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Some water chemistry parameters of breeding habitats of the Caucasian salamander, *Mertensiella caucasica* in the Western Lesser Caucasus

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Abstract

Selection of breeding habitat plays a fundamental role in the reproductive success of urodeles and anurans. We studied the influence of water chemistry variables on the selection of a specific water resource as breeding habitat in *Mertensiella caucasica*. To determine the influence of water chemistry parameters on their habitat selection, we surveyed a total of 45 small river, streams and brooks in the Western Lesser Caucasus (northeastern Turkey and southwestern Georgia). The water samples taken from these localities were analyzed for 14 chemical variables and the results submitted to multiple logistic regression analysis in order to evaluate the influence of these parameters on the presence or absence of the species in the localities. Of these parameters, chloride concentration influenced the breeding habitat selection of *Mertensiella caucasica* significantly. ***To cite this article: F. Sayım et al., C. R. Biologies 332 (2009).***

Résumé

Sur quelques paramètres des sites de développement de la Salamandre caucasienne *Mertensiella caucasica* dans le Petit Caucase occidental. La sélection du site joue un rôle fondamental pour assurer le succès de la reproduction des Urodèles et des Anoures. La présente étude porte sur l'influence des variables chimiques de l'eau pour le choix d'un milieu aqueux spécifique comme site d'habitat de la salamandre *Mertensiella caucasica*. En vue de déterminer l'influence des paramètres chimiques sur le choix de l'habitat, nous avons examiné un total de 45 petits cours d'eau et ruisseaux du Petit Caucase occidental (nord-est de la Turquie et sud-ouest de la Georgie). Des échantillons d'eau prélevés dans ces régions ont été étudiés pour 14 variables chimiques ensuite soumises à une analyse de régression logistique multiple, en vue d'évaluer l'influence de ces paramètres sur la présence ou l'absence locale de l'espèce. Parmi ces paramètres, c'est la concentration en chlorures qui s'est révélée comme conditionnant significativement la sélection du site de développement de *Mertensiella caucasica*. ***Pour citer cet article : F. Sayım et al., C. R. Biologies 332 (2009).***

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1. Introduction

Decline and extinction of amphibian species have been reported in many areas of the world. It has been suggested that infectious diseases [1–3], parasitic infections [4,5], ultraviolet radiation [6], chemical pollutants [7,8], introduced non-native species [9,10], climate change [11] and the destruction of terrestrial and aquatic habitats [12,13] may all be important factors causing the decline of amphibian species. Many urodeles and anurans are extremely dependent on aquatic habitats because of their physiology and natural history. They deposit their eggs in aquatic habitats and the eggs will develop normally only within certain limits of various environmental factors. Moreover, amphibians have highly permeable skin and their larvae may be susceptible to the water chemistry [14]. Thus, some chemicals, such as nitrates and chlorides in water, possibly act as important variables that influence amphibian distribution, abundance and selection of breeding sites [15,16].

Detailed information about the ecological demands of species, including oviposition or breeding sites, is obligatory for successful conservation of endangered amphibians [17–20]. The Caucasian salamander, *Mertensiella caucasica* is a narrow-ranged species found in the western part of the Lesser Caucasus mountain systems in Georgia and Turkey, and has been categorized as "Vulnerable" by the IUCN [21]. Its red list status stresses the importance of studying the ecological characteristics of this species in order to formulate appropriate management and conservation strategies. In general, salamanders are found only in or near running water such as mountain brooks and streams and, potentially, their distribution can be strongly limited by water quality. Despite the fact that some papers focus on the distribution, taxonomy and ecology of the Caucasian salamander, no detailed investigation about breeding habitat selection has been undertaken [22]. Thus, the aim of this study is two fold; first to determine the characteristics of the water chemistry of breeding habitats and second to determine the relationship between the water chemistry parameters and the species' presence.

2. Material and methods

2.1. Field and laboratory work

The field work was carried out in 2007 and covered throughout the entire distribution range of the Caucasian salamander from Borjomi area in Georgia to Ordu area in Turkey. The field trips covered the second half of June and the first half of July, when it is relatively easy to find both the larvae and adult animals [23].

