cultivation), and mining; 2) Environmental contamination, with water courses becoming contaminated by mining and oil extraction; 3) Climate change, which is affecting seasonal weather and flooding patterns and can act synergistically with other threats; 4) Chytridiomycosis, a fungal disease which has caused numerous population declines, often in places with little human disturbance; 5) Overharvesting and pet trade, with some large species collected for local consumption, and many small dendrobatid and hylid species harvested for the pet trade; and finally 6) Introduced species, often comprising domesticated animals which prey on, or destroy the habitats, of amphibians.

Current records suggest that there are 561 amphibian species in Peru, 543 of which are anurans, 15 caecilians and three salamanders (AmphibiaWeb 18/06/2015). In terms of amphibian richness it belongs to one of the three most diverse countries worldwide, i.e. Brazil (1001 species), Colombia (767), and Peru (561) (AmphibiaWeb 18/06/2014). According to the IUCN Red List 101 out of 484 assessed species for Peru were categorized as threatened (IUCN 2014). Catenazzi and May (2014) found no evidence of amphibian declines in undisturbed lowland forests of the Amazon Basin. Yet there is a lack of baseline data and ongoing surveys in lowland sites. This thesis aims to bridge this gap and leave detailed baseline data which can be compared with future surveys. Whilst increasing our understanding of the drivers of amphibian diversity found in Pacaya-Samiria National Reserve (PSNR), and the Amazon Basin.

1.6 Thesis overview

In this thesis I present data collected during 2012 and 2013 on amphibians in the PSNR. Chapter 2 begins with a detailed description of the study site and general survey methods; used throughout the thesis. In Chapter 3 I summarise data which have been collected and published on amphibians in the PSNR since 1996, and present rarefaction curves to determine sampling efficiency. Chapters 4 and 5 examine the floating meadow habitat, and how it has helped to create and maintain the current diversity of Amazonian hylids. This is achieved in Chapter 4 by assessing predictors of the diversity of anurans on the floating meadows, while in Chapter 5 the floating meadow and terrestrial assemblages are compared to determine whether these are distinct or overlap in terms of species composition. The terrestrial assemblage data are then explored further in Chapters 6 and 7. In Chapter 6 I use reproductive modes as a surrogate for other diversity measures and explore how disturbance, environmental and survey variables influence the number of reproductive modes within the assemblage. Finally, in Chapter 7 the impacts of flooding are explored. Climate change may be having a significant impact on the flooded forests and so baseline data and an evaluation of impact on amphibians is vital if we are to understand the potential future effects of climate change on biodiversity in the region.

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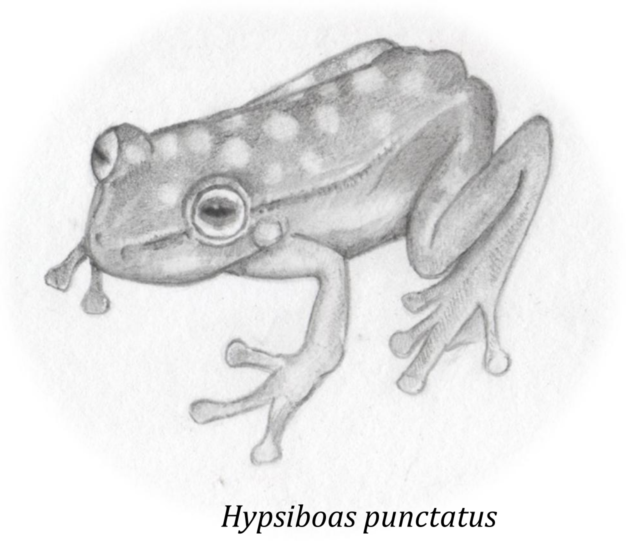
Chapter 2

Study Site & General Methods



Hypsiboas punctatus

2.1 Study Site

Surveys were conducted in the Pacaya-Samiria National Reserve (PSNR), located in the North East of Peru (Fig. 2.1). The Marañon River and the Ucayali River border the reserve, with two smaller rivers flowing through the reserve; the Pacaya River and the Samiria River (Fig. 2.2). The data were collected along the Samiria River, with surveys conducted at four sites: PV1 Shiringal (-4.721173°S, -74.525653°W) (Fig. 2.3), PV2 Tachachocha (-4.879317°S, -74.357028°W) (Fig. 2.4), PV3 Hunguarhai (-5.053327°S, -74.525653°W) and Huisto Cocha (-4.907880°S, -74.474359°W). The three PV sites are guard stations with Huisto Cocha located between PV2 and PV3. The majority of the survey period was spent at PV2 with shorter periods in the other three sites.

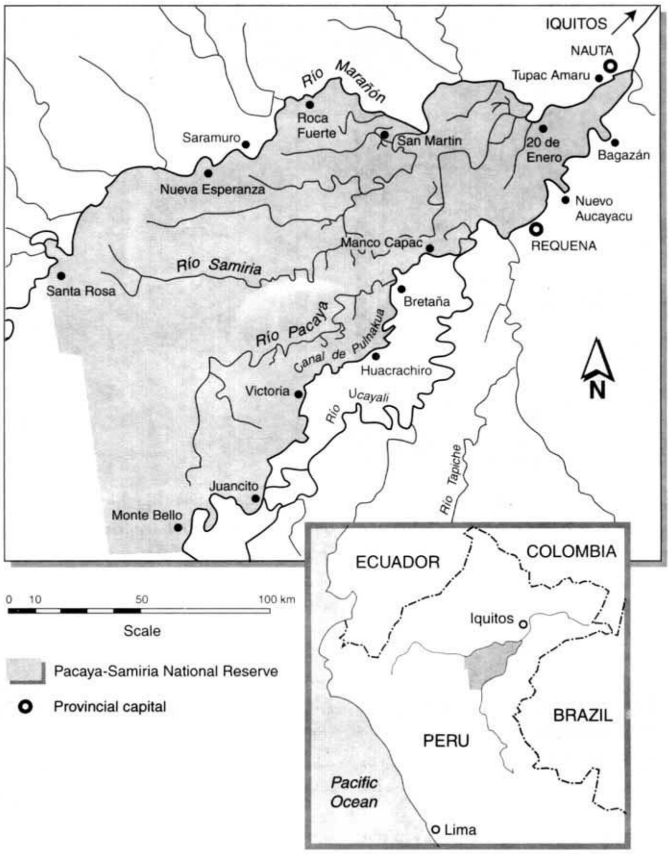


Figure 2.1. The Location of the Pacaya-Samiria National Reserve within Peru, (Reproduced from Barham et al. 1999)

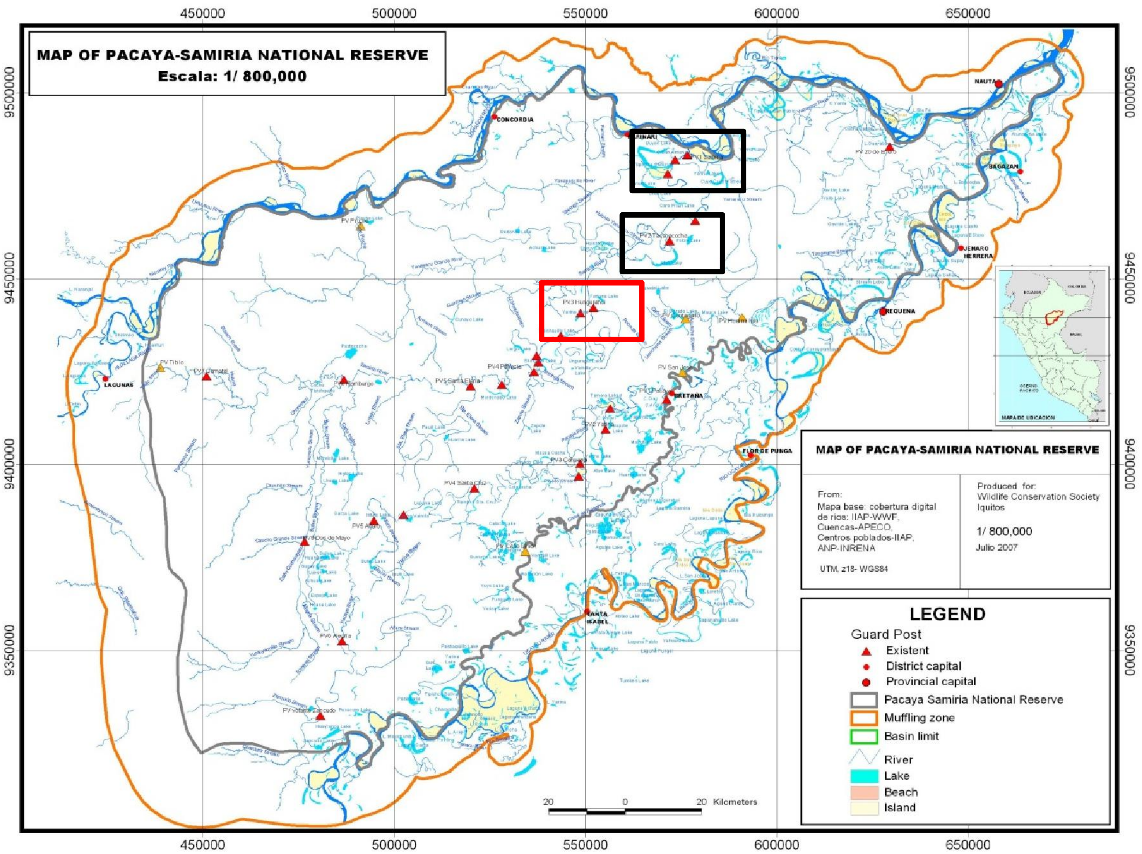


Figure 2.2. Pacaya-Samiria Reserve (the black boxes show the location of figures 2.3 and 2.4 the red shows the location of PV3)

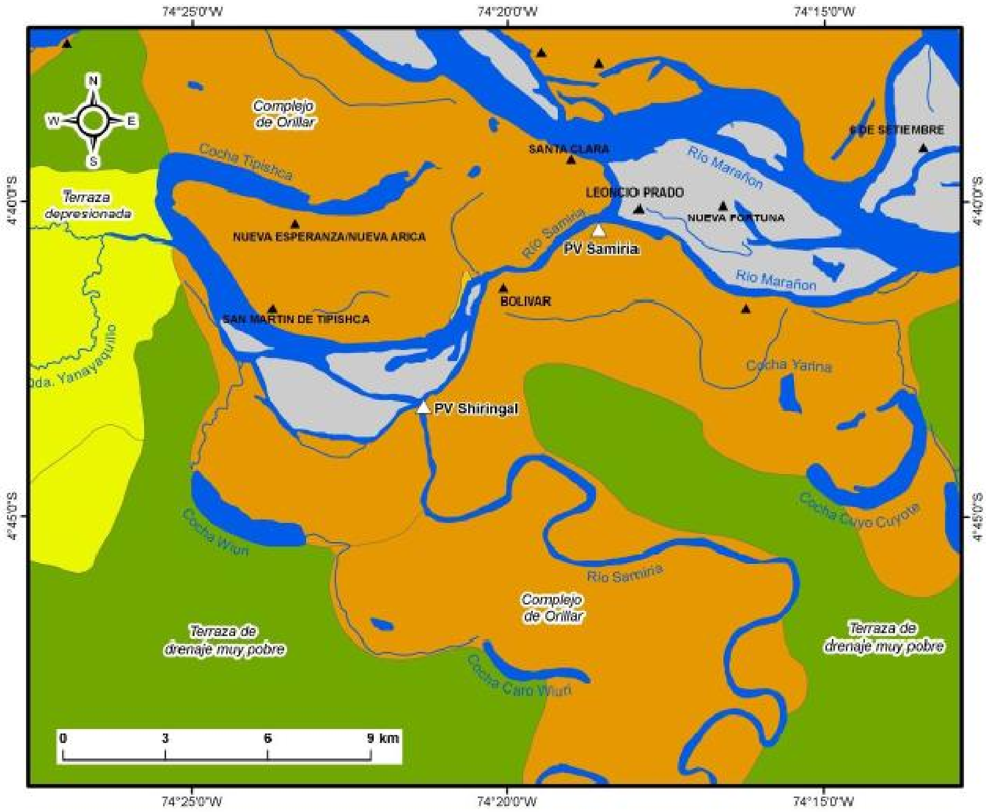


Figure 2.3. The location of PV1 Shiringal at the mouth of the Maranon River

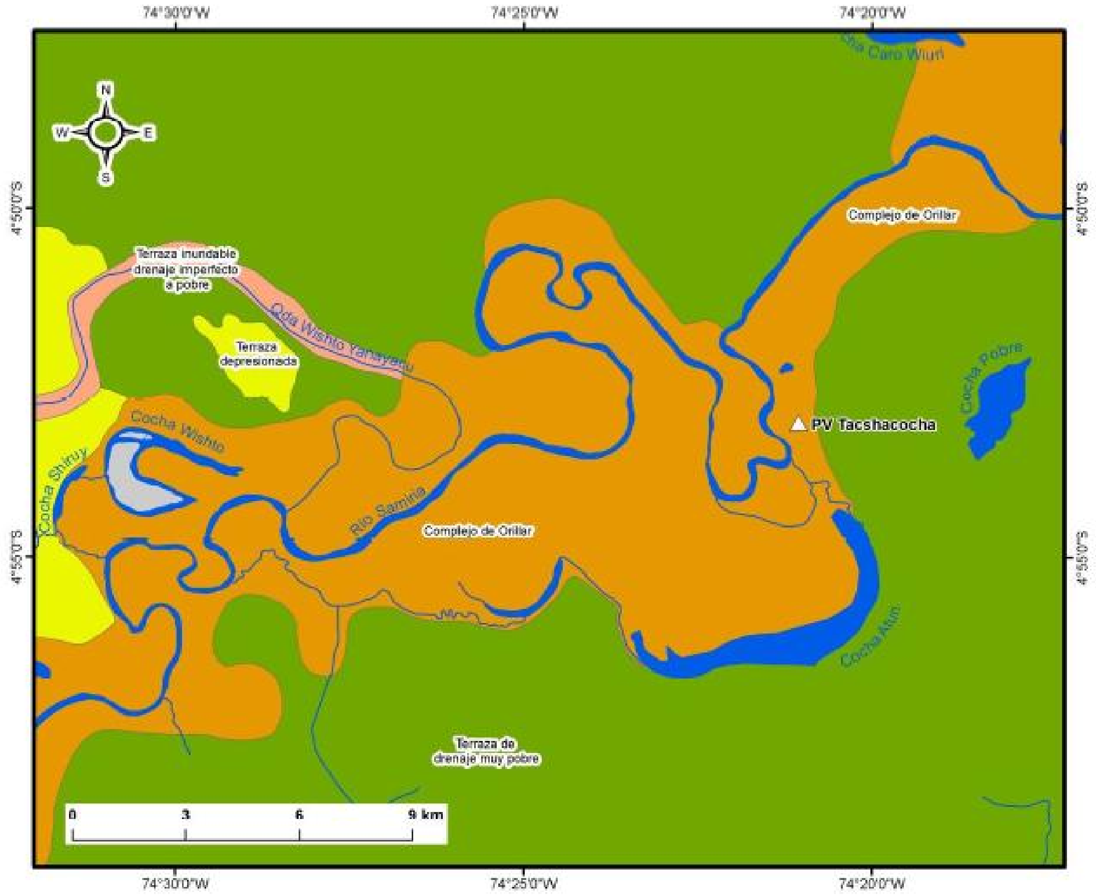


Figure 2.4. The location of PV2 Tacshacocha where the majority of surveys were undertaken

The PSNR is the largest protected reserve in Peru, and one of the largest in South America. The protection of megareserves (at least 1 Mha) in Amazonia is vital for conserving biodiversity (Peres 2005). The PSNR is 2.3 Mha in size and is so vast it is able to support 20 packs of giant river otters (Peres 2005). Unlike most tropical forests the PSNR does not have strict wet and dry seasons. The temperature, humidity and rainfall is fairly constant year round, Figure 2.5 shows the temperature and humidity over a three month period in 2013. Figure 2.6 then shows a subsection of this data showing the daily temperature change.

The PSNR is a flooded forest system which can be inundated with flood water for several months each year. The river level can rise by as much as 10 meters from the low to the high water period. Figure 2.7 shows the yearly water level in 2012 and 2013. Seasonal flooding results in vast areas which are unavailable to terrestrial species, but opens them up to aquatic species. The PSNR forests are of huge economic importance to fisheries along the entire Amazon River (Grockel and Gray 2009). When flood waters enter the forests fish migrate into the flooded forests to spawn (Goulding 1989). These are followed by predators such as pink river dolphins (*Inia geoffrensis)* which have many morphological adaptations useful for fishing in the flooded forests, such as a reduced dorsal fin and the ability to turn their head from side to side (Martin and Da Silva 2004; Eisenberg and Redford 1999; Goulding 1989). In the low water when the forests dry out and river levels drop, fish migrate downriver, followed closely by river dolphins (Martin and Da Silva 2004).

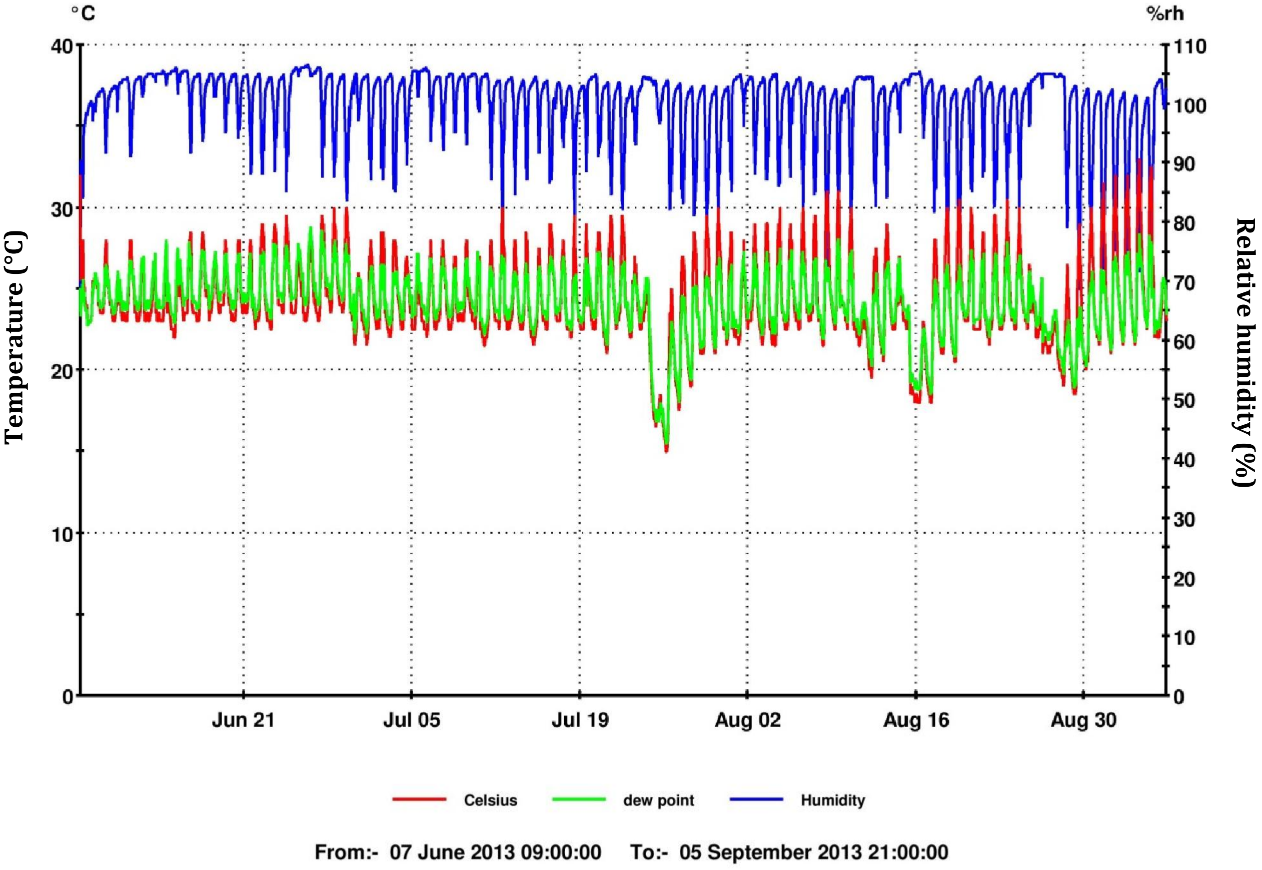


Figure 2.5. Temperature and humidity graph from 7th June 2013 – 5th September 2013.

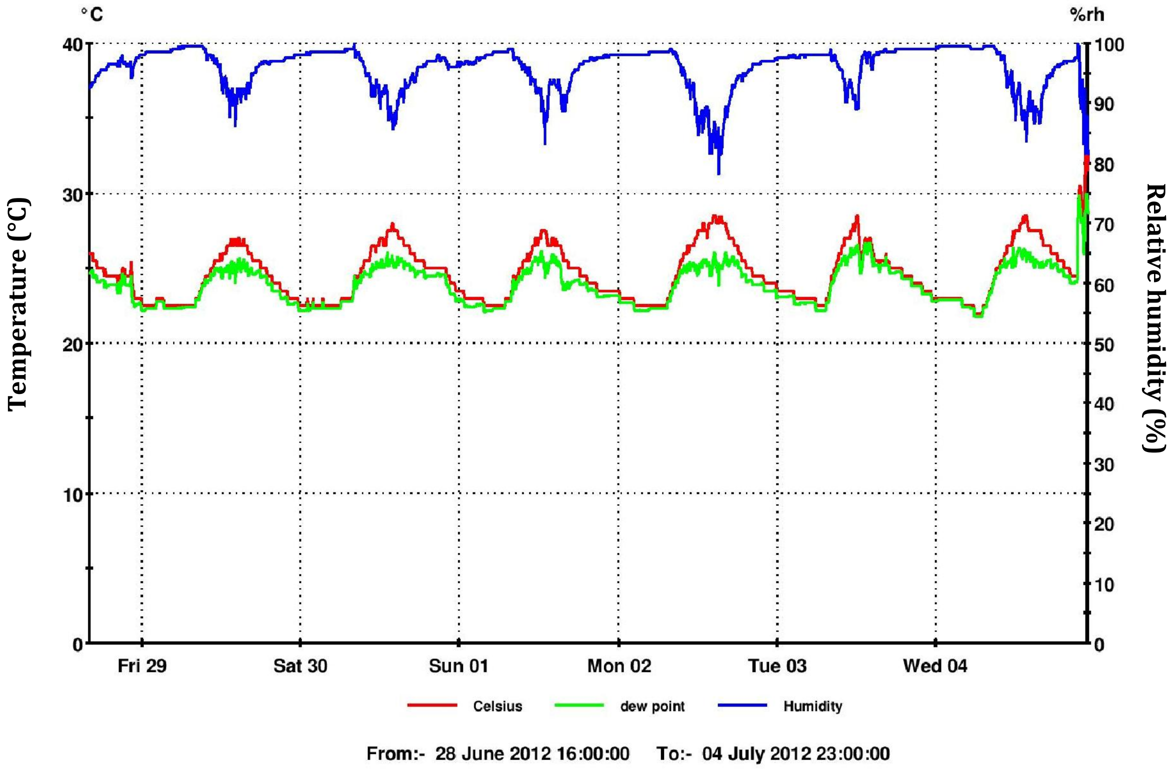


Figure 2.6. Temperature and humidity graph from 26th June 2013 – 4th July 2013.

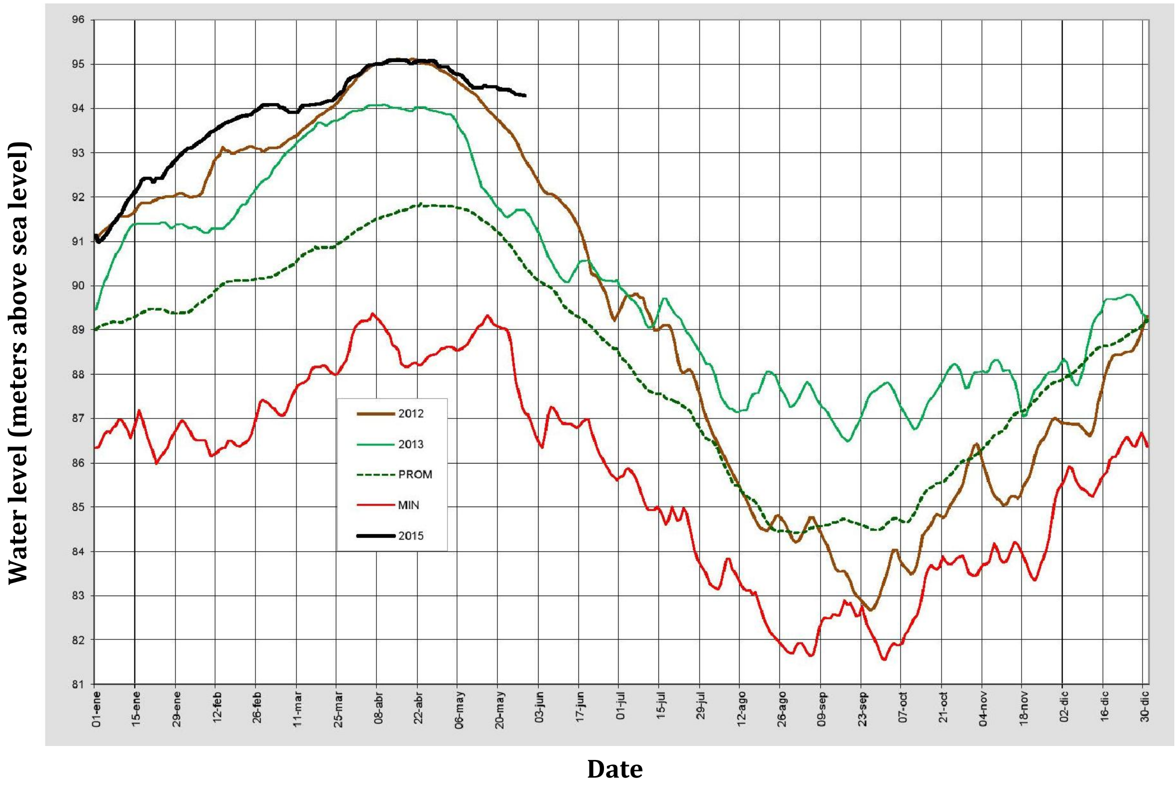


Figure 2.7. Water level of the Maranon River recorded at Nauta from January to December 2012 and 2013 (printed with permission from SEHINAV 2015) PROM represents the average water level.

While flooding may be beneficial to aquatic species, terrestrial species are forced to migrate. Mammals such as peccaries (*Tayassu tajacu* and *T. pecari*), tapir (*Tapirus terrestris*) and red brocket deer (*Mazama americana*) become concentrated within the few remaining non-flooded areas. Often on these 'islands' food can be scarce (Bodmer, 1990). For predators however, such as jaguar (*Panthera onca*) and pumas (*Puma concolor*), this concentration of prey can be beneficial. Seasonal movement is common for many taxa in the flooded forests. Ground dwelling insects, usually found in the leaf litter, will move up into the trees (Pers. obs.). But plants, which cannot migrate, have adaptations enabling them to stay underwater for months at a time. Shoreline shrubs and plants are often submerged for 7-10 months a year, and must flower and fruit in a short period, when above the water (Goulding 1989).

There are approximately 95,000 people living in villages along the boundaries of the PSNR (IRENA 2000). Currently none of these settlements lie within the core area. Yet the impacts of local people, many of whom are Cocama-Cocamilla Indians, have changed significantly in the last few decades (Bodmer and Puertas 2007). Before the area was designated as a reserve many locals lived within the core area along the river banks, utilizing different forest resources (Chocano 2002). However, when the area was designated as a National Reserve the government relocated local communities to the edges. Nevertheless, due to a lack of funding, resources were still heavily used within the reserve. The funding was needed in order to employ guards and set up stations within the reserve to prevent poaching, without this local people were free to enter and utilize all resources. In 1992 a well-funded project began which worked with local communities encouraging participation within the management of resources. This allowed them to use limited amounts legally and different communities managed certain areas which reduced poaching. This resulted in an increase in many species which had previously been persecuted (Bodmer and Purtas 2007).

2.2 Floating Meadow Method

Floating meadows consist of herbaceous water plants and grasses, which grow on the water surface (Junk 1970). They can be found in great abundance in the Amazon, and are quick to colonise areas such as lakes, channels, and rivers. In flooded forests they also grow in tree fall gaps and oxbow lakes. Floating meadows increase the habitat complexity of the aquatic environment (Thomaz et al. 2008), which is normally unavailable for terrestrial species. Floating meadows are therefore a key component of the riverine systems in the Amazon. Floating meadows require high light and nutrient levels, slow moving water and are influenced by the seasonal rise and fall in water levels (Junk 1997). As the water levels rise, bringing with them nutrients, floating meadows rapidly grow into all available space. Around the edges of lakes where water is slow-moving with high light levels, floating meadows are able to form dense mats of vegetation, with extensive root systems. Around the channels and main rivers where water flows faster, vegetation only grows in slow-moving bends, or where it becomes trapped around tree falls. The floating meadow plants that grow in fast-flowing rivers often have reduced root systems, to avoid drifting with the currents (Junk 1970). These root systems are vital for trapping sediments and nutrients for the plants to utilise. Floating meadows growing around the lake edges have longer roots in order to trap sediments more easily in the slow-moving waters (Junk 1970). When sections of floating meadow vegetation dislodge, they become free-floating rafts, which then travel down river with the currents (Schiesari et al. 2003). Heavy rainfall speeds this process breaking down the meadows and dislodging these rafts. After heavy rainfall a larger proportion of vegetation is seen traveling down river (Personal Observation).

Surveys were conducted across three main riverine macrohabitats: (1) the main Samiria River; (2) smaller channels; and (3) open lakes. Where possible the same numbers of surveys were conducted in each macrohabitat. However, few surveys were conducted on the main river as less vegetation was available to survey. The main river only had small sections of vegetation due to the stronger currents that wash floating meadow vegetation down river. The sections that were available to survey had become trapped at the river edge on fallen trees and other debris. Along the channel the slower currents allowed more vegetation to grow along the edges and within slower moving bends (Figs. 2.8, 2.9). Around the lake edge a vast mat of vegetation was able to grow (Figs. 2.10, 2.11); this was possible as the lakes had slow-moving currents. Additionally the lake edge had high light levels, with little tree shade, enabling the vegetation to grow rapidly. This resulted in vegetation which extended for over 50 m beyond the tree-line (Fig. 2.11) the root system of this vegetation was also dense and trapped large amounts of sediment. Finally, rafts of vegetation were also commonly encountered along the channels and main river, as well as some moving across the lake (Fig. 2.12).

Figure 2.8. A small patch of floating meadow within a bend of the channel at PV2

Figure 2.9. Floating vegetation in the low water in the channel at PV2

Figure 2.10. Continuous floating meadow vegetation around the lake edge at PV2

Figure 2.11. Dense floating meadow vegetation on the south facing edge of the lake. In places this extended by over 50 m from the tree line into the lake

Figure 2.12. A floating raft of vegetation which would have broken away from a section of floating meadow upriver

The survey methods were standardised across all sites and macrohabitats. Around lakes survey sites were selected at 100 m intervals. Survey sites refer to the section of floating meadow being surveyed on each separate occasion (details below). Along the channels and main river, where less vegetation was available (Fig. 2.13), all sections of floating meadow were surveyed at least once. As surveys were conducted across two years and different seasons, GPS way-points were recorded and if a survey was close to one undertaken previously, it was recorded as a repeat. This was especially important in the channel and main river where many surveys were considered repeats due to less vegetation available for surveying.

In 2012 preliminary daytime surveys were undertaken (7:00-15:00 hrs). A canoe was used as it enabled the surveyors' closer inspection of the vegetation for hand searching. During these searches the vegetation was also checked for breeding signs such as anuran eggs at the water surface or on the leaves of the vegetation. However no active ampibians were observed at this time.

Ad hoc surveys were also completed when a raft of floating vegetation was encountered or seen from the PV2 guard station (location of 'base camp'). The surveyors would get into a small survey boat and follow the raft down river. Similar methods would be used to the daytime surveys with hand searches to find anurans resting within the vegetation. For every raft encountered the following information was recorded: size, number of plant species, location, number of anuran species and individuals.

All other surveys were undertaken at night (19:00-23:30 hrs) as this is when amphibians were active on this habitat. To complete each survey a boat 10 m in length with an outboard motor was slowly driven into the floating meadow vegetation. This caused the vegetation to part on either side of the boat, with the aim of reducing disturbance. Once the boat was in place the survey would commence, an area of 2 m on either side of the boat was searched for fifteen minutes. For every survey the following variables were recorded: cloud cover, phase of the moon, rainfall, length of boat, number of surveyors, start / end time, maximum / minimum temperature / humidity, GPS way-point, location of meadow, total size of connecting meadow, distance from end of boat to tree-line, number of plant species, maximum height of vegetation and percentage cover of each plant species using two 1 m^2 quadrats. Plant percentage cover did not sum to 100% as the vegetation was measured in layers, meaning water lettuce (*Pistia stratiotes*) could be recorded at 100% while a layer of water hyacinth (*Eichhornia crassipes*) above may be recorded at 50%. By completing the quadrats in this way the variables were not collinear and could therefore be treated as independent in the statistical analysis.

Surveys were undertaken with one field guide, the author and 1-4 field assistants. To locate anurans a spotlight was used by the author (CB2-L1 Clubman Deluxe, LI-ION 9.2AH half-million candle power) with all other researchers using small head torches such as the Petzl tikka. Prior to capture of each individual anuran, the following variables were recorded: plant species, height from water, distance from tree line, distance from boat, and if possible; species, sex and age. The individual was then caught and placed in an individually labelled pot. Once the 15 minute time period was complete each individual was removed and the following recorded; species, age, sex, mass and snout-vent length. Photographs were also taken to confirm identification. After this information was collected each individual was released back onto the habitat where it was initially caught. After the survey the boat was reversed out of the vegetation.

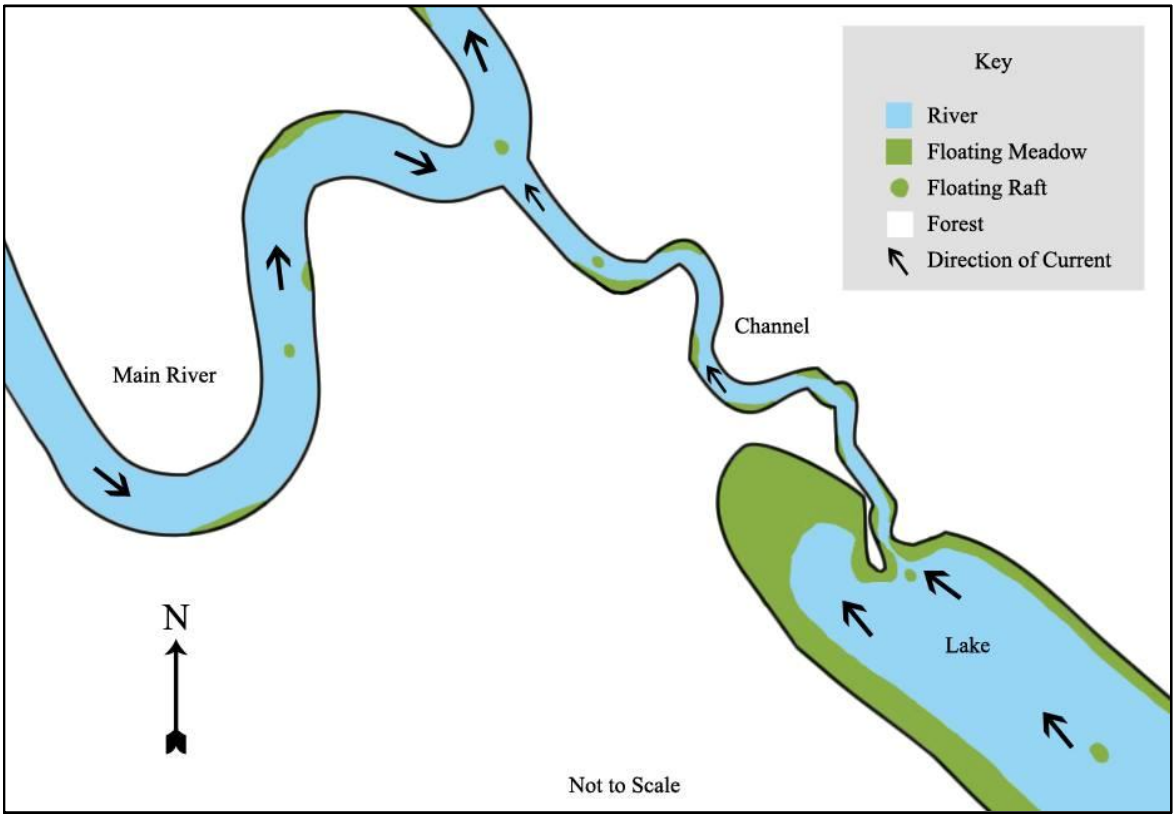


Figure 2.13. The main river, channel and lake at PV2. Although not to scale, this highlights the differences in availability of floating meadows

2.3 Terrestrial Transect Method

Terrestrial transects were undertaken throughout the survey period from April – September 2012 and May – October 2013. Transects were completed during the high, medium and low water period. Diurnal surveys were undertaken between 7:00-11:00 hrs and 15:00-17:00 hrs, with a break during the hottest part of the day as most amphibians were not active during this time. Sunset was around 18:00 hrs throughout the survey period so nocturnal surveys were conducted between 19:00-24:00 hrs. Usually two diurnal and two nocturnal transects were conducted per day. These were alternated so repeats were conducted at different times; see the example timetable below.

	Diurnal		**Nocturnal**	
	First - 7am	**Second**	**First - 7pm**	**Second**
Day 1	Transect 1	Transect 2	Transect 3	Transect 4
Day 2	Transect 2	Transect 1	Transect 4	Transect 3
Day 3	Transect 4	Transect 3	Transect 1	Transect 2
Day 4	Transect 3	Transect 4	Transect 2	Transect 1

The terrestrial habitat was surveyed using 500 m transects. These followed existing paths within the forest used by local people. Most transects were accessed from the main river, on both sides. However, at PV2 four transects were accessed from the channel and one was on the far side of the lake. It was not possible to undertake transects around the Lake due to the dense vegetation and flooding around the edges. The existing transects began at the forest edge and continued into the forest perpendicular to the river. Transects of 500 m were measured using a tape measure and recorded with a GPS; markers were used so that repeats could be undertaken along the same route. A group of 2 – 6 surveyors consisting of one field guide, the author and 1-4 field assistants would slowly walk the transects. Transects were walked slowly ensuring it took at least one hour to cover 500 m. A visual encounter survey method was used to survey for amphibians 2 m either side of the transect and no higher than 2 m above the ground. Sticks were used to move leaf litter on the ground, during diurnal surveys, to hopefully disturb more cryptic leaf litter species (Heyer et al. 1994), avoiding risk of snake bites by hand searching. To locate anurans at night a spotlight was used by the author (CB2-L1 Clubman Deluxe, LI-ION 9.2AH half-million candle power) all other researchers used small head torches such as the Petzl tikka.

Each time an amphibian was located the following was recorded: time, species, microhabitat, height from ground, temperature and humidity. The individual was then captured and further information was recorded including sex, age, snout-vent length, body mass and photographs were taken. Each individual was then released as soon as possible where it was found.

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Chapter 3

Amphibian species richness in the Pacaya-Samiria National Reserve, Peru

Ameerega trivittata

3. Abstract

Species richness is a useful tool for guiding conservation. However, measuring species richness can be difficult, especially in tropical environments. Species accumulation and rarefaction curves are often used to help estimate richness and determine whether survey effort is adequate. In this chapter rarefaction curves were used to estimate the species richness for the terrestrial and floating meadow habitat. Eighty-one species have been recorded in the Pacaya-Samiria National Reserve from 1996 - 2013 representing three orders and eleven families. The most common family is Hylidae with 40 species, followed by Leptodactylidae with 13. The rarefaction curves suggest that further surveys on both habitats may have identified more species. The analysis in subsequent chapters has therefore attempted to account for differences in search effort, sample sizes, methods and possible unobserved species.

3.1 Introduction

Measuring species richness is vital for conservation, as it can help to identify ecosystems, habitats or sites on which to focus limited resources. It can offer a measure of habitat quality, and even degradation, whilst improving understanding of community ecology (Morin 2011). However, measuring species richness can be extremely difficult (Magurran and McGill 2011; Gotelli and Colwell 2001). This is especially true in tropical environments, and with cryptic species which can be hard to detect (Gotelli and Colwell 2001). Indeed, in tropical sites which have been surveyed over many years, new species are discovered which have either not been recorded before, or are new to science (Catenazzi et al. 2013; Schlüter et al. 2004; Duellman 1999; Duellman 1995). In Manu National Park, Peru, for example, 155 amphibian species have been recorded since 1960. However, each year more species are added to this list and the total richness is expected to rise further (Catenazzi et al. 2013).

A species accumulation curve is a useful tool when comparing richness from different sites or habitats. Such curves can be extrapolated to predict actual species richness if not all species are observed in the survey period (Colwell et al. 2012). Species accumulation curves are created by plotting the number of surveys, or individuals, against the number of species observed. This curve will increase rapidly as species are added to the list, but slows when most species have been observed. The shape of the curve depends on the assemblage being surveyed, the more species in an assemblage, such as amphibians in a tropical forest, the more rapidly the curve will rise (Gotelli and Colwell 2011). If the assemblage composition is uneven, with few common species and many rare species; the curve will rise more slowly. This is because the majority of individuals encountered will be the common species, rather than the rare ones which have not yet been detected. There are four types of curves which can be used: individual accumulation, individual rarefaction, samples accumulation and samples rarefaction (Magurran and McGill 2011). An accumulation curve records the number of species during data collection, while rarefaction is produced by repeatedly re-sampling the data and plotting the average (Gotelli and Colwell 2001).

Few amphibian surveys have been undertaken in Pacaya-Samiria National Reserve (PSNR). An amphibian species list was included in Soini et al. (1996) which evaluated the use of wildlife in the PSNR. The IRENA (2000) report also contains a species list for the PSNR, but this is for the entire reserve. Only two papers have been published specifically on amphibian surveys in the PSNR (Upton et al. 2011; Upton et al. 2014). Upton et al. (2011) includes a species list collected over 40 survey days in 2009 and 2010 (Appendix 1). This identified 40 species at the PV3 guard station. Upton et al. (2014) describes anuran activity on floating meadows in the reserve in both 2009 and 2012 (Appendix 1). This paper contains a summary of floating meadow work undertaken in the Amazon Basin, and identifies 27 anuran species and one caecilian using floating meadows across the Amazon Basin. Unpublished data have been collected on amphibians in the PSNR by Durrell Institute of Conservation and Ecology (DICE) undergraduates and Operation Wallacea students (2003-2011). This chapter will collate these data to create a species list for the PSNR.

This chapter aims to test the following hypothesis:

1. This research has observed all amphibian species present in the PSNR
2. Adequate surveys were undertaken to gain comparable estimates of species richness for the different sites, habitats and water levels.

3.2 Methods

3.2.1 Data Collection (2003 – 2013)

Prior to the start of data collection for this thesis in 2012 and 2013, amphibian surveys in the PSNR were conducted by DICE (Durrell Institute of Conservation and Ecology) and Operation Wallacea students. Visual encounter surveys were used for terrestrial amphibians, but the transect length varied from year to year. Transects were undertaken from 2003 – 2013: 2-4 km (2003, 2005), 100 m (2009), 600 m (2010), 500 m (2011, 2012, 2013) (Faulkner 2004; Perez 2005; Upton 2010; Popplewell 2011; Thompson 2012). All terrestrial transects were conducted as per the general methods in Chapter 2.3 Terrestrial Transects; however, nocturnal surveys were not conducted in 2003.

Riverine transects were undertaken by previous students in 2010 (1000 m) and 2011 (600 m). In both years the transects were completed by driving a motorized boat slowly along the river bank. Amphibians were spotted, along the bank, using a large spot light (CB2-L1 Clubman Deluxe, LI-ION 9.2AH half-million candle power). When areas of floating vegetation were located the boat was driven alongside to search for anurans. This method involved some areas being more intensely searched than others so it was adapted in 2012 as per the general methods in Chapter 2.2 Floating Meadow Survey. See Table 3.1 for a breakdown of all surveys conducted since 2003.

Due to the impacts of variation in observer effort and experience across the years only data collected in 2012 and 2013 was used in the analysis. Surveys were always undertaken by the author, one experienced field guide and several student volunteers. The number of surveyors was used in the analysis as a surrogate for search effort.

Table 3.1. A summary of surveys undertaken by DICE and Operation Wallacea students in the PSNR and the number of individuals and species encountered (2003, 2009, 2010, 2012 and 2013). This thesis uses the data collected in 2012 – 2013 (These are highlighted with a *).

