The Egyptian Journal of Hospital Medicine (Jan. 2017) Vol. 66, Page 66- 73

Amphibians as Bioindicators of the Health of Some Wetlands in Ethiopia

Samy Saber[1], Wondwosen Tito[2], Rashad Said[3] , Seyoum Mengistou[2] and Abdulaziz Alqahtani[4]

[1]Department of Zoology, Faculty of Science, Al-Azhar University, Cairo, Egypt
[2]College of Natural Sciences, Addis Ababa University, Ethiopia
[3]Department of Zoology, Faculty of Science, Al-Azhar University, Assiut, Egypt
[4]Department of Biology, Faculty of Science and arts, University of Bisha, Bisha, SaudiArabia
Corresponding author: Samy Saber, Prof. of fauna and animal ecology, Department of Zoology, Faculty of Science, Al-Azhar University, Cairo, Egypt.samy_nn@yahoo.com 00201006995630

ABSTRACT

Background: Ethiopia has a variety of wetlands which provide enormous socio-economic and environmental values. Most of these wetlands are under severe pressure and degradation. Maintaining the health of wetlands is recognized as crucial for protecting biodiversity, ecosystem function, and human health. In order to properly protect and conserve wetlands, it is essential to collect baseline data and establish monitoring programs which can detect change in the health of the wetland over time. One common method for monitoring includes using bioindicators as amphibians.

Materials and Methods: the current study was conducted to assess the health in two areas in Ethiopia, Abijata - Shalla Lakes and Awash National Parks using amphibians as bio-indicators and to predict any changes on the natural environments and resources in the study area.

Results: in Abijata area, less species richness, lower total population index and higher fluctuating asymmetry were observed compared to the second area (Hot spring area) which means that it is more threatened.

Keywords: Diversity, Developmental instability, Malformation, Abijata-Shalla, Awash.

INTRODUCTION

Wetlands not only benefit people by supporting a host of ecological and hydrological functions (e.g. water supply, water purification, flood control), but that they are also critical for the conservation of biological diversity[1]. In spite of their great importance, wetlands continue to decline globally, both in area and in quality[2], he provided the most recent and comprehensive picture of historical wetland losses. In his study of 189 wetland assessments, he estimated that wetland losses in the 20th century were 64-71%, and for some regions, notably Asia, even higher [2]. Ethiopia owns different types of wetlands which provide enormous socioeconomic and environmental values[3]. They are vital in attracting tourists and providing ground for cultural ceremonies[4]. Despite all those and other values, Ethiopian wetlands are under severe pressure and degradation. The health of the wetlands is continuously decreasing from time to time that in doubt their existence in the near future [5,6]. Maintaining the health of wetlands is recognized as crucial for protecting biodiversity, ecosystem function, and human health. In order to properly protect and conserve wetlands, it is essential to collect baseline data and establish monitoring programs which can detect change in the health of the wetland over time. One common method for monitoring includes using bioindicators as proxies (e.g. Amphibians). Ecological indicators can have many purposes, including being used to assess the condition of the environment or monitor trends in condition over time [7,8]. Amphibians are widely considered to be useful as indicator species [9,10,11,12].

Globally; many amphibian populations and species have decreased or disappeared in the last few decades [13]. Environmental impact was suggested to be one of the causative factors of amphibian declines [14]. Anurans, frogs and toads are probably one of the most sensitive animals in the world and react very rapidly to substantial changes in their environments. They also form vital parts of the ecosystems they belong to. That is why they are considered “an indicator species” the species that “indicate” the state of the health of their ecosystems.

On the other hand, Fluctuating asymmetry FA is a measure of the degree to which structures that are bilaterally symmetrical depart from perfect symmetry [15]. A failure of developmental regulation was suggested to increase with levels of environmental stress. Fluctuating asymmetry has been put forward as a biomarker of stress [1, 12,16,17,18].

Received: 03/11/2016
Accepted: 13/11/2016
DOI : 10.12816/0034635

The protection of amphibian's species is not an issue that can yet be meaningfully addressed in Ethiopia [19]. Most of National Parks including Abijata–Shalla Lakes and Awash National Parks are among important wetlands, they are threatened either by anthropogenic or by climate effects but in different degrees. Abijata has been heavily impacted by human activities; the level of Abijata has dropped by about 5 m over three decades [20]. The relatively shallow depth of Lake Abijata and its terminal position make it more susceptible to changes in climate and input from precipitation and river discharge. According to[21], human activities have affected the quality of the waters of the lakes.