Water samples were collected from 37 different breeding habitats where both the adult animals and the larvae are found (see Appendix A). Eight sites were sampled as non-breeding habitats where neither the adult animals and nor the larvae were present, although these are occasionally used for breeding by other amphibians found in the region: *Rana ridibunda, Rana macrocnemis, Bufo viridis, Bufo bufo, Hyla arborea*, and *Pelodytes caucasicus, Triturus vittatus, Triturus karelinii* [22,23]. The breeding sites sampled during the field work covered the entire range of the salamander territory, including its westernmost and easternmost parts, and a broad range of ecological conditions: the elevation of individual locations varied between 40 and over 2400 m a.s.l., and the locations were distributed in various landscapes, including mixed and broadleaf forests, areas near and above timberline. Both "presence" and "absence" samples were taken from running water sources (small rivers, streams, or brooks) within the extent of occurrence of the species. Water conductivity, pH, dissolved oxygen (DO) and salinity were measured in the field using a Hach Portable pH/Conductivity/Dissolved Oxygen Meter, and water temperature was measured with a digital thermometer. Some water chemistry parameters such as iron, manganese, chloride, ammonium, sulphate, potassium, nitrate and hardness (calcium and magnesium) were measured in the laboratory using a DR 2800 VIS Spectrophotometer, following the manufacturer's procedures. In addition, total alkalinity was measured using test strips (Aquacheck, Hach Company, USA). For the list of analyzed variables, see Table 1.

2.2. Statistical analysis

For each variable, as a first step, the statistical significance of differences between the "presence" and "ab-

Table 1
Descriptive statistics of water chemistry variables in breeding and non-breeding habitats of *M. caucasica*.

Variables	**Breeding habitats** ($N = 37$)		**Non-breeding habitats** ($N = 8$)		**Statistical significance**	
	Mean	**STDEV**	**Mean**	**STDEV**		
pH*	7.65	0.370	8.25	0.640	$t = 3.62685$	P-value = 0.0007
DO (mg/L)	8.33	0.816	7.98	0.820	$t = -1.11917$	P-value = 0.2690
Conductivity (µS/cm)*	140.42	106.228	310.24	199.521	$t = 3.3418$	P-value = 0.0017
Salinity (%)*	0.06	0.048	0.14	0.098	$t = 3.51587$	P-value = 0.0010
Iron (mg/L)	0.03	0.052	0.01	0.018	$t = -0.96489$	P-value = 0.3400
Manganese (mg/L)	0.46	0.175	0.55	0.151	$t = 1.28599$	P-value = 0.2053
Chloride (mg/L)*	0.35	0.47	2.27	3.186	$t = 4.05335$	P-value = 0.0002
Ammonia (mg/L)*	0.01	0.032	0.41	0.761	$t = 3.42121$	P-value = 0.0013
Alkanity (ppm $CaCO_3$)*	1.21	1.522	2.87	1.642	$t = 3.27325$	P-value = 0.0020
Sulfide (µg/L)*	2.07	2.518	7.25	9.407	$t = 3.01694$	P-value = 0.0042
Potassium (mg/L)*	0.84	0.452	2.02	2.240	$t = 3.08045$	P-value = 0.0035
Nitrate (mg/L)	0.52	0.645	1.56	3.425	$t = 1.79356$	P-value = 0.0797
Hardness Mg (mg/L)	3.07	0.817	3.34	0.743	$t = 0.81313$	P-value = 0.4206
Hardness Ca (mg/L)	0.66	1.017	0.64	1.015	$t = -0.04645$	P-value = 0.9631

Astericks show parameters significantly ($P < 0.05$ after Bonferroni correction applied) differing the sites where salamanders do and do not present.

sence" water source were evaluated by means of Students t test. To determine which particular water chemistry variables help to separate presence or absence locations, we used stepwise multiple logistic regression analysis [24]. We used species presence or absence as the dependent variable and water chemistry variables as independent variables. The analyses were performed with SPSS Ver. 13 for Windows (SPSS Inc., Chicago, IL, USA) with a forward stepwise entry of independent variables.

3. Results

Descriptive statistics of water chemistry variables of breeding and non-breeding habitats of *M. caucasica*, obtained from analyses were given in Table 1.

