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Contributed Paper

Comparison of Anuran Amphibian Assemblages in Protected and Non-Protected Forest Fragments in Upper Northeastern Thailand

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ABSTRACT

The aim of this three years study was to analyze and compare the richness, composition, abundance and observed frequency of anuran amphibian assemblages in the two forest fragments, Phufoilom (a protected area) and Phuhinlat (a non-protected area), both in the upper northeastern Thailand. The study was the first survey of the anuran amphibian assemblage in these forest fragments. Four Sampling techniques were used which are the visual encounter surveys (VES) using the time constrained count (TCC) technique, the sampling plots, the stream transects and the pitfall-trapping. The Chao-Jaccard Similarity Index was used for the comparison of species richness and composition among habitat types and fragments. Both fragments were very similar in anuran richness and composition, being composed of 5 families representing 15 genera (28 species) in Phufoilom and 13 genera (26 species) in Phuhinlat. However, the differences in richness and composition between habitat types were found within each fragment. Of the 28 species, 26 were shared in both forest fragments. In the protected area 22 anuran species were captured in higher rate than in non-protected, significant differences in 11 species. In addition, relative abundance among species followed a similar pattern within both areas. These results confirm the critical function of habitat in maintaining appropriate population size and proportion of abundance among species in an assemblage.

Keywords: amphibian, anuran, similarity, forest fragments, protected forest area, non-protected forest area, upper northeastern Thailand

1. INTRODUCTION

Currently, amphibians are widely threatened and in decline; with population decreasing over the last several decades until the present. The decline and loss of amphibian populations are problems with complex local causes [1-4]. Habitat transformation, fragmentation and anthropogenic disturbance are accepted as the clearest causes of the reported declines in the global amphibian population [1-3,5,6], particularly in tropical rain forests where the diversity is high and forests are transformed at always-increasing rates [1,7,8]. Habitat transforma-tion and fragmentation occur at relatively higher rates in Southeast Asia and Indo-China than other tropical regions [8,9]. This is an important threat to amphibians in these regions. However, there were few researches that studied the relationship between landscape fragmentation and amphibian diversity in these regions [8-10].

The main effect of fragmentation to anuran amphibians is an impediment to migrated among patches resulting in population isolation, inbreeding, edge effects and disconnection between aquatic and terrestrial environments, both crucial ecology for amphibian reproduction [1,5,6,7]. The relationship between amphibian distributions and habitat fragmentation is available from previous studies as well as the effects of fragmentation upon juvenile dispersal of pond-breeding amphibians [5,6,11,12]. In addition, the habitat fragmentation is an even greater extinction risk for endemics and highly rare anuran species because of their habitat specialization [13, 14]. The transformation and fragmented habitat are often accompanied by a severe loss not only of species diversity but also of genetic diversity. It has been suggested that high genetic diversity was able to maintain by large and interconnected populations [8,15,16].

The fragments, Phufoilom (protected area) and Phuhinlat (non-protected area) are parts of the Phupannoi Mountain Range in upper northeastern Thailand, located in Indo-China sub region, which is a part of Indo-Australian region [17]. In the past, both areas formed a homogeneous, connected forest before being partitioned by human communities, roads and agricultural expansion. Phuhinlat is an area already subject to high levels of disturbance resulting from deforestation for an agricultural expansion and farm animals. The area transformation is forming internal patchy ecosystem structure [18].

Because the threats above mentioned are important problem to the existence of amphibians in tropical forest. Urgent action is required to limit these threats for the conservation of local amphibian biodiversity, including the establishment of strict protection area [8,10,13,16,19]. The creation of protected regions is vital to the long-term viable functioning of ecosystems and the conservation of biodiversity within a region, and their successful establishment requires reliable evaluative data. The term 'protected' applies to an area, established by a national government, within a natural protected area [1,8,13,16,19]. Our objectives were to compare anuran species richness, composition, abundance and frequency between fragmented protected and non-protected areas that allow us to assess the effects of fragmented habitats upon anuran amphibian population and effectiveness of programs on biodiversity conservation.