Site	Start date	End date	Length (days)	Survey hours	No. of individuals	No. of species
PV5	22/06/2003	10/07/2003	19	Unknown	316	25
PV3	30/05/2009	16/06/2009	18	52	301	24
PV3	15/06/2010	10/07/2010	22	40	387	27
Huisto	06/07/2010	15/07/2010	10	16	253	24
PV2	17/07/2010	09/08/2010	13	8	454	28
PV1	31/07/2010	09/08/2010	10	14	246	20
PV3	09/06/2011	21/06/2011	13	41	294	22
Huisto	23/06/2011	06/07/2011	14	Unknown	367	20
PV2*	24/04/2012	28/05/2012	34	69	511	32
Huisto*	05/06/2012	20/06/2012	16	26	268	23
PV2*	20/06/2012	30/07/2012	41	88	561	30
PV2* (down river)	20/07/2012	22/07/2012	3	5	39	8
PV1*	31/07/2012	08/08/2012	8	20	123	14
Samiria River Mouth*	10/09/2012	20/09/2012	10	18	185	16
PV2*	21/09/2012	28/09/2012	9	11	115	14
PV2*	05/06/2013	27/07/2013	53	135	841	40
PV1*	26/08/2013	03/09/2013	9	30	258	26
PV3*	07/09/2013	15/09/2013	9	29	228	17
PV2*	04/09/2013 16/09/2013	06/09/2013 01/10/2013	19	49	664	31
TOTALS			**330**	**651**	**6411**	**69**

3.2.2 Rarefaction curves

Extrapolated rarefaction curves were used to compare the different sites and habitats (Magurran and McGill, 2011). The rarefaction curves were created using EstimateS (Colwell 2013) and were calculated using both the number of individuals and the number of surveys. EstimateS computes this by re sampling the data repeatedly without replacement and averaging these results (Colwell et al. 2012). This means the data can be rescaled so that locations / seasons with different search effort can be compared (Rossi 2011). The rarefaction curves were rescaled by individuals as this creates a smooth curve for comparison between sites and habitats (Chazdon et al. 1998; Gotelli and Colwell 2001). This is particularly important when comparing the floating meadow and terrestrial habitats due to the differences in methods. Rarefaction curves were then extrapolated to see whether all species had been encountered and if adequate surveys had been undertaken. The extrapolation of the data is calculated using Chao2 (Colwell et al. 2012). This was done purely to assess whether surveys were adequate and not to determine how many species are left to find in the area. The rarefaction graphs were created in R using the plot function (Chang 2013).

Rarefaction curves were created for both the floating meadow and terrestrial habitat for comparison. These two habitats were treated separately due to the assemblage differences explored in Chapter 5. Comparisons were then made between different water levels, sites and macrohabitats.

3.3 Results

Eighty-one species have been recorded in the PSNR represented by 11 families and three orders (Table 3.2). The most common family is Hylidae with 40 species, followed by Leptodactylidae (13 species), Strabomantidae (10), Aromobatidae (4), Bufonidae (3), Dendrobatidae (3), Microhylidae (3), Plethodontidae (2), Ceratophryidae (1) and Typhlonectidae (1). Anurans (frogs and toads) comprised 78 species; Caudata (salamanders and newts) was only represented by two species and Gymnophiona (caecilians) by just one species.

Some species were common, encountered in most years such as *Rhinella margaritifera, Dendropsophus haraldschultzi, Dendropsophus triangulum, Hypsiboas punctatus, Osteocephalus taurinus, Sphaenorhynchus lacteus, Leptodactylus discodactylus*, and *Hamptophryne boliviana*. Other species were rare and only encountered on few occasions, such as *Dendropsophus leali, Hypsiboas boans, Phyllomedusa tomopterna, Lithodytes lineatus* and *Pristimantis lacrimosus*. Indeed, even after seven years of surveys new species were still observed in 2012 and 2013 such as *Dendropsophus bokermanni, Dendropsophus koechlini, Scinax cruentommus, Scinax funereus, Pristimantis lacrimosus, Pristimantis ockendeni* and *Pristimantis waoranii.*

Table 3.2. Complete species list for the Pacaya-Samiria Reserve from 1996-2013
(Soini et al. 1996; IRENA 2000; Faulkner 2004; Perez 2005; Upton 2010; Popplewell 2011; Thompson 2012)

Species (81)	IIAP* 1996	IRENA* 2000	Faulkner 2003	Perez 2005	Upton 2009	Opwall DICE 2010	Opwall DICE 2011	Upton 2012	Upton 2013	Total
Aromobatidae (4)										
Allobates brunneus						58				58
Allobates femoralis	x	x	69			2	3			74
Allobates 'samiriensis' sp nov						11		7	1	8
Allobates trilineatus	x		2				2			4
Bufonidae (3)										
Amazophrynella minutes	x	x								2
Rhinella margaritifera (comp)	x	x	104		39	11	54	12	2	222
Rhinella marina	x	x	1	x	1	78		46	9	135
Ceratophryidae (1)										
Ceratophrys cornuta		x		x						2
Dendrobatidae (3)										
Ameerega hahneli		x	41		2			6		49
Ameerega picta	x		1							1
Ameerega trivittata		x	10		5		1	11		27
Hylidae (40)										
Cruziohyla craspedopus		x								1
Dendropsophus bifurcus		x							76	76
Dendropsophus bokermanni									3	3
Dendropsophus brevifrons						1		3	9	13
Dendropsophus haraldschultzi	x	x			3	8	7	42	74	134
Dendropsophus koechlini									1	1
Dendropsophus leali		x	1	x				7		8
Dendropsophus minutus		x					1			1
Dendropsophus parviceps						6		7	5	18
Dendropsophus riveroi		x								1
Dendropsophus rossalleni		x			16	5	6	19	2	48
Dendropsophus timbeba						2		1	1	4
Dendropsophus triangulum	x	x			34	115	95	341	72	657
Hypsiboas boans		x	1	x		13		1		15
Hypsiboas calcaratus		x								1
Hypsiboas cinerascens		x				1		1	2	4
Hypsiboas fasciatus	x	x		x		5		5	1	11
Hypsiboas geographicus	x	x	8			1		27	1	37
Hypsiboas lanciformis	x	x		x		16	4	32	8	60
Hypsiboas microderma			2				4			6
Hypsiboas punctatus	x	x		x	14	86	146	331	515	1092
Osteocephalus cabrerai					2					2
Osteocephalus deridens					1			11	78	90
Osteocephalus leprieurii	x	x				8		33	18	59
Osteocephalus planiceps		x	1			1		18	19	39
Osteocephalus taurinus	x	x	2		2	22	4	74	83	187
Phyllomdeusa vaillantii			1							1
Phyllomedusa tarsius	x	x								2
Phyllomedusa tomopterna		x					2		1	3
Scarthyla goinorum	x	x	3			35	12	184	38	272
Scinax cruentommus								2	1	3
Scinax funereus								3	1	4

Scinax garbei	x	x				1		9	46	56
Scinax pedromedinae	x	x			1	48	2	16	24	91
Scinax ruber	x	x			1	1		4	6	12
Sphaenorhynchus carneus		x			3	4	23	81	47	158
Sphaenorhynchus dorsiae	x	x			44	19	14	24	13	114
Sphaenorhynchus lacteus	x	x			23	4	5	49	29	110
Trachycephalus resinifictrix	x	x				2		1		3
Trachycephalus typhonius	x	x		x				1	1	2
Leptodactylidae (13)										
Adenomera andreae	x	x		x	2	77		10	11	100
Adenomera andreae / hylaedactyla			5				71			77
Adenomera hylaedactyla	x	x		x	5	44			2	51
Engystomops freibergii		x								1
Engystomops petersi	x			x		30				30
Hydrolaetare schmidti		x		x		1				1
Leptodactylus discodactylus	x	x	36	x	35	1	6	30	58	166
Leptodactylus leptodactyloides		x		x	30	267	13	84	95	489
Leptodactylus pentadactylus	x	x		x	2	14	45	16	58	135
Leptodactylus petersii				x	13	127	38	27	35	240
Leptodactylus stenodema			1							1
Leptodactylus wagneri	x	x	1	x	21		42		9	73
Lithodytes lineatus	x	x	2	x		3		1	1	7
Pseudopaludicola ceratophryes		x		x						2
Microhylidae (3)										
Chiasmocleis ventrimaculata		x						1		1
Ctenophryne geayi	x	x				1		1		2
Hamptophryne boliviana	x	x	13		2	5	11	3	12	46
Pipidae (1)										
Pipa pipa		x	1			1		3	2	7
Strabomantidae (10)										
Noblella myrmecoides	x	x		x						3
Oreobates quixensis	x	x		x			1			1
Oreobates saxatilis	x			x						2
Pristimantis altamazonicus			2			14		15	51	82
Pristimantis diadematus	x	x	4	x						4
Pristimantis lacrimosus (comp)								1	1	2
Pristimantis martiae			4							4
Pristimantis ockendeni								1		1
Pristimantis variabilis	x	x		x						3
Pristimantis waoranii									2	2
Plethodontidae (2)										
Bolitoglossa altamazonica		x				5		3	23	31
Bolitoglossa peruviana	x								1	1
Typhlonectidae (1)										
Typhlonectes compressicauda				x						1

*IRENA & IIAP Surveys were conducted across the entire PSNR however all other surveys were only undertaken along the Samiria River

X = present but not counted

Opwall – Operation Wallacea

(Comp) – Complex which is likely to consist of several different species not yet separated

3.3.1 Terrestrial vs floating meadow rarefaction

Rarefaction curves were created using the 2012 and 2013 data. The terrestrial habitat has higher species richness than the floating meadows (Figs. 3.1-3.2). The estimated number of species at 1000 individuals was 59 species for terrestrial and 20 on floating meadows. When extrapolated to 5500 individuals only four more species were added to the terrestrial richness while five were added to the floating meadows. The number of species observed on floating meadows rose rapidly initially suggesting there are many common species with fairly even abundances (Fig. 3.2). However in the terrestrial assemblage the increase is more gradual taking a long time to plateau (Fig. 3.2). This is due to the high species richness, comprising mostly rare species.

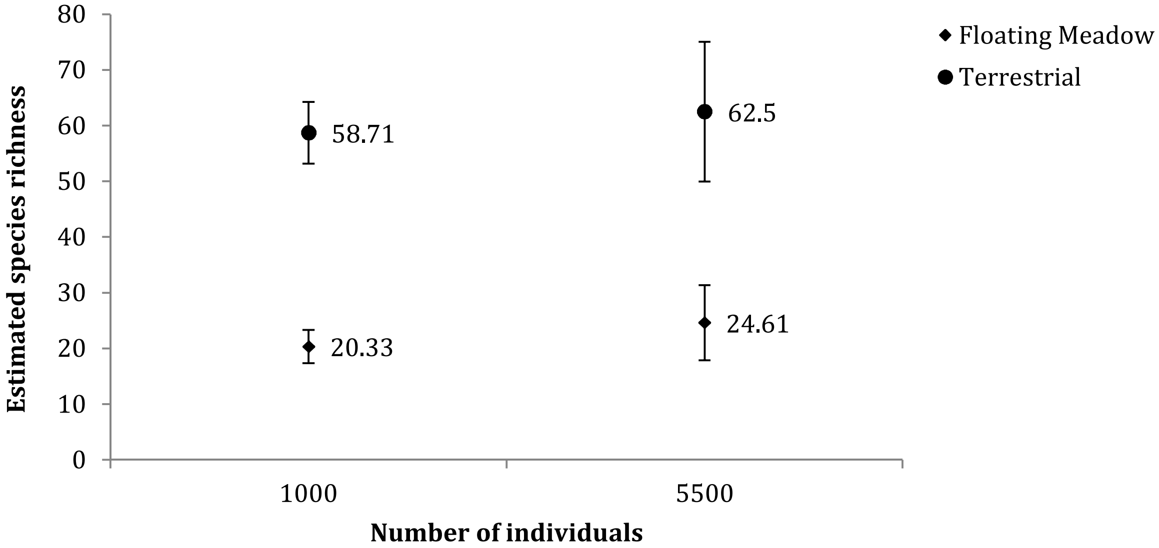

Figure 3.1. Estimated species richness for the floating meadow and terrestrial habitats at both 1000 and 5500 individuals, with 95% confidence intervals. The 1000 individuals estimate represents actual data while the 5500 estimates are extrapolated, hence the larger confidence intervals.

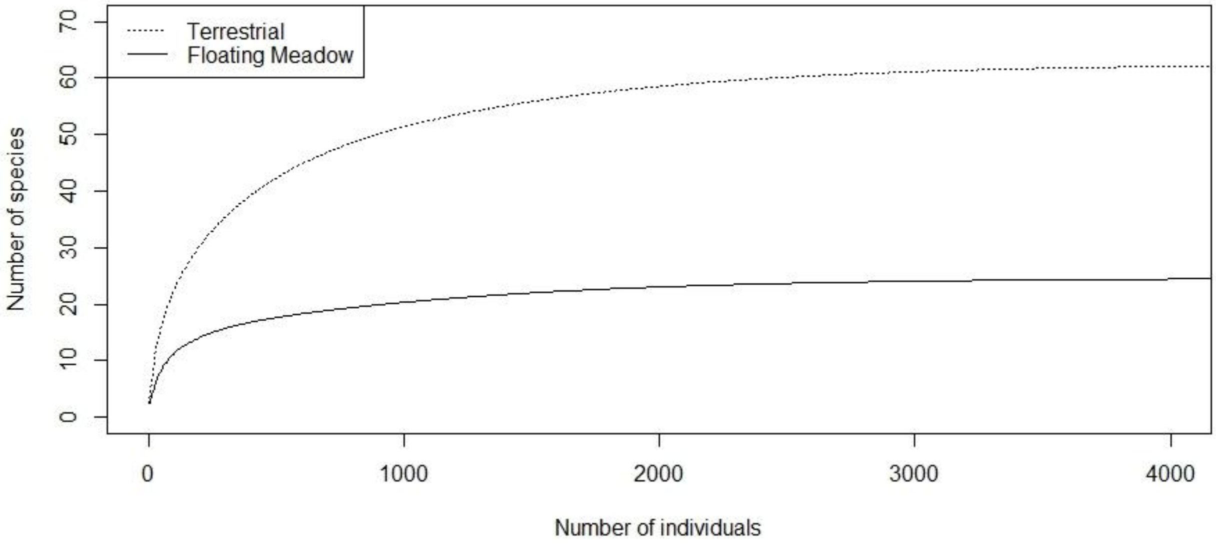

Figure 3.2. Extrapolated rarefaction curves for the floating meadow and terrestrial habitat estimated by number of individuals. 1260 individuals were observed on terrestrial transects while 1498 individuals were observed on floating meadows.

3.3.2 Floating meadow rarefaction

The floating meadow rarefaction curves were further broken down to assess the different water levels, sites and macrohabitats. The species richness is slightly higher in the low (15 species) and high (14) water periods compared to the transition period (11) (Fig. 3.3). This is consistent with the extrapolated results, but the confidence intervals are larger due to the low sample sizes; especially in the high water. The survey rarefaction curve increases rapidly in the low water, while the high and transition periods increase more slowly (Fig. 3.4). However, when calculated by individuals all three periods rise together, yet the transition period plateaus more quickly and at a lower richness (Fig. 3.5).

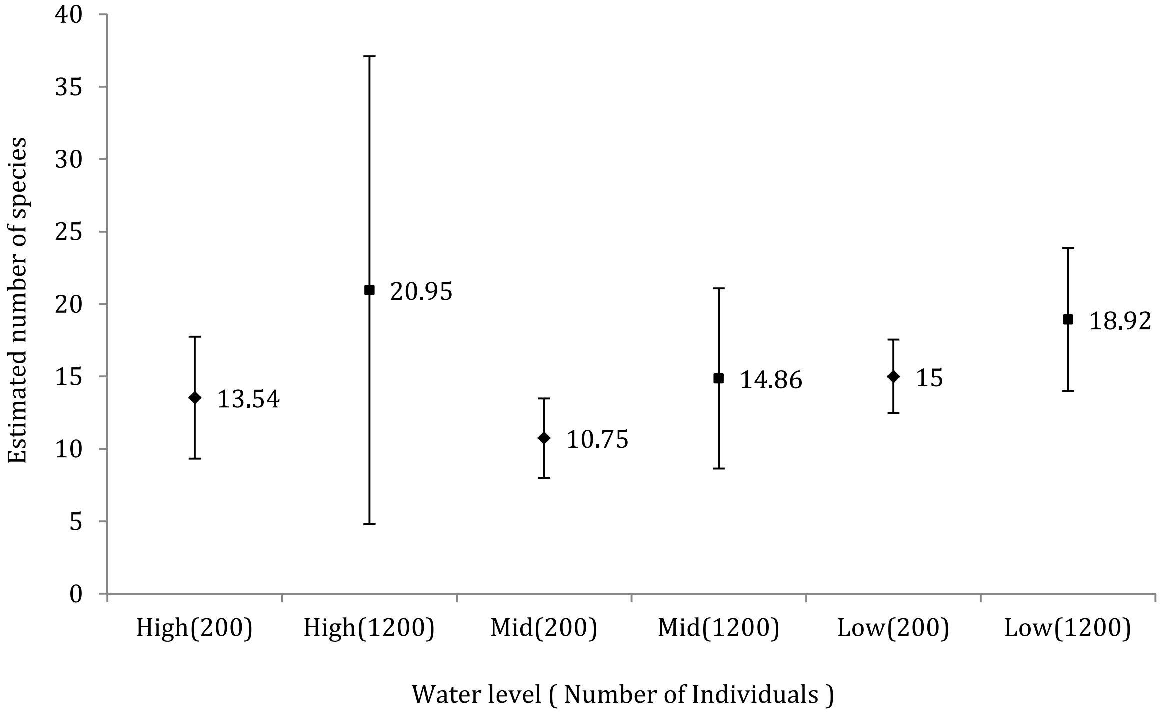

Figure 3.3. Estimated species richness for the three water level periods, with 95% confidence intervals. The 200 individual estimates represent actual data while the 1200 estimates are extrapolated from the data hence the larger confidence intervals.

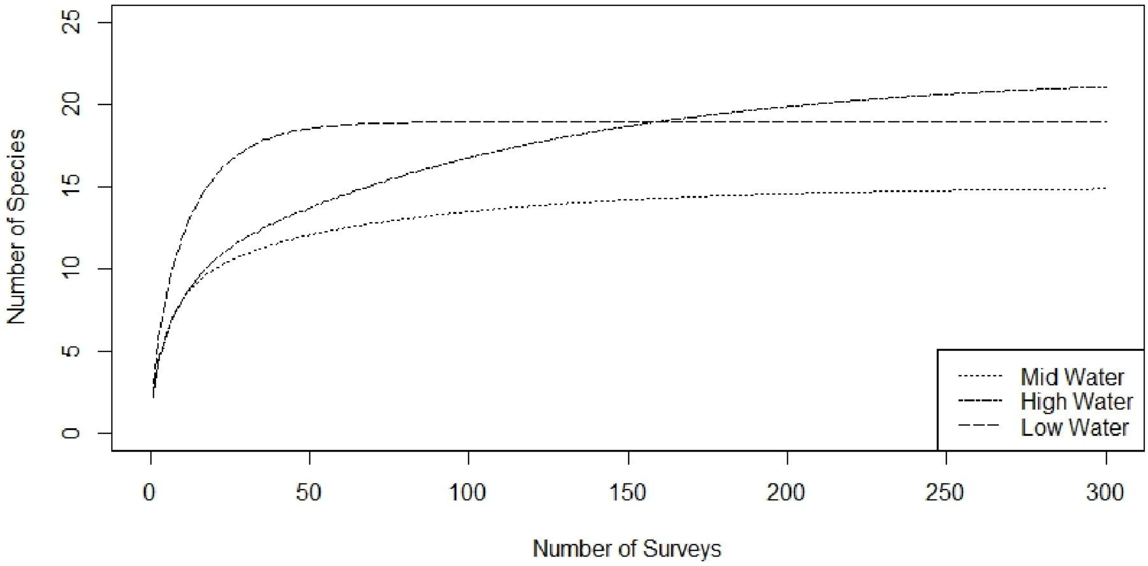

Figure 3.4. Extrapolated rarefaction curves for the three water periods estimated by number of surveys. In the transition period 78 surveys were conducted, with 54 in the high and 28 in the low.

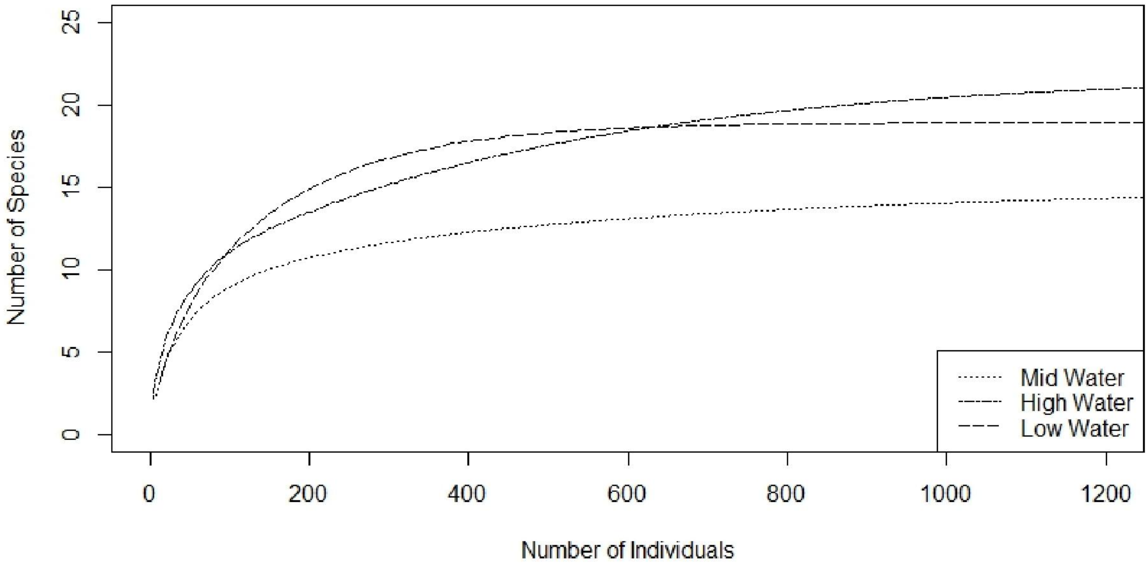

Figure 3.5. Extrapolated rarefaction curves for the three water periods estimated by number of individuals. In the transition water 568 individuals were observed, with 228 in the high and 318 in the low.

The rarefaction curves for the three macrohabitats follow a similar pattern of a rapid increase, slowing after 100 individuals had been encountered (Fig. 3.6). The main river curve plateaus more quickly than the other two macrohabitats and seems to have a slightly lower species richness. The three main sites surveyed have similar curves to start, increasing sharply, but Huisto and PV1 have a lower richness than PV2; this may be influenced by survey effort, as more surveys were conducted at PV2 (Fig. 3.7).

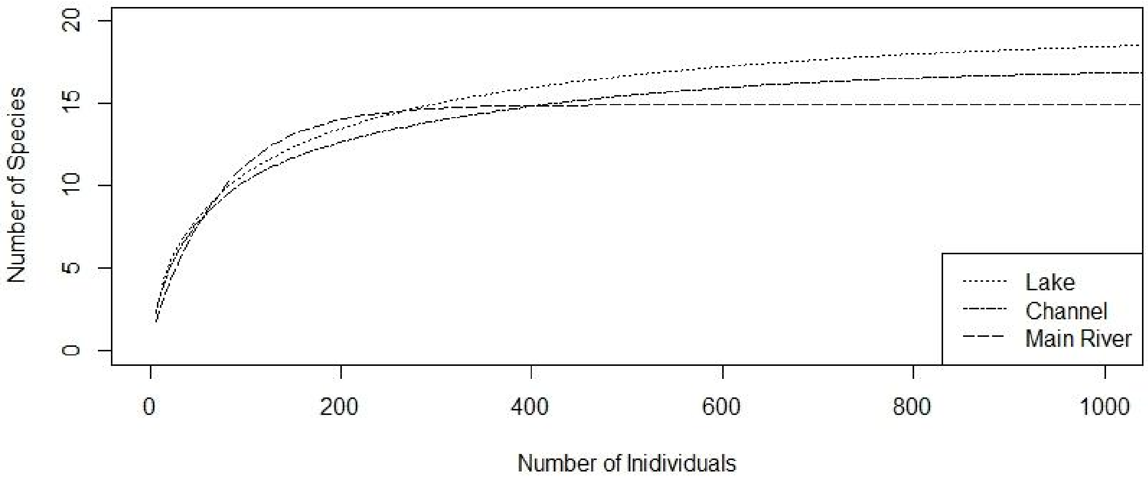

Figure 3.6. Extrapolated rarefaction curves for the three macrohabitats, estimated by number of individuals. In total 554 individuals were observed in the lake, 421 in the channel and 116 on the main river.

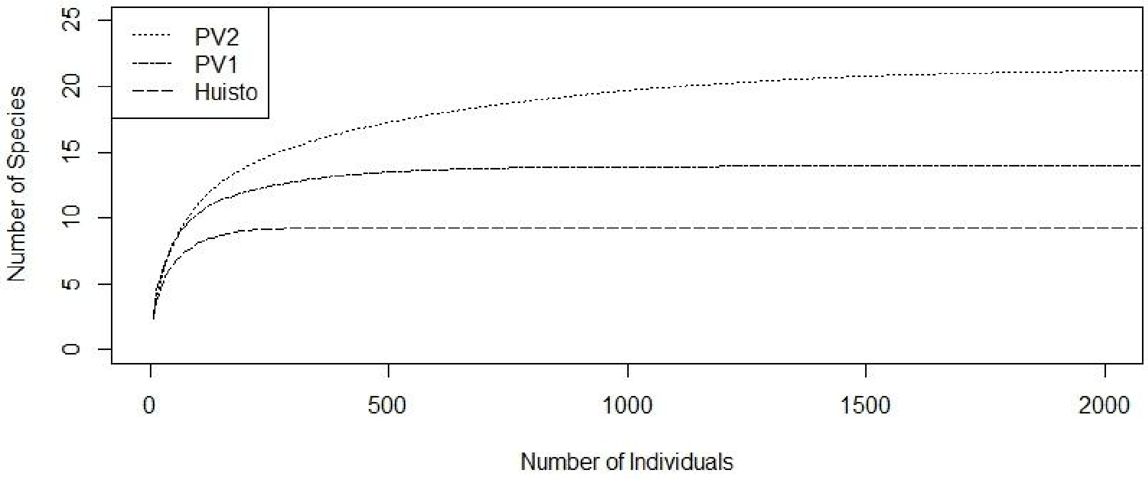

Figure 3.7. Extrapolated rarefaction curves for the three main sites surveyed, estimated by number of individual. In total 1116 individuals were observed at PV2, 189 at Huisto and 203 at PV1.

3.3.3 Terrestrial rarefaction

Rarefaction curves were created for the terrestrial data analysed in Chapters 6 and 7. The PV2 data were split into the three main transects and also the three water periods (Fig. 3.8 + 3.9). The data collected in 2013 in the low water was split into the three sites surveyed, PV1, PV2 and PV3 (Fig. 3.10). Rarefaction curves for the three main transects at PV2 show similar patterns, but transect two continues rising after 100 individuals while the other two transects begin to plateau. This results in higher estimated species richness on transect two. When comparing the different water levels at PV2 the high water had the lowest estimated richness, the curve plateaus after 200 individuals, while both the transition and low water continue to increase. This may be due to search effort as fewer transects were conducted in the high water than the other two periods. Finally when comparing the three sites, PV2 had a much higher estimated richness, while PV3 and PV1 plateau more quickly.

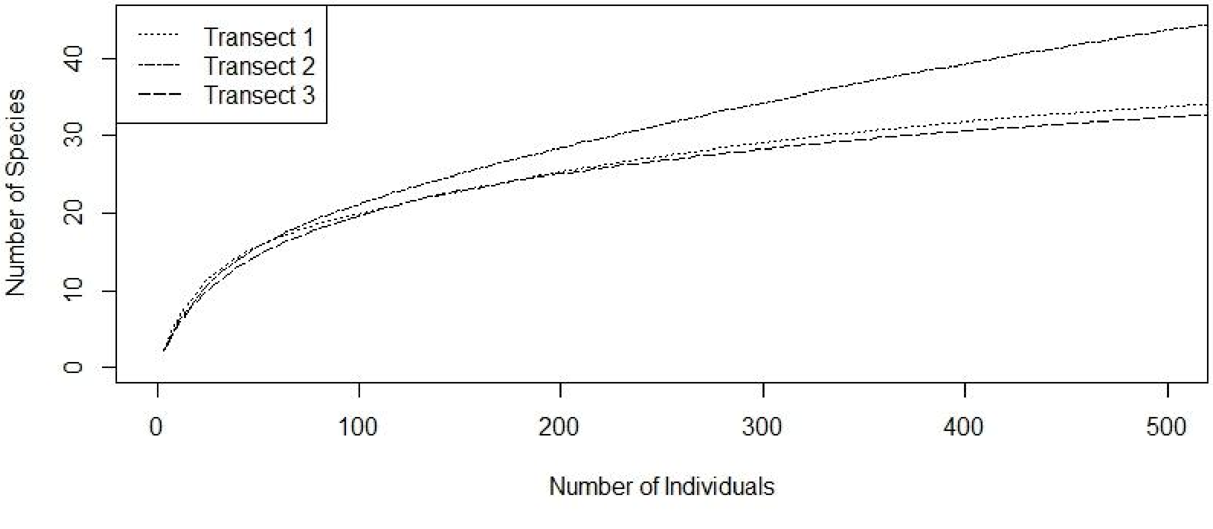

Figure 3.8. Extrapolated rarefaction curves for the three main transects surveyed at PV2 by number of individuals. In total 152 individuals were observed on transect one, 209 on transect two and 256 on transect three.

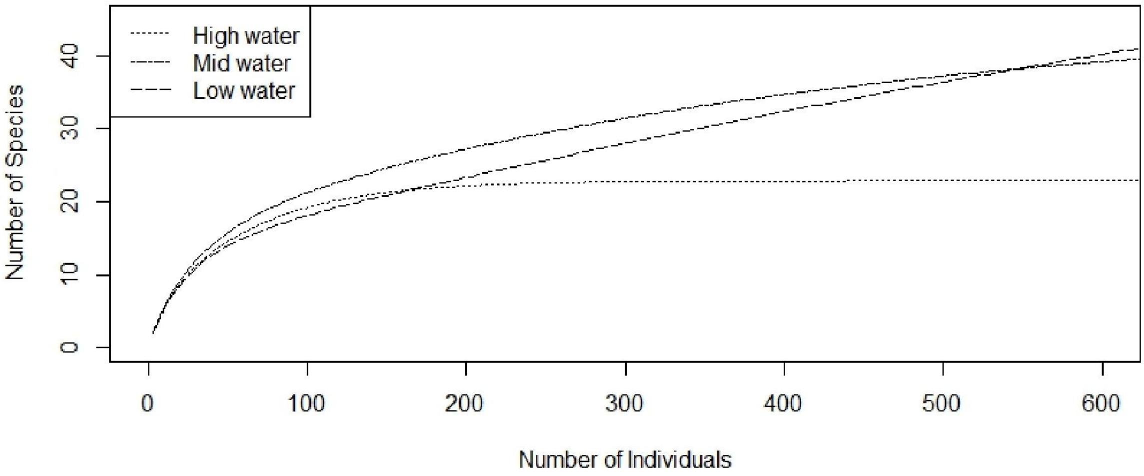

Figure 3.9. Extrapolated rarefaction curves for the three water level periods (see Chapter 6) by number of individuals. In total 71 individuals were observed in the high water, 288 in the transition period and 257 in the low water.

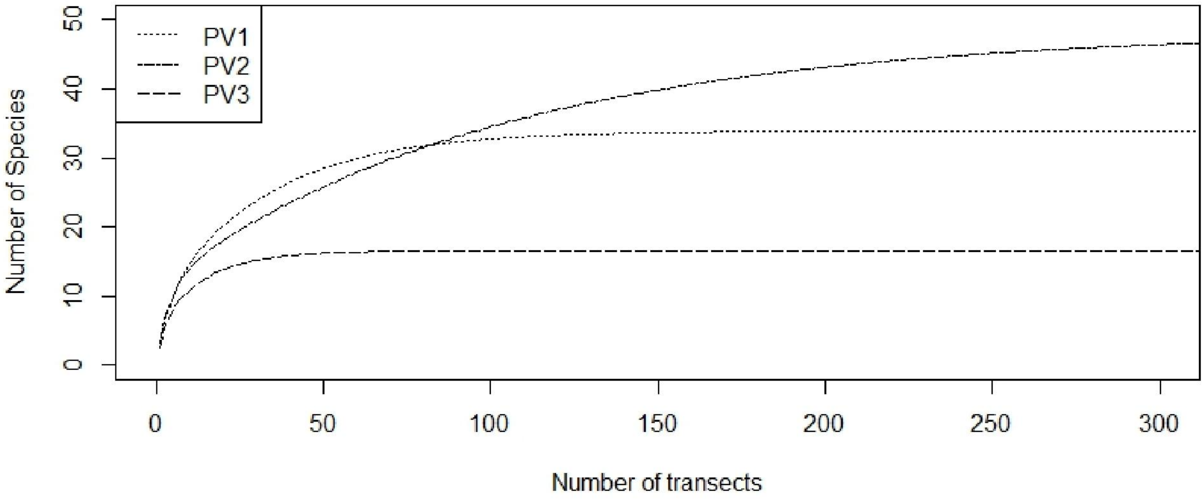

Figure 3.10. Extrapolated rarefaction curves for the three sites surveyed in the low water during 2013 (see Chapter 6) by number of transects. At PV2 42 transects were undertaken, 22 at PV1 and 21 at PV3.

3.4 Discussion

Eighty-one species of amphibians have been recorded in the PSNR since 1996. Whilst some records are for the entire reserve the majority were collected between the PV1 and PV5 guard stations along the Samiria River. Overall Peru has 561 amphibian species (07/06/2015 AmphibiaWeb) of which 14% can be found in the PSNR. Despite the rather limited research conducted in the Loreto region of Peru, 112 amphibian species have been recorded (Perez et al. 2010; Rodriguez and Duellman 1994). Although few long term studies have been conducted within Loreto, rapid inventories have been undertaken for amphibians (Field Museum 2003-2006). These have recorded between 54 and 77 species of amphibians over just 13-22 survey days. Whilst rapid inventories are useful for obtaining an estimate of species richness long term studies are required for more accurate estimates (Magguran and McGill 2011). Catenazzi et al. (2013) showed that even after several years new species can still be added to the species list. This is consistent with the current study as in both 2012 and 2013 new species were observed, including *Pristimantis lacrimosus* and *Pristimantis waoranii* which were represented by just four individuals. These two species are arboreal in nature and rarely seen near the forest floor (McCracken et al. 2007). *Pristimantis waoranii* is believed to be a canopy specialist and has been encountered between 23.5 – 38 m in the rainforest canopy; usually within tank bromeliads (McCracken et al. 2007). It was first described in 2007 and it is currently only known from the type locality in the upper Amazon basin of Eastern Ecuador. The record of this species in the PSNR expands its range significantly. Two *P. waoranii* individuals were observed one was recorded within a bromeliad 6 m above the forest floor; the second was observed on a leaf 150 cm from the ground (see chapter 5 for details). One of the *P. lacrimosus* individuals was found within the same bromeliad as the *P. waoranii*. The other was observed on a terrestrial transect on a leaf at 100 cm. The arboreal nature of hylids means that many species will not be observed within a study area unless canopy specific surveys are undertaken. Two other arboreal tree frogs were observed for the first time in the PSNR in 2013, *Dendropsophus bokermanni* and *Dendropsophus koechlini*. These two species were observed in the low water, after heavy rainfall created large temporary pools. These species were found calling from leaves above the pools 100 – 200 cm from the water surface. It is likely that they moved down from the canopy to breed.

Undetected species may be rare or secretive in nature and may potentially go extinct before discovery (Lees and Pimm 2015). Rarefaction curves were created to assess whether the surveys were adequate for comparing different sites and habitats in the PSNR. When assessing the entire set of terrestrial species the richness in the extrapolated rarefaction curves only increased by four species. This may be because the surveys covered a large area, with many transects and sites surveyed therefore most species were observed. The floating meadow data set increased by five species in the extrapolated rarefaction curves. This is a larger proportion considering fewer species were found on the floating meadows. However, it is not unexpected as some species on floating meadows are transient, only using this habitat in certain seasons (See Chapters 4 and 5).

Further rarefaction curves were created for the floating meadow and terrestrial habitats splitting the data into the different water levels, transects, sites and macrohabitats. All of the rarefaction curves for the floating meadows rise rapidly initially; this is because the floating meadow assemblage contains many common species and only a few rare species. When comparing the richness estimates further surveys may have resulted in more species, but the confidence intervals are large, particularly in the high water. This may be due to the low sample size collected in the high water. The extrapolated curves suggest that the high and low water period have higher species richness than the transition period. The curves also show that the richness is similar in the three main macrohabitats (lake, channel and main river). This is explored further in Chapters 4 and 5. The terrestrial data were also split up to assess the estimated richness for the different transects at PV2, water levels and sites across the reserve. The terrestrial curves all rise more slowly that the floating meadows, this is because the terrestrial assemblage structure is much more uneven, with fewer common species and many rare species. Again the curves suggest that further surveys may have yielded more species. The three transects had similar estimated richness however transect two was slightly higher. The transition and low water period also had similar richness reaching nearly 40 species, but the high water plateaued at around 20 species. This could be because fewer surveys were undertaken in the high water period.

3.5 Conclusions

Eighty-one species have been recorded in the PSNR which represents 14% of the amphibian species found in Peru. Knowing the actual species richness for a tropical site is almost impossible without long-term surveys. However, even after many years of surveys new species can still be detected. It is important to calculate estimates of richness as well as presence / absence of different species so that they can be monitored over time. This is particularly the case for sites that are threatened by disturbance, habitat destruction, diseases and climate change. The following chapters explore the effects of disturbance and climate change on species in the PSNR. Whilst also comparing the floating meadow with the terrestrial assemblage. The results highlight that further surveys would most likely result in more species being detected. Yet surveys were adequate enough to allow comparisons to be made between sites and habitats. The analysis used in the subsequent chapters accounts for potentially undetected species. General Linear Mixed Models are used, these models use survey effort as a predictor variable, and also different response variables are used including the Shannon Diversity Index and the Simson's Diversity Index as surrogates for species richness. Finally the CLAM analysis (Chapter 5 + 7) takes into account potentially undetected species when comparing two assemblages.

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Chapter 4

Floating meadows as an amphibian habitat in the Amazon Basin

Sphaenorhynchus lacteus

4. Abstract

Although the tropics contain more species than temperate regions, understanding the distribution and diversity of species remains a significant challenge. Indeed, even within tropical regions diversity can change dramatically between sites. South America has a high amphibian diversity which has been influenced by the formation of the Amazon. The Amazon Basin once contained a huge wetland ecosystem which may have allowed the diversification of hylid frogs - the most abundant amphibian family in the Amazon. This chapter explores the role of floating meadows in the distribution of hylids, whilst also exploring which factors affect the richness and density of amphibians on floating meadows. Twenty-two anuran species were observed utilising the floating meadow habitat. Hylid species utilize floating meadows for breeding and are found on this habitat all year round. GLMM identified that increases in the plant species diversity increased the number of anuran species and individuals, while certain plant species were more important than others in determining species richness. The anuran assemblage changes little between the lake, channel and main river habitats, and floating meadows that detach and become rafts may be an important mechanism for the dispersal of amphibians. Understanding this process is vital to help explain the diversity and distribution of species within the Amazon Basin.

4.1 Introduction

The worldwide distribution of species has intrigued scientists for generations (Ghazoul and Sheil 2010). Tropical regions usually have a higher species diversity than temperate regions (Ghazoul and Sheil 2010), yet the distribution of species across tropical regions is not even (Duellman 1988). Manu National Park, Peru contains 155 amphibian species (Catenazzi et al. 2013). Yet, rapid biological inventories have been conducted across many other sites in Peru. These have recorded between 32 and 77 amphibian species (Field Museum 2014). The reasons for these diversity hotspots remain elusive. Many theories have been explored such as the impacts of global climate change (Duellman 1982), the length of time species have had to evolve (Wiens et al. 2011), speciation responses to habitat complexity (Fritz and Rahbek 2012), dispersal (Wiens et al. 2011; Santos et al. 2009), and predator-prey interactions (Kricher 1999).

The formation of the Amazon Basin has played a vital role in the distribution and diversity of species (Hoorn and Wesselingh 2010). Three major events in particular have helped to shape the Amazon Basin: 1) the appearance of flowering plants; 2) the rise of the Andes; and 3) the creation and draining of Pebas Lake. Around 105-120 million years ago (MYA) Gondwanaland started to split, separating South America from Africa (McLoughlin 2001). At around the same time, flowering plants start to appear in the fossil records (McLoughlin 2001), opening up new niches for small invertebrates. The Andes formed when the South American and Nazca Plate collided, a slow process which started around 33 MYA and still continues to this day (Hoorn et al. 2010; Hoorn and Wesselingh 2010). Prior to the rise of the Andes, the Amazon River flowed westward into the Pacific. The rise of the Andes subsequently created the largest lake

(Lake Pebas) and wetland system ever recorded (Hoorn et al. 2010; Hoorn and Wesselingh 2010). Figure 4.1 shows the location of Pacaya-Samiria National Reserve (PSNR) in relation to the Pebas Lake. Around 10 MYA the Amazon River broke through into the Atlantic Ocean, allowing the drainage of this wetland ecosystem. Over the subsequent 7 MY, fluctuations in global temperatures caused the sea level to rise, periodically flooding and then draining the Amazon lowlands. The Amazon as we know it today has largely stayed the same over the past 6 MY (Hoorn et al. 2010).

Caecilians are the oldest amphibians, originating in Gondwanaland (Goulding 1989). Most Amazonian species of caecilian are aquatic and have been recorded in the roots of floating vegetation (Tapley and Acosta-Galvis 2010). Caecelians and Pipidae species both of which are aquatic are basal species in the amphibian phylogeny (See Appendix 2 for amphibian phylogenies from; Pyron and Wiens 2011; Weins et al. 2011; Weins et al, 2010). These species may have evolved in the huge Pebas Lake and wetland ecosystem, with their ancestors diversifying into the many other amphibian species we see today. Wiens et al. (2011) suggested that the distribution of amphibians in the Amazon is due to the time-for-speciation-effect principle. This predicts that a group will be more species-rich in a region or habitat where it has been present for a long period of time. Research on dart frogs (*Dendrobates*) suggests that amphibians migrated from the Andes into the Amazon, and subsequently underwent *in situ* radiation during the last 10 MY (Santos et al. 2009). Wiens et al. (2011) argues that although this may be the case for dendrobatids, hylids seem to have originated in the Amazon and later migrated into the Andes. This chapter examines the potential for floating meadows to act as a mechanism for enhancing hylid diversity and distribution across the Amazon.

Figure 4.1. Paleogeographic maps showing the transition of Amazonia A-B as a result of the Andean uplift. (A) Amazonia once extended over most of northern South America. Breakup of the Pacific plates changed the geography and the Andes started uplifting. (B) The Andes continued to rise with the main drainage towards the northwest. (C) Mountain building in the Central and Northern Andes (~12Ma) and wetland propagation into Western Amazonia. (D) Uplift of the Northern Andes. (E) The megawetland disappeared and *terra firme* rainforest expanded. (F) Quaternary.

The ○ symbol represents the location of the PSNR.

From Hoorn et al. (2010) reprinted with permission from AAAS

4.1.1 Floating meadows

The floating meadows of the Amazon provide a vital habitat for many species. The plant species found within meadows provide food to a variety of invertebrate species (Junk 1973). These invertebrates in turn are consumed by a diverse range of taxa including fish (Castatti et al. 2003), amphibians (Hödl 1977), caiman (Pers. Obs.), bats (Eisenberg and Redford 1999) and birds (Schulenberg et al. 2007). Large mammals also make use of this habitat; dolphins can often be observed fishing beneath the meadows (Pers. Obs.). Others, such as capybara and manatees feed on the aquatic plants (Eisenberg and Redford 1999). Birds, including the horned screamer and wattled jacana, are often seen nesting and feeding on floating meadows (Schulenberg et al. 2007; Goulding 1989). Fish use of floating meadows has been extensively studied. Floating meadows are used as feeding sites (Casatti et al. 2003); the fish assemblage on floating meadows is different to the assemblage found within the flooded forest (Correa et al. 2008); rafts of floating meadow vegetation are used as a dispersal vector (Schiesari et al. 2003); and certain factors can be used to predictor fish diversity beneath floating rafts (Dias et al. 2011).