AIM OF THIS WORK

1- To fill a long standing gap in herpetological study for the selected areas.
2- To generate scientific data required for management and sustainable use of the study areas for development.
3- To determine amphibians species diversity in the two study areas.
4- To assess the health of the study area using amphibians as bio-indicators.

MATERIALS AND METHODS

Study Area

The current study was carried out in two Ethiopian natural parks.

1- Abijata-Shalla Lakes National Park (Abijata), 07°40'N, 38°34'E and
2- Awash National Park (The Hot spring), located at 09°06.420'N, 40°00.829'E.

The areas were chosen for this study because both have been anthropogenic threatened but in different rate. The sites of study in both areas have been selected to be of almost of the same habitat type. That of Abijata-Shalla Lakes National Park located near the Lake Abijata and the lake shore, including the place where the two main tributaries of Abijata (Bulbula and Horekello) rivers meet which is of shallow water with grasses.

The hot spring is dominated by grasses and swampy soil in between streams and ponds.

Sampling

A total number of 178 and 324 anuran individuals were collected by hand from Abijatta and the Hot spring, respectively.

Frogs were collected from the study sites using time- constrained Visual Encounter Survey (VES).Visual Encounter Survey (VES) is time honored field technique. It was formalized by [22,23]. VES is conducted by observer walking through a designated area for a prescribed time, visually searching for animals. The number of animals encountered are noted along with time elapsed during the survey.

To be effectives, VES should be conducted when the target species were most likely to be encountered and most active at the surface. The frogs collected were most active at night, so VES was conducted at night using a head lamp to illuminate the search area.

VES can be used along transects, streams, ponds, quadrate or larger area. There are three standard sampling designs for the VES; Randomized walk, transects and quadrate design. In this study, because the area was large, randomized walk was used as a standard sampling design .In addition, randomized walk doesn't need any equipment and can be performed with only one person, walking carefully along the search area, visually searching for terrestrial, emergent, and aquatic surfaces for exposed frogs. All potential breeding habitats of frogs located in the study sites are adequately surveyed for in approximately 2 hours (2 hours per surveyor) each night. Starting time, ending time and total searching time were recorded for all VES. All frogs observed with in the search area during the search time were collected. The sampling was done though 6months from July to December 2009. Sampling in the two studied sites was done in the same week of each month and at the same time.

Frogs Preservation

After collection frogs were immersed into MS22 .MS22 makes frogs to be anesthetized and sleeps without hurting. Then they were transferred in 10% formalin for permanent preservation and stored in laboratory. Specimens were preserved in such a way as to make them least subject to any kind of deterioration. Tag was given for each individual of frog. Labels attached to specimens preserved in formalin were corrosion proof. Any writing attached to specimens was resistant to fading out or washing out.

Species Identification

Species were identified using standard identification keys given in[19].

Species diversity

Species diversity indices were computed for amphibian species collected and identified from each study area. For comparison, both Shannon weaver Index (H') [24] and the inverse of Simpson's index (D) were computed [25].

Total population index

Total population index was calculated by dividing the total population of frogs collected from each site by the total time taken to collect the frogs from each site. The population index of each site is then compared with the environmental factors, mean pH and mean temperature.

$$\text{Total Population Index (TPI)} = \frac{\text{Total population collected}}{\text{Total time for collection}}$$

DI Measurement

To get accurate asymmetry measurements, a random replicates of 178 animals from each site were tested. Measurements were carried out as recommended by[26]. The forelimb measurements were made from the 'elbow' to the palm of the flexed 'hand' and the hind limb measurements were made from the knee to the ankle. The fore limb and hind limbs of both the left and right sides were measured. General distribution for right minus left hind limb and forelimb were done separately.