2. Materials and Methods

2.1 Study Areas

Phufoilom forest is located southwest of Muang District, Udon Thani Province (latitude 17°7.945′ to 17°10.450′ E, longitude 102°39.731′ to 102°41.930′ S), with a total area of approximately 45 km^2 and the highest elevation of about 590 m above Mean Sea Level (MSL). Phuhinlat forest is located west of Muang District, Udon Thani Province (latitude 17°11.220′ to 17°15.894′ E, longitude 102°26.698′ to 102°31.011′ S), with the total area being about 32 km^2 and the highest elevation of about 580 m above MSL (Figure 1). Phufoilom and Phuhinlat are the part of the Phupannoi Mountain Range which is divided into five major areas, including Nampong National Park, Phukaow-Phupankhum National Park, Phuwiang National Park, Phufoilom, which is part of Phundon-Prakho conserved forest [20], and Phuhinlat, the only non-protected area of this group (Figure 1). There are three seasons in Northeastern Thailand each year: summer (February to May), rainy (June to September) and winter (October to January) [21]. The annual precipitation in the region was 1677, 1457 and 1534 mm from 2008-2010, respectively. The annual mean temperature was 30, 28 and 30°C from 2008-2010, respectively [22].

The major vegetation types are tropical dry dipterocarp forest about 54% of the total area in Phufoilom and 61% in Phuhinlat, and mixed deciduous forest (about 25% of the total area in Phufoilom and 27% in Phuhinlat). Dry evergreen forest is also found in both areas, covering about 10% of the total area. Other types of vegetation account for 5-10% [23, 24].

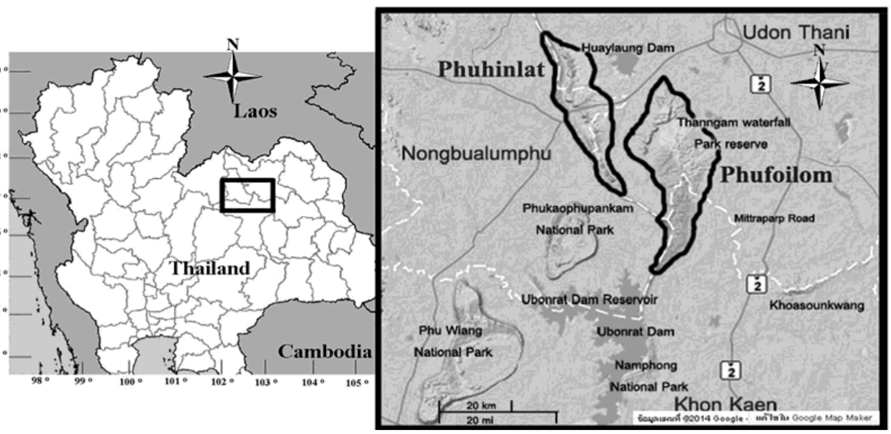

Figure 1. Location of study sites (bordered by dark line), Phufoilom (east) and Phuhinlat (west), are located between Nongbualumphu and Udon Thani Provinces in the Phupannoi Mountain Range in upper northeastern Thailand (edited from www.map.google.co.th.).

2.2 Sampling Methods and Identifications

This amphibian survey was carried out over three years from March 2008 to November 2010. A total of 10 sites were sampled, comprising 5 habitats types present in Phufoilom and Phuhinlat forests. The same habitat type was selected location in both studied localities in the same elevation (± 50 m MSL) [25]. All sites were surveyed three times a year and over three seasons. Habitats types were designated based on three factors, including a dominant vegetation structure, landscape pattern and amphibian habitats association [16,26]. Five habitats under studied were: 1) Permanent ponds (PP), referring to the ponds which have water cages for years in both wet and dry seasons; 2) Temporary ponds (TP), which include road sides, and refer to ponds in which water cages are found only in a hydroperiod; 3) Stream habitats (ST) extending to fifteen meters from stream edges; 4) Woodland habitats (WL) referring to areas located more than 500 m from water source, with tree canopy cover exceeding 50%; 5) Grassland habitats (GL), which are open forest with less than 10% tree cover, with seasonally stagnant areas occurring during the wet season.