Macrophyte habitats have been shown to increase the habitat complexity of aquatic environments worldwide (Thomaz and Cunha 2010). This can benefit amphibians, wetlands in North Dakota and Florida are used by amphibians (Euliss and Mushet 2004; Babbitt and Tanner 2000) yet they do not mention macrophytes which are an important wetland habitat. In Italy Carchini et al. (2005) found that frog presence was correlated with macrophyte coverage. Whilst there are no studies looking at amphibian use on macrophyte habitats outside of South America there are some studies looking at the importance for tadpoles. The complexity of macrophyte root systems can offer refuges for tadpoles and so reduces predation pressures (Hartel et al. 2007; Kopp, et al. 2006; Babbitt and Tanner 1998).

In comparison, amphibian use of floating meadows in the Amazon Basin has received slightly more research attention. The original work undertaken on floating meadows by Junk (1970 and 1973) states that amphibians were rarely encountered. A possible explanation for the lack of amphibians may be the omission of nocturnal surveys. The first amphibian specific survey to be undertaken on floating meadows was by Hödl (1977). This study recorded the acoustic behaviour of 15 species of anuran. Hödl (1977) observed that species were associated with certain types of vegetation, and that females located potential mates via their calls. Following this, Hoogmoed (1993) updated Höld's (1977) list of amphibians on floating meadows. Hoogmoed (1993) combined data from three sites in Brazil, Bolivia and Suriname. This included 26 species of amphibian and concluded that Hylidae was the most commonly encountered family on floating meadows. The most recent study looked at fish and amphibians on floating meadow rafts (Schiesari et al. 2003). They found nine species representing five families, four Anura (Hylidae, Leptodactylidae, Pseudidae and Bufonidae) and one of Gymnophiona (Typhlonectidae). These species were all found on rafts of vegetation which were traveling down-river. See appendix one (Upton et al. 2014) for a summary of this research, including a species list, and updated list; which includes some of the data from this chapter.

This chapter aims to test the following hypotheses

1. Anuran species richness and density on floating meadows is influenced by the plant diversity and composition of the meadows
2. Anuran assemblages are not significantly different between the sites, macrohabitats and water levels
3. The current distribution of hylids across the Amazon Basin can be explained by the movements of floating meadows

4.2 Methods

4.2.1 Floating meadow survey

The methods used were as per Chapter 2.3 Floating meadow survey. For this analysis three water level periods were surveyed: high, low and transition (Mid). The high water period, was from December - May, the transition from June - August and the low September - November. During the high water period the forests were inundated, flooding the edges of rivers and lakes. The low water saw a drop of several meters in water level, lakes dried up and rivers levels were extremely low.

Table 4.1 – Breakdown of surveys across the different sites, years and seasons).

Site	Year	Water level	Start date	End date	No surveys[1]
PV2	2012	High	24/04/2012	26/05/2012	60
PV2	2012	Mid	20/06/2012	28/07/2012	64
PV2	2013	Mid	06/06/2013	23/07/2013	78
PV2	2012	Low	23/09/2012	25/09/2012	2
PV2	2013	Low	16/09/2013	01/10/2013	24
PV1	2012	Mid	31/07/2012	06/08/2012	11
PV1	2012	Low	10/09/2012	19/09/2012	13
PV1	2013	Low	26/08/2013	03/09/2013	12
Huisto	2012	High	05/06/2012	17/06/2012	32
PV3	2013	Low	08/09/2013	15/09/2012	5

[1] This represents the number of individual surveys conducted as described in 2.3 Floating Meadow Survey

The survey methods were standardised across all sites and macrohabitats. Around lakes, survey sites were selected at 100 m intervals. Along the channels and main river, where less vegetation was available, all sections of vegetation were surveyed at least once. As surveys were conducted across two years and different seasons, GPS way points were recorded and if a survey was in the same location as one undertaken previously, it was recorded as a repeat. This was especially important in the channel and main river where many surveys were considered repeats as less vegetation was available. Table 4.1 shows a breakdown of surveys conducted.

4.2.2 Identification

One of the main limitations was that voucher specimens were not collected. Any activities which cause harm to individuals were not allowed under the permits being used to conduct the research (Resolución Jefatural No 005-2013-SENANP-JEF). Voucher specimens are important tools for successful species identification. Samples can be used for DNA analysis which helps to identify cryptic species. This method of identification often reveals more than one morphologically similar species (cryptic species) hidden under one species name (Funk et al. 2011). This is likely to be the case for some Amazonian species such as *Rhinella margaritifera* and potentially the *Dendropsophus triangulum / bifurcus* species. This DNA sequencing analysis requires very intensive sampling in which many individuals of the same species are sampled. Combined with acoustic and morphological assessments identification can be very accurate (Funk et al. 2011). However you need a baseline of data or the permits to collect specimens which was not possible within PSNR instead all identification was done using the following identification guides: Ouboter & Jairam (2012); Duellman (2005); Bartlett & Bartlett (2003); and Rodriguez & Duellman (1994). Many of the Hylidae found on this habitat were easily identified using this method. However, Leptodactylidae were more difficult to identify and as a result, these were often only identified to genus level. All amphibian names used are from AmphibiaWeb (last accessed 16/06/2015).

4.2.3 Statistical analysis

All statistical analysis was completed using the software packages lme4 and ggplot2 with R (R Core Team 2012). Generalized linear mixed effect models were used to analyse which predictor variables influenced the response variables. The data consist of two response variables: (1) the number of species and (2) the number of individuals, per floating meadow survey. Thirteen explanatory variables were used in the models: site (PV1, PV2, PV3, Huisto); water level (low, transition, high); macrohabitat (main river, channel, lake); total survey time; minimum temperature; minimum humidity; survey size (boat length x 4 m); phase of the moon; cloud cover; number of surveyors; total size of connecting meadow; maximum vegetation height and number of plant species. The two survey years were not included as a variable in the analysis, because water level was considered to be more appropriate for analysing possible seasonal variation. Maximum temperature and maximum humidity were also recorded but were omitted from the analysis as they were correlated with the minima and this collinearity may affect the results (Dormann 2013).

Data exploration was completed prior to all statistical analysis to confirm the suitability of the general linear mixed models (Zuur 2010). The following steps were taken:

1) Boxplots and Cleveland dotplots were used to check for outliers
2) Histograms were used to check for normality
3) Scatterplots were used to check for collinearity among the predictor variables (Dormann 2013)
4) Scatterplots were used to check for relationships between the predictor and response variables (Appendix 4.1-4.2)
5) Coplots and interaction plots were used to consider interactions between variables

Generalised linear mixed effect models (GLMM) with Poisson errors were used to analyse which explanatory variables were most significant in influencing the number of anuran species, and number of individuals found per floating meadow survey (Bolker et al. 2008; Faraway 2006). GLMM were chosen as the surveys were not independent of each other, and many repeats were conducted at the same locations for example the channel at PV2. As the data contain repeats in the same locations across different time periods, these are not independent and can result in spatial pseudoreplication (Crawley 2009). In mixed effects models, random effects can be added for these non-independencies. Consequently, as the model now contains both fixed and random effects it is a mixed effects model. The Poisson errors are needed because the two response variables are both count data, rather than continuous data. This ensures that all fitted values are positive and allows the variance to increase with the mean (Crawley 2009). Count data were used as this represents the actual number of individuals and the number of species observed per survey. The aim of the modelling is to see which explanatory variables influence the count of species or individuals and not the overall diversity of that site. Search effort was included in the model using both the number of surveyors and the total survey time.

As the data include repeat surveys across different sites and seasons the assumptions of basic General Linear Models would be violated (Crawley 2009). Consequently, some of the explanatory variables must be put into the model as random effects. This tells the model to expect more than one result per “subject” be that lake, high water or PV2. However, these explanatory variables (site, water level and macrohabitat) are still important and may influence the number of species or individuals, observed per survey, so they will also be included as fixed effects within the model. Fixed effects included were therefore: total survey time, minimum temperature, minimum humidity, area surveyed (boat length x 4 m), moon cycle, cloud cover, number of surveyors, total size of connecting meadow, maximum vegetation height, number of plant species, site (PV1, PV2, PV3, Huisto), water level (Low, Transition, High) and macrohabitat (Main River, Channel, Lake), with a combination of site, water level and macrohabitat used as random effects.

By default, R assigns all numeric values as integers (total survey time, minimum humidity, survey size, moon cycle, cloud cover, number of surveyors, total size of connecting meadow, maximum vegetation height, number of plant species). However, minimum temperature is the only explanatory variable which is numeric as the values are not whole numbers (e.g., 21.5°C). The categorical explanatory variables, site, water level and macrohabitat are dealt with differently. R treats these as "factors" and assigns a number to each level in alphabetical order; these are dealt with as categories and not numerical values.

A full model was run with all explanatory variables included, firstly on the number of individuals, followed by a full model on number of anuran species. The first row of the model output contains the intercept which in all cases is the estimate of the response variable if the explanatory variable on the second row is equal to zero. This value is not necessarily useful but is important for assessing the explanatory variables that follow. The estimate column gives the estimated number of individuals or species if the factor were to increase by one from the intercept. This is followed by the standard error and the p-value which shows the significance of that explanatory variable.

Model simplification was then completed by removing the least significant explanatory variable, which is the one with the highest p-value (Crawley 2009). Once the explanatory variable was removed the new model was compared with the previous model. This was done to validate the removal of that variable. The likelihood ratio test was then used to assess model fit (Winter 2013). The likelihood ratio test was performed using the ANOVA function in R. This gives a chi-squared value, the associated degrees of freedom and the p-value (Winter 2013). The model with the lowest p-value was then selected as the better fit. Model simplification was undertaken as it removed the variables from the model which were not significant in predicting the response variable.

Following analysis with all explanatory variables included, subsequent models were then created with interactions between each of the explanatory variables. Again model simplification was undertaken removing all variables which were not significant. The final model from this analysis was then compared with the original model, without interactions, to establish the best fitting model.

As one of the most significant explanatory variables identified in the original and interaction analysis was the number of plant species, this was explored further. To do this, a subset of data was created using the explanatory variables which were most influential in the initial models. The quadrat data were added to this. A full model was then run on this subset and model selection was undertaken as described above. The new model was then compared with previous models. Finally, to assess the fit of the models, the residuals were plotted using plot, hist and qqnorm (Winter 2013; Zuur 2010).

4.3 Results

Nearly 2000 individuals were captured over the two year survey period on floating meadows, representing 22 species and four families (Bufonidae, Hylidae, Leptodactylidae and Pipidae). Most species were represented by less than 100 individuals with only *Dendropsophus triangulum*, *Hypsiboas punctatus*, *Sphaenorhynchus carneus* and *Dendropsophus haraldschultzi* represented by more. Some species were rarely encountered such as *Hypsiboas boans, Osteocephalus taurinus, Scinax ruber* and *Pipa pipa* (Table 4.2).

Table 4.2. Complete species list for floating meadows (2012 & 2013)

FAMILY	SPECIES	2012	2013	TOTAL
Bufonidae	*Rhinella marina*	7	9	**16**
Hyildae	*Dendropsophus bifurcus*	0	72	**72**
	Dendropsophus bifurcus / triangulum	0	46	**46**
	Dendropsophus haraldschultzi	39	66	**105**
	Dendropsophus leali	7	0	**7**
	Dendropsophus rossalleni	17	1	**18**
	Dendropsophus triangulum	334	73	**407**
	Hypsiboas boans	1	0	**1**
	Hypsiboas lanciformis	26	8	**34**
	Hypsiboas punctatus	292	518	**810**
	Osteocephalus taurinus	2	1	**3**
	Scarthyla goinorum	2	13	**15**
	Scinax garbei	13	44	**57**
	Scinax pedromedinae	2	3	**5**
	Scinax ruber	0	1	**1**
	Scinax sp.	3	0	**3**
	Sphaenorhychus sp.	11	3	**14**
	Sphaenorhynchus carneus	77	45	**122**
	Sphaenorhynchus dorisae	22	9	**31**
	Sphaenorhynchus lacteus	48	28	**76**
	Trachycephalus typhonius (venulosus)	0	1	**1**
Leptodactylidae	*Leptodactylus leptodactyloides*	16	4	**20**
	Leptodactylus petersii	15	4	**19**
	Leptodactylus sp.	26	19	**45**
	Leptodactylus wagneri	0	2	**2**
Pipidae	*Pipa pipa*	1	0	**1**
	TOTAL	961	970	**1931**

4.3.1 Summary statistics

Table 4.3. Summary statistics for the different sites, water levels and macrohabitats

	Number of surveys	Total area surveyed (m^2)	Total survey time (hours)	Total No. Individuals	Total No. species	Mean No. Ind. per survey	Mean No. Sp. per survey	Ind. density (Per $10m^2$)
SITE								
PV1	36	1330	9	203	12	5.7 ±5.5	2.4 ±1.4	1.5
PV2	238	8993	62	1475	20	6.1 ±4.9	2.3 ±1.4	1.6
Huisto	32	1280	8	209	10	6.6 ±5.6	2.4 ±1.3	1.6
PV3	5	160	1.5	44	6	8.8 ±6.1	2.6 ±1.5	2.8
Water Level								
Low	67	2820	17	573	20	8.6 ±6.4	2.7 ±1.4	2
Mid	152	5415	39	809	16	5.5 ±4.4	2.2 ±1.5	1.6
High	92	3528	25	549	15	5.3 ±4.3	2.3 ±1.3	1.3
Macrohabitat								
Main River	52	1757	14	342	18	6.1 ±5.4	2.3 ±1.4	1.8
Channel	102	3878	26	691	15	6.4 ±5.0	2.5 ±1.4	1.6
Lake	116	4848	35	864	17	6.8 ±4.8	2.6 ±1.3	1.6
Rafts	28	726	6	45	10	3.2 ±4.3	1.3 ±1.6	1.2
PV2 Island	4	95	1.5	60	7	15 ±3.7	2.8 ±1.7	6.3
PV2 site - macrohabitat								
Channel	88	3358	22	537	15	5.9 ±4.6	2.4 ±1.5	1.5
Lake	90	3828	26	723	16	7.2 ±4.7	2.7 ±1.2	1.7
Main River	30	1007	8	181	13	5.3 ±5.3	1.9 ±1.2	1.6
PV2 site - water level								
High	60	2260	17	340	15	4.6 ±3.2	2.3 ±1.2	1.2
Mid	152	5415	39	809	16	5.5 ±4.4	2.2 ±1.5	1.5
Low	24	1226	6	326	18	12.6 ±5.9	3 ±1.5	2.5

Survey effort was not constant across all sites, macrohabitats and water levels. This was due to availability of the floating meadow habitat and the timing of surveys. As a result, the average number of individuals and species per survey as well as individual density were calculated (Table 4.3).

The trends in the data show that PV1, PV2 and Huisto have similar densities of individuals and species, while PV3 was considerably higher. Fewer surveys were undertaken at PV3 and only within the low water period, thereby potentially influencing these results (table 4.3).

The water level seems to be influencing individual density with higher numbers encountered in the low water period than in high and transition. More species per survey were also observed in the low water. This is consistent with the results from PV2. The individual density per 10 m^2 increases from 1.2 in the high water to 2.5 in the low water (table 4.3).

Trends in the data show that the main river, channel and lake have similar individual densities, while the island at PV2 is considerably higher (table 4.3). However individual density on rafts is slightly lower. Again this is consistent with the PV2 result.

4.3.2 Floating meadow GLMM predictors of abundance

The full model was initially run with all explanatory variables included with number of individuals as the dependant variable. Model selection was then completed by removing those explanatory variables with the highest p-value one by one. Table 4.4 shows the first model with the following significant explanatory variables: number of plant species, minimum humidity, total survey time, water level and also the phase of moon. To further refine the first model, the explanatory variables were tested for interactions which resulted in the second model in Table 4.4. In this model, none of the explanatory variables were dropped. However, it does highlight the importance of plant species which interact with all other variables. When comparing model one and model two, model two was the best fit (Table 4.5). To explore this relationship further, another dataset was included i.e. the percentage of each plant species present in the survey area. This resulted in model three in Table 4.4.

Table 4.4. Predictors of anuran abundance on floating meadows

	Estimate	**SE**	**p-value**	**AIC[1]**
Abundance model one				
Intercept	1.70	0.35	0.001	1245.5
No. plant species	0.11	0.02	0.001	
Phase of the moon	-0.001	0.001	0.001	
Minimum humidity	-0.02	0.001	0.001	
Total survey time	0.05	0.01	0.001	
Water level low	1.11	0.10	0.001	
Water level transition	0.48	0.09	0.001	
Abundance model two				
Intercept	40.06	10.12	0.001	1226.6
No. plant species	-11.43	3.00	0.001	
Phase of the moon	-0.001	0.001	0.001	
Minimum humidity	-0.53	0.13	0.001	
Total survey time	-2.49	0.68	0.001	
Water level low	1.12	0.10	0.001	
Water level transition	0.52	0.09	0.001	
No. plant*Minimum humidity	0.15	0.04	0.001	
No. plant*total survey time	0.76	0.20	0.001	
Minimum humidity*total survey time	0.03	0.01	0.001	
No. plant*minimum humidity*total survey time	-0.01	0.001	0.001	
Abundance model three				
Intercept	2.65	0.39	0.001	**990.1**
No. plant species	0.11	0.02	0.001	
No. surveyors	0.05	0.02	0.013	
Phase of the moon	-0.001	0.001	0.067	
Minimum humidity	-0.03	0.001	0.001	
Water level low	1.19	0.14	0.001	
Water level transition	0.44	0.10	0.001	
Pistia stratiotes	-0.001	0.001	0.001	
Paspalum repens	-0.001	0.001	0.001	
*Pistia stratiotes *Eichhornia crassipes*	0.001	0.001	0.049	
*Paspalum repens*Eichhornia crassipes*	0.001	0.001	0.017	
*Pistia stratiotes*Paspalum repens*	0.001	0.001	0.001	
*Eichhornia crassipes**grass species two	0.001	0.001	0.001	
*Paspalum repens** four leaf	0.001	0.001	0.004	
*Pistia stratiotes*Paspalum repens* Eichhornia crassipes*	-0.001	0.001	0.033	
*Paspalum repens*Eichhornia crassipes* Oxycorum cubense*	-0.001	0.001	0.001	
*Pistia stratiotes*Paspalum repens* Polygonum acuminatium*	-0.001	0.001	0.015	
*Pistia stratiotes*Paspalum repens* Eichhornia crassipes*Polygonum acuminatium*	0.001	0.001	0.037	

[1] The AIC given is for the entire model adjacent, the lowest number represents the best fitting model

Table 4.5. GLMM comparisons between the first two models

	Df	AIC	BIC	Chi sq	Chi Df	Pr(>Chisq)
Abundance model one	10	1246	1279			
Abundance model two	13	1227	1270	24.9	3	0.001

All three models highlight the importance of plant diversity in determining the number of anuran individuals per survey. Model three shows that each time the number of plant species is increased by one, the estimated number of individuals increases by 0.11±0.02 from the intercept. Other factors which are also important are the minimum humidity, the water level, number of surveyors, and finally the phase of the moon. Model three highlights the importance of interactions between the different plant species in particular the following combinations: (*Pistia stratiotes* + *Paspalum repens)*; (*Eichhornia crassipes* + *Oxycorum cubense)* and (*Paspalum repens* + *Eichhornia crassipes* + *Oxycorum cubense)*.

4.3.3 Floating meadow GLMM predictors of species richness

The full model was initially run with all explanatory variables included. Model selection was then completed by removing those explanatory variables with the highest p-value one by one. Table 4.6 shows the final models with the following significant explanatory variables; number of plant species, water level, minimum temperature and minimum humidity. To further refine the first model, the factors were tested for interactions, but none of these were significant. Again another model was run including the percentage of each plant species present in the survey area (model two).

Table 4.6. Predictors of anuran species richness on floating meadows

	Estimate	**SE**	**p-value**	**AIC**
Species model one				
Intercept	2.45	1.02	0.02	
Number of plant species	0.12	0.03	0.001	
Water level low	0.40	0.14	0.01	729.9
Water level transition	0.27	0.13	0.03	
Minimum temperature	-0.05	0.03	0.09	
Minimum humidity	-0.01	0.01	0.03	
Species model two				
Intercept	0.42	0.68	0.54	
Number of plant species	0.10	0.03	0.001	
Minimum humidity	-0.01	0.01	0.03	
Total survey time	0.07	0.03	0.03	
Pistia stratiotes	-0.001	0.001	0.01	607.8
*Pistia stratiotes*Eichhornia crassipes*	0.001	0.001	0.001	
*Pistia stratiotes*Paspalum repens*	0.001	0.001	0.07	
*Paspalum repens*Polygonum acuminatium*Oxycorum cubense*	0.001	0.001	0.05	

The models for species richness highlight the importance of the number of plant species in particular a combination of *Eichhornia crassipes* and *Pistia stratiotes*. The minimum humidity is also significant as is the total survey time.

4.3.4 Water level, macrohabitat and location

Location (PV1, PV2, Huisto, and PV3) and macrohabitat (lake, channel and main river) were not significant explanatory variables in the GLMM. However, to confirm that there are no significant differences in their species assemblages, the following were explored: species lists and individual / species density. The models did, however, highlight water level as impacting on both individuals and species which were explored further. The majority of surveys were conducted at PV2 therefore the data used for the macrohabitat and water level comparisons only included that from PV2. Tables for all sites / macrohabitats and water levels can be seen in appendix 4.3 – 4.7.

Water Level

In the high water period at PV2, 340 individual anurans were encountered on the floating meadows representing 15 species, with 809 in the mid-water period representing 16 species and a further 326 in the low water period representing 18 species (Appendix 4.4). Most floating meadow species do not show a preference for certain water levels; instead they use the meadows across all seasons. However, rare species were often only found in just one or two seasons. The only family which shows a preference to a certain season is Leptodactylidae which is most commonly encountered in the high water (Appendix 4.4).

Due to differences in survey effort, the median number of individuals and species encountered per survey was calculated. Although there is a slight increase in the number of species per survey in the low water, an ANOVA test showed that there were no significant differences between the different water level periods ($F_{2,\ 308} = 2.77$, $p = 0.06$). However, the number of individuals encountered per survey was significantly different ($F_{2,\ 308} = 11.17$, $p = 0.001$). Post-hoc Tukey testing showed that the most significant differences were between the low and high water and the low and mid water, with no significant difference between the mid and high water. The low water period seems to have a higher number of individuals per survey than the other two seasons (Fig. 4.2). This higher number of individuals increases the density per 10 m^2 from 1.2 in the high water to 2.5 in the low water.

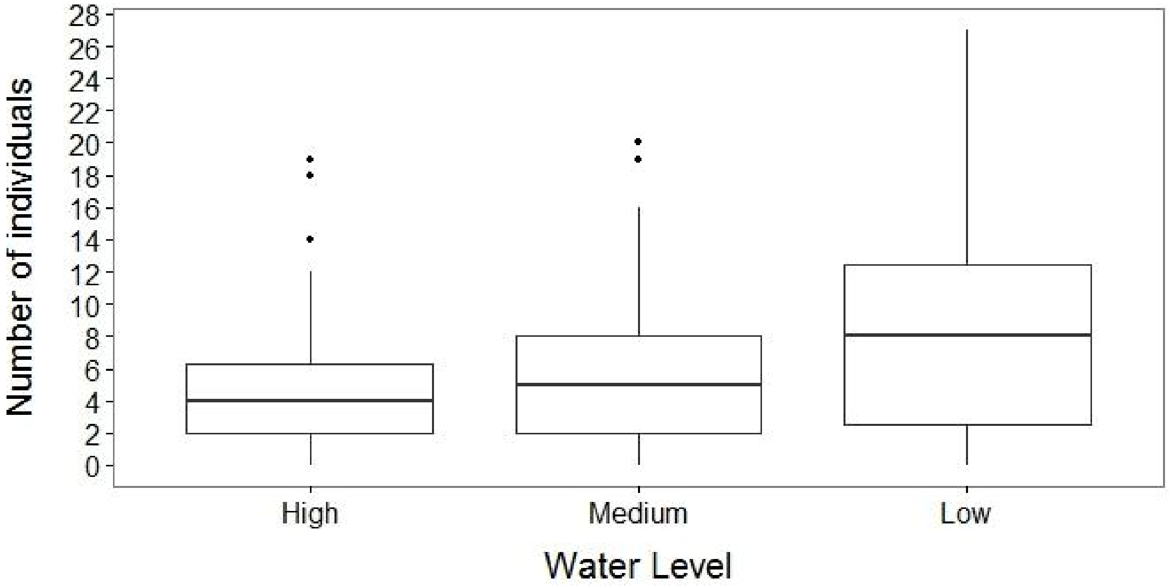

Figure 4.2. Boxplot showing the number of individuals observed per floating meadow survey during the different water levels at PV2 (dark black line = median, boxes show the upper and lower quartiles, whiskers show the variation outside this and the small circles represent outliers.

Macrohabitat

At PV2, 723 individuals were observed on floating meadows in the lake, representing 16 species, while in the channel, 537 individuals were observed of 15 species, and finally on the main river 181 individuals of 13 species (Appendix 4.6).

Due to the differences in survey effort, the median number of individuals and species encountered per survey was calculated (Figs. 4.3 + 4.4).

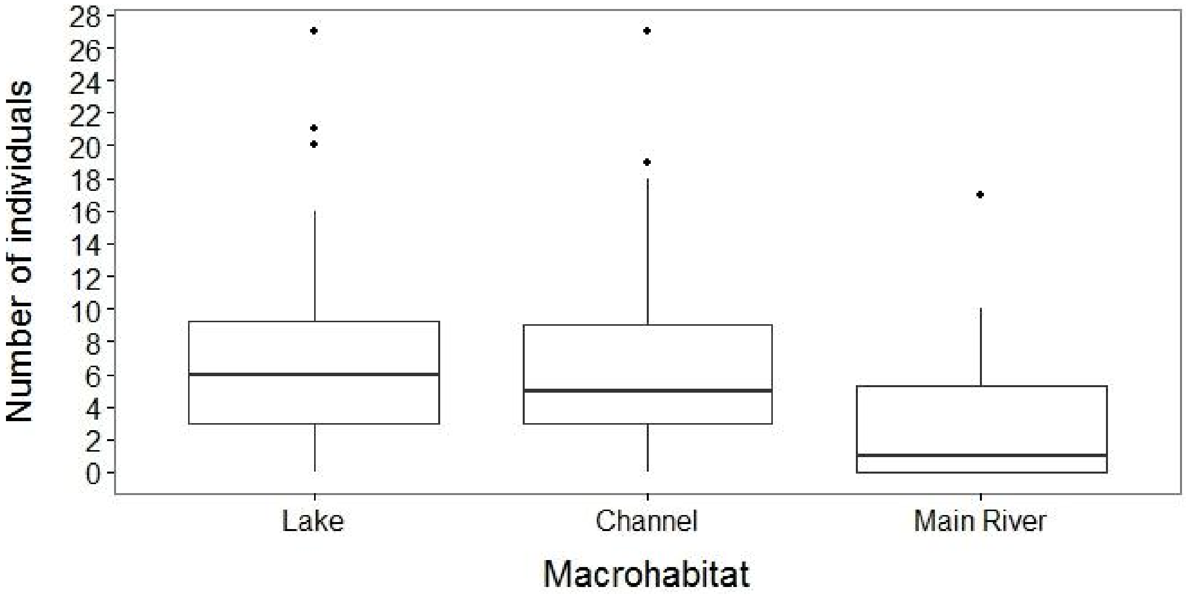

Figure 4.3. Boxplot showing the number of individuals observed per floating meadow survey within the different macrohabitats at PV2 (dark black line = median, boxes show the upper and lower quartiles, whiskers show the variation outside this and the small circles represent outliers)

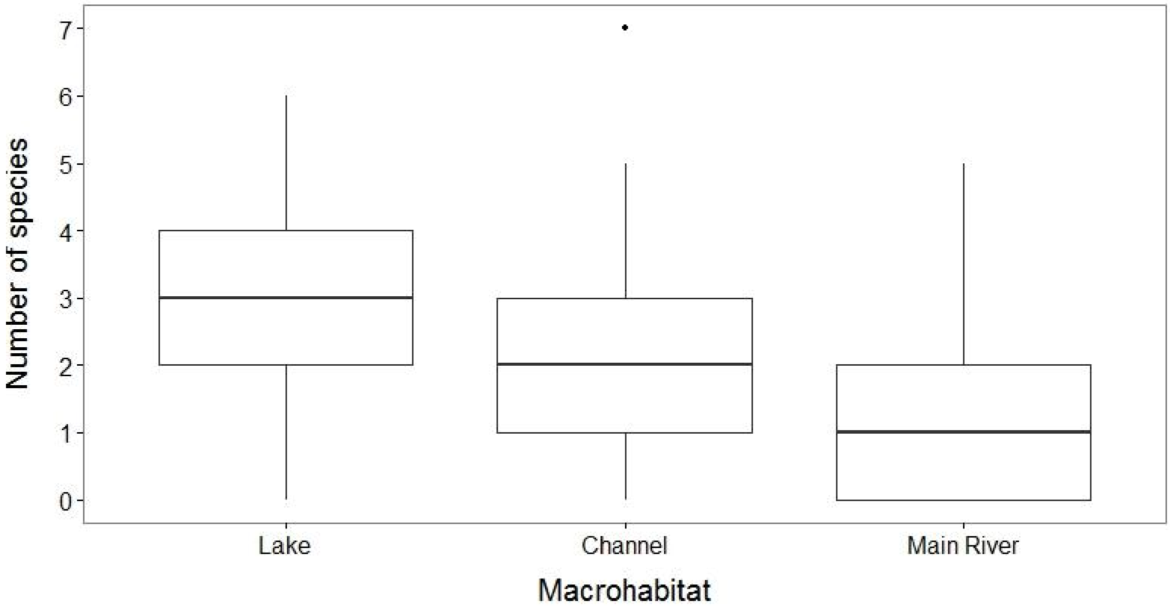

Figure 4.4. Boxplot showing the number of species observed per floating meadow survey within the different macrohabitats at PV2 (dark black line = median, boxes show the upper and lower quartiles, whiskers show the variation outside this and the small circles represent outliers)

The median number of individuals observed was significantly different between the three microhabitats ($F_{2, 243}$ = 6.5, p = 0.001). Post hoc testing revealed that the most significant differences were between the main river + channel and main river + lake. The densities of individuals per 10 m^2 changed little with 1.7 in the lake, 1.5 in the channel and 1.6 on the main river. The median number of species also differed significantly ($F_{2, 243}$ = 10.13, p = 0.001) again, the most significant differences were between the main river and the channel / lake.

Location

The site with the highest number of individuals was PV2 with 1475, representing 20 species, followed by Huisto with 209 and 10 species and then PV1 with 203 and 12 species, and finally PV3 44 and 6 species (Appendix 4.7). The majority of individuals were found at PV2, as this is where surveys were concentrated. However, the most common species were regularly encountered across all sites such as *Dendropsophus triangulum, Hypsiboas punctatus* and *Dendropsophus haraldschultzi,* unlike the rarer species which were only encountered in one or two sites such as *Dendropsophus leali, Osteocephalus taurinus* and *Hypsiboas boans* (Appendix 4.7).

Again the median number of individuals and species encountered per survey was calculated (Fig. 4.5). However, no significant differences were observed between the sites (individuals – $F_{2,303} = 0.297$, $p = 0.743$ / Species – $F_{2,303} = 0.095$, $p = 0.909$). As can be seen in Figure 4.5, the number of individuals was relatively even across the sites.

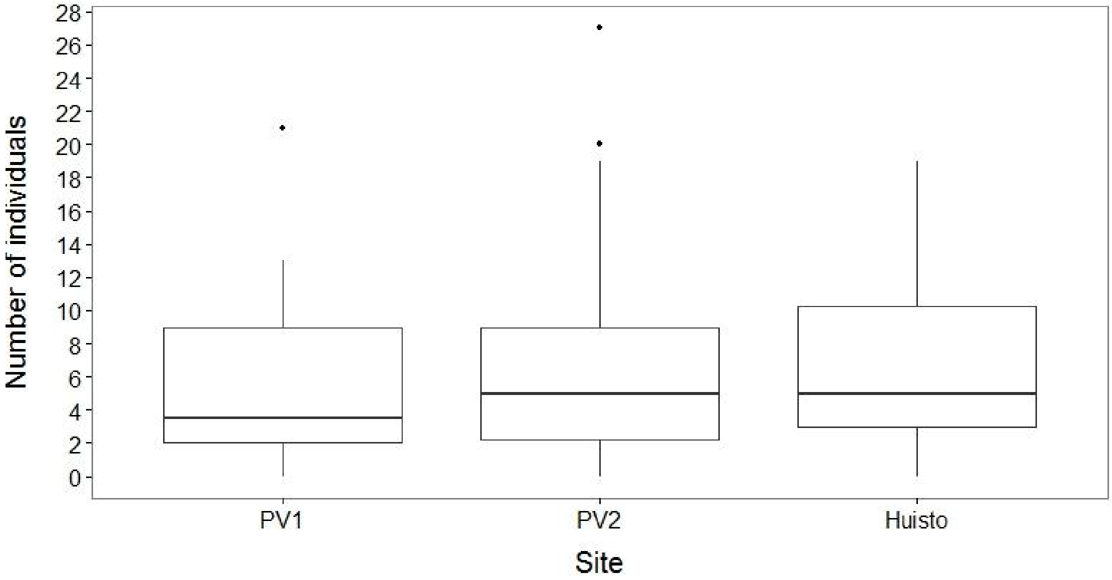

Figure 4.5. Boxplot showing the number of individuals observed per floating meadow survey across the different sites (dark black line = median, boxes show the upper and lower quartiles, whiskers show the variation outside this and the small circles represent outliers)

4.4 Discussion

Twenty-two species were recorded on floating meadows which represented four families: Bufonidae (one species), Hylidae (17), Leptodactylidae (three) and Pipidae (one). All 22 species were anurans with no salamanders or caecilians encountered. Previous studies have found caecilians within floating meadow vegetation (Tapley and Acosta-Galvis 2012; Schiesari et al. 2003), but no salamanders have ever been recorded on this habitat. The most commonly encountered species on floating meadows were *Hypsiboas punctatus* (n=810) and *Dendropsophus triangulum* (n=407), followed by *Sphaenorhynchus carneus* (n=122) and *Dendropsophus haraldschultzi* (n=105). While common on floating meadows, these species were rarely encountered in the terrestrial habitat, and so are likely floating meadow specialists (see Chapter 5 for a comparison). All other species were represented by less than 100 individuals, with some only represented by one individual, e.g. *Hypsiboas boans, Scinax ruber, Trachycephalus typhonius (venulosa)* and *Pipa pipa*. This is not unexpected: *Pipa pipa* is a fully aquatic species, and the individual caught in 2012 was swimming at the edge of the meadows. It may have been using the extensive roots of the floating meadow for foraging or breeding. This aquatic species never chooses to leave the water; it is a sit-and-wait predator, which performs elaborate courtships (Buchacher 1993; Rabb and Snedigar 1960; Rabb and Rabb 1960). *Scinax ruber* was rarely caught in the terrestrial forest; this species is associated with disturbance, and was regularly observed in village clearings. *Hypsiboas boans* is a canopy specialist which comes down to river banks to breed in the low water. Lastly *Trachycephalus typhonius (venulosa)* is a habitat generalist so may have been passing through this habitat, or foraging (Bartlett and Bartlett 2005; Duellman 2005; Rodriguez and Duellman 1994). Other species found in lower abundance than the hylids included those in the Bufonidae and Leptodactylidae. These species may be using the floating meadows as a refuge (from the flood water) or to forage. No evidence of breeding behaviour was found and they were regularly encountered in adjacent terrestrial habitat. In comparison, fourteen of the hylids displayed breeding behaviour on floating meadows (gravid females, calling males, juveniles, eggs and pairs in amplexus). This suggests that unlike bufonids and leptodactylids, hylid species are using the floating meadows for breeding.

The anurans found on floating meadows are nocturnal. Day-time searches revealed no active anurans, and only occasionally disturbed nocturnal species. Anurans used floating meadows across the entire survey period (March – October). They were still active and breeding on the floating meadows in October (during the low water period) and so may continue to use the meadows from November – February. During November and December, water levels may drop slightly, however, after this time the water level will begin to rise and floating meadows will start growing with the rising waters. Hence this habitat may be available all year round, with a reduced availability in the low water period. During low water, floating meadows will either rot in situ on the river bank; be washed down river as rafts; or put roots down into the soil and continue growing. Surveys were conducted on these three low water meadow types and anurans were only present on rafts and within some terrestrial patches when the soils were waterlogged. However, they showed a preference for the small patches still growing on water. Calling males were still active at this time potentially showing that this habitat is used for breeding year round. Floating meadows potentially offer a stable environment in an unstable terrestrial habitat. We have shown that floating meadows are available year round and are continually used by anuran species. However the seasonal flooding (chapter 5) means that the terrestrial forest is inundated with flood waters for several months a year. The floating meadows are available year round for both terrestrial and arboreal species and so this long term stability may have helped drive the diversification of species found on this habitat.

4.4.1 Predictors of anuran abundance

Plant species richness was the most significant explanatory variable of anuran abundance. Floating meadows consist of many different aquatic water plants and grasses (Junk 1973). Some survey areas contained a high diversity of plant species while other locations only had one or two species. The models identified a relationship between an increase in plant species richness and an increase in the number of individuals. This may be due to the increased availability of niches and calling locations. Anurans were observed breeding, with calling males located on particular plant species at specific heights (Upton et al. 2014). As a result, an increase in plant species offers more calling sites, and so more individuals will be present. Foraging opportunities are another possible explanation for this increase in anuran individuals. Floating meadows support a huge diversity of insects; the maximum values of invertebrates found using a net of mesh size 223 microns were 780,000 individuals per m^2 (Junk 1973). This huge invertebrate density offers a wide prey base for many species (Goulding 1989) including anurans. An increase in the plant species on which the insects feed will naturally lead to a higher number and diversity of insects. This increase in plant species increases the habitat complexity which, in turn, may increase the diversity and density of invertebrate species (Thomaz et al. 2008). To determine which plant species were most important, the final model includes the percentages of each plant species. When the survey area contains a high percentage of only *Pistia stratiotes* or *Paspalum repens,* there is a decrease in the number of anurans. However, when these two plant species are combined in the survey area, there is an increase in the number of anurans. Again, this may be due to an increase in available calling heights; *Pistia stratiotes* alone only offers one height as it grows on the water surface. With a combination of *Pistia stratiotes* and *Paspalum repens* there is a greater range of heights.

The water level was another explanatory variable which was significantly related to the number of individuals. Surveys were conducted across the high, transition and low water periods to determine the effects on amphibians. The impacts of flooding on terrestrial species are explored further in Chapter 7. It is important to note that individual density increased in the low water period. The number of individuals per 10 m^2 on PV2 floating meadows increased from 1.2 (high water) to 2.5 (low). This could be due to the limited availability of meadows in the low water period. During the low water, meadows will either rot in situ, be washed down river as rafts, or some small sections which are still floating on the channel and rivers may continue to grow. This reduced availability of meadows and subsequent increase in anuran density shows that anurans do not leave the meadows in the low water period, suggesting this is an important habitat for them year round.

Two variables which are negatively related to the number of individuals are the phase of the moon and humidity. Nights with a full moon led to a reduced number of anurans. On floating meadows, unlike the terrestrial forest, there is no cover to block the intensity of the moon. The full moon can make amphibians conspicuous to predators especially on this habitat. The effect of the lunar cycle on amphibians is well documented in the literature with some amphibian species showing increased activity and some reduced activity during the full moon (Grant et al. 2013). The other significant explanatory variable was the minimum humidity. There was a significant relationship between an increase in humidity and a decrease in the number of anurans. This is likely due to the link between temperature and humidity; when temperatures drop, the humidity rises. This increased humidity and low temperature combination may have reduced the anuran activity.

Other factors which were significant in the final model included the number of surveyors and total survey time. This is to be expected when looking at the number of individuals caught. A longer period of time, and more people searching, is likely to result in more anurans observed and captured. However, when plant species variables were added into the models, total survey time was dropped and number of surveyors became less significant. Total survey time and the number of surveyors may only play a limited role in predicting the number of anurans, this is most likely to be because of the time and size constraints used in the methods. These variables were removed from the models only when they were no longer significant, totally survey time remained in three of the five models with number of surveyors only remaining in one model.

4.4.2 Predictors of anuran richness

The number of anuran species caught per survey was most influenced by the plant species richness; increases in the plant richness increased the number of anuran species. However, single plant species such as *Pistia stratiotes* showed a negative relationship with anuran species richness, although a combination of *Pistia stratiotes* and *Eichhornia crassipes* showed a positive relationship. This is consistent with fish species richness: a greater diversity of plant species increases the root complexity which is beneficial to fish (Petry et al. 2003). During daytime searches of the floating meadow habitat, many anuran individuals were observed resting on the leaves of

Eichhornia crassipes, especially when *Pistia stratiotes* was present below. Daytime searches also revealed anuran eggs, which were always found on *Pistia stratiotes*. This is an ideal location for eggs, as *Pistia stratiotes* grows on the water surface, so stays moist at all times. When tadpoles hatch they move down into the water below and are potentially protected by the root systems of the vegetation. As a result both *Pistia stratiotes* and *Eichhornia crassipes* may be important plant species for anurans on floating meadows. Locations where both these plant species are present may offer an ideal breeding site where individuals do not have to move far during the day to rest. Therefore several species make use of these sites and so increasing species richness. *Pistia stratiotes* alone is exposed to the daytime sun and may not offer suitable daytime resting sites. These high light levels may also lead to tadpole desiccation again not offering a suitable site for egg laying. However, a covering layer of *Eichhornia crassipes* above this offers shade for the spawn below, protecting it from the sun's rays.

Water level is another significant explanatory variable which showed a relationship with increases in the number of species. In the low water period the average number of species present per survey increased from 2.3 (high) to 3 (low). Rarefaction curves in Chapter 3 show a more rapid increase in species richness in the low water than in the other seasons. This increase in richness is not due to an increase in the overall number of species present, but an increase in individual density, resulting in more species captured. Again, this suggests that in the low water individuals choose to stay in a higher density on the floating meadows, rather than moving into adjacent habitats.

4.4.3 Macrohabitat and site

Neither macrohabitat nor site was a significant explanatory variable in the GLMM. Looking specifically at species lists for the macrohabitats and sites, no obvious differences can be seen. Common species are present across all sites and macrohabitats, while rare species are usually present in only one site or macrohabitat. As the rare species were only encountered on a few occasions, they may not be floating meadow specialists. With the exception of the PV2 island (discussed later), density of individuals on the meadows did not vary greatly across the sites and macrohabitats. The similarity of floating meadow assemblages across sites and macrohabitats may be due to the movement of floating meadows. Floating meadows float on the water surface and so can be transported by the river currents, sections will regularly break away and are washed downriver. There is a constant flow of vegetation across the lakes and down channels, with mats eventually reaching the main river where rafts can float for days. These rafts will either become trapped on fallen trees or river bends or continue drifting. Amphibians have been previously recorded on these rafts of vegetation (Schiesari et al. 2003). In the current study, ten species were found on rafts, and seven species found on the PV2 island: a total of 13 species. The PV2 island was a section of vegetation trapped around a fallen tree in the middle of the river, unconnected to the edge. The floating vegetation here changed regularly as new rafts were trapped and old rafts disconnected. The only way anurans would have arrived at this island is via floating rafts. These rafts have the potential to travel over vast distances. A study undertaken by Schiesari et al. (2003) calculated that one raft could travel 80-130 km in one day. The distance between the sites PV1 and PV3 via the river is roughly 110 km and so it is likely that the assemblages are similar due to this dispersal.

The significant difference between the three main macrohabitats at PV2 was when comparing the main river with the channel or lake. The main river had the lowest number of species on average per survey (1.9), while the channel was slightly higher

(2.4), and the lake slightly higher again (2.7). The species rarefaction curves in Chapter 3 showed a slightly higher richness in the lake. The structure of the floating meadows in each macrohabitat may cause these slight differences in richness and averages. The lake contains slow moving water and receives vast quantities of sunlight allowing huge areas of floating meadows to grow all around the lake edge. This can be more than 50 m wide in places, with a high richness of plant species. These form vast root systems which in turn offer food and shelter to a huge diversity of insects. These insects then provide many different species with a prey base, including anurans. The channel does not have such a vast quantity of floating meadows instead they only grow along slow-moving bends in the river or around tree falls where they are not washed away. Finally, on the main river, little floating meadow vegetation is available and most of this is trapped on fallen trees and is regularly washed further down. This system results in a continued shift of vegetation moving out of the lake and into the channel. Over time this then washes out into the main river and further downstream. As a result, the anurans found in the channel and main river likely originated in the lake which helps to explain a reduction in species richness with distance from the lake. This system also helps to explain why floating meadows in other locations further down the Amazon River contain a similar assemblage of anuran species. Of the 15 species Hödl (1977) encountered, we observed nine in the PSNR, although Hödl's surveys were conducted near Manaus over 2000 km downriver. Another study by Hoogmoed (1993) combined data from four sites: one is Hödl's (1977) study, one from Brazil, Suriname and Bolivia. Across all four sites they encountered 26 species, of which we observed 13 in the PSNR. *Hypsiboas punctatus* was the most common species encountered in the current study; this species was also encountered in all of the previous floating meadow surveys (Hoogmoed 1993).