Fluctuating asymmetry was calculated according to[12,27] as:

$$FA = \frac{(R-L)}{\text{Size}}$$

Where: **R** = length of the right limb and **L** = length of left limb,

$$\text{Size} = \frac{(R+L)}{2}$$

Water pH and temperature measurement

Water pH and temperature were measured in the field to determine water quality relative to amphibians using pH -013 high accuracy portable pH meter (since pH is dependent on temperature the pH meter also measures the temperature). In order to eliminate the measurement error arising from the individual characteristic of the sensor, calibrations of the device was carried out prior to measuring the level of pH on site. *The study was approved by the Ethics Board of Al-Azhar University.*

Statistical analysis

To test the present data, computer program SPSS version 16 was applied to produce plots of the test for differences in the levels of fluctuating asymmetry (developmental instability) between Abijata and the Hot spring. The ANOVAS were used to test the difference in levels of signed asymmetry (developmental instability) that exist in between frog populations. To perform the analyses of DI, DI measurement data were laid out as illustrated in appendix 2 and signed asymmetry (right minus left) of fore - and hind limb mean were used to construct the tests of significant difference. The SPSS program processed the ANOVA table automatically.

RESULTS

Species diversity

Within the search effort of 80 man hours (40 man hours for each site), 178 individuals of frogs were collected from lake Abijata belonging to 3 species (species richness) and 324 individuals were collected from the hot spring to 5 species (species richness). *Sclerophrys xeros* was common in the two study sites and a single individual was found from Abijata which was *Tomopterna cryptotis* species (Table 1).

The comparison of amphibian species diversity between the two study sites (Table 2) showed that the Hot spring had more species diversity (H'=1.475, D=0.375) than Abijata (H'=0.807, D=0.187). The results of equitability also showed higher for the Hot spring (J=0.635, V=0.50) than Abijata (J=0.509, V=0.51).

Developmental Instability

The results of the tests of the hypothesis state that signed asymmetry (right–left) fore and hind limb length between the Hot spring and Abijata were significantly different. One-way ANOVA revealed that variation of signed forelimb asymmetry between the two sites were significant ($F=4.527$, $P \leq 0.034$) tested at a significance level of $\alpha=0.05$. Signed hind limb asymmetry variation between the two sites were also significant ($F=4.989$, $P \leq 0.026$) tested at a significance level of $\alpha=0.05$ (Tables 3 and 4 and Figs. 1 and 2).

Measurements of pH and temperature were included as an environmental factor (Table 5). It was found that in Abijata, mean water pH and temperatures were 9.46 and 28.98^{0}C respectively and the total population index was (4.45) and in the Hot spring means water pH and temperatures were 8.08 and 24.4^{0}C respectively and the total population index was (8.1).

Malformation

Two malformed frogs were totally documented, one frog from each study area. Both frogs malformed on their left front limbs. The malformed frog from the Hot spring belongs to *Ptychadina anchietae* and the other one from Abijata belongs to *Sclerophrys regularis.*

DISCUSSION

Maintaining wetland integrity is essential to protect biodiversity, including amphibian species, and the health of human and environment [28]. In order to protect these systems, baseline data and integrated monitoring plans are important to assess existing conditions and observe how these conditions change over [29]. The present study providing an initial observation of two Ethiopian natural parks**,** Abijata-Shalla Lakes National Park (Abijata), and Awash National Park (The Hot spring) using amphibians as bioindicators of the health of the environment.

The data obtained from this study indicate that Hot spring of Awash National Park has more species richness and diversity than Abijata-Shalla Lakes National Park (Abijata). According to[30], mature communities of stable environments typically show high species diversity, and those of disturbed or stressed situations would be less diverse which meets our findings.

The frog community of Abijata also showed fewer populations than the Hot spring. Higher mean water pH (9.46) and temperature (28.98^{o}C.) were recorded from Abijata where the total population index was low (4.45) and in the Hot spring where lower mean water pH (8.08) and temperature (24.4^{o}C.)recorded, the total population index was high (8.1). Elevated pH, water temperature and un-ionized ammonia) may be associated with frog embryo mortality or malformations [31]. Water temperature strongly influences what can live in it. There is also a relationship between temperature and the amount of oxygen that can be dissolved in it. The lower the temperature, the more oxygen can dissolve. Conversely, at higher temperatures, less oxygen can be dissolved.