Sampling techniques followed the standard methods for measurement and monitoring of amphibian diversity [27]. Details of the sampling techniques were followed the procedure for obtain species richness in dry tropical rain forest of Von May *et al.* (2008) and Behangana *et al.* (2009) [16, 28]. Visual encounter surveys (VES) using the time constrained count (TCC) technique, along with sampling plots and stream transects. We used 250 × 250 m plots in grassland and forest with less canopy cover. Transects were used to detect frogs inhabiting streams and stream banks for a limited time (3 hours in each transect). Pitfall traps with drift fences of 40 cm diameter at the mouth and a depth of 50 cm were used for sampling of woodland species and non tree-frog species. Each trap array was checked for capture rate every morning between visiting days. We used nocturnal surveys because all amphibians in our studied are being nocturnal, this method more effective compared to diurnal sampling [16]. Sampling was carried out between 1900 and 2200 hours each day by three observers. Captured amphibians were identified before being released them into the same location. Classification was carried out according to Taylor (1962) [29] and taxonomic nomenclature followed Frost (2014); Pyron & Wiens (2011) [30, 31]. The specimens were deposited, together with related documents, in the Zoological Laboratory, Department of Biology, Faculty of Science, Udon Thani Rajabaht University, Thailand.

2.3 Data Analysis

Because of the different sampling techniques used in the various habitat types, patterns of species richness in the study areas were compared using both individual-based and sample species accumulation curves and species-richness estimators [32, 33]. To derive variables for each fragment, detection data from all sampling techniques and all habitat types were aggregated. Sampling efficiency can be estimated as observed species richness (S_{obs})/species richness estimate (S_{est}) (using average value of all estimators) with values near 1, indicating that most of the species endemic to the area have actually been observed [34]. We used abundance-based richness estimators, the classic Chao 1 and ACE and incidence-based richness estimators, the classic Chao 2 and ICE, which have been recommended as robust species richness estimators using EstimateS 8.2 [35]. To compare species richness and composition among habitat types and fragments, we derived Chao-Jaccard Similarity Index (JSI) [36] using EstimateS 8.2. We calculated estimates for species richness estimators and JSI using 500 randomizations, with replacement, from X-metrics of initial detection data, which included recaptures [35,37]. To compare the differences in abundance and individual capture rate with respect to habitat, years and seasons, we compared observed data using the two-sample t-test, one-way ANOVA and the multiple comparisons Tukey test [38]. Statistical tests were conducted using statistic program R version 2.15.1.

3. Results and Discussions

3.1 Evaluation of Sampling Effort

A total of 2811 individuals were collected, 1623 captures (28 anuran species) in Phufoilom and 1188 captures (26 anuran species) in Phuhinlat. Evaluating the potential effects of the sampling effort, using pooled data from all samples from each forest fragment (45 each from Phufoilom and Phuhinlat), sample-based and individual-based accumulation curves reached a plateau after 43 samples and 1549 individuals for the Phufoilom curve, and after 42 samples and 1105 individuals for the Phuhinlat curve. All species accumulation curves approached an asymptote, suggesting that most species of the anuran assemblages within the fragment areas could be practically detected [32,33]. With regard to the Chao 1 and Chao 2 estimates for total richness, the Phufoilom values were 30 ± 1 (SD); 28 ± 2, and Phuhinlat 27 ± 1; 27 ± 1. The ACE and ICE estimates for total richness were 29 ± 2; 29 ± 1 for Phufoilom and 28 ± 1; 26 ± 1 for Phuhinlat. The sampling efficiency, estimated by S_{obs}/ average S_{est}, was 0.96 in Phufoilom and 0.98 in Phuhinlat, confirming that observed species richness within the two areas was very similar to estimated richness. It may be assumed that the sampling effort was approaching completeness, the results being directly comparable [34,35]. The results also suggest that estimates of richness based on incidence data are closer to estimates from abundance data. Thus, the surveys should record both abundance and incidence data to ensure that maximum accuracy is achieved in the estimates of richness [36,37].