4.4.4 Hylidae Distribution

Ten MYA when the Amazon contained the huge Pebas Lake and wetland ecosystem it may have looked similar to high water in the PSNR. Vast areas of floating meadow cover the lakes, channel and rivers when the floodwater inundates the forest for several months. The Pebas Lake and wetland system may have offered perfect growing conditions for free-floating vegetation, potentially resulting in mats similar to the floating meadows found today. Hylid species are well adapted to the floating meadows, and may have rapidly evolved in this vast wetland. They are well suited to life above ground and the wetland floating meadows would have opened a large diversity of niches to them, allowing rapid speciation to occur. Reptiles also underwent a huge diversification at this time including gharials, caimans and turtles all taking advantage of the wetland system (Hoorn et al. 2010). The vast wetland ecosystem would have contained fragmented islands of forest. Records suggest that these fragments were similar in plant composition to the current forest today (Hoorn et al. 2010). Floating meadows may also have been present in this system growing at the edges of these forest fragments. When the vast lake system drained, the floating vegetation may have been washed downriver, again as they are today, transporting hylids down the Amazon. Unlike the reptiles, which declined with the draining of the wetlands (Hoorn et al. 2010), hylids would have diversified into the new available terrestrial habitats. As the water levels receded, creating upland areas, terrestrial forests became available to them. They would have moved into these new areas with fewer competitors allowing rapid speciation to occur. Other species such as the dendrobatids are not well suited to a wetland system which may explain why they moved into the Amazon later (Santos et al. 2009). Hylids, on the other hand, could have evolved and radiated within the vast wetland system to which they are well-adapted. By studying the floating meadows in the PSNR and other wetland systems in the Amazon, we might be able to uncover the drivers of the vast diversity of the Hylidae family.

4.5 Conclusions

This study shows floating meadows to be an important habitat for several anuran species. Most of the hylid species found on meadows rely on them for breeding and foraging, while other families may use meadows as a refuge during high water. Many of the hylids encountered are considered to be floating meadow specialists which were rarely encountered in the terrestrial forest (explored further in chapter 5). Anurans are likely to stay on floating meadows all year round, increasing in density during the low water. This is when less floating meadow is available, suggesting that anurans become concentrated in a smaller area rather than leaving this habitat. The most important factor in predicting both anuran richness and density on meadows is plant species richness; the more plant species, the more anurans are likely to be present. Structure seems to play an important part of this as when certain plant species are found in combination, they lead to an increase in anuran species. Floating meadows can be transported by the river currents as rafts, and may provide an important mode of dispersal for anurans throughout the Amazon basin. This in turn could help explain why hylids are found in such a high abundance in the Amazon and have a large distribution range. It is vital that we understand the importance of this habitat which is heavily influenced by the rise and fall of the seasonal flooding, in order for it to be conserved along with the many species that rely on it for breeding and foraging.

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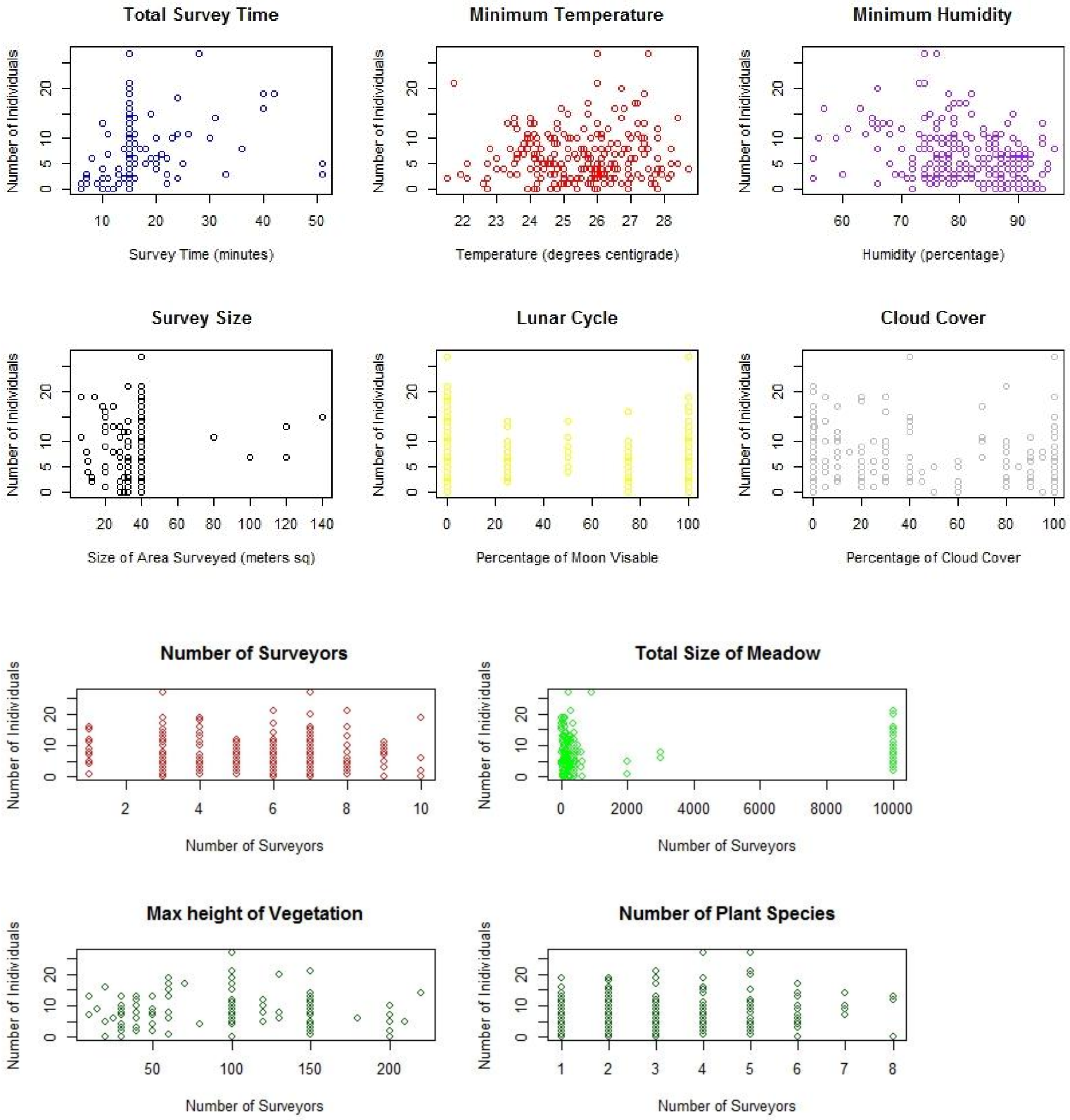
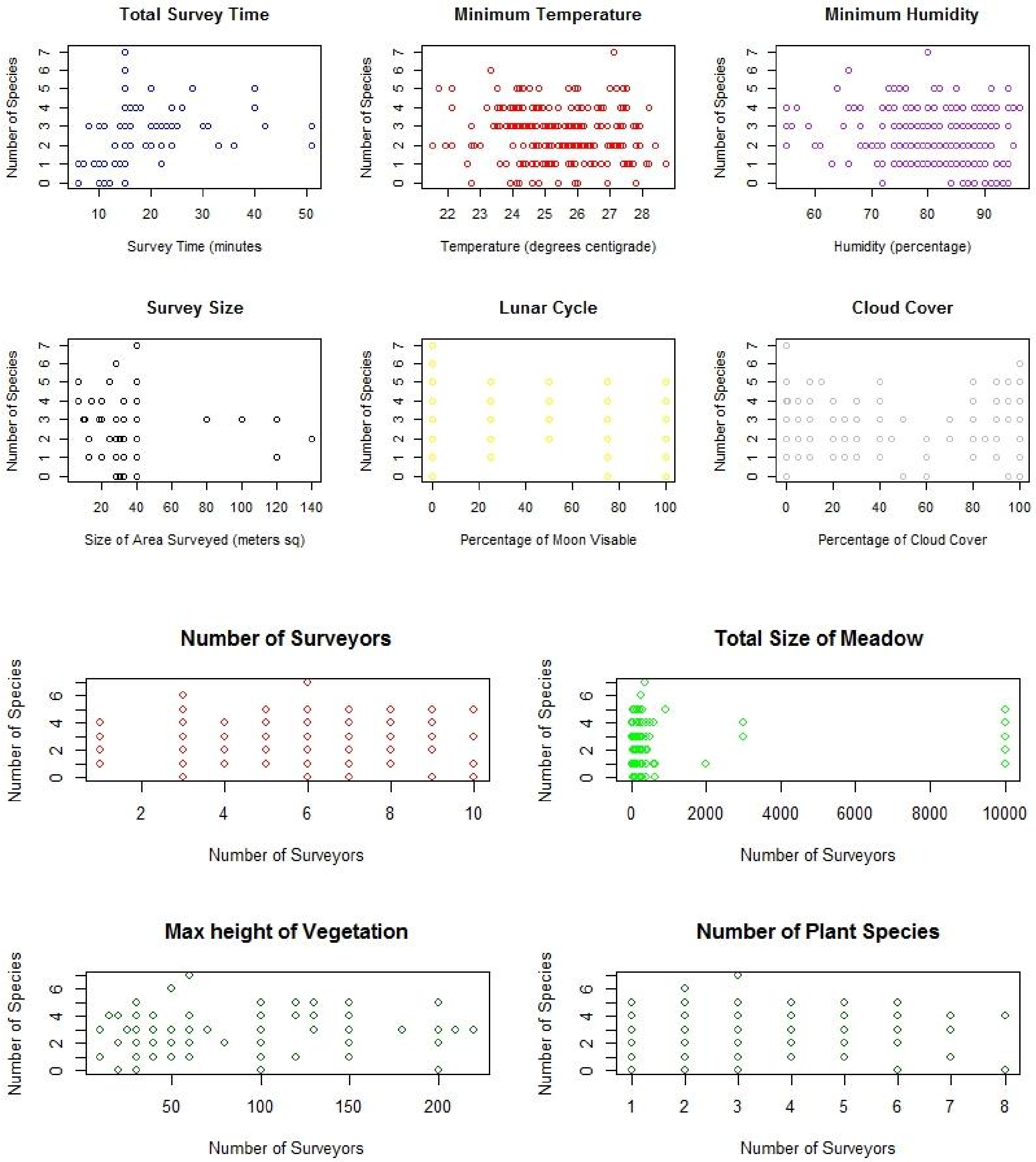
Appendix 4.1

Scatterplots showing the relationships between each explanatory variable and the response variable (number of anuran individuals)



Appendix 4.2

Scatterplots showing the relationships between each explanatory variable and the response variable (species richness)



Appendix 4.3

Species list for the different water level periods across all sites and habitats

FAMILY	SPECIES	High	Medium	Low
Bufonidae	*Rhinella marina*	2	5	9
Hyildae	*Dendropsophus bifurcus*	0	43	29
	Dendropsophus bifurcus / triangulum	0	18	28
	Dendropsophus haraldschultzi	8	54	43
	Dendropsophus leali	0	0	7
	Dendropsophus rossalleni	17	1	0
	Dendropsophus triangulum	218	141	48
	Hypsiboas boans	0	0	1
	Hypsiboas lanciformis	2	15	17
	Hypsiboas punctatus	109	390	311
	Osteocephalus taurinus	2	0	1
	Scarthyla goinorum	1	4	10
	Scinax garbei	2	48	7
	Scinax pedromedinae	2	1	2
	Scinax ruber	0	0	1
	Scinax sp.	3	0	0
	Sphaenorhychus sp.	10	4	0
	Sphaenorhynchus carneus	63	50	9
	Sphaenorhynchus dorisae	20	4	7
	Sphaenorhynchus lacteus	35	24	17
	Trachycephalus typhonius (venulosus)	0	0	1
Leptodactylidae	*Leptodactylus leptodactyloides*	14	4	2
	Leptodactylus petersii	15	2	2
	Leptodactylus sp.	26	0	19
	Leptodactylus wagneri	0	0	2
Pipidae	*Pipa pipa*	0	1	0

Appendix 4.4

Species list for the different water periods at PV2 only

Family	Species	High	Mid	Low
Bufonidae	*Rhinella marina*	2	5	6
Hyildae	*Dendropsophus bifurcus*	0	43	24
	Dendropsophus bifurcus / triangulum	0	18	28
	Dendropsophus haraldschultzi	4	54	8
	Dendropsophus leali	0	0	0
	Dendropsophus rossalleni	15	1	0
	Dendropsophus triangulum	124	141	12
	Hypsiboas boans	0	0	1
	Hypsiboas lanciformis	1	15	4
	Hypsiboas punctatus	52	390	192
	Osteocephalus taurinus	2	0	1
	Scarthyla goinorum	1	4	3
	Scinax garbei	2	48	1
	Scinax pedromedinae	2	1	2
	Scinax ruber	0	0	1
	Scinax sp.	3	0	0
	Sphaenorhychus sp.	9	4	0
	Sphaenorhynchus carneus	48	50	9
	Sphaenorhynchus dorisae	13	4	5
	Sphaenorhynchus lacteus	12	24	5
	Trachycephalus typhonius (venulosus)	0	0	0
Leptodactylidae	*Leptodactylus leptodactyloides*	12	4	2
	Leptodactylus petersii	12	2	2
	Leptodactylus sp.	26	0	18
	Leptodactylus wagneri	0	0	2
Pipidae	*Pipa pipa*	0	1	0

Appendix 4.5

Species list for the different macrohabitats across all sites

FAMILY	SPECIES	Main River	Channel	Lake	Rafts	Flooded forest clearing	Log PV2
Bufonidae	*Rhinella marina*	4	5	7	1	0	0
Hyildae	*Dendropsophus bifurcus*	14	39	19	0	0	1
	Dendropsophus bifurcus / triangulum	2	33	11	0	0	1
	Dendropsophus haraldschultzi	37	43	24	3	1	6
	Dendropsophus leali	7	0	0	0	0	0
	Dendropsophus rossalleni	5	3	3	0	7	0
	Dendropsophus triangulum	48	187	168	11	1	6
	Hypsiboas boans	1	0	0	0	0	1
	Hypsiboas lanciformis	5	9	19	4	0	0
	Hypsiboas punctatus	179	260	365	19	0	43
	Osteocephalus taurinus	1	1	0	0	1	0
	Scarthyla goinorum	3	4	8	1	0	0
	Scinax garbei	5	16	36	1	0	0
	Scinax pedromedinae	0	0	5	0	0	0
	Scinax ruber	1	0	0	0	0	0
	Scinax sp.	0	1	2	0	0	0
	Sphaenorhychus sp.	0	3	11	0	0	0
	Sphaenorhynchus carneus	5	36	78	0	3	1
	Sphaenorhynchus dorisae	2	13	16	0	0	0
	Sphaenorhynchus lacteus	12	30	33	1	0	0
	Trachycephalus typhonius (venulosus)	0	0	1	0	0	0
Leptodactylidae	*Leptodactylus leptodactyloides*	1	2	17	2	0	1
	Leptodactylus petersii	3	0	9	2	7	0
	Leptodactylus sp.	7	5	30	0	3	0
	Leptodactylus wagneri	0	0	2	0	0	0
Pipidae	*Pipa pipa*	0	1	0	0	0	0

Appendix 4.6

Species list for the main macrohabitats at PV2 only

FAMILY	SPECIES	Channel	Lake	Main river
Bufonidae	*Rhinella marina*	5	7	1
Hylidae	*Dendropsophus bifurcus*	37	19	11
	Dendropsophus bifurcus / triangulum	33	11	2
	Dendropsophus haraldschultzi	36	15	14
	Dendropsophus leali	0	0	0
	Dendropsophus rossalleni	2	2	5
	Dendropsophus triangulum	106	141	26
	Hypsiboas boans	0	0	1
	Hypsiboas lanciformis	9	10	0
	Hypsiboas punctatus	228	298	102
	Osteocephalus taurinus	1	0	1
	Scarthyla goinorum	4	4	0
	Scinax garbei	16	35	0
	Scinax pedromedinae	0	5	0
	Scinax ruber	0	0	1
	Scinax sp.	1	2	0
	Sphaenorhychus sp.	3	10	0
	Sphaenorhynchus carneus	26	73	5
	Sphaenorhynchus dorisae	10	12	0
	Sphaenorhynchus lacteus	13	26	1
	Trachycephalus typhonius (venulosus)	0	0	0
Leptodactylidae	*Leptodactylus leptodactyloides*	2	15	1
	Leptodactylus petersii	0	6	3
	Leptodactylus sp.	4	30	7
	Leptodactylus wagneri	0	2	0
Pipidae	*Pipa pipa*	1	0	0

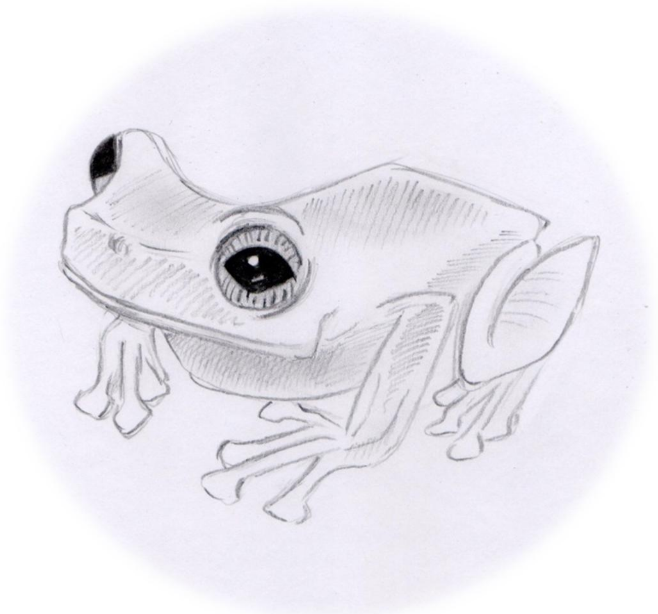
Appendix 4.7

Species list for the four sites surveyed along the Samiria River

FAMILY	SPECIES	PV1	PV2	Huisto	PV3
Bufonidae	*Rhinella marina*	3	13	0	0
Hylidae	*Dendropsophus bifurcus*	2	67	0	3
	Dendropsophus bifurcus / triangulum	0	46	0	0
	Dendropsophus haraldschultzi	31	66	4	4
	Dendropsophus leali	7	0	0	0
	Dendropsophus rossalleni	0	16	2	0
	Dendropsophus triangulum	25	277	94	11
	Hypsiboas boans	0	1	0	0
	Hypsiboas lanciformis	13	20	1	0
	Hypsiboas punctatus	95	634	57	24
	Osteocephalus taurinus	0	3	0	0
	Scarthyla goinorum	7	8	0	0
	Scinax garbei	6	51	0	0
	Scinax pedromedinae	0	5	0	0
	Scinax ruber	0	1	0	0
	Scinax sp.	0	3	0	0
	Sphaenorhychus sp.	0	13	1	0
	Sphaenorhynchus carneus	0	107	15	0
	Sphaenorhynchus dorisae	1	22	7	1
	Sphaenorhynchus lacteus	12	41	23	0
	Trachycephalus typhonius (venulosus)	1	0	0	0
Leptodactylidae	*Leptodactylus leptodactyloides*	0	18	2	0
	Leptodactylus petersii	0	16	3	0
	Leptodactylus sp.	0	44	0	1
	Leptodactylus wagneri	0	2	0	0
Pipidae	*Pipa pipa*	0	1	0	0

Chapter 5

Comparison of amphibian assemblage structure between floating meadows and terrestrial habitats



Osteocephalus taurinus

5. Abstract

Understanding how amphibian diversity originated and is maintained is a vital aspect of community ecology. By comparing the terrestrial assemblage with that found on floating meadows this chapter explores floating meadows as a dispersal vector for hylid amphibians. Ten species were floating meadow specialists rarely encountered in the terrestrial forest. The floating meadow habitat also contained a higher density of individuals than the equivalent area of terrestrial forest. Several floating meadow specialists have large geographical ranges across the Amazon Basin, suggesting that floating meadows may be an important mechanism for dispersal. Nevertheless, the terrestrial forest had a much higher species richness and species diversity. The flooded forests of the PSNR are vital for the creation of floating meadows and may offer an insight into what the huge Pebas wetland ecosystem was like prior to its drainage 10 million years ago.

5.1 Introduction

Understanding community ecology, especially in complex habitats like tropical rainforests, is vital to understanding the impacts of the many threats amphibians face. A main aim of community ecology is to understand how the diversity of species originates and is maintained within them (Morin 2011). Von May et al. (2010) highlighted flooded forests as containing a high amphibian richness and diversity, relative to other habitat types in the Amazon Basin. Chapter 4 highlighted floating meadows in Pacaya-Samiria National Reserve (PSNR) as possibly contributing to the diversity of hylid amphibians across the Amazon Basin. This chapter aims to build on this hypothesis by comparing the floating meadow assemblage with that found in adjacent terrestrial forest.

The Varzea (flooded forests) of the PSNR may resemble the huge Pebas Lake and wetland ecosystem; discussed in Chapter 4. The PSNR is the largest area of seasonally inundated forest in Peru, spanning over 60,000 km^2 (Kvist and Nebel 2001). However, flooded forests only make up 3-4% of the entire Amazon Basin, 2% of which is varzea forests (Kricher 1999). The other 96-97% is terra firma, or upland forest. The increases in river level in the PSNR results in flooding which can inundate up to 92% of the reserve (Talling and Lemoalle 1998; Myers 1990). There are two types of rivers in the Amazon Basin, white and black water. The rivers in the PSNR are black water, but, unlike most black water systems those in the PSNR are high in nutrients (Kvist and Nebel 2001). The Samiria River, along which surveys were undertaken, is an old channel of the Maranon River. Its river bed contains nutrient rich alluvial soils which enrich the water (Kvist and Nebel 2001). This creates a unique ecosystem, which supports a wide range of habitats and species.

Floating meadows are regularly encountered in flooded forests, along rivers, channels, and lakes, and are heavily influenced by the rise and fall of water levels. Many species have adapted to life on and around floating meadows, including commercially important fish species (Henderson and Hamilton 1995), fishing dolphins (pers. obs.), and breeding birds (Schulenberg et al. 2007; Goulding 1989). Some species even synchronise reproduction with the high water such as capybaras (*Hydrochoerus hydrochaeris*) and manatees (*Trichechus inunguis*), as this is when most vegetation is available. Manatees rely on floating meadow vegetation as it makes up a large proportion of their diet (Goulding 1989). When the waters recede, and floating meadows are unavailable, manatees must fast using up the fat reserves accumulated in the high water.

In order to further explore floating meadows, as a possible mechanism for the dispersal of Amazonian hylid species, the floating meadow assemblage was compared with terrestrial assemblage. If floating meadow species are found solely on this habitat – rather than being a subset of the terrestrial assemblage - this will highlight the importance of this habitat for maintaining diversity.

This chapter aims to test the flowing hypotheses:

1) The floating meadow amphibian assemblage is not a subset of the terrestrial
2) Some of the species found on floating meadows are habitat specialists whilst others are generalists
3) Floating meadow specialists are large ranging species due to the dispersal of floating meadows down river
4) Floating meadows can only support a limited number of species due to competition and lower habitat complexity

5.2 Methods

5.2.1 Floating meadows

For a full description of the floating meadow survey methods see Chapter 2.3 Floating Meadow Survey. Data were collected throughout the survey period from April – September 2012 and May – October 2013. Surveys were conducted during the high, transition and low water period. Nocturnal surveys were conducted between 19:00 – 24:00 hrs with usually three or four surveys conducted per night. A complete list of floating meadow species, from all sites surveyed was used in this analysis. See Table 5.1 for a comparison of search effort in both habitats.

5.2.2 Terrestrial

For a full description of the terrestrial transect survey methods see Chapter 2.4 Terrestrial Survey Methods. Terrestrial transects were undertaken throughout the survey period from April – September 2012 and May – October 2013. Transects were completed during the high, transition and low water period. Diurnal surveys were undertaken between 7:00-11:00 hrs and 15:00-17:00 hrs. Nocturnal surveys were conducted between 19:00-24:00 hrs. Usually two diurnal and two nocturnal transects were conducted per day.

Table 5.1. Total survey time, area and number of surveys for the terrestrial and floating meadow habitats across all sites

	Terrestrial (All sites)	**Floating meadow (All sites)**
Survey time (hours)	384	80.5
Survey area (m^2)	607,260	11,763
Number of surveys*	359	311

***Surveys refer to 500 m transects for terrestrial and individual floating meadow surveys (each 15 minute period)**

5.2.3 Survey method differences

Because of the differences in habitat structure survey methods varied slightly between the two habitats. The main difference was the length and intensity of the survey (Table 5.1). Floating meadow surveys were 10 m in length compared with the 500 m terrestrial transects. Terrestrial transects were walked at a consistent pace while floating meadow surveys were searched for a set time period (15 minutes). This may result in slightly higher search intensity on the floating meadows. The methods used to analyse the results have been chosen accordingly to compensate.

No amphibians are active by day on the floating meadows as this habitat is exposed to both sun and diurnal predators (Upton et al. 2014). Therefore only nocturnal surveys were undertaken. However, both diurnal and nocturnal surveys were undertaken on the terrestrial transects. This was particularly important in determining whether the floating meadow species are also found in the terrestrial environment. For example, *Scarthyla goinorum* was active at night on floating meadows but was active by day on terrestrial transects. It is unknown whether this is a single population which migrates between the two habitats, across seasons, or if this is two distinct populations with different activity patterns.

5.2.4 Bromeliad survey methods

Seventeen of the species found on floating meadow are hylids so searches of bromeliads were conducted in the forest canopy. Suitable trees were first identified for surveying, based on the following criteria:

- Number and size of bromeliads
- Location of bromeliads within the tree (was it possible to access them)
- Suitability of the tree, was it safe to climb / survey
- Any possible hazards such as wasp nests, dead branches and snakes

Bromeliads were either accessed using single-rope techniques (SRT) or with a ladder. The bromeliads searched ranged in height from 2-24 m above the forest floor. In total 32 trees containing 121 bromeliads were hand-searched for amphibians. These were surveyed on an ad hoc basis when suitable trees were identified. All were surveyed at the PV2 site. Some were located in close proximity to floating meadow vegetation along the channel whilst others were at the end of transect one, 500m from the river edge.

5.2.5 Statistical analysis

5.2.6 Species diversity

The Shannon Diversity Index and the Simpson's Diversity Index were calculated using the software *Species Diversity and Richness version 4.2.1* (Seaby and Hendson 2006). The indices were calculated for each individual survey (floating meadows), or transect (terrestrial), and averaged across all seasons and years. Confidence intervals were then calculated using Excel. Both indices were used as the Simpson's Index is weighted towards the abundances of the commonest species, while the Shannon Index is weighted towards the rarer species (Magurran and McGill 2011; Hill 1973). The maximum values of both indices may not be the same therefore it is difficult to make direct comparisons, yet they do highlight the difference in assemblage structure which is explored further using the CLAM analysis.

5.2.7 Species richness

Estimations of species richness were calculated using rarefaction with the software package EstimateS (Colwell 2013). Both survey and individual based rarefaction curves were created as the individual based curves control for the different sample lengths (floating meadows 10x4 m and transects 500x4 m per survey). Individual rarefaction curves are useful for comparing samples with different survey methods as they calculate the number of species observed by the number of individuals caught rather than the number of surveys undertaken. EstimateS calculates the species richness without replacement. This does not violate the assumption that the process of taking the sample itself did not change the relative abundance of species (Magurran and McGill 2011).

5.2.8 Habitat Generalists and Specialists

To determine which species in the two habitat assemblages were generalists or specialists the program CLAM was used (Chao and Lin 2011; Chazdon et al. 2011). CLAM is a classification method which uses both the R and C language to classify and then plot species. CLAM uses a multinomial model based on estimated species relative abundance. This method was chosen as it minimises bias due to differences in sampling intensities between the two habitats, as well as bias due to insufficient sampling of rare species in each habitat (Chazdon et al. 2011). This is suitable for the current data set as the floating meadows surveys were more intense than the terrestrial surveys, and some of the rare species were only represented by just one or two individuals. This analysis is also suitable for surveys spanning large areas which can be pooled into two main habitat types (Chazdon et al. 2011). The floating meadow surveys encompass the lakes, channel and rivers while the terrestrial covered many transects across different macrohabitat types. This model allows for some species to be present in one or both habitats, even if they were not detected during surveys. This is important as observed values (especially in the tropics) are often based on incomplete samples (Chazdon et al. 2011). This model assumes that the observed relative abundance in the samples reflects the true relative abundances, but is subject to random sampling error (Chazdon et al. 2011).

CLAM categorises the different species into four groups: (1) habitat A specialists, (2) habitat B specialists, (3) generalists, and (4) species that are too rare to classify as either specialists or generalists. When running the analysis in CLAM two settings can be chosen. Firstly the significance probability (P) can be set to one of four values; 0.05, 0.01, 0.005 and 0.001. For this analysis P = 0.005 was chosen as the objective was to classify all species in the assemblage (Chazdon et al. 2011). The second setting to choose is the threshold of K (K = 0.667 for super-majority threshold or K = 0.5 for simple-majority threshold). K represents the cut-off point for classification of a species as either a generalist or specialist. Super-majority was chosen as this is most suitable for smaller samples (Chazdon et al. 2011). CLAM firstly calculates the number of individuals in each habitat for each species. It them compares these using the K threshold. CLAM then tests whether the value calculated for the first habitat is significantly higher / lower than the value for the second habitat. If both tests correspond then that species is deemed a habitat specialist. The raw data consists of two species lists with the number of individuals representing each species for each habitat. The output consists of lists of species in each category, percentages of species in each category and a figure showing the two habitat types and the spread of species across the four categories.

5.3 Results

5.3.1 Species lists for all sites

Fifty-four species were observed representing nine families and two orders (Anuran and Caudata) in the PSNR (Table 5.2). Twenty-two species were found on floating meadows representing four families and 51 species on terrestrial transects representing nine families. Only three species were found exclusively on the floating meadow habitat while 32 were found exclusively on the terrestrial habitat. There were 19 species which were found in both habitats but were always more common in one habitat. *Hypsiboas punctatus* was the most common species on floating meadows with 810 individuals, yet only one individual was observed on terrestrial transects. The second most common species on floating meadows was *Dendropsophus triangulum* with 407 individuals yet only two were observed on the terrestrial transects. The same can be seen with common terrestrial species such as *Scarthyla goinorum:* 205 individuals were observed on terrestrial transects while only 15 were observed on the floating meadows.

Table 5.2. Floating meadow and terrestrial species lists for all sites surveyed in the PSNR in 2012 and 2013 (PV1, PV2, PV3 and Huisto)

FAMILY	SPECIES	Floating meadow	Terrestrial
Aromobatidae	*Allobates "samiriensis"* sp nov		3
	Allobates sp.		6
Bufonidae	*Rhinella margaritifera*		12
	Rhinella marina	16	4
Dendrobatidae	*Ameerega hahneli*		6
	Ameerega sp		1
	Ameerega trivittata		11
Hylidae	*Dendropsophus timbeba*		2
	Dendropsophus bifurcus	72	4
	Dendropsophus bifurcus / triangulum	46	
	Dendropsophus bokermanni		3
	Dendropsophus brevifrons		12
	Dendropsophus haraldschultzi	105	9
	Dendropsophus koechlini		1
	Dendropsophus leali	7	
	Dendropsophus parviceps		11
	Dendropsophus rossalleni	18	1
	Dendropsophus triangulum	407	2
	Hypsiboas boans	1	
	Hypsiboas cinerascens		3
	Hypsiboas fasciatus		1
	Hypsiboas geographicus		26
	Hypsiboas lanciformis	34	2
	Hypsiboas punctatus	810	1
	Osteocephalus deridens		86
	Osteocephalus leprieurii		51
	Osteocephalus planiceps		37
	Osteocephalus sp.		10
	Osteocephalus taurinus	3	147
	Phyllomedusa tomopterna		1
	Scarthyla goinorum	15	205
	Scinax cruentommus		3
	Scinax funereus		4
	Scinax garbei	57	7
	Scinax pedromedinae	5	38
	Scinax ruber	1	2
	Scinax sp.	3	
	Sphaenorhychus sp.	14	
	Sphaenorhynchus carneus	122	3
	Sphaenorhynchus dorisae	31	4
	Sphaenorhynchus lacteus	76	2

	Trachycephalus resinifictrix		1
	Trachycephalus typhonius (venulosus)	1	
Leptodactylidae	*Adenomera andreae*		21
	Adenomera andreae/hylaedactyla		1
	Adenomera hylaedactyla		2
	Leptodactylus discodactylus		87
	Leptodactylus leptodactyloides	20	157
	Leptodactylus pentadactylus		71
	Leptodactylus petersii	19	51
	Leptodactylus sp	45	357
	Leptodactylus wagneri	2	7
	Lithodytes lineatus		2
Microhylidae	*Chiasmocleis ventrimaculata*		1
	Ctenophryne geayi		1
	Hamptophryne boliviana		15
Pipidae	*Pipa pipa*	1	3
Plethodontidae	*Bolitoglossa altamazonica*		26
	Bolitoglossa peruviana		1
Strabomantidae	*Pristimantis altamazonicus*		66
	Pristimantis lacrimosus		2

5.3.2 Individual Density

Floating meadows had a considerably higher density of individuals, with 1.6 individuals per 10 m^2, compared with 0.026 individuals per 10 m^2 on terrestrial transects (Table 5.3).

Table 5.3. Species density on the floating meadows and terrestrial transects per 10 m^2, and 500 m^2 (T = Terrestrial, FM = Floating meadow)

			Number of individuals	
	No. of individuals	**No. of species**	**10 m^2**	**500 m^2**
All sites T	1603	52	0.026	1.32
All sites FM	1931	22	1.6	82

5.3.3 Species Richness Estimates

The estimated species richness is higher on terrestrial transects with 53 species estimated compared to floating meadows with 21 species (Table 5.4).

Table 5.4. Estimated species richness for 214 surveys and 1250 individuals (T = Terrestrial, FM = Floating meadow)

	Estimate of richness for 214 surveys	**Estimate of richness for 1250 individuals**
All sites T	50	53
All sites FM	22	21

5.3.4 Bromeliad anurans

Thirty-two trees were searched for amphibians containing 121 bromeliads. Twelve amphibians were caught comprising of six species which represented two families and only one order (Table 5.5). The most commonly encountered species was *Pristimantis lacrimosus* with three individuals. Three individuals escaped prior to identification.

Table 5.5. Species list for the bromeliad surveys

Species	No. of individuals	Height in tree (meters)	Tree number
ANURAN (6)			
Hylidae (3)			
Osteocephalus deridens	1	6	2
Osteocephalus taurinus	1	6	2
Scinax pedromedinae	1	2	10
Strabomantidae (3)			
Pristimantis altamazonicus	2	2.5	21
Pristimantis lacrimosus	3	6	2
Pristimantis waoranii	1	6	2
Unidentified	3	7/4/7	1/14/16

5.3.5 Species Diversity

The Simpson's Diversity Index was significantly higher on terrestrial transects (3.64 ± 0.44) than on floating meadows (2.65 ± 0.30) (t = 3.26, df = 156, p = 0.001). However, the Shannon Diversity Index was not significantly different between the two habitats (t = 1.27, df = 156, p = 0.21) (Fig. 5.1).

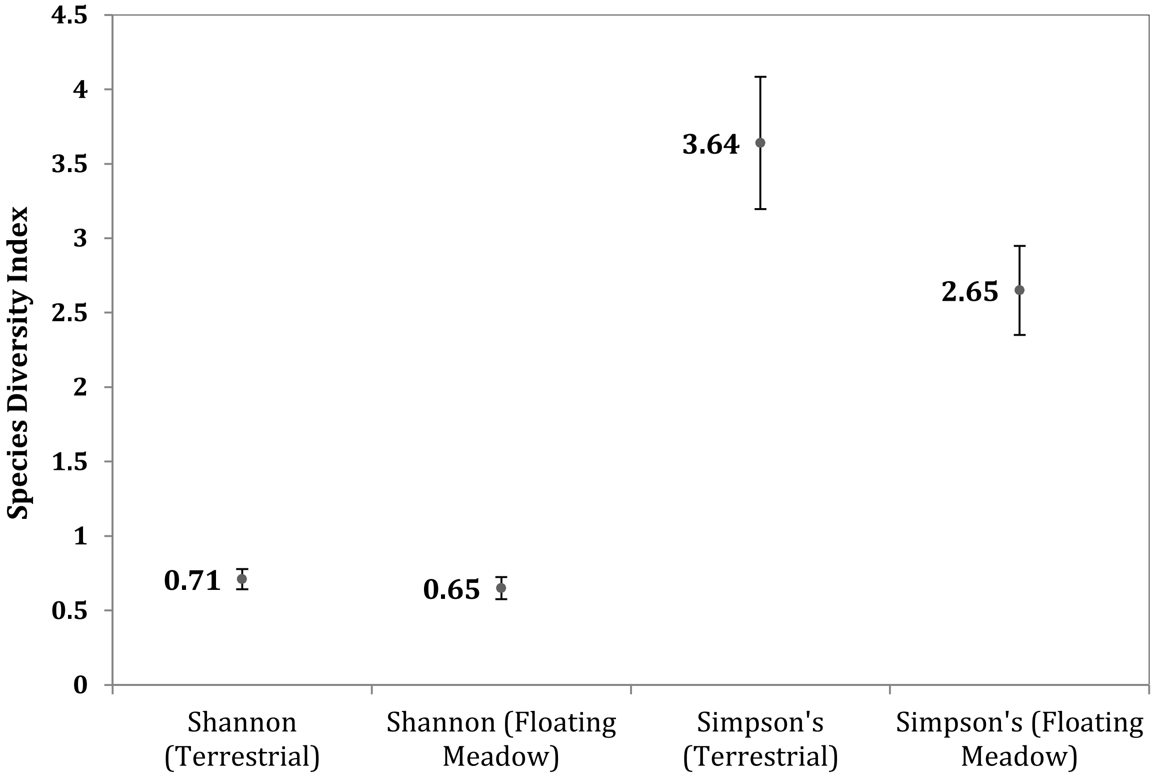


Figure 5.1. Mean Shannon & Simpson's Diversity Indices for the (157) floating meadow and (285) terrestrial surveys with 95% confidence intervals.

5.3.6 Habitat generalists and specialists

Nineteen of the 54 species (34.5%) were shared between the terrestrial and floating meadow habitat. Using the software package CLAM 24 species (45.5%) were too rare to classify. Ten (18.2%) were classified as floating meadow specialists, 19 (34.5%) as terrestrial specialists and one (1.8%) generalist (P = 0.005; Fig. 5.2). The species identified as a habitat generalist was *Rhinella marina.* See Table 5.6 for the full list of classifications.

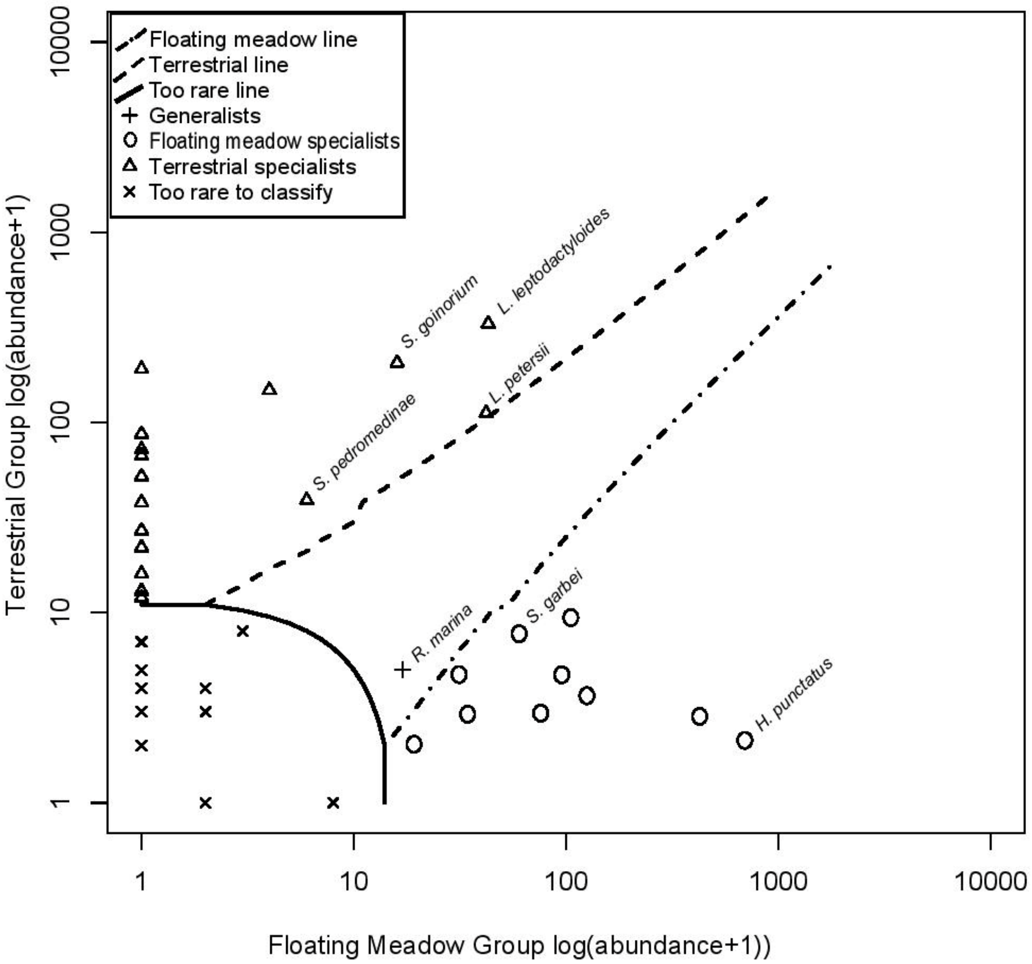


Figure 5.2. Classification of the terrestrial and floating meadow assemblages across all sites surveyed in the PSNR.

Table 5.6. A list of all species for the floating meadow and terrestrial habitats split into the classifications assigned by CLAM analysis

Generalists	Floating meadow specialists	Terrestrial specialists	Too rare to classify
Rhinella marina	*Dendropsophus bifurcus*	*Rhinella margaritifera*	*Allobates "samiriensis" sp nov*
	Dendropsophus haraldschultzi	*Ameerega trivittata*	*Allobates sp*
	Dendropsophus rossalleni	*Dendropsophus brevifrons*	*Ameerega hahneli*
	Dendropsophus triangulum	*Dendropsophus parviceps*	*Dendropsophus timbeba*
	Hypsiboas lanciformis	*Hypsiboas geographicus*	*Dendropsophus bokermanni*
	Hypsiboas punctatus	*Osteocephalus deridens*	*Dendropsophus koechlini*
	Scinax garbei	*Osteocephalus leprieurii*	*Dendropsophus leali*
	Sphaenorhynchus carneus	*Osteocephalus planiceps*	*Hypsiboas boans*
	Sphaenorhynchus dorisae	*Osteocephalus taurinus*	*Hypsiboas cinerascens*
	Sphaenorhynchus lacteus	*Scarthyla goinorum*	*Hypsiboas fasciatus*
		Scinax pedromedinae	*Phyllomedusa tomopterna*
		Adenomera andreae	*Scinax cruentommus*
		Leptodactylus discodactylus	*Scinax funereus*
		Leptodactylus leptodactyloides	*Scinax ruber*
		Leptodactylus pentadactylus	*Trachycephalus resinifictrix*
		Leptodactylus petersii	*Trachycephalus typhonius (venulosus)*
		Hamptophryne boliviana	
		Bolitoglossa altamazonica	*Adenomera hylaedactyla*
		Pristimantis altamazonicus	*Leptodactylus wagneri*
			Lithodytes lineatus
			Chiasmocleis ventrimaculata
			Ctenophryne geayi
			Pipa pipa
			Bolitoglossa peruviana
			Pristimantis lacrimosus

5.4 Discussion

The terrestrial habitat has much higher species richness with 54 species compared to only 22 species on floating meadows. This is supported by the rarefaction curves in Chapter 3 which showed an increasing richness even after 1200 individuals had been caught. Whilst the floating meadow curves plateaued shortly after 200 individuals and this was consistent across sites and macrohabitats. The Simpson's diversity indices were higher in the terrestrial habitat (Simpson's: 3.46) compared with the floating meadows (Simpson's: 2.65). The Simpson's Index in its simplest form represents the likelihood of two randomly selected individuals being from the same species. The terrestrial assemblage contained more species that the floating meadow assemblage, which had two common species (*Hypsiboas punctatus* and *Dendropsophus triangulum)* that made up 63% of the individuals caught. This increased the probability that two individuals caught in the floating meadows would be from the same species. In comparison, on the terrestrial transects there were 35 rare species (represented by fewer than 10 individuals). Thus the likelihood of encountering two individuals of the same species was much lower on the terrestrial transects resulting in high a Simpson's diversity index.