Abijata is under serious threats due to water abstraction for Soda Ash enterprise, increased irrigation, expansion of settlement, cultivated land and grazing land, deforestation and over grazing [32,33]. As a result, the aquatic habitat has confronted problem of water resource exploitation and decline of lake water capacity. According to**[33]**, the lake lost 46% of its area between 2000 and 2006. In addition, the water level of the lake is dropping [32]. If current trends in natural and socio economic conditions continue, Lake Abijata is likely to disappear by 2021 [34]. Similarly, the terrestrial habitats faced degradation and fragmentation due to major agricultural activities which compete with wildlife for food, cover and space. Subsequently, the biodiversity of both terrestrial and aquatic habitats are under great challenge [35]. Awash National Park is currently facing also major threats because of the growing pressure of the local communities in search of resources. Policy unfairness was identified as the main threatening cause of the park resources. The impact of expansion of private and state farms was also reported as another impact [36].

It is generally acknowledged that levels of fluctuating asymmetry, the final form of asymmetry, provide a good index of levels of developmental instability within populations and the levels of DI increased as stress increases or health decreases in many species [27,37]. Several studies have shown that DI levels increase before or during population declines [26]. Frogs from Abijata showed higher signed asymmetry level than those from the Hot spring (Figs. 1 and 2).

If ideal (perfectly symmetrical) phenotype exist in population of frogs, the population mode and mean would be perfect symmetry of bilateral structures, with left- right fore- and hind limb differences normally (or at least unimodally and symmetrically, distributed with a mean of zero[27]. The frequency distribution of signed asymmetry forelimb in Abijata was less perfectly symmetrical compared to the frequency distribution of signed asymmetry forelimb shown by frogs from the Hot spring (Fig. 3 and 4).

The higher fluctuating asymmetry, the less species diversity and the less population index in Abijata suggests that environmental stress level experienced by the frog populations were much higher than the frog population of the Hot spring. Because amphibians are important predators and prey in many ecosystems, declines in their populations may affect many other species that live within the same ecological community. For example, populations of aquatic insect and amphibians predators such as snakes, birds, mammals, and fish may be especially affected by a loss in amphibians. Moreover, the populations of animals that amphibians feed on, such as mosquitoes, may increase as the amphibian population decrease which may have a negative effect on human health. Water quality degradation has been linked to severe physical malformations (including missing, malformed, and extra limbs) reported in dozens of amphibian species from diverse aquatic habitats [38]. On the other hand, fluctuating asymmetry was found to correlate positively with the degree of impact on natural habitat at which *Sclerophrys regularis* thrive [10, 12].

Generally, as can be understood from different studies most of the environmental problems of Abijata are anthropogenic in nature.

CONCLUSION

Evidence of higher DI, less species diversity and less total population index were observed in Abijata compared to the Hot spring. These suggests that stress increases on the natural frog populations of Abijata which is another example of an environmental sentinel providing a warning of environmental deterioration in Abijata and the frog populations are also at risk of decline. The total population index of *Sclerophrys xeros* was higher in Abijata, this suggests that higher temperature and elevated pH were suitable environmental factors for *Sclerophrys xeros.* Two frogs, one frog from each study area showed malformation on their left front limbs. The malformed frog from the hot spring belongs to *Ptychadina anchietae* and the other one from Abijata belongs to *Sclerophrys regularis.* This malformation might be linked with water quality degradation, higher pH, in both study areas. Higher environmental stress level on frogs dwelling in Abijata reflects the need for close monitoring of amphibians of the study area and determination of the source of the increased stress on amphibians of the study area. Therefore, the reduced health of frogs and malformation in Abijata and the hot spring in general may be caused because of the environmental problems of the area that threaten amphibian communities.

ACKNOWLEDGMENTS

We gratefully acknowledge the managers and workers of Abijata-Shalla Lakes and Awash National Parks for allowing and assisting the work freely in the parks, Horn of Africa and the Zoological Museum of Addis Ababa University for providing research fund.

REFERENCES

1- Ramsar COP7 (1999): 7th Meeting of the Conference of the Contracting Parties to the Convention on Wetlands (Ramsar, Iran, 1971).

2 -Davidson NC (2014): How much wetland has the world lost? Long-term and recent trends in global wetland area. Mar. Freshwater Res.**,** 65 (10): 934-941.

3- Gebresllassie H, Gashaw T, Mehari A (2014): Wetland Degradation in Ethiopia: Causes, Consequences and Remedies. J Environ Earth Sci., 4(11): 40-48.

4- Finlayson M, Moser M (1991): Wetlands' international waterfowl and wetlands research bureau. Facts on File Ltd. Oxford, UK 224pp.