3.2 Comparison of the Species Richness and Composition

The two fragments were composed of 5 families representing 15 genera (28 species) in Phufoilom and 13 genera (26 species) in Phuhinlat. There were 26 species found in both forests, except *Hylarana taipehensis* and *Pelophylax lateralis* were only captured in Phufoilom. The highest number (11) of species was represented by Microhylidae, followed by Dicroglossidae (6 species), Rhacophoridae (4 species), Ranidae (5 species in Phufoilom and 3 in Phuhinlat) and Bufonidae (2 species) (Table 1). The results clearly indicate that species richness and composition in anuran amphibian communities in the protected and non-protected studied areas are highly similar (JSI = 0.92). Such similarity is not surprising, as both fragments resemble each other in geography, topography, climate patterns (temperature, precipitation and season) [21, 22], and vegetation type [23, 24], as these factors have a strong influence upon richness and distribution of amphibious fauna [16, 25, 26, 28]. According to the two species were not observed in Phuhinlat, *H. taipehensis* is a pond breeding species, breeding occurs in surface water with that dense tree cover at water's edge [39]. They are observed in permanent ponds of Phufoilom, which provide better breeding site characteristics than Phuhinlat as the single permanent pond is covered with lower tree density at water's edge. Since it is newly was originated by human excavation for sandstone mining. The second species only recovered in Phufoilom, *P. lateralis* is associated to woodland habitat, in our study is found in woodland (WL, dry dipterocarp forest). *P. lateralis* is the largest frog reported in WL in our study, as dipterocarp forest patches are small sized in non-protected forest probably will be not enough to support populations of *P. lateralis* as it big size need more ecological resources than small frogs [34]. Phufoilom has provided an excellence habitat that is not patches with less anthropogenic disturbance. Previous report suggested that the rare species from naturally fragmented habitats much remains in the suitable fragment to responsible for breeding pressure [5,15].

Table 1. Families recorded and species listed of anuran amphibians in Phufoilom and Phuhinlat, with representative relative abundance and percentages of observed frequencies.

Families and Species	Phufoilom		Phuhinlat	
	Relative abundance	% Observed frequencies	Relative abundance	% Observed frequencies
I. Bufonidae				
1. *Duttaphrynus melanostrictus*	1.85	31.11	2.69	31.11
2. *Ingerophrynus macrotis*	0.19	4.44	0.18	2.22
II. Dicroglossidae				
3. *Fejervarya limnocharis*	6.78	77.78	6.24	55.56
4. *Hoplobatrachus rugurosus*	1.55	26.67	2.10	20.00
5. *Limnonectes gyldenstolpei*	1.42	13.33	2.47	17.78
6. *Occidozyga lima*	8.20	80.00	7.44	66.67
7. *Occidozyga magnapustulosa*	0.80	13.33	1.09	8.89
8. *Occidozyga martensii*	9.74	73.33	10.10	66.67
III. Microhylidae				
9. *Calluella guttulata*	1.85	33.33	1.44	15.56
10. *Glypholossus molossus*	1.17	24.44	1.26	17.78
11. *Kalophrynus interlineatus*	0.99	22.22	1.11	11.11
12. *Kaloula mediolineata*	1.59	26.67	1.64	24.44
13. *Kaloula pulchra*	2.77	44.44	2.47	35.56
14. *Microhyla berdmorei*	2.78	48.89	2.31	31.11
15. *Microhyla butleri*	5.41	64.44	7.35	55.56
16. *Microhyla heymonsi*	3.26	55.56	3.28	31.11
17. *Microhyla fissipes*	11.57	86.67	11.77	75.56
18. *Microhyla pulchra*	3.03	44.44	3.37	35.56
19. *Micryletta inornata*	2.21	40.00	1.91	24.44
IV. Ranidae				
20. *Hylarana erythraea*	2.67	31.11	2.37	22.22
21. *Hylarana macrodactyla*	2.65	40.00	2.04	28.89
22. *Hylarana nigrovittata*	4.56	20.00	4.40	17.78
23. *Hylarana taipehensis*	0.93	11.11	-	-
24. *Pelophylax lateralis*	0.31	6.67	-	-
V. Rhacophoridae				
25. *Chiromantis doria*	1.92	31.11	2.11	31.11
26. *Chiromantis nongkhoensis*	3.01	44.44	3.77	42.22
27. *Raorchestes parvulus*	4.67	31.11	3.43	24.44
28. *Polypedates leucomystax*	12.14	75.56	11.67	75.56