These differences in both diversity and richness are not unexpected results as the two habitats differ in microhabitat availability. Tropical rainforests comprise many layers including, but not limited to, the forest floor, understory, canopy and emergent trees (Kricher 1999). These layers are formed by the high diversity of plant and tree species and in turn offer many different microhabitats to a variety of taxa and species. The floating meadows on the other hand are formed of a limited number of plant species and thus do not have the structural complexity of the terrestrial forests. Only three species were found exclusively on the floating meadows, yet 33 species were found exclusively on terrestrial transects. It is possible that floating meadows can only support a limited number of species, due to competition and lower habitat complexity. For example, *Leptodactylus pentadactylus* is a large terrestrial species which lays its foam nests underground. This species could not utilize the floating meadows due to its large size and specific reproductive strategy.

Although the diversity and richness within the terrestrial habitat was considerably higher, individual density was lower. The average number of individuals on terrestrial transects was 1.32 individuals per 500 m^2 compared to 82 per 500 m^2 on floating meadows. This equates to about 5 individuals per terrestrial transect and 382 individuals in the equivalent area of floating meadow. The terrestrial forest is able to support a higher diversity of species than the floating meadows, but they are found in lower abundances. This may be due to the habitat structure, as while floating meadows are only found around lakes, rivers and channel edges, the forest spans a much larger area. Floating meadows are essentially island of isolated habitat, surrounded by less suitable terrestrial habitat, whilst the terrestrial is continuous and connected. The theory of island biogeography and species area relationships is very relevant here. Chapter four noted differences in species richness between the lake, channel and main river. The lake contained a much larger island of floating meadows whilst in comparison the channel and main river were much smaller. The number of species corresponds to this with fewer observed in the channel and main river. Generally larger islands will contain more species than smaller islands and this pattern is followed here. Whilst the terrestrial habitat is vast and continuous allowing many more species to exist.

Structurally the two habitats are different floating meadows are limited in height by the plant species of which they are comprised, often no higher than 3 m. In comparison, the forest can be upwards of 30 m in height with hundreds of plant species. This means the amphibians on the terrestrial habitat have a vast area available. On the floating meadows hylids congregate to breed in a much smaller area which means they can be found in a much higher density. It may also be explained by the density of prey items, which are more concentrated, on the floating meadows (as highlighted in Chapter 4). A greater diversity of plant species, on which invertebrates feed, could result in a higher concentration of prey species. This could help to explain the abundance of anurans on floating meadows. Predation risk may also influence the density on floating meadows, especially diurnally; no anurans are active on floating meadows during the day. Whilst this may be explained by the exposure to the sun, it is also likely to be influenced by the diversity and density of aquatic birds present on this habitat by day. These birds will readily catch and eat active anurans on the floating meadows. This means all anurans are active nocturnally and so concentrating them at night. This may explain the unusual activity patterns of *Scarthyla goinorum* which was found active diurnally in the terrestrial forest yet was active nocturnally on floating meadows.

Rarefaction curves in Chapter 3 showed that adequate sampling had been conducted on floating meadows, yet the curves for the terrestrial transects were still increasing. This may mean, however, that some of the hylid floating meadow species could be found in the terrestrial forest but were not present in the survey area, or too rare to be detected using the transect method. Arboreal searches of bromeliads were therefore undertaken to search for hylid floating meadow specialists. Although 121 Individual bromeliads were searched only 12 anurans were encountered representing six species. None of these were floating meadow specialists - *Osteocephalus taurinus* and *Scinax pedromedinae* were both found on floating meadows, but were classified as terrestrial specialists. These surveys were only undertaken on an ad hoc basis when suitable trees and bromeliads were located, so could not be considered as an extensive survey. Other hylid species are likely to be found in the canopy but conducting arboreal surveys is difficult. Anurans were also hard to find within bromeliads and they would often escape or take refuge inside the leaf axils.

The most commonly encountered family in both habitats was the Hylidae family, represented by both the highest number of species (32), and individuals (3349). Chapter 1 discussed how floating meadows could help to explain the diversity of hylids across the Amazon. This chapter has explored whether those species found on floating meadows are restricted to this habitat. Only one species was classified as a habitat generalist, *Rhinella marina*, which was found in both habitats. *Rhinella marina* is an invasive species in many countries, but is native in the Amazon region (Bartlett and Bartlett 2003). This species was often encountered in villages and disturbed habitats, even in the city of Iquitos (pers. obs.). As a habitat generalist it is able to survive and breed in a wide range of habitats, hence why it is an invasive species (Phillips et al. 2007). The classification analysis identified ten species as floating meadow specialists, all were hylids. Five of these ten species have large ranges spanning much of the Amazon Basin (AmphibiaWeb 2015). *Hypsiboas punctatus* was the most commonly encountered species on floating meadows and seems to have the largest range of all the floating meadow species. It has been recorded throughout the Amazon Basin and is even present on Trinidad and Tobago (AmphibiaWeb 2015). This species is often recorded around flooded lakes and rivers as well as permanent and semi-permanent water bodies (Bartlett and Bartlett 2003; Rodriguez and Duellman 1994). Males have been recorded calling from floating vegetation and bushes around the edges of pools with eggs deposited in water (Bartlett and Bartlett 2003; Rodriguez and Duellman 1994). It has even been observed along the water's edge (within floating vegetation) in Iquitos city away from any suitable forest habitat (Bartlett and Bartlett 2003; Pers. Obs.). This suggests that *Hypsiboas punctatus* does not rely on the terrestrial forest and is able to reproduce and live entirely on the floating meadows. *Dendropsophus triangulum* was the second most common species on the floating meadows and again has a large range which seems to closely follow the Amazon River (AmphibiaWeb 2015). This species is heavily associated with permanent and semi-permanent water bodies with males recorded calling from aquatic vegetation (Bartlett and Bartlett 2003; Rodriguez and Duellman 1994). Three species in the genus *Sphaenorhynchus* were also floating meadow specialists, *S. lacteus, S. dorisae* and *S. carneus*. Relatively little is known about their breeding activity. The few that have been recorded were again observed around the edges of permanent and semi-permanent pools on emergent and floating vegetation (Bartlett and Bartlett 2003; Rodriguez and Duellman 1994). These three species are referred to as semi-aquatic due to their close associations with water, often when disturbed they will jump down into the water and swim away (Bartlett and Bartlett 2003; pers. obs.).

Many of the floating meadow specialists have the ability to breed in rivers, lakes, and temporary pools which enables them to occupy many aquatic habitats. When the huge Pebas wetland system drained expanding the terrestrial forest ancestral hylids could have migrated into the forests and diversified into the many species we see today. Appendix two contains several Hylidae phylogenies which contains both floating meadow and terrestrial specialists. Some of the relationships are quite interesting, for example *Dendropsophus koechlini; brevifrons;* and *parviceps* (terrestrial specialists) are closely related species which share a common ancestor with *Dendropsophus bifurcus* and *triangulum* two floating meadow specialists. These species may have split and evolved separately on these two habitat types after the drainage of the wetland system. Another interesting observation is that Sphaenorhynchus dorisae and Sphaenorhynchus lacteus are much more basel in the phylogeny than other species. These are both floating meadow specialists which are well adapted to this habitat, and are likely to have speciated within the floating meadows.

There are many small hylid species which could have evolved from floating meadow species entering the terrestrial habitat, such as *Dendropsophus brevifrons* and *D. parviceps,* terrestrial specialists which are never encountered on the floating meadows. Both of these species breed in temporary pools after heavy rainfall in upland forests or when flood water recedes, in the flooded forest (Bartlett and Bartlett 2003; Rodriguez and Duellman 1994; pers. obs.). Both species are likely to spend their time in the canopy only coming down to breed when temporary pools are available. The diversification of anuran species within the terrestrial forest has allowed the evolution of a wealth of different reproductive modes. Many species still reproduce by depositing eggs on leaves above water, with aquatic tadpoles developing in temporary pools. However, other modes have developed allowing amphibians to occupy different niches. For example, *Pristimantis altamazonicus* (a terrestrial specialist) removes the need for water as the tadpoles undergo direct development within the egg sac. Some species have evolved specific niches such as *Trachycephalus resinifictrix* which is most often found within tree holes where it lives and breeds (Bartlett and Bartlett 2003). Others even show parental care for young such as *Ameerega hahneli,* males firstly protect the eggs. When they hatch the male carries tadpoles to individual pools of water such as bromeliads to continue their development (Bartlett and Bartlett 2003). Many of these reproductive modes may have developed after the drainage of the huge Pebas wetland ecosystem allowing amphibians, particularly hylids, to diversify and occupy many different niches within the terrestrial forests. Chapter 6 will further explore the diversity of reproductive modes in the PSNR.

5.5 Conclusion

This chapter further supports the hypothesis that floating meadows may explain the huge diversity and wide distribution of hylid species across the Amazon Basin. Ten species were identified as floating meadow specialists; these were rarely encountered in the terrestrial forest. Yet these species have large ranges spanning much of the Amazon Basin. Chapter 1 has shown that floating rafts of vegetation can transport hylids large distances. This would explain why the floating meadow specialists are found across the entire Amazon Basin. The flooded forests of the PSNR are vital for the creation of floating meadows and may offer an insight into what the huge Pebas wetland ecosystem was like prior to its drainage 10 million years ago. The high density of anurans found on floating meadow vegetation in the PSNR may play an important role in dispersal downriver, aiding gene flow.

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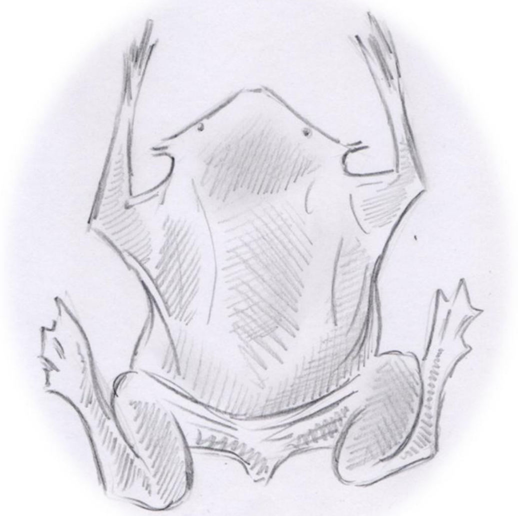
Chapter 6

Predictors of anuran reproductive modes in a Peruvian flooded forest



Pipa pipa

6. Abstract

Anurans have a greater diversity of reproductive strategies than any other vertebrate group. These have evolved from the primitive strategy of laying eggs within water, to the other extreme of direct development within a nest, with a wide range of intermediates. Thirty-nine reproductive modes have been described in anurans worldwide, 31 of which have been recorded in the Neotropics. This chapter uses reproductive modes as a surrogate for other diversity measures, and using General Linear Mixed Effect Models explores potential predictors of the number of reproductive modes observed in Pacaya-Samiria National Reserve (PSNR). Fifty-four anuran species were encountered representing fourteen reproductive modes. The most common modes involve eggs laid within and overhanging water with tadpoles dropping down into the water on hatching. Some species show plasticity in their modes, and are able to change depending on the available habitats. This could be beneficial in response to climate change which is affecting the natural flood cycle. In habitats that are subject to seasonal flooding reproductive modes that allow eggs to be laid over either still or moving water may be more advantageous than those that require temporary ponds. However, fewer reproductive modes were observed in disturbed habitats.

6.1 Introduction

Anurans have a higher diversity of reproductive strategies than any other vertebrate class (Santoroa and Brandae 2014). The most primitive strategy involves laying eggs in water, which hatch as tadpoles, eventually undergoing metamorphosis (Gomez-Mestre et al. 2012; Duellman 1985). However, predation, competition, and habitat availability has resulted in the evolution of many different reproductive strategies (Duellman 1989). Indeed, some anurans do not require aquatic habitats for reproduction at all. Tadpoles of *Pristimantis* species undergo direct development within a nest, emerging as fully formed froglets (Duellman and Trueb 1986). Direct development has evolved independently in at least 12 anuran groups, representing 24% of all species (Duellman 2007; Duellman and Trueb 1986). There is a wide range of intermediate strategies, such as dendrobatid species that lay eggs in leaf litter and then transport tadpoles to bromeliads where they continue their development (Brown et al. 2008). Certain strategies, such as parental care, have been selected for as a result of predation on tadpoles, eggs and adults; competition for resources; or a move towards terrestrial development (Duellman and Trueb 1986). There are many forms of parental care. Some species transport tadpoles (many dendrobatids), others swallow their tadpoles allowing them to develop in their vocal sac (*Rhinoderma darwinii*), whilst some carry the eggs embedded in their dorsum (*Pipa pipa*) (Crump 2015). Some anurans even have the ability to change their reproductive strategy depending on environmental conditions. Species may change the location of their egg deposition site. For example, *Hypsiboas boans* usually constructs nests in sandy river banks, but in rocky locations they deposit eggs in pools of water between the rocks or within leaf litter (Caldwell 1992).

Reproductive modes (the name given to reproductive strategies) have been extensively studied: Santoro and Brandao (2014); Bitar et al. (2012); Gomez-Mestre et al. (2012); Hadden and Prado (2005); Magnusson and Hero (1991); Hödl (1990); Duellman (1989); Duellman and Trueb (1986); Crump (1974); and Salthe and Duellman (1973). Salthe and Duellman (1973) first defined the concept of reproductive modes in anurans. The traits they used to define a reproductive mode were "oviposition site, ovum and clutch characteristics, rate and duration of development, stage and size of hatchling and type of parental care, if any". Duellman and Trueb (1986) later identified 29 separate reproductive modes. More recently, however, Hadden and Prado (2005) further increased the list of reproductive modes, bringing the total number of anuran reproductive modes worldwide to 39, 31 of which have been recorded in the Neotropics. Novel modes are still being discovered, such as the recently described fanged frog which gives birth to live tadpoles (Iskandar et al. 2014).

Whilst our understanding of anuran reproductive modes has increased greatly, there are still many questions left to answer (Crump 2015). We need to further investigate the evolutionary drivers of terrestrial reproduction, as well as how reproductive modes will respond to changes in environmental conditions, and whether plasticity can affect the ability to survive climate change (Crump 2015). Certain reproductive modes may make species more vulnerable to population declines (Lips 1998; Pounds et al. 1997). Equally, a reduction in the number of reproductive modes may occur in relation to the size of forest fragments (Almeida-Gomes and Rocha 2015). Smaller fragments contain fewer modes, probably due to a reduction in suitable breeding sites. Habitat loss, climate change and diseases have the potential to affect certain reproductive modes more than others. Increasing breeding sites in fragmented habitats could increase populations of declining species (Crump 2015). This chapter aims to use reproductive modes as a surrogate for other diversity measures. By exploring the predictors of reproductive modes in the PSNR we can start to understand how anurans respond to disturbance, environmental and survey variables. This chapter will also document the range of reproductive modes found in the PSNR which will create baseline data that can be used in further work to assess the impacts of climate change.

This chapter aims to test the following hypotheses:

1. The Pacaya-Samiria will contain a large number of reproductive modes due to the large amphibian diversity
2. The number of reproductive modes will be influenced by disturbance, environmental and survey variables

6.2 Methods

Two data sets were analysed for this chapter. The first data set was collected at the PV2 site, with three terrestrial transects surveyed both day and night in equal numbers as per the methods described in Chapter 2.3 Terrestrial Transects. Data were collected in the high, low and transition water periods in both 2012 and 2013.

The second set of data was collected from the 27th August 2013 until 30th September 2013 at three sites, PV1 (river mouth), PV2 and PV3. Three terrestrial transects were surveyed at each site as per the methods in Chapter 2. These transects were varying distances from the guard station at each site, on both sides of the river. Eight replicated surveys were conducted, four diurnal surveys and four nocturnal. These surveys were undertaken within five weeks of each other to minimise the influence of seasonal and water level changes.

6.2.1 Disturbance

Localised disturbance many influence the number of reproductive modes observed. The following five disturbance variables were used: 1) distance from the guard station; 2) distance from the village; 3) distance from the mouth of the Samiria River; 4) the number of trees on a transect over 50 cm DBH; and 5) the average canopy score for each transect. The canopy score was conducted as per the methods in Brown et al. (2000). The canopy score is calculated using an array of 25 dots printed on a transparent screen (canopy scope). Every 50 m, along the transect, canopy score was calculated. The canopy scope is held 15 cm from the eyes and pointed at the largest canopy gap, the number of dots within the sky (rather than tree canopy) are counted. This score was then averaged across each transect. This is used to assess canopy openness (Brown et al. 2000). The three distance variables were used because transects closer to human habitation are likely to suffer higher disturbance. Distance from the Samiria River mouth was used as the PSNR is a protected reserve yet some resource extraction is allowed. Local people tend to use small boats, which limits the distance they can travel. Consequently the resource use at the edge of the reserve is likely to be higher than further up river into the reserve. The number of large trees over 50 cm DBH was used as this represents the impacts of logging (Rödel and Ernst 2004). The number of trees was counted within 2 m either side of each transect (the survey area). Larger trees are cut for firewood, house and canoe building. A transect with many large trees is likely to have a lower logging level than one with few large trees and a dense undergrowth. This then influences the canopy score; a lower count represents a full canopy while a high score represents an open or patchy canopy.

6.2.2 Reproductive modes

Reproductive modes were used as a surrogate for other diversity measures to explore how disturbance, environmental and survey variables influences diversity. The reproductive modes used in this chapter were described by Hadden and Prado (2005). Each species was assessed individually by checking the literature, AmphibiaWeb, and the following ID guides (Ouboter and Jairam 2012; Duellman 2005; Bartlett and Bartlett 2003; Rodriguez and Duellman 1994). When possible this was then confirmed by personal observation in the field. Only anuran data were included in the analysis due to the limited number of salamanders observed (only two species), and the lack of information on their reproductive modes. A description of the different reproductive modes can be found in Table 6.1. The number of reproductive modes was calculated by counting the number of modes a species or group of species displayed for example one species which used three modes, or ten species which used three modes would both only count as three reproductive modes.

Table 6.1. All reproductive modes currently described (Hadden and Prado 2005)

Aquatic eggs	
Eggs deposited in water	
Mode 1	Eggs and exotrophic[1] tadpoles in lentic water
Mode 2	Eggs and exotrophic tadpoles in lotic water
Mode 3	Eggs and early larval stages in constructed subaquatic chambers; exotrophic tadpoles in streams
Mode 4	Eggs and early larval stages in constructed subaquatic chambers; exotrophic tadpoles in streams
Mode 5	Eggs and early larval stages in subterranean constructed nests; subsequent to flooding, exotrophic tadpoles in ponds or streams
Mode 6	Eggs and exotrophic tadpoles in water in tree holes or aerial plants
Mode 7	Eggs and endotrophic[2] tadpoles in water-filled depressions
Mode 8	Eggs and endotrophic tadpoles in water in tree holes or aerial plants
Mode 9	Eggs deposited in a stream and swallowed by female; eggs and tadpoles complete development in stomach
Eggs in bubble nest	
Mode 10	Bubble nest floating on pond; exotrophic tadpoles in ponds
Eggs in foam nest (aquatic)	
Mode 11	Foam nest floating on pond; exotrophic tadpoles in ponds
Mode 12	Foam nest floating on pond; exotrophic tadpoles in streams
Mode 13	Foam nest floating on water accumulated in constructed basins; exotrophic tadpoles in ponds
Mode 14	Foam nest floating on water accumulated on the axis of terrestrial bromeliads; exotrophic tadpoles in ponds
Eggs embedded in dorsum of aquatic female	
Mode 15	Eggs hatch into exotrophic tadpoles
Mode 16	Eggs hatch into froglets
Terrestrial or arboreal eggs (not in water)	
Eggs on ground, on rocks, or in burrows	
Mode 17	Eggs and early tadpoles in excavated nests; subsequent to flooding, exotrophic tadpoles move to water
Made 18	Eggs on ground or rock above water; upon hatching, exotrophic tadpoles move to water
Mode 19	Eggs on humid rocks, in rock crevices, or on tree roots above water, exotrophic semi terrestrial tadpoles living on rocks and rock crevices in a water film or in the water-land interface
Mode 20	Eggs hatching into exotrophic tadpoles that are carried to water by adult
Mode 21	Eggs hatching into endotrophic tadpoles that complete their development in the nest
Mode 22	Eggs hatching into endotrophic tadpoles that complete their development on the dorsum or in the pouches of the adults
Mode 23	Direct development of terrestrial eggs
Arboreal eggs	
Mode 24	Eggs hatching into exotrophic tadpoles that drop in lentic water

Mode 25	Eggs hatching into exotrophic tadpoles that drop in lotic water
Mode 26	Eggs hatching into exotrophic tadpoles that develop in water-filled cavities in trees
Mode 27	Eggs hatching into froglets
Eggs in foam nests (terrestrial or arboreal)	
Mode 28	Foam nest on the humid forest floor; subsequent to flooding, exotrophic tadpoles in ponds or streams
Mode 29	Foam nests with eggs and early larval stages in basin; subsequent to flooding, exotrophic tadpoles in ponds or streams
Mode 30	Foam nests with eggs and early larval stages in subterranean constructed nests; subsequent to flooding, exotrophic tadpoles in ponds
Mode 31	Foam nest with eggs and early larval stages in subterranean constructed nests; subsequent to flooding, exotrophic tadpoles in streams
Mode 32	Foam nest in subterranean constructed chambers; endotrophic tadpoles complete development in nest
Mode 33	Arboreal foam nest; hatchling tadpoles drop into ponds or streams
Eggs carried by adult	
Made 34	Eggs carried on legs of male; exotrophic tadpoles in ponds
Made 35	Eggs carried in dorsal pouch of female; exotrophic tadpoles in ponds
Mode 36	Eggs carried on dorsum or in dorsal pouch of female; endotrophic tadpoles in bromeliads or bamboo
Mode 37	Eggs carries on dorsum or in dorsal pouch of female; direct development into froglets
Eggs retained in oviducts	
Mode 38	Ovoviviparity; nutrition provided by yolk
Mode 39	Viviparity; nutrition provided by oviductal secretions

[1] Exotrophic tadpoles – feeding on other sources not from parents

[2] Endotrophic tadpoles – food is acquired from parents e.g. yolk in stomach or infertile feeder eggs provided by female

6.2.3 Statistical Analysis

Generalized linear mixed effect models were used as per the methods in chapter 4. For this analysis the data consist of one response variable - the number of reproductive modes. With the following explanatory variables: distance from the river mouth; distance from the guard station; distance from villages; the number of large trees (over 50cm DBH); canopy score; water level; minimum temperature; minimum humidity; cloud cover; number of surveyors; nocturnal or diurnal survey; and phase of the moon. Maximum temperature and maximum humidity were also recorded but were omitted from the analysis as they are correlated with the minima (Dormann 2013). Distance from villages and distance from the river mouth were also correlated so only village distance was used in the analysis.

Data exploration was completed prior to analysis confirming the suitability of general linear mixed models (Zuur 2010), as per chapter 4 methods.

Generalised linear mixed effect models (GLMM) with Poisson errors were used to analyse which explanatory variables were most significant in influencing the number of observed reproductive modes (Bolker et al. 2008; Faraway 2006). GLMM were chosen as the surveys were not independent of each other (transects and sites were repeatedly surveyed), and could result in spatial pseudoreplication (Crawley 2009). In mixed effect models random effects can be added for these non-independencies. The Poisson errors are needed because the response variable is count data, rather than continuous data (Crawley 2009). GLMM allow the use of random effects which tells the model to expect more than one result for certain variables (Crawley 2009) as a result transect and/or site will be included as random effects. Two full models were run: one using the data from PV2 only, the second used a small subset of data from PV1, PV2 and PV3 over a six week period. All variables were included initially in the models and model simplification was completed by removing variables which were not significant. Models were compared using the likelihood ratio test. Model simplification was completed by removing the least significant explanatory variable, which is the one with the highest p-value (Crawley 2009). Once the explanatory variable was removed the new model was compared with the previous model. This was done to validate the removal of that variable. The likelihood ratio test was then used to assess model fit (Winter 2013). Model simplification was undertaken as it removed the variables from the model which were not significant in predicting the response variable. These were only removed where suitable, for example the lunar cycle was used originally in the models however as one of the least significant variables was removed. Under the forest canopy changes in light levels were unlikely to impact on the number of modes observed.

See Chapter 4 methods for further details on the use of GLMM. Four models were run as follows:

Model one (Low water dataset) – Number of reproductive modes ~ Distance from village + distance from guard station + number of large trees + average canopy score + minimum temperature + minimum humidity + phase of the moon + percentage of cloud cover + number of surveyors (Fixed effects – transect + site)[1]

[1] This model did not include water level as a variable as the data were collected over a short time period.

Model two (complete PV2 dataset) – Number of reproductive modes ~ distance from guard station + average canopy score + minimum temperature + minimum humidity + phase of the moon + percentage of cloud cover + number of surveyors + water level + nocturnal or diurnal (Fixed effect – transect)[2]

[2] This model did not include the number of large trees as this was correlated with the distance from guard station. Distance from village was omitted as all data were collected at PV2 so the distance is the same for each transect.

Model three (PV2 nocturnal dataset) – Number of reproductive modes ~ distance from guard station + average canopy score + minimum temperature + minimum humidity + phase of the moon + percentage of cloud cover + number of surveyors + water level (Fixed effect – transect)

Model four (PV2 diurnal dataset) – Number of reproductive modes ~ distance from guard station + average canopy score + minimum temperature + minimum humidity + percentage of cloud cover + number of surveyors + water level (Fixed effect – transect)

6.3 Results

During the 2012 and 2013 surveys fifty-four anuran species were observed in the PSNR representing eight families (Table 6.2). Fourteen reproductive modes were observed, the most common of which was used by 27 of the 54 species. This mode (Mode 1) involves eggs being laid in still water where the tadpoles stay and complete metamorphosis. The second most common mode (Mode 2) was represented by 14 species. Five modes are only represented by one or two species such as Mode 16 where the eggs are embedded in the dorsum of the aquatic female and the eggs hatch into froglets.

There is a significant relationship between the number of reproductive modes and four species diversity measures: Shannon diversity index; Simpson's diversity index; number of species and; number of individuals (Fig. 6.3). These diversity measures are all positively correlated with the number of reproductive modes (Figure 6.1). Simple linear regression using General linear mixed models was used to test this relationship: an increase in the Shannon index by one increased the number of reproductive modes by 1.03 ($p = 0.001$); an increase in the Simpsons index by one increased the number of reproductive modes by 0.07 ($p = 0.001$); an increase in the number of species by one increased the number of reproductive modes by 0.29 (0.001); and an increase in the number of individuals by one increased the number of reproductive modes by 0.10 (0.001).

Table 6.2. Complete species list for Pacaya-Samiria Reserve during 2012 and 2013 with reproductive modes

Family	Species	No. Ind.	Reproductive Modes
AROMOBATIDAE	*Allobates "samiriensis"* sp nov	1	20
	Allobates sp.	9	20
BUFONIDAE	*Rhinella margaritifera*	15	1 + 2
	Rhinella marina	69	1
DENDROBATIDAE	*Ameerega hahneli*	5	20
	Ameerega trivittata	11	20
HYLIDAE	*Dendropsophus timbeba (allenorum)*	2	1
	Dendropsophus bifurcus	82	24 + 25
	Dendropsophus bokermanni	3	24
	Dendropsophus brevifrons	12	24
	Dendropsophus haraldschultzi	116	1 + 2
	Dendropsophus koechlini	1	1
	Dendropsophus leali	7	1 + 2
	Dendropsophus parviceps	12	1
	Dendropsophus rossalleni	21	24 + 25
	Dendropsophus triangulum	421	24 + 25
	Hypsiboas boans	1	4
	Hypsiboas cinerascens	3	1 + 2
	Hypsiboas fasciatus	6	1
	Hypsiboas geographicus	28	1 + 2
	Hypsiboas lanciformis	38	1 + 2
	Hypsiboas punctatus	842	1 + 2
	Osteocephalus deridens	90	6
	Osteocephalus leprieurii	51	1
	Osteocephalus planiceps	37	6
	Osteocephalus taurinus	159	1
	Phyllomedusa tomopterna	1	1
	Scarthyla goinorum	224	1 + 2
	Scinax cruentommus	3	1
	Scinax funereus	4	1
	Scinax garbei	65	1 + 2
	Scinax pedromedinae	42	1 + 2
	Scinax ruber	7	24 + 25
	Sphaenorhynchus carneus	128	1 + 2
	Sphaenorhynchus dorisae	35	1 + 2
	Sphaenorhynchus lacteus	78	1 + 2
	Trachycephalus resinifictrix	1	6
	Trachycephalus typhonius (venulosus)	2	1 + 2
LEPTODACTYLIDAE	*Adenomera andreae*	21	32
	Adenomera hylaedactyla	2	32
	Leptodactylus discodactylus	88	29
	Leptodactylus leptodactyloides	182	11
	Leptodactylus pentadactylus	72	29 + 30 + 32
	Leptodactylus petersii	63	11

	Leptodactylus sp.	409	11
	Leptodactylus wagneri	9	11
	Lithodytes lineatus	2	32
MICROHYLIDAE	*Chiasmocleis ventrimaculata*	1	1
	Ctenophryne geayi	1	1
	Hamptophryne boliviana	15	1
PIPIDAE	*Pipa pipa*	5	16
STRABOMANTIDAE	*Pristimantis altamazonicus*	68	23
	Pristimantis lacrimosus	4	27 + 23
	Pristimantis ockendeni	1	27
	Pristimantis waoranii	3	27

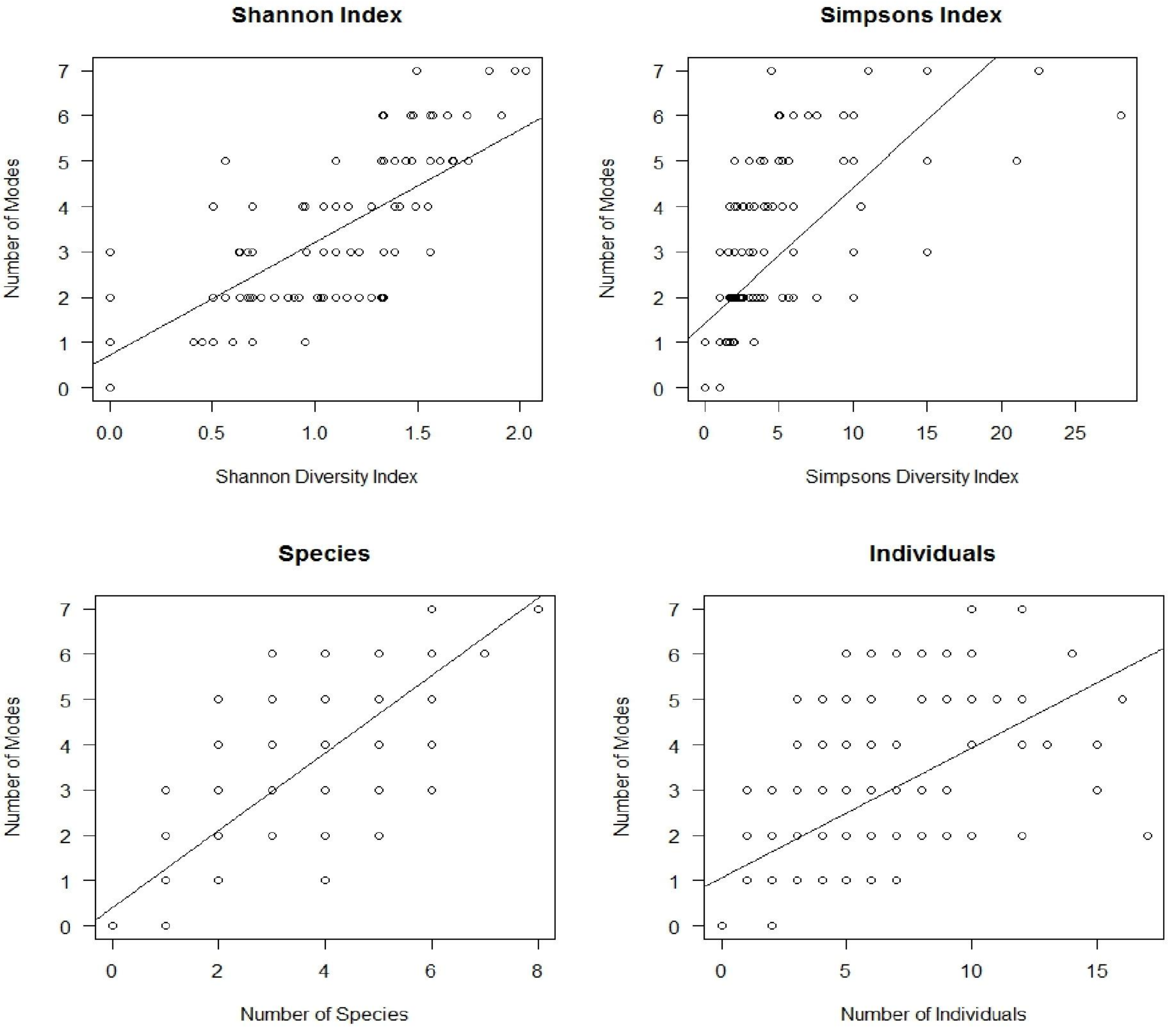


Figure 6.1. The relationship between the number of reproductive modes and four diversity measures: number of species; number of individuals; Shannon diversity index and; Simpson's diversity index. The line represents the line of best fit using the intercept and slope values from the GLM output. The data used in these figures was accumulated across transects.

6.3.1 Low water reproductive modes

Table 6.3. Reproductive modes and species list for the three sites

Family	**Species**	**PV1**	**PV2**	**PV3**
Bufonidae	*Rhinella margaritifera*	1 + 2		
Hylidae	*Dendropsophus bifurcus*	24 + 25	24 + 25	24 + 25
	Dendropsophus bokermanni		24	
	Dendropsophus brevifrons		24	
	Dendropsophus haraldschultzi	1 + 2	1 + 2	1 + 2
	Dendropsophus koechlini		1	
	Dendropsophus parviceps		1	
	Dendropsophus rossalleni	24 + 25		
	Hypsiboas fasciatus			1
	Hypsiboas punctatus		1 + 2	
	Osteocephalus deridens	6	6	6
	Osteocephalus leprieurii	1	1	1
	Osteocephalus planiceps	6	6	6
	Osteocephalus taurinus	1	1	1
	Scarthyla goinorum	1 + 2	1 + 2	
	Scinax cruentommus	1		
	Scinax garbei	1 + 2		
	Scinax pedromedinae	1 + 2	1 + 2	
	Scinax ruber			24 + 25
	Sphaenorhynchus carneus	1 + 2		
	Sphaenorhynchus dorisae	1 + 2	1 + 2	
Leptodactylidae	*Adenomera andreae*	32	32	32
	Adenomera hylaedactyla		32	
	Leptodactylus discodactylus	29	29	29
	Leptodactylus leptodactyloides	11	11	11
	Leptodactylus pentadactylus		29 + 30 + 32	29 + 30 +32
	Leptodactylus petersii	11	11	11
	Leptodactylus wagneri		11	11
	Lithodytes lineatus	32		
Microhylidae	*Hamptophryne boliviana*	1	1	
Strabomantidae	*Pristimantis altamazonicus*	23	23	
Number of species	**31**	**21**	**23**	**14**
Number of families	**5**	**5**	**4**	**2**
Number of reproductive modes	**10**	**9**	**10**	**9**

Thirty-one species were observed across the three sites over a five week period. Twenty-one at PV1, 23 at PV2 and 14 at PV3 (Table 6.3). The number of reproductive modes observed was nine at both PV1 and PV3 with 10 observed at PV2. The most common reproductive mode was again Mode 1, followed by Mode 2. All modes were represented at all three sites, except for Modes 30 and 23.

6.3.2 Low water reproductive mode predictors

The estimated number of reproductive modes in the low water period was most significantly influenced by the minimum temperature (Table 6.4). Decreases in temperature result in a slight decrease in the number of reproductive modes observed. Two other variables influenced the number of observed modes; the average canopy score and the percentage of cloud cover. Increases in both decreased the estimated number of reproductive modes. In sum, once variables that account for anurans activity and detectability are accounted for (I.e. temperature and cloud cover), a higher number of reproductive modes are found in forests with a more closed canopy.

Table 6.4. General linear mixed effect model reproductive mode predictors (low water). The estimated number of modes should in each case be added/removed from the intercept (i.e. canopy score decreases the number of modes by 0.13)

	Estimated number of modes	**SE**	**p-value**
Reproductive model one			
Intercept	4.24	0.733	0.001
Canopy score	-0.13	0.058	0.021
Minimum temperature	-0.09	0.026	0.001
Cloud cover	-0.003	0.001	0.033

6.3.3 PV2 reproductive modes

Table 6.5. Reproductive modes and species list for the three transects surveyed at PV2

Family	Species	T1	T2	T3	Reproductive modes
AROMOBATIDAE	*Allobates "samiriensis" sp nov*			1	20
	Allobates sp.		5	1	20
BUFONIDAE	*Rhinella margaritifera*	1			1 + 2
	Rhinella marina		2		1
HYLIDAE	*Dendropsophus allenorum (timbeba)*	1		1	1
	Dendropsophus bifurcus			1	24 + 25
	Dendropsophus bokermanni			2	24
	Dendropsophus brevifrons		4	5	24
	Dendropsophus haraldschultzi	1		2	1 + 2
	Dendropsophus koechlini		1		1
	Dendropsophus parviceps	4	1	1	1
	Dendropsophus triangulum		1		24 + 25
	Hypsiboas cinerascens			3	1 + 2
	Hypsiboas geographicus	2	3	5	1 + 2
	Hypsiboas lanciformis	1	1		1 + 2
	Hypsiboas punctatus		1		1 + 2
	Osteocephalus deridens	8	14	10	6
	Osteocephalus leprieurii	6	19	4	1
	Osteocephalus planiceps	6	9	4	6
	Osteocephalus taurinus	15	28	29	1
	Phyllomedusa tomopterna			1	1
	Scarthyla goinorum	12	23	37	1 + 2
	Scinax cruentommus		2		1
	Scinax funereus		4		1
	Scinax pedromedinae	5	4	15	1 + 2
	Sphaenorhynchus carneus		1		1 + 2
	Sphaenorhynchus dorisae		1		1 + 2
	Sphaenorhynchus lacteus	1			1 + 2
	Trachycephalus resinifictrix			1	6
LEPTODACTYLIDAE	*Adenomera andreae*	7	1	3	32
	Adenomera hylaedactyla	2			32
	Leptodactylus discodactylus	12	13	32	29
	Leptodactylus leptodactyloides	22	16	32	11
	Leptodactylus pentadactylus	1	9	31	29 + 30 + 32
	Leptodactylus petersii	9	1	18	11
	Leptodactylus wagneri	4	3		11
MICROHYLIDAE	*Ctenophryne geayi*			1	1
	Hamptophryne boliviana		1	2	1
STRABOMANTIDAE	*Pristimantis altamazonicus*	21	20	8	23

	Pristimantis lacrimosus	1			27
Total number of species		**22**	**27**	**26**	**40**
total number of reproductive modes		**10**	**12**	**12**	**13**

Forty species were observed on three transects at PV2 utilizing 13 reproductive modes. Of these 22 species and 10 modes were observed on transect one, 27 species and 12 modes on transect two and finally 26 species and 12 modes on transect three (Table 6.5. The most common mode at PV2 was Mode 1, followed by Mode 2.

6.3.4 PV2 reproductive mode predictors

The number of reproductive modes observed on transects at PV2 was most significantly influenced by survey time (nocturnal or diurnal) and the average canopy score. Nocturnal surveys had a higher number of reproductive modes (Table 6.6). The number of modes increased as the number of surveyors increased. The low water period had a higher number of observed reproductive modes. In sum, once survey effort is considered, a higher number of reproductive modes are found at night and in the low water.

Table 6.6. General linear mixed effect model reproductive mode predictors (PV2) The estimated number of modes should in each case be added/removed from the intercept (i.e. canopy score decreases the number of modes by 0.207)

	Estimated number of modes	**SE**	**p-value**
Reproductive model two			
Intercept	0.046	0.346	0.178
Canopy score	-0.207	0.059	0.001
Low water	0.600	0.201	0.003
Transition water	0.056	0.153	0.716
Surveyors	0.090	0.040	0.023
Nocturnal	0.785	0.109	0.001

Due to the impact of survey time on the number of observed modes the data were analysed separately for diurnal and nocturnal surveys.

On nocturnal transects the number of reproductive modes decreased as the canopy score increased, while increases in the number of surveyors increased the number of modes (Table 6.7). The number of modes observed was again higher in the low water and transition periods.

Table 6.7. General linear mixed effect model reproductive mode predictors (PV2 nocturnal) The estimated number of modes should in each case be added/removed from the intercept (i.e. canopy score decreases the number of modes by 0.221)

	Estimated number of modes	**SE**	**p-value**
Reproductive model three			
Intercept	1.173	0.420	0.005
Canopy score	-0.221	0.071	0.002
Low water	0.614	0.237	0.009
Transition water	0.171	0.181	0.346
Surveyors	0.103	0.045	0.021

On diurnal transects increases in both the distance from the guard station and the number of surveyors increased the number of observed reproductive modes (Table 6.8).

Table 6.8. General linear mixed effect model reproductive mode predictors (PV2 diurnal). The estimated number of modes should in each case be added/removed from the intercept (i.e. the number of surveyors increases the number of modes by 0.157)

	Estimated number of modes	**SE**	**p-value**
Reproductive model four			
Intercept	0.863	0.259	0.001
Distance from guard station	0.0001	0.0001	0.136
Surveyors	0.157	0.06	0.008

6.3.5 Modelling summary

The four models highlight several key factors influencing the number of reproductive modes. Some influence the detectability of anurans such as the number of surveyors which increased the number of modes. As well as minimum temperature and cloud cover which both affect anuran activity thus reducing the number of modes observed.

Others however are likely to influence diversity of anurans such as increases in the canopy score, which represents a patchy canopy, led to a decrease in the number of observed modes. While more reproductive modes were observed on transects further from the guard station. Finally more modes were observed in the low water period which had a significantly higher number of modes than the other seasons.

6.4 Discussion

Fourteen out of the 39 reproductive modes described by Hadden and Prado (2005) were recorded in the PSNR. This is similar to other areas of Amazonian forests: Manaus (Amazonas, Brazil) 66 species recorded representing 17 reproductive modes (Hödl 1990); Parque Nacional da Serra do Divisor (Acre, Brazil) 124 species, representing 12 modes (Souza 2002); Santa Cecilia (Ecuador) 88 species, representing 16 reproductive modes (Duellman 1978; Hödl 1990); Panguana (Peru) 66 species, representing 14 modes (Hödl 1990). Hadden and Prado (2005) reported 22 modes in Amazonian forests; we found one further mode that they did not report. This was mode 29: "foam nests with eggs and early larval stages in basin; subsequent to flooding" (Table 6.1). Two species from the PSNR have been reported using this mode, *Leptodactylus discodactylus* and *Leptodactylus pentadactylus*.

The two most common modes involve eggs deposited in water and exotrophic tadpoles in either lentic (still) water (Mode 1) or lotic (moving) water (Mode 2). The next most commonly encountered modes involved eggs laid on vegetation overhanding lotic (Mode 25) and lentic (Mode 24) water. Once tadpoles have developed they drop down into the water below. These four modes are often utilised by hylids (Duellman and Trueb 1986), which were the most common anurans in the PSNR. Many species in the PSNR were recorded using both still and moving water, yet anurans usually show a preference to just one. However, species in the PSNR use both due to the dynamic nature of the flooded forests. *Dendropsophus triangulum* (Mode 24 + 25) and *Hypsiboas punctatus* (Mode 1 + 2) use both lotic and lentic water. These species are both floating meadow specialists (Chapter 4) who use the meadows for breeding, yet floating meadows grow in both lotic and lentic water. Only four hylids have different reproductive modes. Mode 6 was used by *Osteocephalus deridens*, *Osteocephalus planiceps* and *Trachycephalus resinifictrix*. Mode 6 involves eggs and endotrophic tadpoles in water within tree holes or aerial plants. Males of these three species were observed calling from tree holes to attract mates (Pers. Obs.). The final species *Hypsiboas boans* uses Mode 4 in which eggs are laid in basins constructed by the males, along river banks (Bartlett and Bartlett 2003).