5- Zerihun D (2003): Challenges and opportunities of Ethiopian wetlands: the case of Lake Awassa and its feeders, Debub University, Awassa College, Awassa, Ethiopia.

6- Yilma D (2003): Wetlands of Ethiopia: proceedings of a seminar on the resources and status of Ethiopia's wetlands, edited by Abebe, Y. D. and Geheb, K, IUCN.

7- Cairns J, McCormick PV, Niederlehner BR (1993): A proposed framework for developing indicators of ecosystem health. Hydrobiologia, 236: 1–44.

8- Dale VH, Beyeler SC (2001): Challenges in the development and use of ecological indicators. Ecol Indic., 1: 3-10.

9- Sheridan CD, Olson DH (2003): Amphibian assemblages in Zeroorder Basins in the Oregon Coast Range. Can. J. For. Res., 33:1452-1477.

10- Said REM (2013): Using of some amphibians as bioindicator of aquatic pollution of the Egyptian river Nile. PHD thesis, department of zoology Faculty of science-Al-Azhar University- Assiut branch.

11- Said R EM, Saber SAA , Gadel Rab AG, Abouel Fadl KY (2015): Hepatic Alteration of the Egyptian Toad *Amietophrynus Regularis*, as Biomarker to Environmental Deterioration. Int. J. Environ. Monit. Anal., **3:** 22-29.

12- Saber SA, El Salkh BA, Said AS, Said, REM, Gadel-Rab, AG (2016): Limbs asymmetry as biomarker in the Egyptian toad *Amietophrynus regularis* Exposed to Atrazine and Nitrates. Inter. J. Ecotoxicol. Ecobiol., 1(3): 103-110.

13- Houlahan JE, Findlay CS (2000): Quantitative evidence for global amphibian population declines. Nat. **404:** 752–55.

14- Carey C, Bryant J (1995): Possible interrelations among environmental toxicants, amphibian development, and decline of amphibian populations. Environ. Health Perspect., **103 (4):**13-17.

15- Sherman B, Amsalu A, Abebe E (2009): Land Use and Land Cover Changes in Awash National Park, Ethiopia: Impact of Decentralization on the

Use and Management of Resources . Open .J. Ecol**.,** **4:** 950-960.

16- Lauck B (2006): Fluctuating asymmetry of the frog Crinia signifera in response to logging. Wildl. Res., **33:**313-320.

17- Amaral M J, Carretero MA, Bicho R C, Soares A M, Manna R M (2012): The use of a lacertid lizard as a model for reptile ecotoxicology studies - Part 1 Field demographics and morphology. Chemosphere, **87(7):** 757-64.

18- Zhelev ZM, Popgeorgiev GS, Arnaudov A D, Georgieva K N, Mehterov N H (2015): Fluctuating asymmetry in *Pelophylax ridibundus* (amphibia: ranidae) as a response to anthropogenic pollution in south Bulgaria. Arch. Biol. Sci., Belgrade, **67(3):** 1009-1023.

19- Largen M J (2001): Catalogue of the amphibians of Ethiopia, including a key for their identification. Trop. Zool., **14:**307-402.

20- Ayenew T (2007): Water management problems in the Ethiopian rift: Challenges for development. Journal of African Earth Sciences, 48(2-3): 222-236**.**

21- Gebremariam Z (1989): Water Resources and Fisheries Management in the Rift Valley Lakes. SINET: Ethiopian Journal of Science, 12(2): 95-109.

22- Campbell HW, Christman SP (1982): Field techniques for herpetofaunal community analysis. Pp. 193–200. In: Scott, NJ (Jr.) (Ed.), Herpetological communities. Wildlife Research Report (United States Fish and Wildlife Service), 13: 1–239.

23- Corn PS, Bury R B (1990): Sampling methods for terrestrial amphibians and reptiles. Pp. 1–28. In: Carey, AC & Ruggiero, LF (Eds.), Wildlife-habitat relationships: Sampling procedures for Pacific northwest vertebrates. United States Department of Agriculture, Forest Service, General Technical Report, NW-GTR-256.

24- Shannon CE, Weaver W (1949): The Mathematical Theory of Communication. Urbana, IL: University of Illinois Press.

25- Simpson EH (1994): Measurement of diversity. Nat.163:688.

26 Alford RA, Bradfield KS, Richards S (1999): Measuring and analyzing developmental instability as a tool for monitoring frog populations. In: Declines and Disappearances of Australian Frogs, p. 34-43. Campbell, A., Ed., Environment Australia, Canberra.