A total of 141 amphibian species were recorded in Thailand, comprising 1 family, 1 species of order Caudata, 1 family, 6 species of order Gymnophiona and 7 families, 134 species of order Anura [40,41]. The number of anuran amphibians found in this study were 28 species belonging to 5 families, this being the lowest level of species richness compared to the area based studies existing from other regions of Thailand, for example in the west it recorded was 6 families, 42 species [42], in the south was 6 families, 39 species [43], lower central was 6 families, 43 species [17], and lower northeast was 6 families, 45 species [44]. The lowest diversity of anuran species in our study can be reflect to dominant forest types, topography that are importance factors for anuran distributions [25,28,45]. The evergreen forest, which was generally found more in the other regions of Thailand, has more diverse amphibious fauna than that of the deciduous forest, which has more diversity in the area upper northeast [44].

3.3 Comparison of the Individuals Captured, Abundance and Observed Frequencies

The total number of captures (2811 individuals) was divided into 1623 captures (57.7%) in Phufoilom and 1188 captures (42.3%) in Phuhinlat. These were significantly different [only total individuals number of shared species was tested by two-sample t-test ($t = 5.263$, $p < 0.05$) and also significantly different when compared to two areas by survey years (two-sample t-test; 2008 $t = 6.628$, 2009 $t = 3.506$, 2010 $t = 4.834$ and $p < 0.05$)]. Individual capture rates showed a significant difference (using two-sample t-test), 11 of a shared total of 26 species being obtained in Phufoilom, more than the total number in Phuhinlat and total 22 species of Phufoilom being more than number in Phuhinlat (Figure 2).

However, the proportion of relative abundance among species followed a very similar pattern in both areas. Only 2 species, *L. gyldenstolpei* and *M. butleri*, of 26 shared species were significantly different. A possible reason for these results is provided by a basic concept of ecology, that the carrying capacity of an environment or habitat is its ability to support and limit the population size of a species within a community [16,46]. We therefore assumed that Phufoilom, as a protected area, had higher ability to support an anuran amphibian population than Phuhinlat, a non-protected area. Several factors influence the carrying capacity of anuran amphibian habitats in Phuhinlat. Firstly, this area has a lower tree density and average percentage canopy cover within each habitat than Phufoilom, which makes this area receive a higher UV level, affecting the survival of amphibian eggs and larva [1,3]. Phuhinlat has a lower average leaf litter depth, which makes it less effective in retaining moisture for woodland or stream banks. Previous reports indicated that tree density and leaf litter depth may affect the distribution of amphibian species [16,26,45] and that the anuran amphibian population tended to be higher with increasing forest canopy cover [28,34,45]. Secondly, anthropogenic activities can decrease the area of amphibians distribution, hence affecting reproductive success [1,3,7,47].

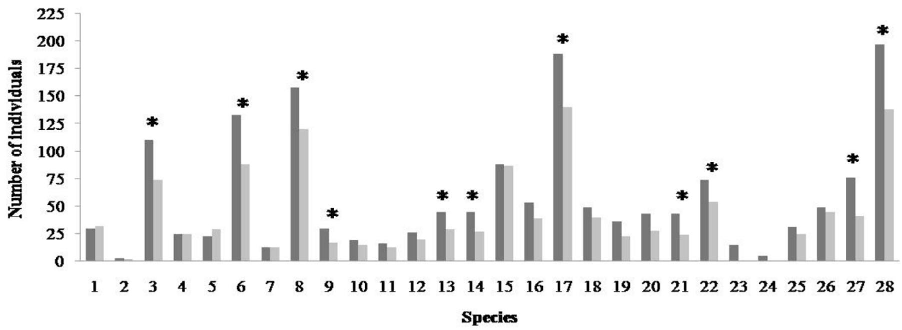

Figure 2. Comparison of individuals captured from the two fragment areas, Phufoilom (dark gray) and Phuhinlat (light gray). The significantly for individuals captured indicated by an asterisk. The species name, which is indicated by a number, is represented in table 1.