Leptodactylus pentadactylus is the only species which has the ability to use more than two reproductive modes (Bartlett and Bartlett 2003; Rodriguez and Duellman 1994; Muedeking and Heyer 1976). All three modes involve eggs within a foam nest, Mode 29 involves eggs and early larval stages deposited within a basin that will subsequently be washed into pools or streams. Mode 30 is similar to Mode 29, but the nest is subterranean. Finally Mode 32 involves tadpoles completing development within the subterranean chamber potentially utilising the egg yolk to complete metamorphosis (Muedeking and Heyer 1976).

One of the more unusual modes recorded in the PSNR is utilised by *Pipa pipa*. This species undertakes an elaborate courtship dance in which the male rubs eggs across the female's back while fertilising them (Rabb and Rabb 1960; Rabb and Snedigar 1960). The female's skin then grows over the top of them completely encasing them where they undergo development, finally emerging as fully formed froglets (Rabb and Rabb 1960; Rabb and Snedigar 1960). Another unusual mode is the relationship between *Lithodytes lineatus* and leaf cutter ants. This species is often observed calling and sitting at the entrance to leaf cutter ant nests, the ants usually attack animals yet they completely ignore this anuran. Tadpoles have been recorded within a water filled depression inside an active nest (Schlüter et al. 2009). Foam nests were observed in a tunnel above the water, but little is known about this relationship (Schlüter et al. 2009).

Some species show plasticity in the modes that they use, and are able to change their mode depending on the habitat available to them. In large ecosystems like the Amazon rainforest species which can change their mode depending on the habitats have an advantage over others. There are two types of plasticity in this case, 1) species which have different modes (which are fixed) in different locations (e.g. *Hypsiboas boans*) and 2) species which are able to change their modes depending on the habitat they encounter. This second form of plasticity can be observed in the floating meadow specialists who can use both still and moving water. The use of both still and moving water may have evolved within flooded forests, as during the low water period, water becomes trapped in lakes and channels, no longer connected to the main river (and is therefore still). Floating vegetation continues to grow here and the anurans continue to breed. This would have aided them as they were transported downriver on floating rafts eventually arriving in upland areas where they could breed in still temporary pools.

6.4.1 Reproductive mode predictors

As 'number of reproductive modes' was used as a surrogate measure of diversity, whilst the disturbance, environmental and survey variables may influence the number of modes detected. This is due to an increase in diversity not because these variables are evolutionary drivers of reproductive modes. All PV2 models contained the number of surveyors as a significant factor. This is unsurprising as more surveyors increase the chances of detecting anurans. This variable was not significant for the low water data (Model 1) as the same number of surveyors conducted all transects. However, the low water data highlighted temperature and cloud cover as significant factors. Silva et al. (2012) showed that sites with high humidity levels have a higher number of reproductive modes; as both temperature and cloud cover influence the humidity levels this is an unsurprising result. The PV2 data, which were collected over several months, covered the different water level periods. Water level was therefore a significant factor affecting the number of reproductive modes observed. Both Model 2 and 3 included low water level as a significant factor; an increased number of reproductive modes were observed in the low water (this is explored further in Chapter 7).

In three of the four models average canopy score was a significant factor in determining the estimated number of reproductive modes. An increase in the canopy score decreased the number of modes. This result indicates that a more open canopy decreases the anuran diversity. Natural canopy gaps occur after a tree fall, but these are quickly replaced with pioneering species (Kricher 1997). Canopy openness may be a result of habitat disturbance; patchy canopies could be the result of selective logging (Asner et al. 2004; Slik et al. 2002). Which decreases the habitat complexity, that in turn decreases the number of suitable breeding and foraging sites. As a result fewer species can be supported. Hylids made up a large percentage of the species observed on terrestrial transects, arboreal species rely on the canopy complexity for breeding and foraging. Breeding hylids utilise bromeliads, tree holes and temporary pools; a reduction in the number of large trees reduces the availability of these microhabitats.

Distance from the guard station also significantly influenced the number of reproductive modes. The guards living in the station are given small quotas of resources they are able to extract (Naughton-Treves et al. 2006). This means that transects closer to the guard station have a slightly higher level of disturbance than those further away.

This variable was significant in the PV2 diurnal model. Many of the species observed on diurnal transects were found in the leaf litter. Disturbance on these transects could cause erosion to the pathways, reducing the depth of leaf litter, fallen branches and logs which offer spawning sites for anurans. Thus they will move into the more suitable surrounding forest.

6.5 Conclusion

Overall a reduction in the number of reproductive modes was observed in areas with higher levels of disturbance. This disturbance is localised and so anurans can potentially move out of the area into the more suitable surrounding forest. It is unlikely that species that show plasticity in their modes will be better adapted to disturbance; most of the species recorded in the PSNR are only able to switch between still and moving water, which is not of great benefit in response to disturbance. However, this could be beneficial in response to climate change, as climate change is affecting the seasonal flooding in the reserve (Chapter 7) causing droughts and extreme flood events. Species that have reproductive modes that involve breeding over either still or moving water can still breed in both drought and flood conditions. This flexibility may offer an advantage over species that rely on temporary pools.

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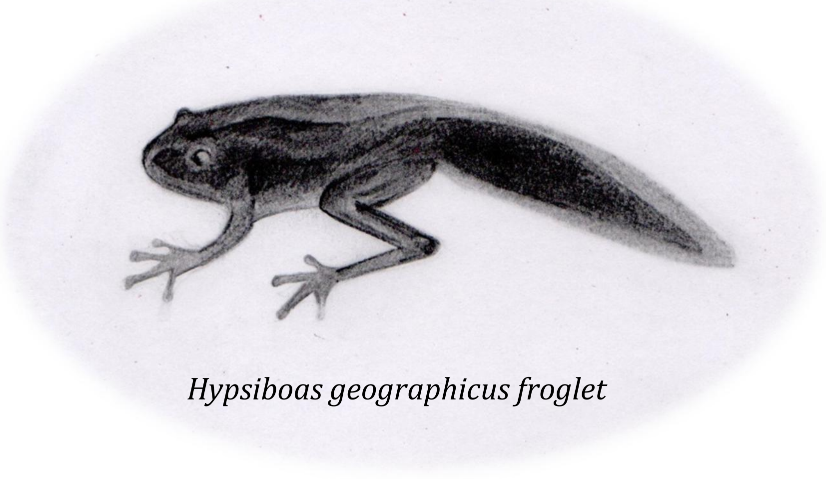
Chapter 7

The impacts of seasonal flooding on amphibian diversity in a Peruvian lowland flooded forest



Hypsiboas geographicus froglet

7. Abstract

The Pacaya-Samiria National Reserve (PSNR) is greatly influenced by two main rivers, the Maranon and the Ucayali. Seasonal fluctuations in water level mean that 92% of the reserve is classed as low-lying flooded forest known as varzea. Over the past five years both flooding and droughts have increased in intensity in Western Amazonia. This chapter shows that there are strong associations between different genera and water level periods. Many species were found in greater numbers in certain seasons; leptodactylids, for example, were more common in the low water. The impact of water level on both the Shannon and Simpson's Diversity Index was highly significant. Both indices were higher in the low water period, likely due to species moving back into the area once flood waters have receded. Seventeen species were classified as seasonal specialists within the PSNR. The seasonal flooding of the PSNR has a huge impact on amphibian diversity. The extremes in seasonal flooding are likely to have severe consequences, especially for species which rely on the natural flood pulse for breeding.

7.1 Introduction

The Amazon River is the largest river system in the world, and is continually meandering and transforming its landscape (Osborne 2000). It has a huge catchment area spanning much of South America. The annual rise and fall of the river level is influenced by rainwater in its upper tributaries. In lowland areas this fluctuation leads to the creation of vast floodplains which are seasonally inundated with flood water. Floodplains are a diverse habitat which are utilised by many different species (Goulding 1989). Floodplains help to bridge the gap between the terrestrial and aquatic environments, as flood water transports nutrients between them (Junk et al. 1989).

The Pacaya-Samiria National Reserve (PSNR) is greatly influenced by two main rivers: the Maranon and the Ucayali. These rivers border the reserve, but in the past flowed through the area. Two tributaries - the Samiria River and the Pacaya River - are old river beds of these main rivers. These are continually shifting as they flow through the reserve, creating ephemeral lakes and channels. Unlike many other tropical forests the PSNR does not have strict wet and dry seasons, instead there are high and low water periods. The flood pulse in the PSNR is caused by rainfall high in the Andes, falling within the catchment area of both the Maranon and Ucayali. As a result of these seasonal fluctuations in water level, 92% of the reserve is classed as low-lying flooded forest known as varzea (Talling and Lemoalle 1998; Myers 1990). The Ucayali and Maranon are both white-water rivers, full of nutrient-rich sediments originating in the Andes (Osborne 2000; Kricher 1999). As the forest floods with the white waters of the Maranon and Ucayali sediments are deposited and tannins are leached from the leaf litter. As a result the Samiria River is characterised by the nutrient high white water which feeds it, yet is classed as a black water river.

The natural cycle of flooding can fluctuate from year to year, with extended periods of drought or flood. Particularly high water years can result in reduced availability of land which can negatively impact terrestrial mammals (Bodmer 1990). On the other hand, a drought year can negatively impact fisheries, because the flooded forests are important fish nurseries (Correa et al. 2008). Recent climate models for the Amazon Basin predict that the western side will become wetter with greater flooding during high water seasons, with extreme weather patterns becoming more common (Cook et al. 2012; Langerwisch et al. 2012). These models also predict drought events during the low water periods (Cook et al. 2012). Over the past five years a greater intensity of flooding and increased droughts in Western Amazonia has occurred (Bodmer et al. 2014). Fluctuations in river level are becoming more intense with periods of record highs and lows. The Loreto region of Peru, where the PSNR is located, saw record highs in 2009 - 2010, followed by a record low. The following two years (2011-2012) then exceeded record highs, resulting in the relocation of hundreds of indigenous Cocama people (Bodmer et al. 2014). In 2013 the level was again unusually high although did not exceed the 2012 record.

The effect of seasonal flooding on biodiversity has been studied over the past eight years in the PSNR (Bodmer et al. 2014). The drought periods had a negative effect on the populations of dolphins, fish and caiman. However, the record breaking high years resulted in population increases of the same species. These periods of high water had a negative effect on terrestrial ground dwelling mammals such as peccaries, brocket deer, giant anteaters and armadillos (Bodmer et al. 2014). Arboreal and semi-arboreal mammals were not directly affected as they feed on resources in the canopy, untouched by the floods. Amphibians could act as a good indicator of the impacts of flooding, because the diversity embraces species which are arboreal, terrestrial, fossorial and aquatic. This chapter aims to determine how the amphibian diversity and assemblages changed over the different water periods. This will help us to understand the possible impacts future changes in the flood pulse may have on amphibians in the PSNR.

This chapter aims to test the following hypotheses:

1. Amphibian species diversity on terrestrial transects will be influenced by disturbance, environmental and survey variable
2. Terrestrial amphibian assemblages will be influenced by the seasonal flood patterns in the PSNR

7.2 Methods

Data were collected at the PV2 site from April – September 2012 and June – October 2013. Three transects were surveyed spanning the different water levels. These transects were located on both sides of the river. They were 500 m in length and were surveyed as per section 2.3 Terrestrial transect methods. Table 7.1 shows the number of replicates for each transect, across the different water periods.

Table 7.1. Transects undertaken over the different water level periods (2012 & 2013).

Transect one	**No. Night repeats**	**No. day repeats**	**Total distance (m)**	**Total time (hrs)**
High	6	3	4400	10
Transition	15	15	15000	31
Low	11	9	9510	14
Transect two				
High	6	3	4400	9
Transition	18	15	15500	36
Low	10	9	9500	16
Transect three				
High	4	3	3500	8
Transition	16	12	15350	39
Low	10	12	11000	19

7.2.1 Flooding

For this analysis three water level periods were used, high, low and transition. The high water period was from April - May, the transition from June - August and the low September - October. During the high water period terrestrial transects were inundated with flood water, requiring a canoe to undertake surveys. During the transition period, June - August, the water level dropped and transects drained. By September the water level had dropped by 8 meters (in 2013), drying out the terrestrial transects.

Two variables were used to quantify flood levels: (1) the water level of the Maranon recorded at Nauta - this town is located down-river from the PSNR but fluctuations in the river level here represent those in the PSNR (SEHINAV 2015); (2) average 'water-mark' on each transect. When the forest floods a mark remains on the trees which can be measured and averaged for the transect. This gives an indication of the depth of flood waters on that transect.

7.2.2 Statistical Analysis

All statistical analysis was completed using the software package R (R Core Team, 2012). The packages lme4 and ggplot2 were used within R. Generalized linear mixed effect models were used to analyse which explanatory variables influenced the response variables. The data consist of four response variables the Shannon Diversity Index, the Simpson's Diversity Index, the number of individuals and the number of species. Both indices were used as the Simpson's Index is weighted towards the abundances of the commonest species while the Shannon Index is weighted towards the rarer species (Magurran and McGill 2011; Hill 1973). A combination of the following explanatory variables were used in the models: water level period; average water mark, Maranon River water level; distance from the river mouth; distance from the guard station; the number of large trees (over 50 cm DBH); canopy scope score (see Chapter 6.2.1 for description); minimum temperature; minimum humidity; cloud cover; number of surveyors; nocturnal or diurnal survey; and phase of the moon. All variables were checked for collinearity as per Chapter 4.2. Maximum temperature and maximum humidity were omitted from the analysis are they were correlated with the minima (Dormann 2013). Transect was used as a fixed effect in all models.

Generalised linear mixed effect models were run as per the methods in Chapter 4 with data exploration completed prior to analysis. Mixed models were again chosen due to the repeats undertaken on each transects. Poisson errors were used for the count data (individual and species models) but were not used for the diversity index models (continuous data).

7.2.3 Species Diversity

The Shannon Diversity Index and the Simpson's Diversity Index were calculated using EstimateS (Colwell 2013). Both indices were calculated for each transect undertaken and were used as the response variables in two models.

7.2.4 Generalists and Specialists

To determine which species in the different water seasons were generalists or specialists the program CLAM was used (Chao and Lin 2011; Chazdon et al. 2011). For a full description of the CLAM analysis see Chapter 6 methods. This analysis was chosen as it minimises bias due to differences in sampling intensities between the seasons, as well as bias due to insufficient sampling of rare species in each habitat (Chazdon et al. 2011). This analysis is suitable for surveys which can be pooled into two categories such as the high and low water seasons (Chazdon et al. 2011). CLAM categorises the different species into four groups: (1) High water specialists, (2) Low water specialists, (3) generalists, and (4) species that are too rare to classify as either specialists or generalists.

CLAM firstly calculates the number of individuals in each habitat for each species. It then compares these using the K threshold (Set at 0.5 for this analysis). K is the cut-off point for classification of a species as either a generalist. CLAM then tests whether the value calculated for the first habitat is significantly higher / lower than the value for the second habitat (P=0.05). If both tests correspond then that species is deemed a habitat specialist (Chazdon et al. 2011). The raw data consists of two species lists with the number of individuals representing each species for each water level. The output consists of lists of species in each category, percentages of species in each category and a figure showing the two habitat types and the spread of species across the four categories.

7.3 Results

Forty-two species were recorded representing seven families. Twenty species were recorded in only one water period, 12 were recorded in two periods and 11 were recorded across all three water periods (Table 7.2). There are strong associations between different genera and water level periods. For example, *Dendropsophus* species were only found in the transition and low water period. The two *Pristimatis* species and *Bolitoglossa* species were also only found in the transition and low water period. Many species were found in greater numbers in certain seasons such as leptodactylids which were more common in the low water. Others were rare across all seasons such as three *Sphaenorhynchus* species, *Hypsiboas punctatus, Dendropsophus bifurcus,* and *Dendropsophus triangulum.*

Table 7.2. The number of each species observed on terrestrial transects in the three seasons

FAMILY	Species	High	Transition	Low
AROMOBATIDAE	*Allobates 'samiriensis'* sp nov	0	1	0
	Allobates sp.	0	5	1
BUFONIDAE	*Rhinella marina*	2	0	0
	Rhinella margaritifera	1	0	0
HYLIDAE	*Dendropsophus timeba (allenorum)*	0	1	1
	Dendropsophus bifurcus	0	0	1
	Dendropsophus bokermanni	0	0	2
	Dendropsophus brevifrons	0	5	4
	Dendropsophus haraldschultzi	0	2	1
	Dendropsophus koechlini	0	0	1
	Dendropsophus parviceps	0	6	1
	Dendropsophus triangulum	0	1	0
	Hypsiboas cinerascens	1	2	0
	Hypsiboas geographicus	3	7	0
	Hypsiboas lanciformis	2	0	0
	Hypsiboas punctatus	0	0	1
	Osteocephalus deridens	1	20	11
	Osteocephalus leprieurii	3	23	3
	Osteocephalus planiceps	1	11	7
	Osteocephalus taurinus	4	48	19
	Phyllomedusa tomopterna	0	1	0
	Scarthyla goinorum	22	34	16
	Scinax cruentommus	0	2	0
	Scinax funereus	0	4	0
	Scinax pedromedinae	3	5	16
	Sphaenorhynchus carneus	1	0	0
	Sphaenorhynchus dorisae	0	0	1
	Sphaenorhynchus lacteus	0	1	0
	Trachycephalus resinifictrix	1	0	0
LEPTODACTYLIDAE	*Adenomera andreae*	0	5	6
	Adenomera hylaedactyla	0	1	1
	Leptodactylus discodactylus	8	6	43
	Leptodactylus leptodactyloides	14	24	44
	Leptodactylus pentadactylus	0	29	12
	Leptodactylus petersii	2	6	24
	Leptodactylus sp.	13	15	155
	Leptodactylus wagneri	0	0	3
MICROHYLIDAE	*Ctenophryne geayi*	0	1	0
	Hamptophryne boliviana	0	2	1
PLETHODONTIDAE	*Bolitoglossa altamazonica*	1	11	10
	Bolitoglossa peruviana	0	1	0
STRABOMANTIDAE	*Pristimantis altamazonicus*	0	22	27
	Pristimantis lacrimosus	0	1	0

7.3.1 Water level impact on diversity and abundance

The impact of water level on both the Shannon and Simpson's Diversity Index was highly significant. The water level period (high, transition or low) was significant in both models (Table 7.3 and 7.4) while the recorded water level of the Maranon was only significant in the Shannon model (p=0.06). The models show that as the water level drops the species diversity increases, the estimated Shannon index increases by 1.03 in the low water while the Simpson's index increases by 2.69. Yet, decrease in temperature decreased the observed diversity (p= 0.0001 / 0.001). Other significant factors included the percentage of cloud cover which decreased the diversity with increased cover (p= 0.02 / 0.03) and the number of surveyors which increased diversity (p= 0.01).

Table 7.3. GLMM showing predictors of the Shannon Diversity Index

	Estimate of species diversity	**SE**	**p-value**
Shannon Index Predictors			
Intercept	-4.14	3.68	0.26
Low water	1.03	0.28	0.0001
Transition water	0.24	0.18	0.17
Maranon water level	0.06	0.03	0.06
Minimum temperature	-0.10	0.02	0.0001
Cloud cover	-0.002	0.001	0.02
Number of surveyors	0.09	0.03	0.01

Table 7.4. GLMM showing predictors of the Simpson's Diversity Index

	Estimate of species diversity	**SE**	**p-value**
Simpson's Index Predictors			
Intercept	14.06	4.79	0.004
Low water	2.69	1.19	0.03
Transition water	0.23	0.92	0.80
Minimum temperature	-0.51	0.16	0.001
Cloud cover	-0.02	0.01	0.03
Number of surveyors	0.56	0.23	0.01

The impact of water level on the number of species and individuals was also highly significant (Tables 7.5-7.6). The low water period increased both the number of species by 0.52 and the number of individuals by 1.49. The level of the Maranon River only increased the number of individuals (p= 0.01). The distance from the guard station (p= 0.0001) and the lunar cycle (p= 0.03) both also increased the numbers of individuals. Canopy scope decreased the number of species (p= 0.08), the number of surveyors increased both individuals and species (p= 0.001 / 0.01).

Table 7.5. GLMM predictors of the number of species caught per terrestrial transect

	Estimated No. species	SE	p-value
Species Predictors			
Intercept	3.30	0.80	0.0001
Low water	0.52	0.20	0.01
Transition water	-0.16	0.15	0.30
Minimum temperature	-0.10	0.03	0.0001
Canopy scope	-0.10	0.06	0.08
Cloud cover	-0.003	0.001	0.01
Number of surveyors	0.13	0.04	0.001

Table 7.6. GLMM predictors of the number of individuals caught per terrestrial transect

	Estimated No. individuals	SE	p-value
Individual Predictors			
Intercept	-9.76	3.20	0.002
Low water	1.49	0.24	0.0001
Transition water	0.25	0.16	0.12
Maranon water level	0.09	0.03	0.001
Distance from guard station	0.0001	0.0001	0.0001
Lunar cycle	0.002	0.001	0.03
Number of surveyors	0.09	0.03	0.01

7.3.2 Water level specialists and generalists

The Clam analysis can only compare two sets of data at once so the analysis was run three times to compare the high + low, high + transition and low + transition periods.

Table 7.7. Species classification by CLAM for the: high and low water comparison; high and transition comparison; and transition and low water comparison.
(See figures 7.1-7.3 for a visual display)

Generalist	**High water specialist**	**Low water specialist**
Osteocephalus leprieurii	*Hypsiboas geographicus*	*Leptodactylus pentadactylus*
Osteocephalus taurinus	*Scarthyla goinorum*	*Leptodactylus petersii*
Scinax pedromedinae		*Pristimantis altamazonicus*
Leptodactylus discodactylus		

Generalist	**High water specialist**	**Transition specialist**
Hypsiboas geographicus	*Scarthyla goinorum*	*Osteocephalus deridens*
Leptodactylus petersii	*Leptodactylus discodactylus*	*Osteocephalus leprieurii*
Scinax pedromedinae	*Leptodactylus leptodactyloides*	*Osteocephalus planiceps*
		Osteocephalus taurinus
		Leptodactylus pentadactylus
		Bolitoglossa altamazonica
		Pristimantis altamazonicus

Generalist	**Transition specialist**	**low water specialist**
Dendropsophus brevifrons	*Allobates* sp.	*Scinax pedromedinae*
Adenomera andreae	*Dendropsophus parviceps*	*Leptodactylus discodactylus*
Leptodactylus leptodactyloides	*Hypsiboas geographicus*	*Leptodactylus petersii*
Bolitoglossa altamazonica	*Osteocephalus deridens*	
Pristimantis altamazonicus	*Osteocephalus leprieurii*	
	Osteocephalus planiceps	
	Osteocephalus taurinus	
	Scarthyla goinorum	
	Scinax funereus	
	Leptodactylus pentadactylus	

Seventeen species were classified as seasonal specialists within the PSNR (Table 7.7). This analysis identified six species as seasonal specialists across all comparisons: high water, *Scarthyla goinorum*; low water, *Leptodactylus petersii*; and transition, *Osteocephalus deridens, O. leprieurii, O. planiceps* and *O. taurinus* (Fig. 7.1 - 7.3). Some species were specialists in two seasons, but this was always the transition period and either high or low. For example *Leptodactylus pentadactylus* was classified as a low water specialist but also classified as a transition specialist. However, when comparing the high water with the low water two species were classified as high specialists, *Hypsiboas geographicus* and *Scarthyla goinorum* and three species were classified as low specialists, *Leptodactylus pentadactylus, Leptodactylus petersii* and *Pristimantis altamazonicus*. Four species were classified as generalists across the high and low water season, *Osteocephalus leprieurii*, *Osteocephalus taurinus, Scinax pedromedinae* and *Leptodactylus discodactylus*.

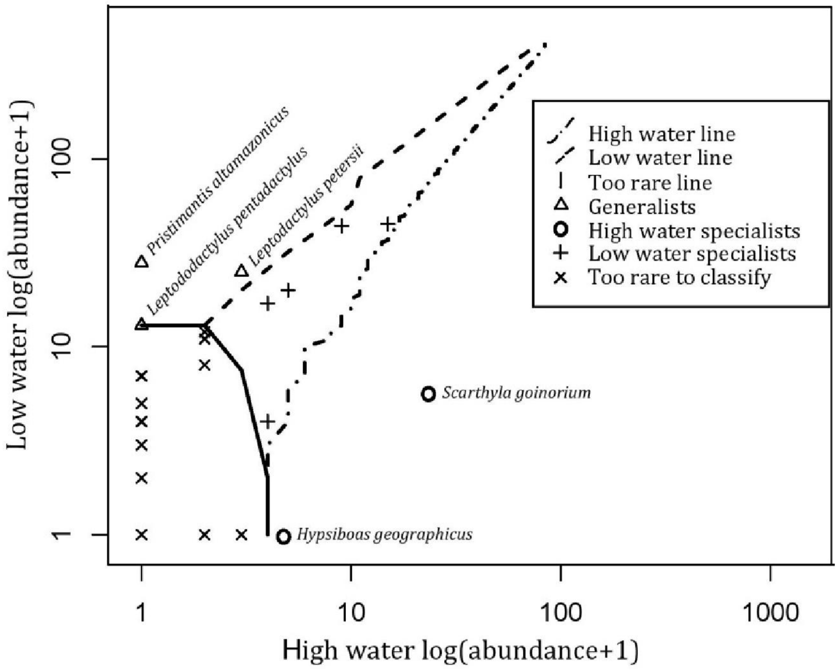


Figure 7.1. CLAM Species classification graph displaying the high water specialists, low water specialists and seasonal generalists. The three species above the dashed line are the low water specialists whilst the two species below the dash-dot line are the high water specialists, between these two lines are the habitat specialists. Finally and species below the dark black line were too rare to classify.

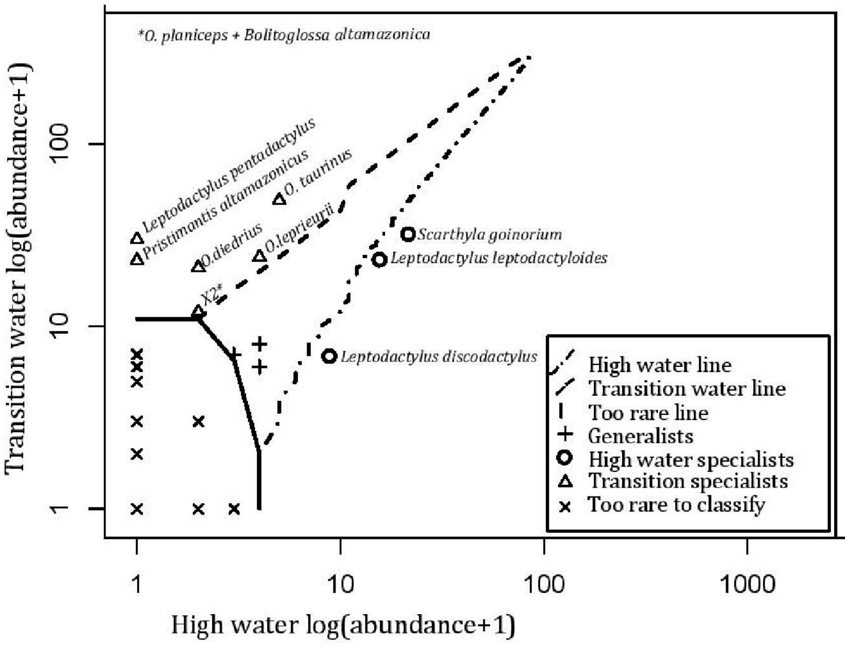


Figure 7.2. CLAM Species classification graph displaying the high water specialists, transition specialists and seasonal generalists. The seven species above the dashed line are the transition specialists whilst the three species below the dash-dot line are the high water specialists, between these two lines are the habitat specialists. Finally and species below the dark black line were too rare to classify.

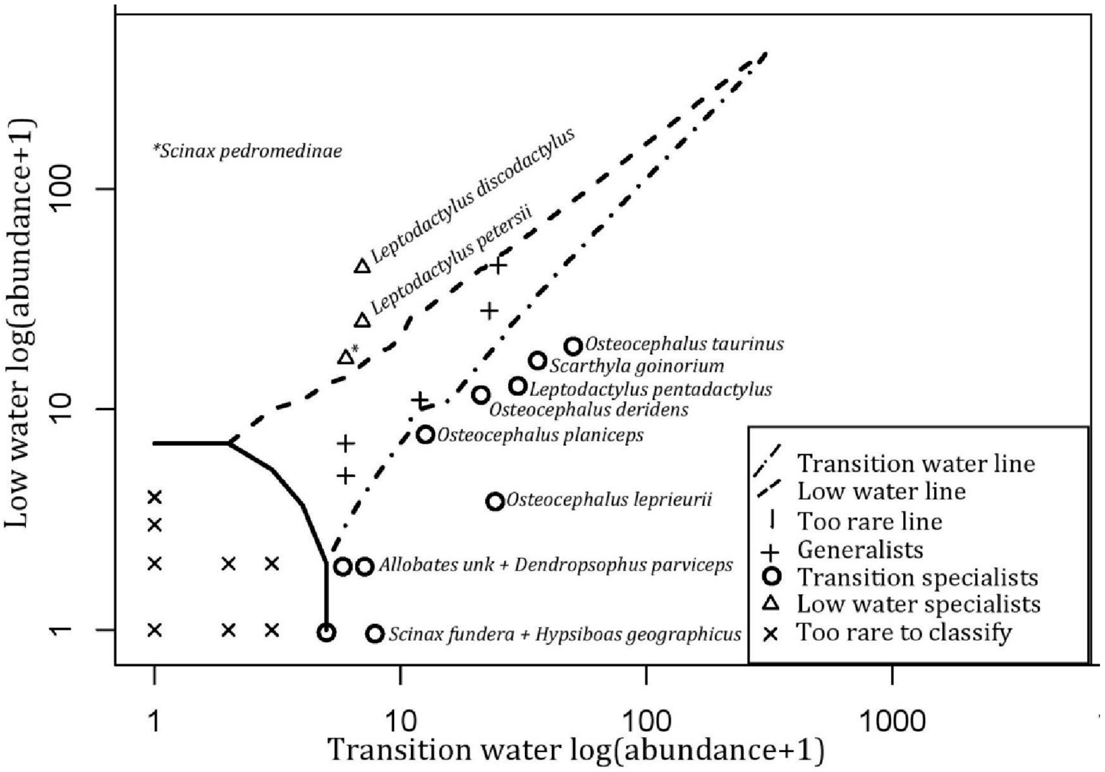


Figure 7.3. CLAM Species classification graph displaying the transition specialists, low water specialists and seasonal generalists. The three species above the dashed line are the low water specialists whilst the ten species below the dash-dot line are the transition specialists, between these two lines are the habitat specialists. Finally and species below the dark black line were too rare to classify.

7.4 Discussion

Forty-two amphibian species were observed, representing seven families: Aromobatidae (2); Bufonidae (2); Hylidae (25); Leptodactylidae (7); Microhylidae (2); Plethodontidae (2); and Strabomantidae (2). The amphibian assemblage, abundance and diversity in the PSNR are greatly influenced by seasonal flooding. Whilst some species are generalists encountered across all seasons many others are only found during certain water periods. The high species diversity in the PSNR represents terrestrial, fossorial, arboreal and aquatic species, whilst some species can move between these environments in response to water level.

The most commonly encountered family was Hylidae with 25 species and 300 individuals. Due to their arboreal nature they are unlikely to be directly affected by the flooding, although they may be indirectly affected. Some species were only observed in one or two water periods including *Dendropsophus bifurcus, Dendropsophus triangulum, Hypsiboas punctatus, Sphaenorhynchus carneus, S. lacteus,* and *S. dorisae*. These species have been identified as floating meadow specialists (Chapter 5). Four of these species were observed on transects in the transition and low water period when less floating vegetation was available, so they may have migrated into the terrestrial habitat. Interestingly, different genera show different responses to the water level. No *Dendropsophus* species were observed in the high water period, five species were encountered in the transition period and seven in the low water. *Dendropsophus* species have been recorded breeding in temporary pools on the forest floor (Bartlett and Bartlett 2003; Rodriquez and Duellman 1994; Hödl 1990). *Dendropsophus brevifrons, D. bokermanni* and *D. koechlini* were observed calling in the low water, above a temporary pool (Pers. Obs.). These small arboreal tree frogs are usually found in the canopy, and may have moved down to breed. Temporary pools are a preferred reproductive habitat for most amphibians rather than flood waters; which have a higher predation risk as they are utilised as a fish nursery (Goulding 1989). In contrast *Hypsiboas* species were encountered in the high and transition period and not in the low water. *Hypsiboas geographicus* was classified by the CLAM analysis as a high water specialist. *Hypsiboas geographicus* has been recorded calling and breeding along streams and lakes (Bartlett and Bartlett 2003; Rodriquez and Duellman 1994). Their tadpoles form schools and are distasteful to fish, enabling breeding in the high water period (Rodriquez and Duellman 1994). Some species in the Hylidae family were identified as generalists, such as *Osteocephalus leprieurii* and *Osteocephalus taurinus*. *Osteocephalus* are canopy specialists which live in bromeliads (Bartlett and Bartlett 2003), but they may move down to breed or forage. Changes in the natural flooding of the reserve could have detrimental impacts on many of the Hylidae species; mainly affecting their breeding habits. The small *Dendropsophus* species would be affected by longer and higher flood periods which may limit the number of temporary pools they can use for breeding. Other species will however be affected by drought years, which could reduce the availability of breeding habitats; this will particularly affect floating meadow specialists.

The second most common family was Leptodactylidae which was represented by seven species and 411 individuals. All species were more abundant during the low water period. *Adenomera andreae*, *Adenomera hylaedactyla* and *Leptodactylus pentadactylus* were not observed in the high water: *L. pentadactylus* was categorised as a low water specialist. The first two species are direct developers, meaning they lay eggs which develop into froglets inside a foam nest (Bartlett and Bartlett 2003; Rodriquez and

Duellman 1994). These species are terrestrial, spending much of their time in the leaf litter, were they construct nests (Bartlett and Bartlett 2003; Rodriquez and Duellman 1994). During the high water periods these two species may migrate into more upland areas. *Leptodactylus pentadactylus* is a large, terrestrial species, with terrestrial or fossorial nests (Muedeking and Heyer 1976). The other *Leptodactylus* species *L. discodactylus, L. leptodactyloides* and *L. petersii* were encountered across all water periods, yet were more common in the low water. However, they were also observed in the high water period. Unlike the other *Leptodactylus* species *L. discodactylus, L. leptodactyloides* and *L. petersii* are adapted to the flood periods, rather than migrating out, they move upwards away from the flood waters. In the high water period all *Leptodactylus* species were observed on floating debris on the water surface (leaf litter, logs etc.) and also on roots and branches just above the water (Pers. Obs.).

Species in the Strabomantidae family were encountered in the low and transition period. No *Pristimantis altamazonicus* individuals were observed in the high water period. This species is often found in bromeliads, however, it was observed depositing eggs in the leaf litter on the forest flood (Pers. Obs.). *Pristimantis altamazonicus* is also a direct developer (Duellman and Paramuk 1999). Arboreal species are less likely to be affected by the rise and fall of the water as they can migrate upwards away from the floods. Terrestrial species are more likely to be directly affected, such as the two Microhylidae species *Ctenophryne geayi* and *Hamptophryne boliviana* which are both terrestrial and fossorial (Bartlett and Bartlett 2003; Rodriquez and Duellman 1994). These species were only recorded in the low and transition periods; again they may migrate during the high water into upland areas. Interestingly no Dendrobatid species were encountered during these terrestrial surveys. Whilst some have been recorded in the reserve (Chapter 3) such as *Ameerega hahneli*, these species are more common in upland sites. As terrestrial species which often defend small territories they seem to be excluded from the flooded forest.

7.4.1 Predictors of amphibian diversity and abundance on terrestrial transects

The Shannon Index, Simpson's Index, number of species and number of individuals all increased in the low water period. This may mean that species favour the low water for breeding, either moving down from the canopy or back into the area from non-flooded localities. Equally, leaf litter species may return to the area after the flood waters have receded. Fewer species were encountered in the high water period especially during diurnal transects. This could be influenced by the survey methods, as surveys during the high water were conducted from canoes. This made it more difficult to find amphibians, especially leaf litter species which can take refuge within debris on the water surface.

Other variables which influenced the species diversity and abundance included the minimum temperature and cloud cover. Both variables were also significant factors when looking at the floating meadow predictors in Chapter 4. Again this is not an unexpected result as decreases in temperature and increases in cloud cover are closely related and often lead to a reduction in amphibian activity and therefore detectability. Another variable which was significant in all models was the number of surveyors. This again is unsurprising as more surveyors meant more amphibians were spotted and captured.

Two disturbance variables were also significant. As canopy score increased, representing a patchier canopy, the number of species decreased. This suggests that areas with higher disturbance levels have fewer species. Equally, transects further from the guard station had more individuals, suggesting disturbance may influence amphibian abundance. This is consistent with the results in Chapter 6 which showed decreases in the number of reproductive modes in relation to disturbance.

7.5 Conclusion

The seasonal flooding of the PSNR has a huge impact on amphibian diversity. The extremes in seasonal flooding are likely to have severe consequences, especially for species which time their breeding with seasonal flooding. This chapter has shown that amphibian families will respond differently to extreme season events. *Dendropsophus* species will be adversely affected by periods of extreme flooding due to a lack of suitable breeding habitats, while floating meadow specialists will benefit. However, droughts will reduce the habitat of floating meadow specialists yet benefit species such as *Dendropsophus bokermanni* which rely on temporary pools, for reproduction, that are only available in the low water period. Amphibian declines can only be reported with baseline data and long term monitoring. Further long-term surveys are needed particularly focused on the species identified in this chapter as being sensitive to seasonal flooding.

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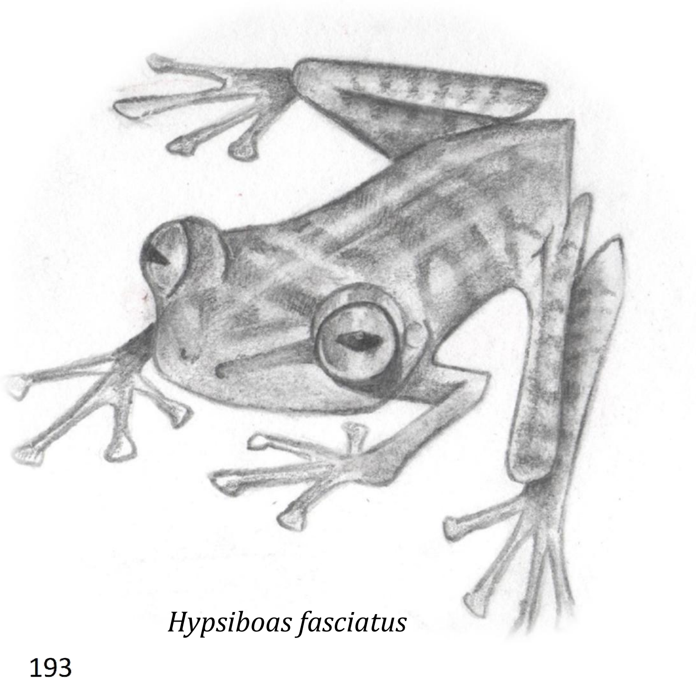
Chapter 8

General Discussion



Hypsiboas fasciatus

8. General Discussion

The Pacaya-Samiria National Reserve (PSNR) contains a diverse amphibian fauna with 81 species recorded since 1996. Rarefaction curves identified that further surveys could have resulted in more species being detected. On the floating meadow habitat 22 species were recorded, of these most were hylids breeding throughout the year (Chapter 4). The anuran assemblage changed little between the channels, lakes and main river. This is because floating rafts transport species down river from the lake into the channel and main river so the assemblage is similar. Chapter 5 showed that the floating meadow assemblage contained several hylid species which are floating meadow specialists, rarely encountered in adjacent terrestrial habitat. These specialists have large geographical ranges likely due to their dispersal on floating rafts. Chapter 6 highlights the impacts of disturbance on the number of reproductive modes (used as a surrogate for species richness and diversity). Increased human disturbance decreases the number of reproductive modes on terrestrial transects. Flooding emerged as a predictor of the number reproductive modes and diversity on floating meadows and terrestrial transects, so was explored in Chapter 7. There are distinct associations between certain genera and water level periods, and water level has a significant effect on species diversity.

The diversity of amphibians in the Amazon Basin is greater than any other location worldwide. It is likely that many amphibian families originated here and so were able to diversify into the species we see today (Weins et al. 2011; Santos et al. 2009; Vanzolini and Heyer 1985). Species richness is ultimately driven by speciation, extinction, and dispersal (Ricklefs 1987). There are many factors which influence these drivers such as: time (time-for-speciation effect - Stephens and Weins 2003); humidity and temperature

(Buckley and Jetz 2007); range expansion (Bocxlaer et al. 2010); body size (Wollenberg et al. 2011); phenotypic plasticity (Pfenning et al. 2010); isolation (Hoskin et al. 2005); and geological events (Munoz-Ortiz et al. 2014; Yan et al. 2012). Hylids are the most commonly encountered species in the Amazon, with 40 species observed in the PSNR since 1996. Floating meadow rafts disperse hylids downriver, and this mechanism may explain the dispersal of amphibians from the huge Pebas lake wetland ecosystem which was present here around 23 MYA (Hoorn et al. 2010). Wiens et al. (2011) suggested that the diversity of hylids was due to the time-for-speciation effect, hylids having had a long time to diversify into the many species we see today. Yet the question still remains as to what drives the variation in their diversity across the Amazon Basin. The dispersal of hylids on floating meadow rafts, coupled with the different habitats of wetland and forest may have aided speciation. This may explain the current distribution of hylids with ranges spanning the entire Amazon Basin (AmphibiaWeb 2015). Originally rivers were believed to be a barrier to dispersal for many Amazonian vertebrates (Peres et al. 1996; Ayres and Clutton-Brock 1992), but Gascon et al. (2000) analysed the phylogeography of frogs and showed that rivers did not act as a significant barrier. The dispersal of floating meadow species via rafts of vegetation confirms that rivers are not a barrier for many amphibian species. This dispersal may also be important for downriver populations, aiding gene flow across the Amazon Basin. However, this natural dispersal could be compromised by the current threats amphibians face.

Chapters 4 and 5 explored the impacts of seasonal flooding on floating meadow which are reduced in size in the low water. This is a vital habitat for many hylids, as instead of dispersing, they are found in a greater density in the low water. Chapter 7 discussed the problems of climate change and how the seasonal rise and fall of the water level is becoming more severe with both drought and unusually high flooding (Bodmer et al. 2014). Drought years reduce the availability of floating meadows which could cause floating meadow specialists to suffer population declines. The natural flood cycle of the PSNR could also be affected by the proposed dams that the Peruvian government have planned. They aim to build 70+ dams in the Peruvian Amazon over the next 20 years, 20 of which are planned for the Maranon River (International Rivers 2015), which directly feeds the PSNR. Yet the impacts of these dams on the ecosystem are largely unknown. Not only are they likely to affect the natural rise and fall of the river level, they may cause pollution downriver, prevent fish migrations and drastically reduce sediment loads. The nutrient rich sediment suspended in the rivers is vital for the PSNR. The nutrients are used by terrestrial plants and incorporated into the ecosystem, they are also trapped in the roots of floating meadows, and enable rapid growth. Without suspended sediments in the water column, and the natural flood cycle, floating meadows could decline which would not only affect the local anurans but could reduce the gene flow and cause a decline in floating meadow specialists across the entire Amazon Basin.

Other threats outside the reserve such as mining for gold and palm oil production could have negative impacts on amphibians within the reserve. Recently Peru has seen a surge in gold mining due to an increase in price. This not only directly affects the location where forests are being cleared but pollution (mainly mercury) is used in the process and is left to enter the ecosystem. Mercury pollution in the water will easily travel downriver and could have devastating consequences in the flooded forests of PSNR. Amphibian use of both the terrestrial and aquatic environment could help the mercury move between the two systems and could increase its concentration further up the food chain in higher predators which may then be consumed by local people. Palm oil production is also threatening the Loreto region of Peru, four very large plantations have been scheduled which will clear a vast area of pristine rainforest. Whilst outside the boundary of the PSNR they could isolate the reserve from the surrounding habitat. It could potentially result in pollution from the production of the palm oil, pesticides and fertilisers used in the growing process and air pollution from clearing and burning of the original forest. This could greatly impact on the amphibians and other species found within the PSNR.

Further threats outside the reserve include emerging infectious diseases, floating meadow rafts could enable the spread of diseases such as chytridiomycosis. If a chytrid positive anuran is transported downriver the spread will be rapid, as rafts are able to quickly cover vast distances (Schiesari et al. 2003). Whilst some species can be killed by the chytrid fungus others such as the cane toad (*Bufo marinus*) are not affected by the fungus, but can act as a carrier (Lips et al. 2006). This species was recorded on floating meadows in the PSNR. Intermittent swabbing has been undertaken it 2009, 2010 and 2012 to check for the presence of chytrid yet so far there have been no confirmed cases, although this does not prove the absence of chytrid in the PSNR. Chytrid is highly likely to enter the reserve as the water feeding the Samiria River originates in the Andes; where chytrid is present. Screening for chytrid by Kosch et al. (2012) showed that nine out of 39 sites along the eastern slopes of the Andes harboured the fungus. Both *Leptodactylus petersii* and *Scinax garbei* tested positive (Kosch et al. 2012), and both of these species were present on floating meadows in the PSNR.