27- Palmer AR Strobeck C (1986): Fluctuating asymmetry: Measurement, analysis, patterns. Annu. Rev. Ecol. Syst., **17**: 391-421.

28- Moser M, Prentice C, Frazie, S (1996): A global overview of wetland loss and degradation. In: Technical Session B of the Sixth Ramsar Conference of Parties, Brisbane,21–31.URL.http://www.ramsar.org/about/about_wetland_loss.htm [consulted 21 April 2009].

29- Davidson NC, Finlayson CM (2007): Earth Observation for wetland inventory, assessment, and monitoring. Aquatic Conservation: Mar. Freshwater Ecosyst., **17(3):** 219-228.

30- Zand S M (1976): indexes associated with information theory in water quality. J. water. Poll. Contr. Fed., **48:** 2026-2031.

31- Boyer R, Grue CE (1995): The Need for Water Quality Criteria for Frogs. Environ. Health Perspect., 103:352-357.

32- Flower R (2011): Abijata-Shalla Lakes National Park: Assessment of Factors Driving Environmental Change for Management Decision-Making. Report to the Ethiopian wildlife conservation authority'ssustainable development of the protected area system of Ethiopia program 81.

33- Temesgen H, Nyssen, J, Zenebe A Haregeweyn N, Kindu M, Lemenih M Haile M (2013): Ecological succession and land use changes in a lake retreat area (Main Ethiopian Rift Valley). J. Arid. Environ,. **91:** 53-60.

34- Meshesha T, Tsunekawa A, Tsubo M (2012): Continuing land degradation: Cause-effect in Ethiopia's Central Rift Valley. Land Degrad. Develop., **23:** 130–143

35 -Tafesse Kefyalew Estifanos (2008): Integrated Assessment of ecosystem services and stakeholders analysis of Abijata-Shalla Lakes National Park, Ethiopia. M Sc. Thesis in Environmental Sciences, Washington University. April, 2008.

36 -Zerga B (2015): Status of degradation in Awash national park: Its degradation status and protection measures. Palgo J. Agric., **2:** 57 – 66.

37 -Palmer AR, Strobeck C (1992): Fluctuating asymmetry as a measure of developmental stability: Implications of non-normal distributions and power of statistical tests. Acta. Zool. Fenn., **191:** 57-72.

38- Guerra C Aráoz E (2016): Amphibian malformations and body condition across an agricultural landscape of northwest Argentina. Diseases of aquatic organisms, 121(2):105-116

Table 1. Species composition identified from the study areas and their abundance

Site	Species	Number of specimens	Total number of specimens
Abijata	*Sclerophrys xeros*	39	
	Tomopterna cryptotis	1	***178***
	Amietophrynus regularis	138	
Hot spring	*Ptychadina filwoha*	93	
	Duttaphrynus dodsoni	2	
	Sclerophrys xeros	4	***324***
	Sclerophrys blanfordii	40	
	Ptychadina anchietae	185	

Table 2. Amphibian diversity indices

Index	Abijata	Hot Spring
Species richness	3	5
Number of Individuals	178	324
Shannon Index, H’	0.807	1.475
Simpson Index, D	0.187	0.375
Shannon Equitability (J) Index	0.509	0.635
Simpson Equitability (V) Index	0.51	0.50

Table 3. ANOVA table showing variation of signed forelimb asymmetry

Right- left forelimb	Sum of squares	df	Mean square	F	Sig.
Between groups	.028	1	.028	4.527	0.034
Within groups	2.221	354	.006		
Total	2.250	355			

Table 4. ANOVA table showing variation of signed hind limb asymmetry

Right- left forelimb	Sum of squares	df	Mean square	F	Sig.
Between groups	.029	1	.029	4.989	0.026
Within groups	2.041	354	.006		
Total	2.070	355			

Table 5. Water pH, temperature and population index data

Site	Water body	Temp.	pH	**TPI**
Abijata	Stretch of lake shore	26.4	9.30	4.45
	Stretch of lake shore	27.0	9.42	
	Stretch of lake shore	26.0	10.10	
	Stretch of lake shore	26.5	9.03	
Mean		**28.98**	**9.46**	
Hot spring	Pond	22.0	7.20	8.1
	Pond	25.7	8.85	
	Small streams	22.2	8.17	
	Small streams	27.7	8.08	
Mean		**24.4**	**8.08**	