Phuhinlat is an open forest, where local villagers have the advantage of having access to wild products, hunting small animals and fish, vegetables, orchids, logging and cattle. These activities result in disturbances to pond-breeding amphibians and limit distribution of terrestrial amphibians [1,37,47]. Thirdly, in Phuhinlat, frogs are constantly threatened by capture. During the study we interviews with a group of villagers and survey of local markets found that they captured an average of 6 kg/man per day of frogs from Phuhinlat for food and trade in the reproductive season. An example of such over-capturing activity was reported by Rowley *et al* (2010) and Lau *et al* (1999) [8, 48], as six million Chinese edible frogs (*Hoplobatrachus rugulosus*) were exported from Thailand to Hong Kong in a single year, most of these frogs being collected from the wild. Over-capturing has resulted in the decline and extinction of various vertebrate species [49].

In Phufoilom, there were 6 species namely *Fejervarya limnocharis*, *Occidozyga martensii*, *O. lima*, *Microhyla butleri*, *M. fissipes* and *Polypedates leucomystax*, having a high relative abundance (above 5), 18 species with intermediate (between 1-5) and 4 species namely *Ingerophrynus macrotis*, *O. magnapustulosa*, *Kalophrynus interlineatus* and *Hylarana taipehensis* with a low percentage of relative abundance (< 1). In Phuhinlat, 6 species (the same Phufoilom species) were found with a high relative abundance, 19 species were intermediate and 1 species, *I. macrotis*, with a low percentage. *F. limnocharis*, *O. martensii*, *O. lima*, *M. butleri*, *M. fissipes* and *P. leucomystax* were considered to be common species (observed frequencies greater than 50%) to both areas and as dominant species in their own habitat (Table 1).

H. taipehensis and *P. lateralis* were considered to be rare species (observed frequencies lower than 10%, see Table 1 for other anuran species) to Phufoilom. Certainly, these two amphibian populations were of very small population size within both areas, while they were generally recorded as common in north of SE Asia. Currently, *H. taipehensis* status is that of a stable population [39] and *P. lateralis* has unknown status. This low observed frequency needs further investigation.

I. macrotis and *O. magnapustulosus* in Phuhinlat were considered to be rare species. According to the IUCN red list data, *I. macrotis* is generally distributed throughout Thailand and SE Asia and now its population is tending toward decline

[50]. *O. magnapustulosus* is a native species in the north of SE Asia in Laos, Thailand and Vietnam, and now it has unknown status [51]. This study is allows to increase the previous poor knowledge of this two anuran species. The small population size (usually less than 100 individuals) was adverse consequences for low effective breeding population and loss of genetic diversity [5,15,16]. The result demonstrated that these four are threatened species for this area, and population dynamics should be monitored in the long-term for status determination.

3.4 Comparison of Anuran Amphibian Assemblage Across Habitat Types

The number of individuals captured across the five habitat types differed in Phufoilom ($F = 10.28$, $p < 0.05$). Woodland (WL) was significantly lower than for the Permanent Pond (PP) and Temporary Pond (TP) sites by using Tukey test for WL & PP and WL & TP, the p-values were 0.001 and 0.0038, respectively. There were no significant differences in Phuhinlat ($F = 0.996$, $p > 0.05$), showing all habitat types a very similar in total number of captured individuals. The highest number of individuals at both areas was the Stream (ST) and PP sites, this may be attributed to a high moisture and water content persisting over many years, creating an appropriate, all-season habitat for a group of amphibians as has been reported in previous studies [28,38,45].