Two of the greatest threats amphibians currently face are habitat loss and anthropogenic climate change. Habitat loss is often caused by humans clearing forests for agriculture, housing, and resource extraction (Catenazzi and May 2014). Whilst amphibian declines as a result of habitat loss have been well document (Jongsma et al. 2014; Hamer and McDonnell 2008; Pearman 1997), there is a lack of data documenting the effects on lowland flooded forest amphibian species (Catenazzi and May 2014). By using reproductive modes as a surrogate for other diversity measures we discovered that small scale localised disturbance reduces the number of observed modes. Whilst this represents decreases in the species diversity, the number of reproductive modes should be highlighted as an important measure of habitat quality. More reproductive modes will be utilized within a complex forest with plenty of egg deposition sites, temporary and permanent pools, and structural complexity (Crump 2015). The incorporation of reproductive mode information is useful for increasing amphibian populations, increasing the number of reproductive sites can increase breeding activity. This is a technique used worldwide from restoration of ponds in the UK (Phillips et al. 2002) to using oil drums as temporary pools at the Costa Rican Amphibian Research Centre. This technique is increasing the population size of critically endangered Lemur leaf frogs (*Hylomantis lemur*) (Pers. obs.).

Research in the PSNR has identified that climate change is affecting the natural flood cycle. Recently, both record highs and record lows have been recorded in the Loreto region (Bodmer et al. 2014). These extreme flood events will have significant impacts on species which live in the flooded forest. Terrestrial leaf litter amphibians must migrate upwards or out of the flooded areas during the high water. Many of them breed in temporary pools which are only available in the low water and so could suffer population declines during long periods of inundation. The natural flood cycle is vital for the creation of breeding sites in the forest, and this has enabled the evolution of numerous reproductive modes. Consequently, reproductive modes – and associated frog diversity - may be lost because of these extreme seasonal events.

Although the PSNR is protected it is important to understand the potential implications of extraneous threats, such as dams, emerging infectious disease, mining, oil palm production and climate change. Protection of an area is not necessarily effective in protecting all species and habitats it encompasses (Mora and Sale 2011). The data presented here can therefore be incorporated into the reserve management in order to identify potential impacts at an early stage and design appropriate mitigation measures. Further research within the PSNR is also vital, long term surveys are needed to identify population declines. Further understanding the threats that the floating meadow habitat faces including detailed swabbing for the chytrid fungus should be undertaken. A detailed assessment of the planned dams and potential effects on the seasonal flooding is important as this could have dramatic impacts not only on the amphibians but all other species found within the reserve. Baseline data on amphibians in the lowland Amazon is lacking yet this thesis highlights the importance of detailed studies in understanding the threats they face. Floating meadows are an important driver of hylid diversity which contain a separate assemblage from the surrounding terrestrial forests and may offer a stable habitat in an otherwise unstable environment. The loss of this mechanism could affect populations of hylids along the entire Amazon, as well as other species such as fish and invertebrates which also disperse on rafts of vegetation. However, floating meadows are threatened by environmental and climate change. Biodiversity conservation needs to focus not just on species but the processes that drive the diversity we see today. These drivers of diversity play an important role in maintaining Amazonian anuran assemblages and need to be protected along with species and habitats, to conserve this unique and amazing ecosystem.

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Appendix 1 – Publications

Upton, K. Warren-Thomas, E., Rogers, I., and Docherty, E. (2014). Amphibian diversity on floating meadows in flooded forests of the Peruvian Amazon. *Herpetological Review,* 45, 209-212.

Upton, K. et al. (2011). Amazonian frog diversity and microhabitat use. *Herpetological Bulletin*, 118, 10-17.

Upton, K. (2011). Frog diversity in Amazonian Peru. *Herpetological Journal*, 21, 1-3.

Herpetological Review, 2014, 45(2), 209–212.

Amphibian Diversity on Floating Meadows in Flooded Forests of the Peruvian Amazon

Floating meadows are often associated with Amazonian white-water flooded forests (varzea), where they grow between the tree line and open-water. Seasonal flooding in varzea results in an unstable forest floor for terrestrial species. However, floating meadows may offer a refuge for some species that would otherwise be displaced by rising water. Floating meadows consist of herbaceous water plants that begin growing at the end of the low water period, taking root in the waterlogged soils of river, channel and lake banks. As the water rises, some plant species grow rapidly upwards, others become free-floating and grow horizontally, expanding the surface area they occupy (Junk 1970, 1997). As water levels begin to recede, currents and rainfall can dislodge sections of floating meadows to create rafts that are then transported via the river current.

The importance and diversity of floating meadows has been highlighted for several taxa (Goulding et al. 1996; Junk 1997; Schiesari et al. 2003; Dias et al. 2011; Ferreira et al. 2011), yet studies focusing on amphibian use of floating meadows are relatively scarce. Junk (1973) found that amphibians were rarely encountered on floating meadows. However, methodology was not provided by Junk (1973), and if nocturnal surveys were not undertaken, amphibians were unlikely to have been adequately sampled. Carrying out specific amphibian surveys, Hödl (1977) found 15 anuran species on floating meadows and concluded that this habitat was a potential breeding site. Hoogmoed (1993) published a list of the herpetofauna known to occur on or near to floating meadows in Suriname, Bolivia, and Brazil, adding to Hödl's (1977) list. This research brought the total number of amphibian species recorded on floating meadows to 26 (Hoogmoed 1993). On the Solimoes River, Schiesari et al. (2003) observed 42 individuals comprising eight anuran and one caecilian species, all on floating meadow rafts. They highlighted the importance of rafts of floating meadow vegetation as dispersal vectors for fish and potentially also for amphibians (Schiesari et al. 2003). In a preliminary study of only 18 days, Upton et al. (2011) found 16 anuran species on floating meadows. The amphibians recorded on floating meadow habitats to date are listed in Table 1.

This paper aims to: 1) Update the current list of amphibians found on floating meadows in Peruvian varzea flooded forest, and 2) update the information on reproductive habitat use provided by Hödl (1977), which showed evidence of reproductive behavior on the floating meadow habitat (see Hödl 1977; Fig. 3, Table 1).

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MATERIALS AND METHODS

Study site.—Our study was conducted in the Samiria River basin of the Pacaya-Samiria National Reserve, Loreto, Peru

TABLE 1. List of amphibian species that have been recorded on, or near, floating meadows (x = on, [x] = near). Numbers for localities: 1. Brazil, Solimões (Hödl 1977); 2. Suriname, Para River (Hoogmoed 1993); 3. Bolivia, Perserverancia (Hoogmoed 1993); 4. Brazil, Caxiuanã (Hoogmoed 1993); 5. Brazil, Solimões (Schiesari et al. 2003).

Species	1	2	3	4	5
ANURANS (27)					
BUFONIDAE (1)					
Rhinella marina		x		[x]	x
HYLIDAE (23)					
Dendropsophus haraldschultzi	x				
Dendropsophus leucophyllatus		x	x		x
Dendropsophus minusculus		x			
Dendropsophus nanus	x		[x]		
Dendropsophus rossalleni	x				
Dendropsophus triangulum	x				
Dendropsophus walfordi					x
Hypsiboas boans	x	[x]			
Hypsiboas geographicus		[x]	x		
Hypsiboas lanciformis	x				
Hypsiboas punctatus	x	x	x		x
Hypsiboas raniceps	x		[x]		x
Hypsiboas wavrini				x	
Dryaderces pearsoni			x		
Lysapsus boliviana			x		
Lysapsus caraya				x	
Lysapsus laevis	x				x
Scinax boesemani	x				
Scinax nebulosus	x	x	x	x	
Scinax ruber		x			
Sphaenorhynchus carneus	x				x
Sphaenorhynchus dosisae	x				
Sphaenorhynchus lacteus	x		x		
LEPTODACTYLIDAE (2)					
Leptodactylus leptodactyloides					x
Leptodactylus wagneri	x	[x]	x		
PIPIDAE (1)					
Pipa pipa		x			
CAECILIANS (1)					
TYPHLONECTIDAE (1)					
Typhlonectes compressicauda					x
Total species on floating meadows (and adjacent)	15(15)	7(10)	8(10)	3(4)	9(9)

TABLE 2. Species list including number of individuals captured and life stages on the floating meadows of the Samiria River in Pacaya-Samiria National Reserve, during May–June 2009 and March–October 2012. F = gravid female; M = calling male; A = adult (sex unknown); J = juvenile; *above the floating meadow either on a branch or tree trunk; ° not previously recorded on floating meadows.

Species	F	M	A	J	Total 2009	Total 2012
BUFONIDAE (1)						
Rhinella marina			9		1	8
HYLIDAE (15)						
Dendropsophus haraldschultzi		1	29	10	2	38
Dendropsophus leali	1	1	3			5°
Dendropsophus rossalleni		3	23		16	10
Dendropsophus triangulum	12	106	232	21	33	338
Hypsiboas boans		1				1*
Hypsiboas lanciformis		14	8	3		25
Hypsiboas punctatus	30	38	184	62	14	300
Osteocephalus taurinus			1			1°
Scarthyla goinorum			2			2°
Scinax garbei		2	8	4	1°	13
Scinax pedromedinae				2		2°
Scinax ruber			3		1	2
Sphaenorhynchus carneus	2	28	44	6	5	75
Sphaenorhynchus dorisae	2	6	50	2	38	22
Sphaenorhynchus lacteus	1	16	50	3	22	48
LEPTODACTYLIDAE (2)						
Leptodactylus leptodactyloides			23		7	16
Leptodactylus petersii			20		12°	8
PIPIDAE (1)						
Pipa pipa			1			1
Total number of species	6	11	17	10	12	19

(4.893256°S, 74.355526°W). The Pacaya-Samiria National Reserve is one of the largest varzea forests in western Amazonia spanning over 20000 km^2 between the confluence of the Ucayali and Marañon Rivers.

Data collection.—Preliminary data for this study were collected in May and June 2009, with more extensive surveys conducted from March to October 2012. Most surveys were carried out at night from 1800–2400 h, with some daytime searches between 1400–1700 h.

The floating meadows surveyed were located in the Samiria River basin at PV1 Shiringal, PV2 Tacshacocha, Huishto Cocha, and PV3 Hungurahui. These sites cover a small proportion of the Samiria River Basin, which spans a wide area; however, they were chosen as they have varying levels of disturbance by local fishermen and tourism. At each site, surveys were conducted across all river systems, in both the main Samiria River and in adjacent channels and lakes. In total, 221 surveys were conducted, 52 in 2009 and 169 in 2012. The surveys in 2012 were split as follows: 71 in lakes, 60 in channels, and 38 on the main Samiria River. Around the lake edges, floating meadow surveys were conducted at 100-m intervals. Within the channel and main river, less floating vegetation was available to survey, so all sections were surveyed at least once. A 10-m boat with outboard motor was slowly driven into the floating meadow vegetation, causing it to part on either side of the boat with the aim of reducing disturbance. On each sampling occasion, an area of 2 m on either side of the boat (50 m^2 in total) was searched for fifteen minutes. During this time, all frogs encountered were captured and placed in individually marked pots. Time of capture, species, behavior (e.g., calling male) and location (including plant species and height above water), were recorded. All surveys were completed with one local field guide, one biologist, and three or four student volunteers. To locate frogs at night, one main flashlight was used by either a guide or the biologist (CB2-L1 Clubman Deluxe, LI-ION 9.2AH half-million candle power); all students used smaller flashlights such as the Petzl Tikka to search. Species identification was undertaken using several field guides (Ouboter and Jairam 2012; Duellman 2005; Bartlett and Bartlett 2003; Rodriquez and Duellman 1994). Although identification of Amazonian amphibians can be very difficult, many of the species observed on floating meadows are distinctive and can be quickly identified in the hand using identification guides. Voucher specimens were not taken as the collection of specimens from within this protected reserve was prohibited under the permit and authorization being used (Resolucion Jefatural No 005-2013-SENANP-JEF).

Data analysis.—To analyze microhabitat use, the data were separated into the four main genera: *Dendropsophus, Hypsiboas, Scinax,* and *Sphaenorhycnhus.* Kruskal-Wallis tests were then used to determine if there were significant differences in the median calling heights among species in the same genus.

RESULTS

Nineteen amphibian species and 1090 individuals were recorded on the floating meadow habitat representing four families (Table 2). Six species have not been previously recorded on floating meadows: *Dendropsophus leali, Osteocephalus taurinus, Scarthyla goinorum, Scinax garbei, Scinax pedromedinae,* and *Leptodactylus petersii.* Most of the species recorded on floating meadows were hylids (15) compared with only two leptodactylids, one bufonid, and one pipid. The most abundant species were *Dendropsophus triangulum* and *Hypsiboas punctatus* with 371 and 314 individuals, respectively. No other species was represented by more than 100 individuals. *Scarthyla goinorum* and *Scinax pedromedinae* were both represented by only two individuals while *Pipa pipa* and *Hypsiboas boans* were represented by just one individual each (Table 2).

The median height in which amphibians were found and the most commonly used (over 50% of encounters) plant species are presented for all species observed (Fig. 1, modelled after Hödl 1977, figure 3). One species, *Pipa pipa,* was found swimming at the water surface; all others were on the floating meadow or adjacent emergent vegetation. All leptodactylid and bufonid species were most often found on Water Lettuce (*Pistia stratiotes*), a species that floats on the water surface. *Paspalum repens* was the most commonly used plant species on the floating meadows and also the most abundant in the survey area. Only in *Sphaenorhychus* were there significant differences in perch height between species (Kruskal-Wallis chi squared = 16.62, $P < 0.05$, df = 2), with *S. carneus* occupying lower heights than *S. lacteus.* Calling males,

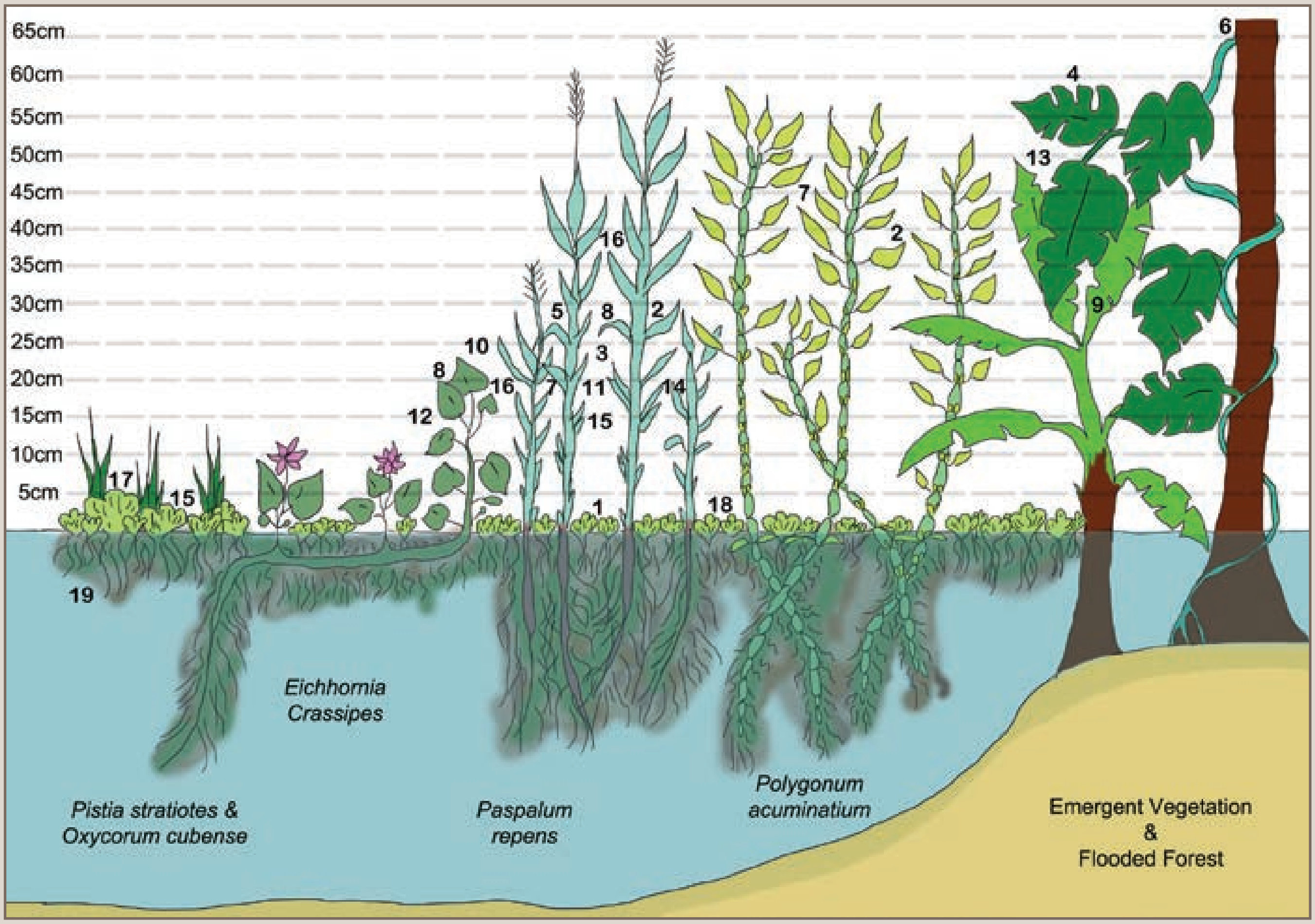

Fig. 1. Anuran species found on floating meadows, the most common plant species on which they were found, and the median height at which they were found. 1) *Rhinella marina* 2) *Dendropsophus haraldschultzi,* 3) *Dendropsophus leali,* 4) *Dendropsophus rossalleni,* 5) *Dendropsophus triangulum,* 6) *Hypsiboas boans,* 7) *Hypsiboas lanciformis,* 8) *Hypsiboas punctatus,* 9) *Osteocephalus taurinus,* 10) *Scarthyla goinorum,* 11) *Scinax garbei,* 12) *Scinax pedromedinae,* 13) *Scinax ruber,* 14) *Sphaenorhynchus carneus,* 15) *Sphaenorhynchus dorisae,* 16) *Sphaenorhynchus lacteus,* 17) *Leptodactylus leptodactyloides,* 18) *Leptodactylus petersii,* 19) *Pipa pipa.*

gravid females, and juveniles were all found on floating meadows, although only for hylids (Table 2, modelled after Hödl 1977). Calling males were observed for 11 of the 15 hylid species while ten species were represented by juveniles and six represented by gravid females. Leptopdactylids were only represented by adults, as were *Rhinella marina* and *Pipa pipa*.

Discussion

Hylids were the most frequently encountered family on the floating meadows. The two most common species, *Dendropsophus triangulum* and *Hypsiboas punctatus*, are either rare or absent from the adjacent terrestrial habitat (pers. obs.), highlighting the importance of floating meadows for some hylid species. Other species were only represented by one or two individuals. One example is *Pipa pipa*, a fully aquatic species that is rarely encountered on land; this individual was caught swimming at the surface of the water. The floating meadow root system potentially offers refuge and cover to aquatic species such as *Pipa pipa*.

Few leptodactylids and bufonids were observed on the floating meadows, as these species are usually terrestrial and are regularly encountered on the forest floor within the leaf litter (Allmon 1991) or on river banks (Bartlett and Bartlett 2003), and it is therefore possible that frogs in these families are only using floating meadows as a refuge during high water periods. In both 2009 and 2012 the water level in Pacaya-Samiria exceeded all records from the last 100 years (Bodmer et al. 2012). During these high water periods up to 95% of the reserve can be inundated, with water levels reaching a depth of several meters within the forest. This reduced availability of terrestrial habitat may have displaced some individuals onto floating meadows.

Hödl (1977) noted frogs showing call site segregation, with most species associated with specific plant species. Calling males were regularly observed on the floating meadows in this study, with most species calling from a certain plant species at similar heights. For example the three *Sphaenorhynchus* species called from significantly different heights on *Paspalum repens*. Anuran morphology may influence plant choice, for example, the small *S. carneus* was sometimes observed calling from *Oxycoryum cubense*, a small grass species. Male *S. carneus* are usually between 15–18 mm and females 22–23 mm SVL, compared to *S. lacteus* males which are 26–29 mm and females 36–40 mm SVL (Rodríguez and Duellman 1994). The latter, larger species may be unable to physically climb up on this smaller grass species. Other species that showed preference for certain plants includes *D. triangulum*, which was most commonly found on *Paspalum repens*, and

H. punctatus, which was found on either *Paspalum repens* or *Eichhornia crassipes*. This is consistent with Hödl's (1977) findings in which *D. triangulum* and *H. punctatus* were both observed on plants in the genus *Paspalum*.

Hylids may potentially be using floating meadows for breeding, as calling males, gravid females, egg masses, and newly metamorphosed juveniles of twelve species were observed. These findings are consistent with Hödl's (1977) observations of calling males and pairs in amplexus on floating meadows. Many hylids are arboreal and are documented to move from higher strata to breed in temporary or permanent pools of water in the terrestrial habitat (Rodríguez and Duellman 1994; Bartlett and Bartlett 2003; Dodd 2010). However the flooded forest in the Pacaya-Samiria Reserve can be inundated for up to 6–9 months a year, resulting in an unstable terrestrial habitat. Additionally, the draining of floodwaters occurs rapidly, with fluctuations of up to 30 cm a day. Thus, temporary pools are only available for short periods, if at all.

Floating meadows may be a more stable habitat, available for the 6–9 months that the terrestrial habitat is flooded, and also offers an opportunity to study species that are not often encountered within the terrestrial habitat. In addition, floating meadows may offer an important refuge for other anuran families during extreme flooding. Marked oscillations in annual water levels are becoming more common due to climate change (Bodmer et al. 2012). The availability of floating meadows is dependent on the water level. Therefore it will be important to further elucidate the role of floating meadows in maintaining amphibian diversity in flooded forests as seasonal flooding becomes more extreme.

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Amazonian frog diversity and microhabitat use

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ABSTRACT - Upper Amazonian forests offer some of the highest species diversity in the world due in part to their complex habitats created by fluctuating water levels. In the Pacaya-Samiria National Reserve within the upper Amazonian forest of Peru, forty species of anuran belonging to seven families were recorded in 2009 and 2010 over forty survey days. A species accumulation curve indicated that most species present were detected after ten days of surveying. On land, frogs were most frequently observed among leaf litter. In the river, floating rafts of vegetation may be an important mechanism for the dispersal of frogs.

THE Amazon rainforest contains some of the greatest species diversity on Earth (Salo et al., 1986; Osborne, 2000; Bodmer, 2008). It is a complex ecosystem combining different strata from emergent layer through to canopy, shrubs and forest floor. This wealth of niches has enabled many species to evolve specialist adaptations to their environment. Consequently a huge diversity of amphibian and reptile species exist in the Amazon, with over 250 amphibian and reptile species described as "commonly seen" (Bartlett & Bartlett, 2003).

Surveys indicate that the upper Amazonian forests offer high species diversity due to complex habitats created by fluctuating water levels (Salo et al., 1986; Gentry, 1988; Bodmer, 2008). Gentry (1988) surveyed a series of 1 ha plots in Peru, and found 580 individual trees representing 283 species per plot. The Amazon rainforest would not function without the Amazon river which forms at the confluence of the Maranon and Ucayali rivers. These rivers border the Pacaya-Samiria National Reserve, a 8,042 km^2 protected area located in the upper Amazonian forests of Loreto, Peru. This region contains one of the highest anuran diversities in the world. Rodriguez & Duellman (1994) describe 112 species from the Iquitos region alone. The number of anuran species in this area is constantly increasing as new species are discovered (Perez-Pena et al., 2010). The Pacaya-Samiria reserve has been degraded in the past through overhunting, deforestation and overfishing (Bodmer, 2008). However, wildlife monitoring in the reserve has noted increases in woolly monkeys *Lagothrix lagothrica*, black caiman *Melanosucus niger*, manatees *Trichechus inunguis*, dolphins *Inea geoffrensis* and macaws (Bodmer, 2008).

Despite ongoing monitoring of wildlife in this reserve, little research on diversity and populations of amphibians has been published. The aims of this research were to create a baseline anuran species list for the Pacaya-Samiria reserve and describe the habitat and microhabitat use by them.

MATERIALS AND METHODS

Site Description

This study was undertaken in the Pacaya-Samiria National Reserve, a site with a complex ecosystem. The reserve does not have strictly defined wet and dry seasons and more often has high and low water seasons. As a result of extreme seasonal water changes 92% of the reserve comprises low lying flooded forest know as varsea (Myers, 1990; Talling & Lemoalle, 1998). Inundation and run-off of tannins from trees likely creates the blackwaters of the Samiria River (Bodmer et al., 2010). Periodically, the forest becomes flooded with white water from the Maranon river. The sediment from this water is dropped and tannins from decomposing leaves are taken in. This water then flows back out of the forest into the Samiria River as tannin rich blackwater (Bodmer, pers. comm.).

The Samiria River is an old channel of the Manranon River, therefore the Samiria river bed contains nutrient rich alluvial soils (Kvist & Nebel, 2001). This hydrological system, combined with the alluvial soils, helps create an environment that is very nutrient rich and therefore able to support a diverse range of species across many taxa.

Methods (2009)

Surveys were carried out adjacent to a location known as PV3, a guard post on the Samiria River, at Hungurahui. Land (walking) and river (canoe) transects were conducted within the vicinity of PV3. Data were collected over 18 days between the 30 May to 16 June 2009. During this time 104 transects of 100 m were completed in 52.5 hours. Transects were alternated between land (52 surveys) and river (52 surveys) with equal numbers at day and night in a variety of habitats and temporal zones. River transects were alternated between banks, with a GPS used to calculate distance travelled. For land transects, a tape measure was used with random numbers applied to a compass to determine the direction of travel. Sampling was undertaken no higher than 2 m from the ground or river surface and transect width was 4 m. Day surveys began at 08:00 lasting until approximately 13:00. Night surveys were from 19:00 to 22:00. A team of three to four people walked each land transect and canoed each river transect using a visual encounter survey method (VES) which has been shown to give a good representation of species in tropical forests over a short time period (Doan, 2003). There was no time limit on each transect. They were travelled at the same speed of 0.5 km an hour. Each individual amphibian was captured to collect data. Date, time and transect number were recorded as well as habitat, microhabitat, and substrate. The individuals were then measured (1 mm precision) and weighed (0.1 g precision). Additional factors including temperature, rainfall, detection method, light level and ecologically relevant notes (e.g. sitting on a foam nest) were also recorded. Identification was undertaken using three guide books; Rodriguez & Duellman (1994), Bartlett & Bartlett (2003) and Duellman (2005). Where possible identification was confirmed by local experts.

Methods (2010)

Data were collected from the 15 June to 10 July 2010 (22 survey days). A total of 31 sampling transects was undertaken comprising four permanent land and five permanent river transects, each of 1000 m, surveyed both nocturnally and diurnally. A total of 64 hours of survey was completed. Transects began at 10:00 for the dawn transects and 20:00 for the night transects. VES method was used. The land surveys involved scanning leaf litter and vegetation whilst walking along the transect, using sticks to tap the leaf litter during the day and using torches to spot frogs at night. River surveys involved using torches to scan the riverbank and floating vegetation. All other methods were the same as described for 2009.

RESULTS AND DISCUSSION

Diversity of Amphibians

Forty amphibian species belonging to seven families were recorded in Pacaya-Samiria during 2009 and 2010. They included; Arobatidate (1 species), Bufonidae (3 species), Dendrobatidae (2 species), Hylidae (23 species), Leptodactylidae (8 species), Microhylidae (1 species), Strabomantidae (2 species). Appendix 1 shows a full list of species and the corresponding years in which they were recorded. The highest number of species was recorded in 2009 (29 species). Twenty-seven species were recorded at the same site in 2010. Between these two studies a total of 845 anurans were caught in just 40 days of surveys.

The species list compiled from the 2009 and 2010 research shows possible absences as well as new discoveries in some species. However, the differences in methods and timing make comparisons in abundance difficult without long-term monitoring. Nevertheless, the Pacaya-Samiria reserve has an extremely high anuran diversity (40 species recorded), which can be compared with other anuran hotspots. For example, 52 amphibian species have been recorded in just 45 hectares of Costa Rica (Kubicki, 2010), 27 species representing 5 families were found in Borneo (Keller at al., 2009) and studies on woodlands in western Tanzania found 4247 individuals representing 28 amphibian species (Gardner et al., 2007). The Gibraltar Range National Park in Australia is also

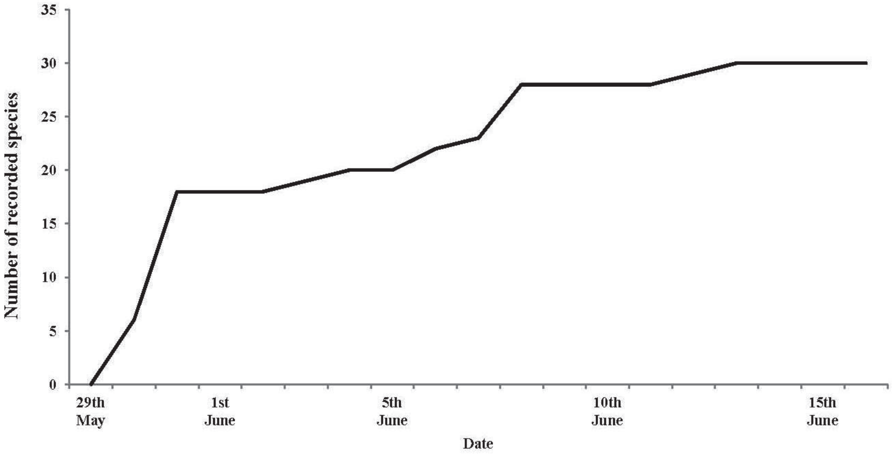

Figure 1. The species accumulation curve for the 2009 Pacaya-Samiria study

home to 30 anuran species (Mahony, 2006). These studies all had longer survey periods than that of the Pacaya-Samiria research and are therefore more extensive. Despite these caveats, 40 amphibian species were recorded in just 40 days; representing a higher diversity than three of these four studies. Fig. 1 shows the species accumulation curve for the 18 days spent in the field in 2009. The curve stabilised after 10 days of surveying. This suggests the majority of species present in the habitats surveyed had been observed.

Microhabitat Use

Fig. 2 shows the number of individuals of the five most abundant species found in the terrestrial habitat in each of the three main micro-habitats on the forest floor (2009 data only). The five species were found in differing frequencies across the three microhabitats suggesting differential usage (Chi-squared = 24.09, df = 8, $P < 0.01$). Most frogs were found in leaf litter. *Leptodactylus discodactylus* showed no preference for a single habitat type. *Rhinella margaritifera* was most commonly found in the leaf litter.

The high diversity of species may present the possibility of resource partitioning on a spatial scale. Many microhabitats were available within the terrestrial habitat including leaf litter, bare ground, puddles, tree trunks and fallen logs. When foraging, frogs may utilise a range of microhabitats as they travel through their range. Leaf litter was the microhabitat utilised most often in this study, a finding supported by Morales & McDiarmid (1996). Leaf litter may reduce the risk of detection by predators (Vonesh, 2001). *Rhinella margaritifer* and *Rhinella daphillis* were often recorded in the leaf litter and have coloration and morphology that resembles leaves of the region (Marent, 2008).

All but one dendrobatid species found in 2009 were active in open spaces during the day. This is commonly recorded behaviour for frogs of the family as they produce toxins which are unpalatable to potential predators; a point broadcast by their striking colours (Symela et al., 2001). A single *Ameerega trivittata* was observed on the same log for three consecutive days. As dendrobatids defend small territories that contain good breeding sites (Poelman & Dicke 2008), this *Ameerega trivittata* may have been the same individual, however, without marking for recapture this could not be confirmed.

The Floating Meadows

Due to the high level of flood water in Pacaya-Samiria in 2009 the only habitat available on the river was floating meadow (2009 data only). Fig. 3 shows the number of individuals representing each species in each of the three main microhabitats found on the floating meadow (Fig. 4 illustrates these microhabitats). The floating meadow habitat and its microhabitats were able to support a large number of species. Sixteen species were found

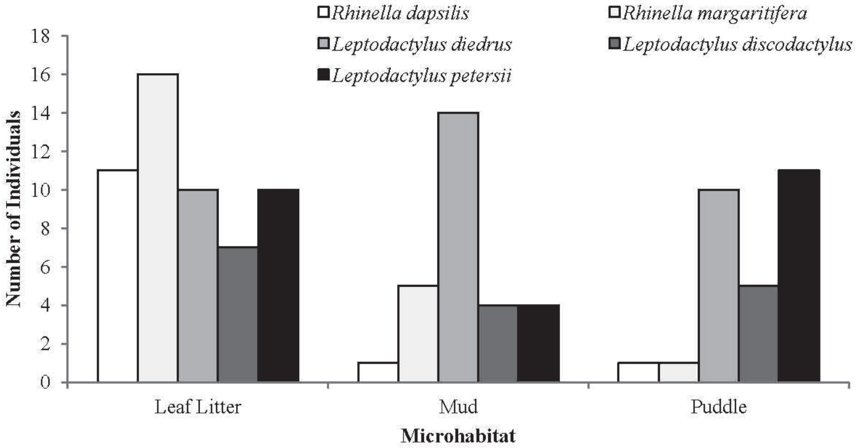

Figure 2. The number of individuals, of each species, recorded on the three main microhabitats on the land transects (2009).

using this habitat, while both Hödl (1977) and Goulding (1989) recorded 15 species on floating meadow at different Amazon sites. These meadows are created from extensive macrophyte stands that grow along the banks of rivers and in lakes (Schiesari et al., 2003). In some parts the meadows covered the entire water channel from one bank to the other, a feature that could aid dispersal across the river. The floating meadows may have been formed at a lake up-river from the study site and therefore facilitate dispersal downstream as well. However, further research is required to confirm this.

The water lettuce microhabitat was dominated by *Sphaenorpyhchus dorisae* and *Sphaenorhynchus lacteus*. Both of these were found most often on this microhabitat, with small numbers recorded in the other two microhabitats. *S. lacteus* was found mainly on this microhabitat possibly due to its morphology. *S. lacteus* was one of the largest species found on the floating meadows. It also lacks adhesive disks on its fingertips (Rodriguez & Duellman, 1994). The emergent vegetation and water hyacinth were very spindly and weak and therefore may only be able to support smaller hylid species.

Calling site partitioning has been observed on floating meadows (Hödl, 1977). Four of the species recorded herein also featured in Hödl's (1977) study, with each observed frog calling from one

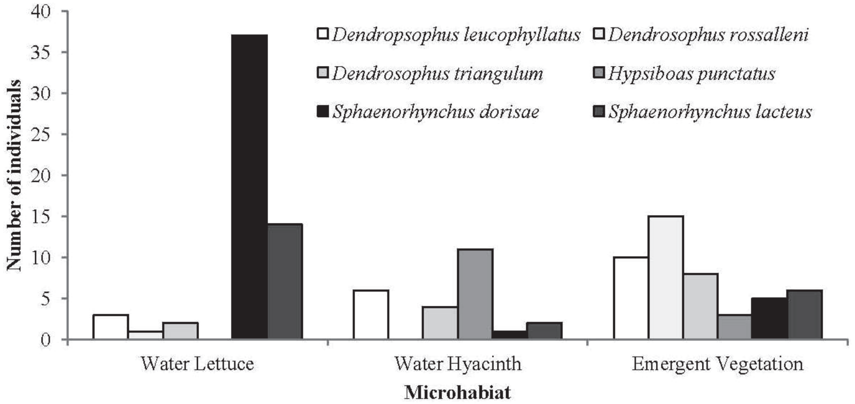

Figure 3. The number of individuals, of each species, recorded on the three main microhabitats on the river transects (2009).

Figure 4. A small section of the floating meadow habitat connected to the flooded forest. In this photograph water lettuce, water hyacinth and emergent vegetation are all present.

particular microhabitat, possibly attracting mates for breeding. In addition to calling adults, froglets were also observed on the floating meadow habitat. Many species in the central Amazon tropical forest breed all year round (Hödl, 1990). Frogs may also have been exploiting the abundance of insect prey available on the floating meadow habitat (Schiesari et al., 2003). The meadows grow very rapidly thus producing a lot of detritus and shelter in the root zone that provides suitable habitat and food for a wide variety of invertebrates (Schiesari et al., 2003). Many frogs observed on the floating meadow habitat were hylid species that would usually be expected to be found in the canopy. Thus this habitat could offer a rare opportunity to study their ecology.

Floating meadows are not permanent habitats. Sections break away, creating floating rafts of vegetation carried down river after rainfall (Schiesari et al., 2003) (Fig. 5). This transport of individuals can be very important to downriver dispersal, facilitating gene flow (Schiesari et al., 2003). Species found on these floating rafts include *Rhinella marinus*, *Leptodactylus leptodactyloides*, *Dendropsophus leuchophyllatus*, *Hypsiboas punctatus* and *Sphaenorhynchus carneus* (Schiesari et al., 2003), all of which were present in this habitat during this study. A further four species were found on floating rafts by Schiesari et al. (2003). However, their survey methods were more intensive. Surveying included eight floating rafts collected in their entirety, with all vertebrates counted and identified. These rafts were collected in Brazil on the Solimões River, which prompts the question of whether such rafts could travel this far. Schiesari et al. (2003) calculated that a vegetation raft could travel 4000 km in as little as 31 days. These rafts also have a great abundance of prey species as the submerged root zone of 1 m^2 of floating meadow will usually support over 500,000

Figure 5. Rafts observed floating down river transporting anuran species. This raft contained *Dendropsophus triangulum* and *Hypsiboas punctatus* individuals.

invertebrate individuals (Goulding, 1989). Therefore, rivers may not be barriers to the dispersal of terrestrial amphibians, but actually aid population dispersal.

Further impacts like disease should be considered potential threats to herpetofauna of floodplains, especially chytridiomycosis. If present in aquatic environments, infected frogs could spread the disease easily when they are breeding further downstream. The potential impact of climate change in the area could also threaten dramatic changes in the water levels and flooding patterns that may have far-reaching impacts on amphibian diversity and abundance.

Further research would be required to fully investigate amphibian population trends in Pacaya-Samiria National Reserve. Such work will hopefully form the basis of a Ph.D. conducted by the senior author commencing September 2011, that seeks to assess the suitability of amphibians in tropical environments as indicator species.

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APPENDIX

Numbers of individuals of each species observed in Pacaya-Samiria National Reserve.

Family	**Scientific Name**	**2009**	**2010**
Arobatidate	*Allobates femoralis*	1	2
Bufonidae	*Rhinella dapsilis*	14	-
	Rhinella margaritifera	25	2
	Rhinella marina	1	17
Dendrobatidae	*Ameerega hahneli*	1	-
	Ameerega trivittata	3	1
Hylidae	*Dendropsophus haraldschultzi*	-	2
	Dendropsophus leucophyllatus	20	-
	Dendropsophus parviceps	-	5
	Dendropsophus rossalleni	16	7
	Dendropsophus triangulum	15	58
	Dendropsophus allenorum	-	1
	Hypsiboas boans	-	9
	Hypsiboas fasciatus	2	2
	Hypsiboas geographicus	-	1
	Hypsiboas lanciformis	-	7
	Hypsiboas punctatus	14	22
	Osteocephalus buckleyi	1	-
	Osteocephalus cabrerai	1	-
	Osteocephalus leprieurii	1	-

	Osteocephalus planiceps	-	1
	Osteocephalus taurinus	3	11
	Scarthyla goinorum	-	7
	Scinax ruber	1	-
	Scinax pedromedinae	-	19
	Sphaenorhynchus carneus	5	-
	Sphaenorhynchus dorisae	43	8
	Sphaenorhynchus lacteus	22	-
	Trachycephalus resinifictrix	-	2
Leptodactylidae	*Leptodactylus andreae*	3	9
	Leptodactylus diedrus	43	-
	Leptodactylus discodactylus	16	24
	Leptodactylus hylaedactyla	6	6
	Leptodactylus leptodactyloides	13	265
	Leptodactylus mystaceus	5	-
	Leptodactylus pentadactylus	1	6
	Leptodactylus petersii	31	36
Microhylidae	*Hamptophryne boliviana*	2	-
Strabomantidae	*Pristimantis altamazonicus*	-	4
	Pristimantis carvalhoi	2	-

Conference report

The joint scientific meeting of Amphibian and Reptile Conservation (ARC) and the British Herpetological Society took place in the lecture hall of the Bournemouth Natural Science Society on 5 December 2010, and encompassed the following contributions.

Population genetics of sand lizards, *Lacerta agilis,* in Dorset

Liam Russell, School of Life Sciences, University of Sussex, UK
E-mail: liam.russell@talk21.com

The sand lizard *Lacerta agilis* is a widely distributed reptile with a range that stretches from northwest Europe across Asia into Mongolia. It reaches the western edge of its range in the United Kingdom, where its specific habitat requirements have left it restricted to three separate geographical areas, the most significant populations occurring within heathland habitats in the southwestern county of Dorset. Lizards from these three areas have been shown previously to be genetically distinct from each other.

The current range of sand lizards in Dorset is crossed by six rivers of differing sizes, which may constitute a historical barrier to dispersal. Sand lizards from seven sites separated by rivers were sampled and genotyped at 15 microsatellite loci. F_{st} estimations indicated significant levels of genetic differentiation between sampling sites. Genotype data were analysed using a Bayesian approach (the STRUCTURE program), which infers population clusters based on genetic information alone; this identified six sand lizard populations across the Dorset range. The relationship between genetic differentiation and geographical distance, and other landscape features, between populations is investigated.

Frog diversity in Amazonian Peru

Katy Upton, Durrell Institute of Conservation and Ecology, University of Kent, UK
E-mail: k80upton@hotmail.com

The diversity of frogs in Peru is extraordinary but very poorly understood in terms of both ecology and conservation. This research was undertaken in the Pacaya-Samiria National Reserve in Peru during May–June 2009. The research aims were to discover what species were present at this site, and to determine their habitat use.

Eighteen days were spent in the field during which 104 transects, each measuring 100 metres, were undertaken on the river and on land. A total of 326 individual frogs, comprising 30 species from six families (Bufonidae, Dendrobatidae, Hylidae, Leptodactylidae, Microhylidae and Strabomantidae) were recorded in this period. This was compared with a previous study undertaken in 2003. Slightly more species were found in 2009 than in 2003. When comparing the number of individuals representing each family, however, there were declines in the number of bufonids and dendrobatids, and increases in the number of hylids and leptodactylids. Eighteen species were represented by less than ten individuals, with only two species, *Leptodactylus diedrus* and *Sphaenorhynchus dorisae*, found in high abundances of more than 30 individuals. My study of the habitats used by these species highlights the importance of floating meadows, which were utilized by 12 different species.

Further research may include 1) longer-term studies to observe real declines in species abundances rather than general fluctuations; 2) the impacts of climate change on long-term population trends; and 3) evaluating whether tropical amphibian assemblages can act as surrogates for environmental change.