TPI*= Total population index*
Temp. = Temperature

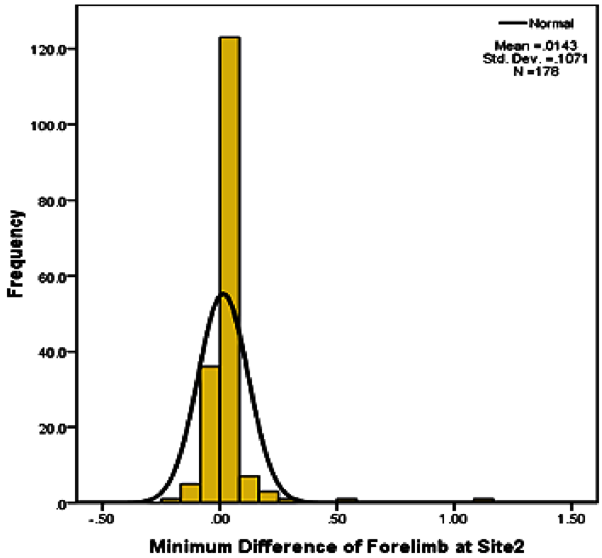

Figure 2. Signed asymmetry (right-left) forelimb frequency distribution in Abijata (site 2)

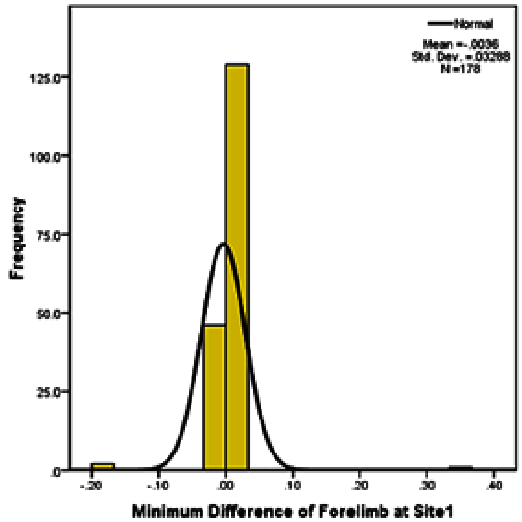

Figure 1. Signed asymmetry (right-left) forelimb frequency distribution in the hot spring (site 1)

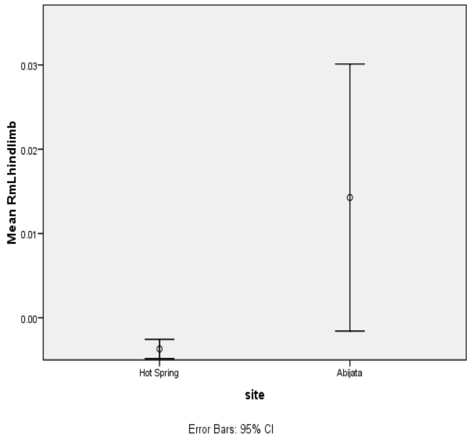

Figure 4. Signed asymmetry of the hind limb, illustrating the increase in asymmetry levels between the hot spring and Abijata.

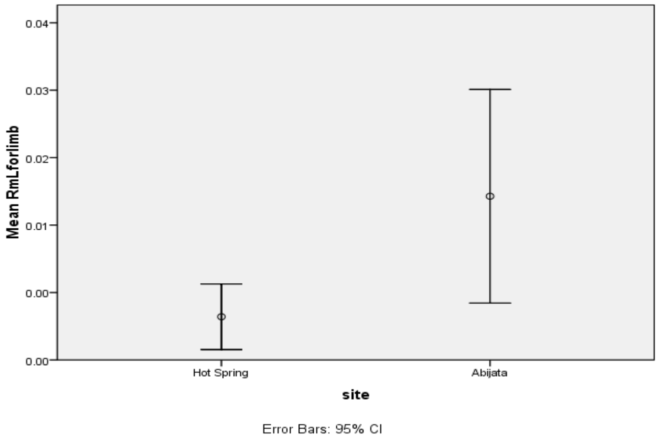

Figure 3. Signed asymmetry of the fore limb, illustrating the increase in asymmetry levels between the hot spring and Abijata