High degree of similarity, where Chao-Jaccard Similarity Index (JSI) ≥ 0.80, in Phufoilom was founded among Grassland (GL) & WL, GL & TP, WL & PP, WL & TP and PP & TP, while the rest of habitat pairs showed moderate degree of similarity ($0.40 \geq$ JSI ≤ 0.79). In Phuhinlat, the high degree of similarity was found among GL & WL, WL & TP and PP & TP, while a low degree of similarity (JSI ≤ 0.39) was found among PP & ST. The result has the same trend in both areas (Figure 3). High composition similarity among PP & TP was not surprising because 17 shared species (in Phufoilom) needed pond for their breeding. Of the 17 shared anuran species in GL & WL (in Phufoilom) requires forest or dense grassland for adult summer habitat and winter hibernate [12,26,39]. Also, of the 16 amphibians of GL & WL (in Phufoilom) were ponds breeding species. These amphibians were migrating to PP & TP in breeding season before their adults and juveniles were migrating back to GL & WL. Migration can play a role in distribution of frog in different habitat types [11,12,52]. The migration was normally situation in each observed years resulting in altering the composition of amphibian in these habitats assemblages among seasons. Certainly, fragmentation effect has influence to the migration when it has breaking up of a formerly connected habitat resulting in increased isolation of habitat patches [7,14,15].

The results showed differentiation of ST site in Phufoilom and Phuhinlat compared to others habitats, being a significantly lower species numbers than the other habitat types. Moreover, ST had low shared species when paired with other habitat types (Figure 3). ST showed the highest number of 4 species unique species (*Limnonectes gyldenstolpei*, *O. magnapustulosa*, *Micryletta inornata* and *H. nigrovittata*), these species tend to be a strict habitat types and associated with lotic ecosystem and they do not have to migrate to PP or TP for breeding because this site is appropriate all-season habitat for this amphibians [28, 38, 45].

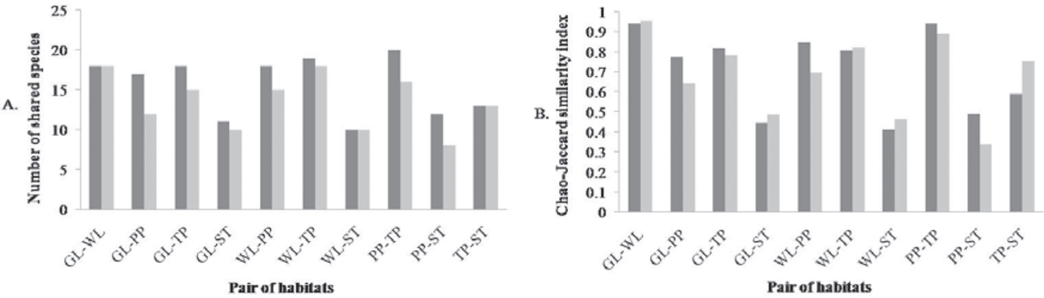

Figure 3. Comparison of shared anuran species numbers (A) and Chao-Jaccard similarity indix values (B) among the different habitat types paired from Phufoilom (dark gray) and Phuhinlat (light gray). Abbreviations in line axis paired are habitat types: GL = Grassland, WL = Woodland, PP = Permanent Pond, PP = Temporary Pond, and ST = Stream.

4. CONCLUSIONS

Highly similary for species richness and composition were noted within the two area fragments, indicating that amphibian fauna resources may exhibit such similarities with respect to protected (Phufoilom) and non-protected areas (Phuhinlat) except some species with special ecological requirement, were not found in Phuhinlat. The different numbers of individuals captured and similar proportions of relative abundance among species within the two area fragments are the characteristic of a habitat maintaining appropriate size and proportions of populations among species in an assemblage. The differences in total numbers of captured individuals suggest that most anuran amphibian species in this protected area has larger population size than those in the non-protected area. This finding emphasizes the value of protected area which is a contributing factor for maintaining effective amphibian population sizes [2,8, 13,16].

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