Assessment of an established population of atypical grass snakes, *Natrix natrix*, in the Aire Valley, UK

Darryn J. Nash, University of Leeds, UK
E-mail: darrynnash@hotmail.com

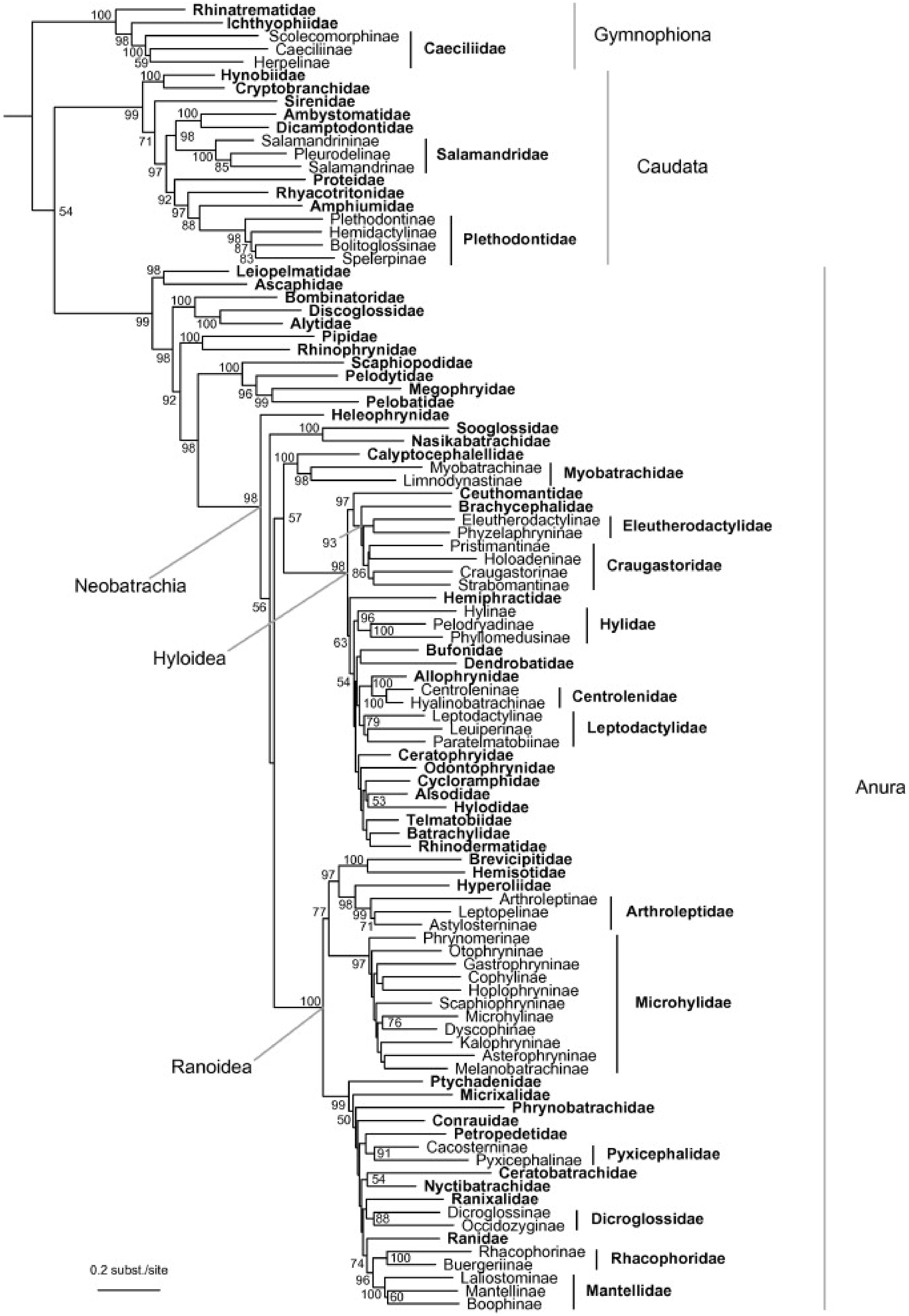
During the past 20 years, an atypical population of grass snakes, *Natrix natrix*, has been observed within and around the Esholt Sewage Treatment (EST) facility, West Yorkshire, UK. Based solely on phenotypic appearance (the presence of dorso-lateral striping and a relatively indistinct collar), the snakes were suspected to originate from southern or eastern Europe. Principally, this study sought to ascertain the origin of the snakes through genetic means. Mitochondrial DNA samples, collected using buccal swabs, were extracted, amplified and sequenced. Sequences were then inserted into the existing natricine phylogeny, thus enabling inferences to be made about the origin of the snakes. An estimate of the population size (Nc) was calculated using a capture/mark/recapture (CMR) programme. Population estimates were generated using a Peterson–Schnabel maximum likelihood census model. Based on the output of this model, an effective population size (Ne) was also produced. Comparative morphometric studies investigated the possibility of discriminating between races using multivariate statistics (principal component analysis). Phylogenetic trees indicated that the Esholt population had originated from eastern Romania and was genetically distinct from native snakes. The CMR programme calculated an Nc of approximately 46 snakes (95% C.I. 28–106), although the actual value was expected to be higher. This figure translated to an Ne of 25, assuming constant sex ratio and excluding individuals of less than 300 mm SVL. No native snakes were encountered during the surveys and historical records for the area are unclear. Morphometric studies indicated that snakes could be discriminated based on a combination of morphometric measurements and phenotypic descriptions. Despite the fact that individuals from non-native populations were significantly

Appendix 2 – Amphibian Phylogenies

Page 206 – Amphibian phylogeny tree. Reprinted from: *Pyron, R.A. and Wiens, J.J. (2011). A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders and caecilians. Molecular Phylogenetics and Evolution. 61, 543-583.*

Page 207 – Hylid frog phylogeny with a map identifying location and diversity of species. Reprinted from: *Wiens, J.J. et al. (2011). Phylogenetic origins of local scale diversity patterns and the causes of Amazonian megadiversity. Ecology Letters, 12, 643-652.*

Page 208-213 – Detailed hylid phylogeny based on 362 hylid species out of 885 (currently described at the time). Reprinted from: *Wiens, J.J. et al. (2010). An expanded phylogeny of treefrogs (Hylidae) based on nuclear and mitochondrial sequence data. Molecular Phylogenetics and Evolution. 55, 871-882.*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Scolecomorphinae
Caeciliinae
Herpelinae
Caeciliidae
Gymnophiona
100
98
100
59
Hynobiidae
Cryptobranchidae
Sirenidae
Ambystomatidae
Dicamptodontidae
Salamandrininae
Pleurodelinae
Salamandrinae
Salamandridae
Proteidae
Rhyacotritonidae
Amphiumidae
Plethodontinae
Hemidactylinae
Bolitoglossinae
Spelerpinae
Plethodontidae
Caudata
100
99
100
71
98
100
85
97
92
97
88
98
87
83
54
Leiopelmatidae
Ascaphidae
Bombinatoridae
Discoglossidae
Alytidae
Pipidae
Rhinophrynidae
Scaphiopodidae
Pelodytidae
Megophryidae
Pelobatidae
Heleophrynidae
Sooglossidae
Nasikabatrachidae
Calyptocephalellidae
Myobatrachinae
Limnodynastinae
Myobatrachidae
98
99
100
100
100
98
92
100
96
99
98
100
100
98
Neobatrachia
98
57
56
Ceuthomantidae
Brachycephalidae
Eleutherodactylinae
Phyzelaphryninae
Eleutherodactylidae
Pristimantinae
Holoadeninae
Craugastorinae
Strabomantinae
Craugastoridae
Hemiphractidae
Hylinae
Pelodryadinae
Phyllomedusinae
Hylidae
Bufonidae
Dendrobatidae
Allophrynidae
Centroleninae
Hyalinobatrachinae
Centrolenidae
Leptodactylinae
Leuiperinae
Paratelmatobiinae
Leptodactylidae
Ceratophryidae
Odontophrynidae
Cycloramphidae
Alsodidae
Hylodidae
Telmatobiidae
Batrachylidae
Rhinodermatidae
97
93
98
86
Hyloidea
96
100
63
54
100
100
79
53
Anura
Brevicipitidae
Hemisotidae
Hyperoliidae
Arthroleptinae
Leptopelinae
Astylosterninae
Arthroleptidae
Phrynomerinae
Otophryninae
Gastrophryninae
Cophylinae
Hoplophryninae
Scaphiophryninae
Microhylinae
Dyscophinae
Kalophryninae
Asterophryninae
Melanobatrachinae
Microhylidae
100
97
98
99
71
77
97
76
100
Ranoidea
Ptychadenidae
Micrixalidae
Phrynobatrachidae
Conrauidae
Petropedetidae
Cacosterninae
Pyxicephalinae
Pyxicephalidae
Ceratobatrachidae
Nyctibatrachidae
Ranixalidae
Dicroglossinae
Occidozyginae
Dicroglossidae
Ranidae
Rhacophorinae
Buergeriinae
Rhacophoridae
Laliostominae
Mantellinae
Boophinae
Mantellidae
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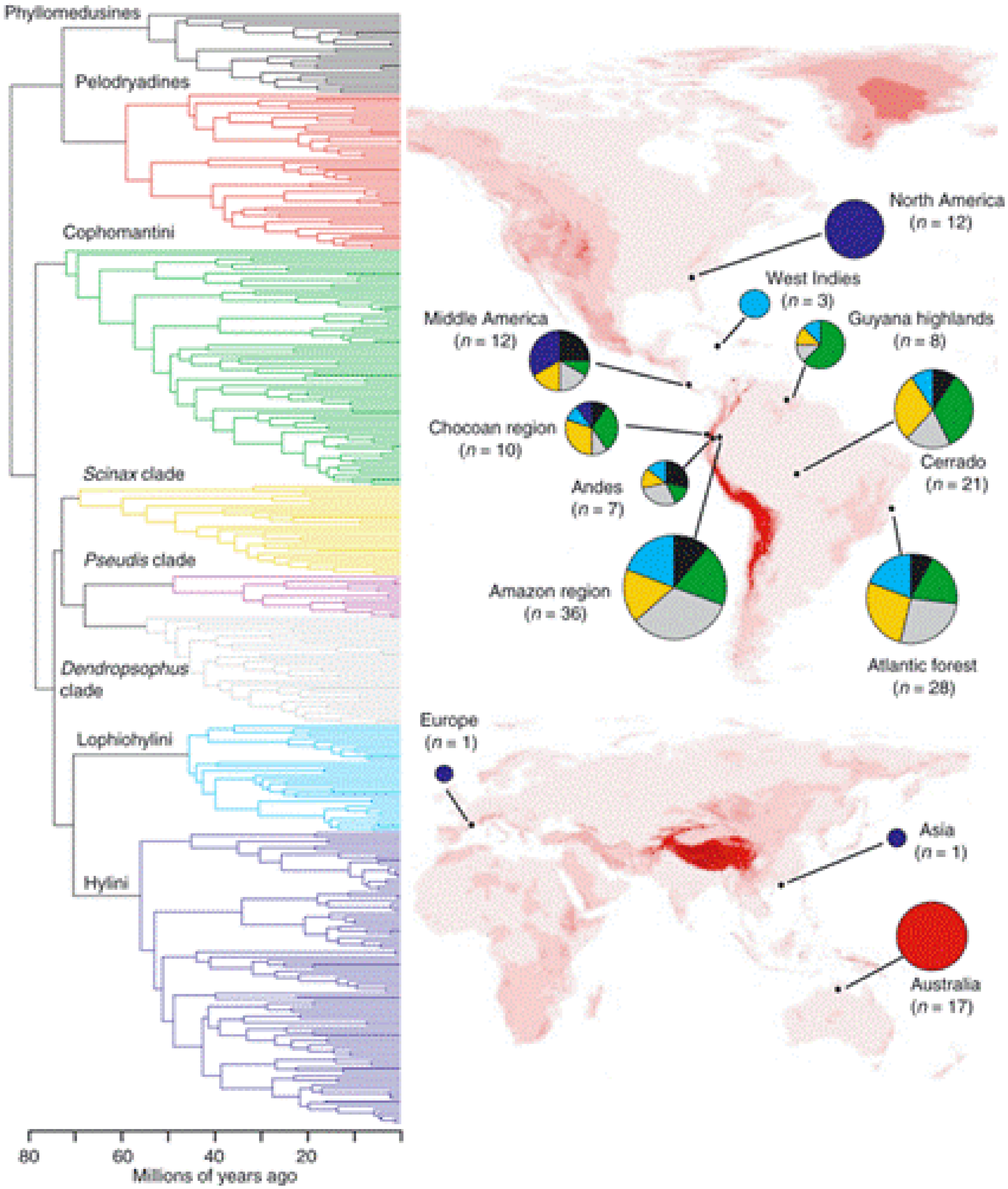
50
91
54
88
74
100
96
100
60
0.2 subst./site


Phyllomedusines
Pelodryadines
Cophomantini
Scinax clade
Pseudis clade
Dendropsophus clade
Lophiohylini
Hylini
80
60
40
20
Millions of years ago
North America
(*n* = 12)
West Indies
(*n* = 3)
Middle America
(*n* = 12)
Guyana highlands
(*n* = 8)
Chocoan region
(*n* = 10)
Cerrado
(*n* = 21)
Andes
(*n* = 7)
Amazon region
(*n* = 36)
Atlantic forest
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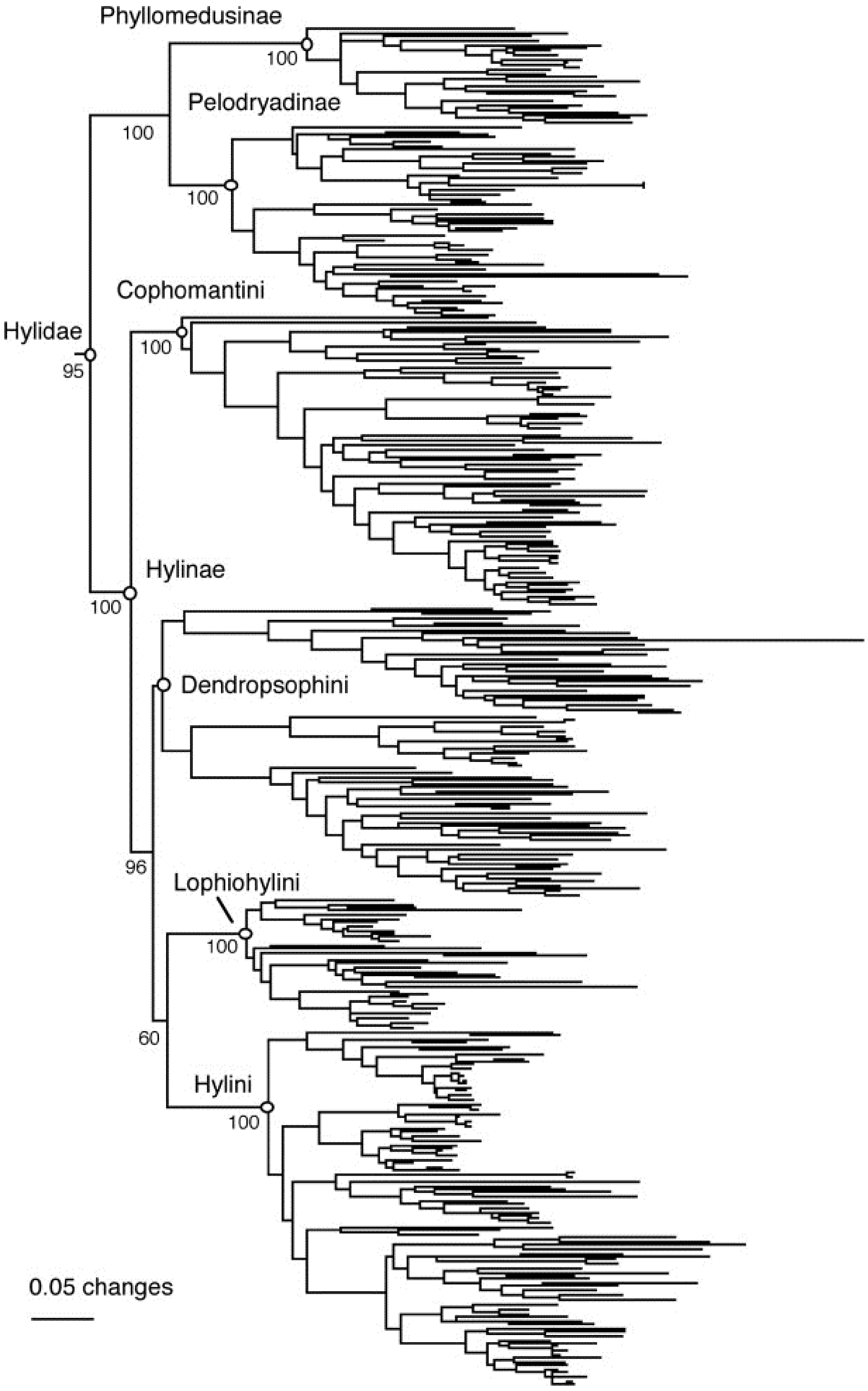
Europe
(*n* = 1)
Asia
(*n* = 1)
Australia
(*n* = 17)


Phyllomedusinae
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Pelodryadinae
100
100
Cophomantini
Hylidae
100
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Hylinae
100
Dendropsophini
96
Lophiohylini
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Hylini
100
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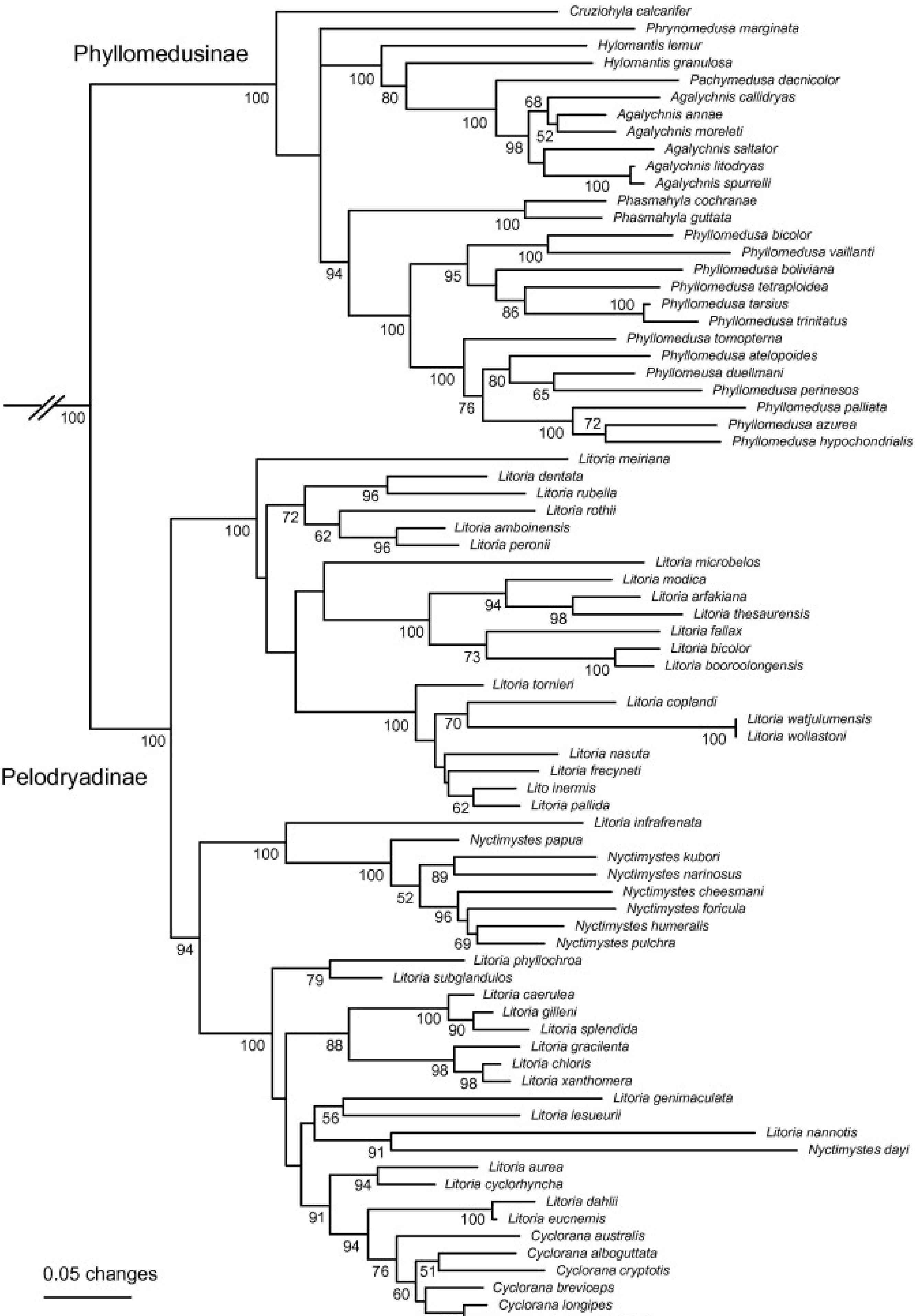
Phyllomedusinae
Cruziohyla calcarifer
Phrynomedusa marginata
Hylomantis lemur
Hylomantis granulosa
Pachymedusa dacnicolor
Agalychnis callidryas
Agalychnis annae
Agalychnis moreleti
Agalychnis saltator
Agalychnis litodryas
Agalychnis spurrelli
Phasmahyla cochranae
Phasmahyla guttata
Phyllomedusa bicolor
Phyllomedusa vaillanti
Phyllomedusa boliviana
Phyllomedusa tetraploidea
Phyllomedusa tarsius
Phyllomedusa trinitatus
Phyllomedusa tomopterna
Phyllomedusa atelopoides
Phyllomeusa duellmani
Phyllomedusa perinesos
Phyllomedusa palliata
Phyllomedusa azurea
Phyllomedusa hypochondrialis
100
100
80
100
68
52
98
100
100
94
95
100
86
100
100
100
80
65
76
100
72
100
Pelodryadinae
Litoria meiriana
Litoria dentata
Litoria rubella
Litoria rothii
Litoria amboinensis
Litoria peronii
Litoria microbelos
Litoria modica
Litoria arfakiana
Litoria thesaurensis
Litoria fallax
Litoria bicolor
Litoria booroolongensis
Litoria tornieri
Litoria coplandi
Litoria watjulumensis
Litoria wollastoni
Litoria nasuta
Litoria frecyneti
Lito inermis
Litoria pallida
Litoria infrafrenata
Nyctimystes papua
Nyctimystes kubori
Nyctimystes narinosus
Nyctimystes cheesmani
Nyctimystes foricula
Nyctimystes humeralis
Nyctimystes pulchra
Litoria phyllochroa
Litoria subglandulos
Litoria caerulea
Litoria gilleni
Litoria splendida
Litoria gracilenta
Litoria chloris
Litoria xanthomera
Litoria genimaculata
Litoria lesueurii
Litoria nannotis
Nyctimystes dayi
Litoria aurea
Litoria cyclorhyncha
Litoria dahlii
Litoria eucnemis
Cyclorana australis
Cyclorana alboguttata
Cyclorana cryptotis
Cyclorana breviceps
Cyclorana longipes
Cyclorana manya
100
96
72
62
96
94
98
100
73
100
100
70
100
62
100
100
89
52
96
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94
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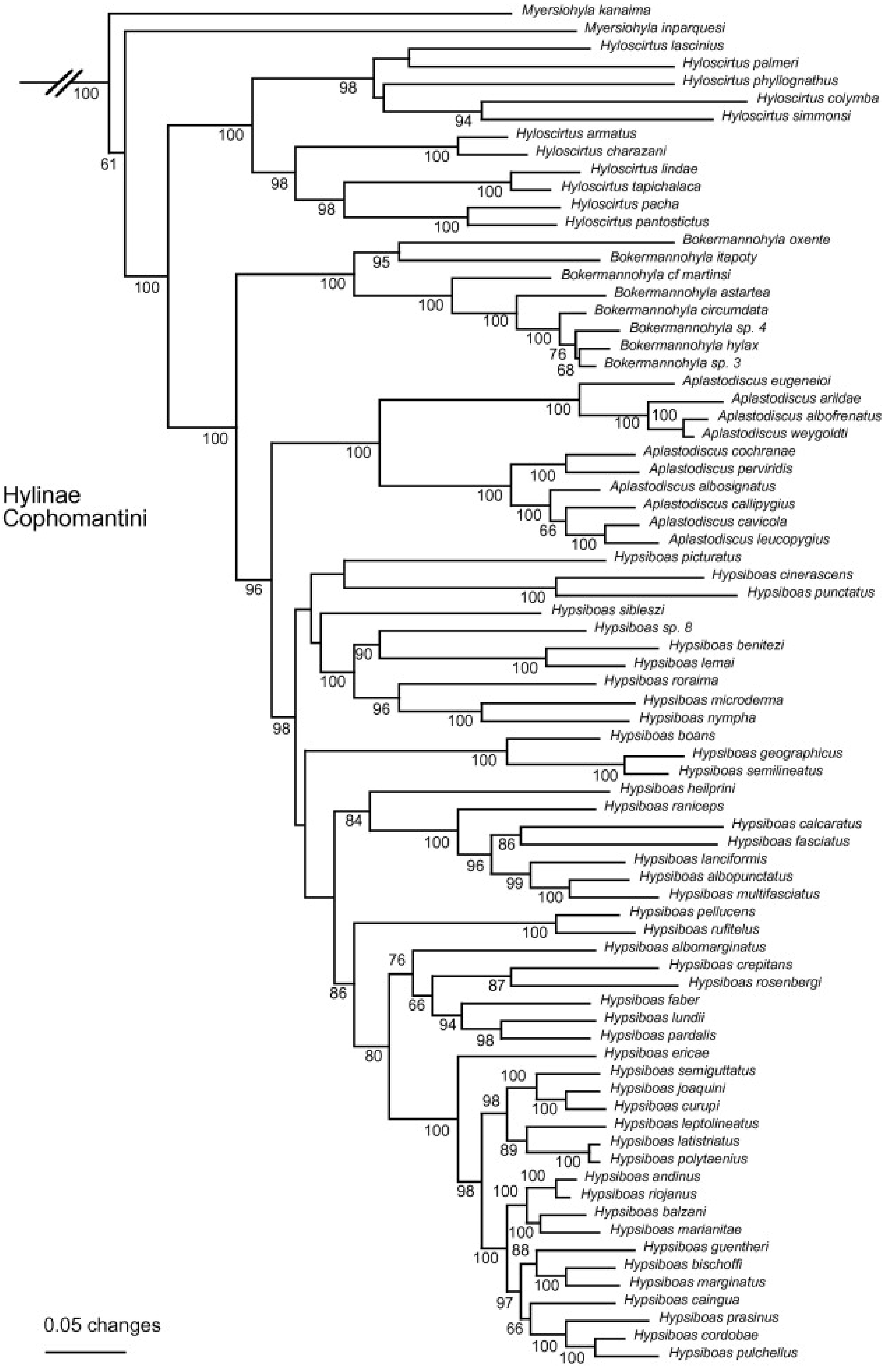
51
60
82
0.05 changes



Myersiohyla kanaima
Myersiohyla inparquesi
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Hyloscirtus palmeri
Hyloscirtus phyllognathus
98
100
Hyloscirtus colymba
Hyloscirtus simmonsi
94
Hyloscirtus armatus
100
61
Hyloscirtus charazani
100
Hyloscirtus lindae
98
Hyloscirtus tapichalaca
100
Hyloscirtus pacha
98
Hyloscirtus pantostictus
100
Bokermannohyla oxente
Bokermannohyla itapoty
95
100
Bokermannohyla cf martinsi
100
Bokermannohyla astartea
100
Bokermannohyla circumdata
100
Bokermannohyla sp. 4
100
Bokermannohyla hylax
76
Bokermannohyla sp. 3
68
Aplastodiscus eugeneioi
Aplastodiscus arildae
100
Aplastodiscus albofrenatus
100
100
Aplastodiscus weygoldti
100
Aplastodiscus cochranae
100
Aplastodiscus perviridis
100
Aplastodiscus albosignatus
Hylinae
Cophomantini
100
Aplastodiscus callipygius
100
Aplastodiscus cavicola
66
Aplastodiscus leucopygius
100
Hypsiboas picturatus
Hypsiboas cinerascens
Hypsiboas punctatus
100
96
Hypsiboas sibleszi
Hypsiboas sp. 8
Hypsiboas benitezi
90
Hypsiboas lemai
100
Hypsiboas roraima
100
Hypsiboas microderma
96
Hypsiboas nympha
100
98
Hypsiboas boans
Hypsiboas geographicus
100
Hypsiboas semilineatus
100
Hypsiboas heilprini
Hypsiboas raniceps
84
Hypsiboas calcaratus
Hypsiboas fasciatus
100
86
Hypsiboas lanciformis
96
Hypsiboas albopunctatus
99
Hypsiboas multifasciatus
100
Hypsiboas pellucens
Hypsiboas rufitelus
100
Hypsiboas albomarginatus
76
Hypsiboas crepitans
Hypsiboas rosenbergi
87
86
Hypsiboas faber
66
Hypsiboas lundii
94
Hypsiboas pardalis
98
80
Hypsiboas ericae
100
Hypsiboas semiguttatus
Hypsiboas joaquini
98
Hypsiboas curupi
100
Hypsiboas leptolineatus
100
89
Hypsiboas latistriatus
Hypsiboas polytaenius
100
Hypsiboas andinus
100
100
Hypsiboas riojanus
98
Hypsiboas balzani
Hypsiboas marianitae
100
Hypsiboas guentheri
88
100
Hypsiboas bischoffi
Hypsiboas marginatus
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Hypsiboas prasinus
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Hypsiboas cordobae
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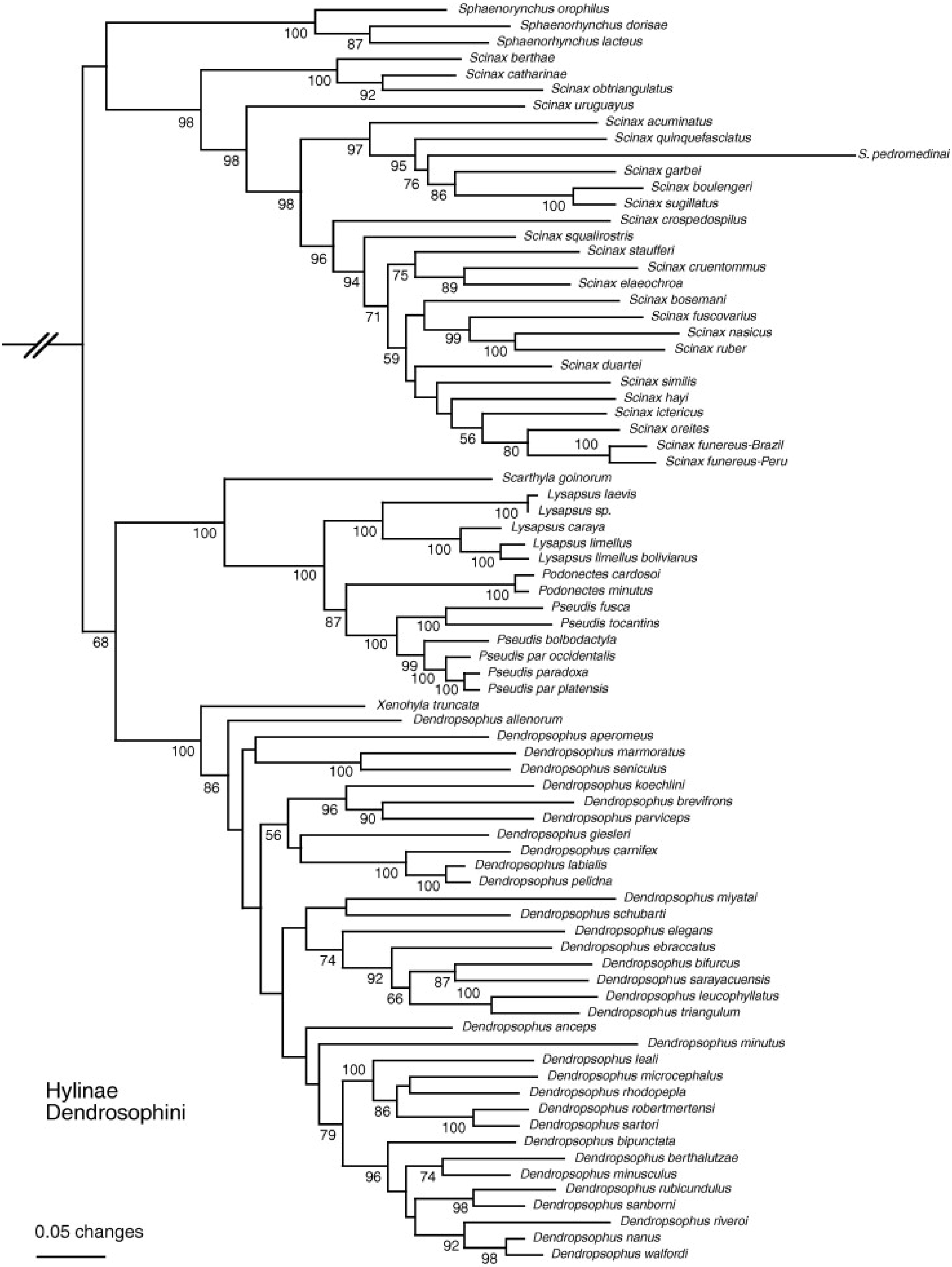
Hypsiboas pulchellus
100
0.05 changes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Sphaenorhynchus dorisae
87
Sphaenorhynchus lacteus
Scinax berthae
100
Scinax catharinae
92
Scinax obtriangulatus
Scinax uruguayus
98
Scinax acuminatus
Scinax quinquefasciatus
97
98
S. pedromedinai
95
Scinax garbei
76
Scinax boulengeri
86
Scinax sugillatus
100
98
Scinax crospedospilus
Scinax squalirostris
Scinax staufferi
Scinax cruentommus
96
75
Scinax elaeochroa
94
89
Scinax bosemani
71
Scinax fuscovarius
Scinax nasicus
99
Scinax ruber
100
59
Scinax duartei
Scinax similis
Scinax hayi
Scinax ictericus
Scinax oreites
56
100
Scinax funereus-Brazil
80
Scinax funereus-Peru
Scarthyla goinorum
Lysapsus laevis
100
Lysapsus sp.
100
Lysapsus caraya
100
Lysapsus limellus
100
Lysapsus limellus bolivianus
100
100
Podonectes cardosoi
Podonectes minutus
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Pseudis fusca
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Pseudis tocantins
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Pseudis bolbodactyla
Pseudis par occidentalis
99
100
Pseudis paradoxa
100
Pseudis par platensis
Xenohyla truncata
Dendropsophus allenorum
Dendropsophus aperomeus
100
Dendropsophus marmoratus
100
Dendropsophus seniculus
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Dendropsophus koechlini
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96
90
Dendropsophus parviceps
56
Dendropsophus giesleri
Dendropsophus carnifex
Dendropsophus labialis
100
100
Dendropsophus pelidna
Dendropsophus miyatai
Dendropsophus schubarti
Dendropsophus elegans
74
Dendropsophus ebraccatus
Dendropsophus bifurcus
92
87
Dendropsophus sarayacuensis
66
100
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Dendropsophus triangulum
Dendropsophus anceps
Dendropsophus minutus
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Dendropsophus leali
Dendropsophus microcephalus
Hylinae
Dendrosophini
Dendropsophus rhodopepla
86
Dendropsophus robertmertensi
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Dendropsophus sartori
79
Dendropsophus bipunctata
Dendropsophus berthalutzae
96
74
Dendropsophus minusculus
Dendropsophus rubicundulus
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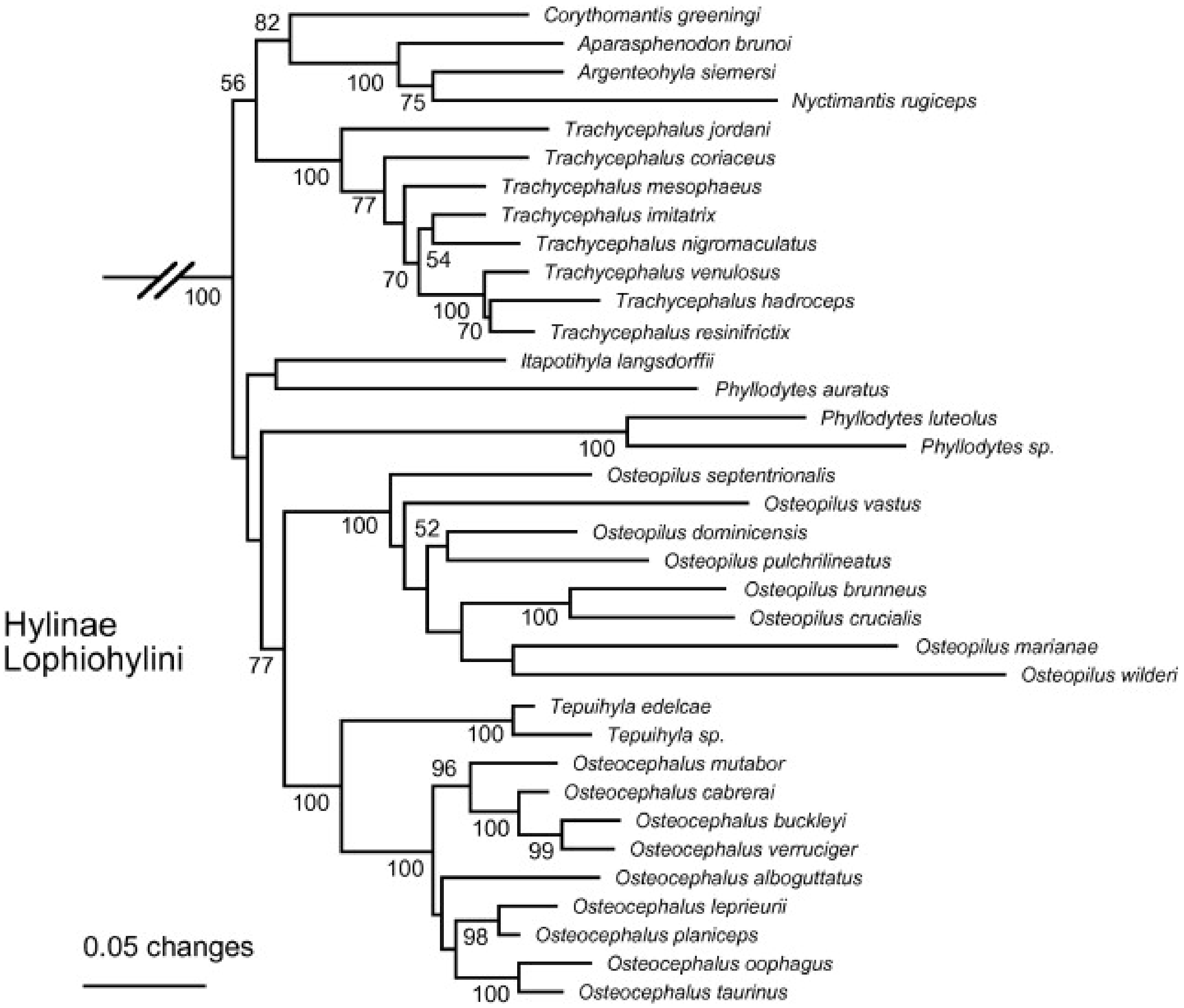
Dendropsophus walfordi


Corythomantis greeningi
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Trachycephalus imitatrix
Trachycephalus nigromaculatus
Trachycephalus venulosus
Trachycephalus hadroceps
Trachycephalus resinifrictix
Itapotihyla langsdorffii
Phyllodytes auratus
Phyllodytes luteolus
Phyllodytes sp.
Osteopilus septentrionalis
Osteopilus vastus
Osteopilus dominicensis
Osteopilus pulchrilineatus
Osteopilus brunneus
Osteopilus crucialis
Osteopilus marianae
Osteopilus wilderi
Tepuihyla edelcae
Tepuihyla sp.
Osteocephalus mutabor
Osteocephalus cabrerai
Osteocephalus buckleyi
Osteocephalus verruciger
Osteocephalus alboguttatus
Osteocephalus leprieurii
Osteocephalus planiceps
Osteocephalus oophagus
Osteocephalus taurinus
82
56
100
75
100
77
54
70
100
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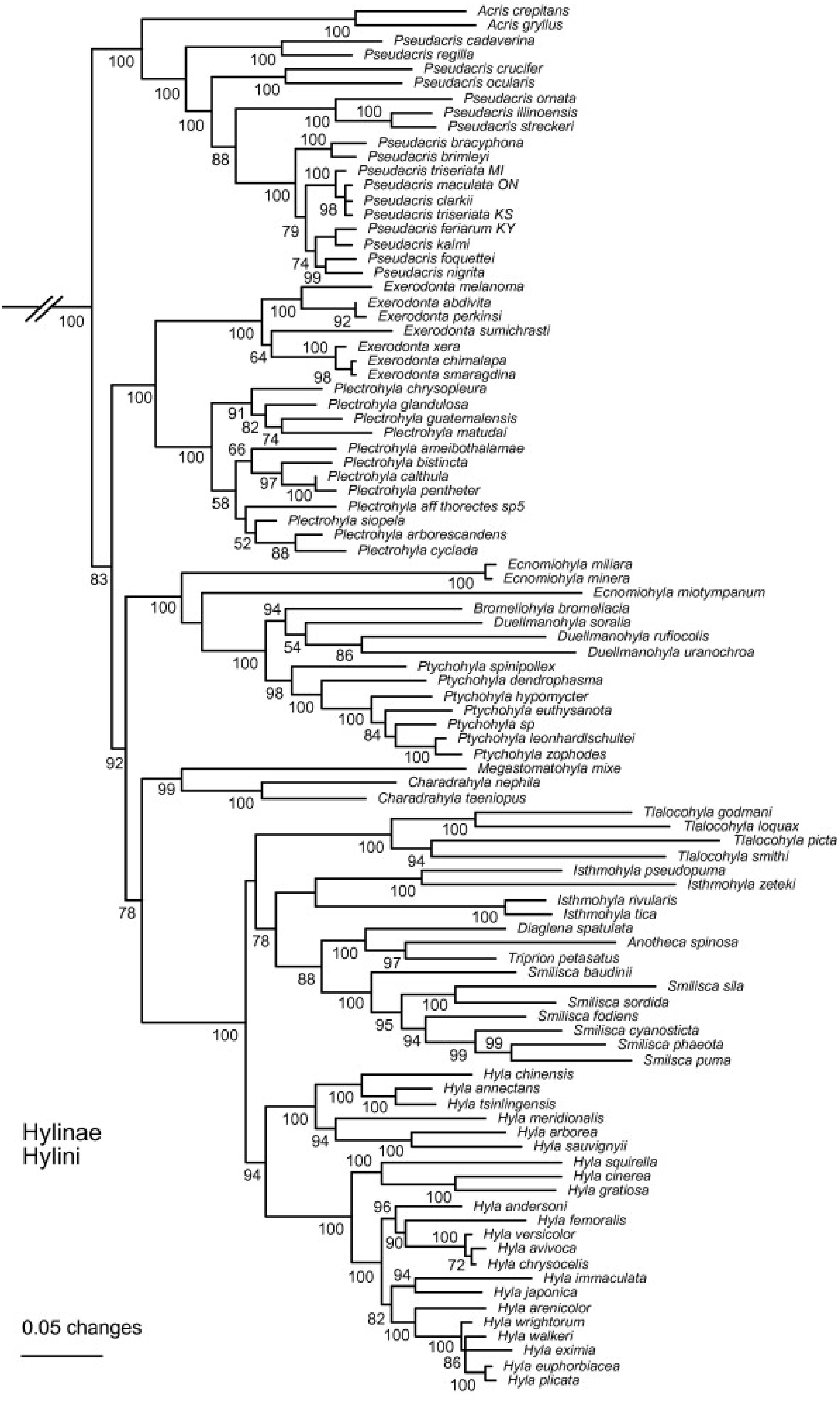
100
Hylinae
Lophiohylini
0.05 changes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Acris gryllus
Pseudacris cadaverina
Pseudacris regilla
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Pseudacris ocularis
Pseudacris ornata
Pseudacris illinoensis
Pseudacris streckeri
Pseudacris bracyphona
Pseudacris brimleyi
Pseudacris triseriata MI
Pseudacris maculata ON
Pseudacris clarkii
Pseudacris triseriata KS
Pseudacris feriarum KY
Pseudacris kalmi
Pseudacris foquettei
Pseudacris nigrita
Exerodonta melanoma
Exerodonta abdivita
Exerodonta perkinsi
Exerodonta sumichrasti
Exerodonta xera
Exerodonta chimalapa
Exerodonta smaragdina
Plectrohyla chrysopleura
Plectrohyla glandulosa
Plectrohyla guatemalensis
Plectrohyla matudai
Plectrohyla ameibothalamae
Plectrohyla bistincta
Plectrohyla calthula
Plectrohyla pentheter
Plectrohyla aff thorectes sp5
Plectrohyla siopela
Plectrohyla arborescandens
Plectrohyla cyclada
Ecnomiohyla miliara
Ecnomiohyla minera
Ecnomiohyla miotympanum
Bromeliohyla bromeliacia
Duellmanohyla soralia
Duellmanohyla rufiocolis
Duellmanohyla uranochroa
Ptychohyla spinipollex
Ptychohyla dendrophasma
Ptychohyla hypomycter
Ptychohyla euthysanota
Ptychohyla sp
Ptychohyla leonhardschultei
Ptychohyla zophodes
Megastomatohyla mixe
Charadrahyla nephila
Charadrahyla taeniopus
Tlalocohyla godmani
Tlalocohyla loquax
Tlalocohyla picta
Tlalocohyla smithi
Isthmohyla pseudopuma
Isthmohyla zeteki
Isthmohyla rivularis
Isthmohyla tica
Diaglena spatulata
Anotheca spinosa
Triprion petasatus
Smilisca baudinii
Smilisca sila
Smilisca sordida
Smilisca fodiens
Smilisca cyanosticta
Smilisca phaeota
Smilsca puma
Hyla chinensis
Hyla annectans
Hyla tsinlingensis
Hyla meridionalis
Hyla arborea
Hyla sauvignyii
Hyla squirella
Hyla cinerea
Hyla gratiosa
Hyla andersoni
Hyla femoralis
Hyla versicolor
Hyla avivoca
Hyla chrysocelis
Hyla immaculata
Hyla japonica
Hyla arenicolor
Hyla wrightorum
Hyla walkeri
Hyla eximia
Hyla euphorbiacea
Hyla plicata
Hylinae
Hylini
0.05 changes