Although eight of the studied variables showed significant differences between the presence and absence locations, the logistic regression analysis showed the best classification results with only two variables, chloride and O_2 retained in the equation at the step 2 (Table 2). Overall, 88.9% of investigated locations were correctly classified (Table 3). The most important parameter was concentration of chloride in water (Table 2).

4. Discussion

Many factors have responsibilities for declining amphibian populations and it has been shown by a great deal of studies that these factors contribute to their decline alone or synergistically. Of these factors, habitat loss and fragmentation are among the largest threats to amphibian populations. Here we study some aspects of the breeding habitat of Caucasian salamander, which have a crucial part in breeding and survival of their life history.

Table 2
Results of logistic regression analysis for water chemistry variables; variables in the equation.

		B	**S.E.**	**Wald**	**df**	**Sig.**	**Exp(B)**
Step 1 (a)	Chloride	1.519	0.627	5.865	1	**0.015**	4.569
	Constant	−2.692	0.661	16.577	1	0.000	0.068
Step 2 (b)	O_2	−0.891	0.507	3.090	1	0.079	0.410
	Chloride	1.631	0.708	5.306	1	**0.021**	5.107
	Constant	4.467	4.001	1.246	1	0.264	87.100

(a) Variable(s) entered on step 1: Chloride.
(b) Variable(s) entered on step 2: O_2.

Table 3
Classification table of logistic regression analysis for water chemistry variables.

			Predicted		
			Presence		**Percentage correct**
			1	2	
Step 1	Presence	1	37	1	97.4
		2	5	2	28.6
	Overall percentage				86.7
Step 2	Presence	1	37	1	97.4
		2	4	3	42.9
	Overall percentage				88.9

The cut value is ,500.

Ecological features influencing the occurrences of amphibians in certain habitats have been subjected to numerous studies [25–31]. Some characteristics of water including turbidity, pH, density, chlorine, chloramines, nitrate, nitrite, ammonium, heavy metals and also surrounding vegetation cover, permanency and presence of tadpole predators can influence anuran breeding site choice [32–39]. We used 14 water chemistry variables in order to investigate which ecological parameters better explained Caucasian salamander breeding site selection. Mean values of eight parameters were significantly different between presence and absence locations (Table 1). The values of pH, water conductivity, salinity, alkalinity and concentrations of ammonia, sulfide and potassium of the salamander breeding sites were significantly lower than in streams and brooks where the salamanders do not breed. However, according to the results of the logistic regression analysis, concentration of chloride and, perhaps, oxygen consumption are variables sufficient for separating the sites appropriate and inappropriate for Caucasian salamander reproduction (Tables 2 and 3).

Chloride ions play important role in vertebrate physiology. It is a chemical the body needs for energy metabolism. It also helps to keep the body's acid-base balance. The ebb and flow of chloride ions into and out of cells can alter cells electrical properties, influencing nerve function, muscle contraction and a variety of other processes [40]. According to Kaushal et al. [41] chloride concentrations are increasing at a rate that threatens the availability of fresh water in the northeastern United States. Concentrations of chloride in soils as low as 30 mg/L have been found to damage terrestrial plants. Increased chloride concentrations in surface waters could lead to adverse effects on water quality. Increases in salinity up to 1000 mg/L can have lethal and sublethal effects on aquatic plants and invertebrates [42], and chronic concentrations of chloride as low as 250 mg/L have been recognized as harmful to freshwater life and not suitable for human consumption [43,44]. Most urodeles and anurans have a highly permeable skin, largely unprotected aquatic eggs and aquatic larvae. Their adults use wetlands for foraging, hibernation and breeding. Because of these peculiar characteristics, they are considered as excellent indicators of ecosystem health. In our study, among the investigated water chemistry parameters affecting the presence of Caucasian salamander, chloride concentration was shown to be the most important one. The threshold level of chloride in salamander habitats (range = 0–2.0 mg/L) appears to be lower than in other amphibians of the region that breed, usually or occasionally, in running waters and presence of these animals could be used as a perfect biological indicator of water quality.

According to Cushman [45], in most part of the world, there is very limited knowledge of the species–environment relationships of amphibians, their responses to habitat loss and fragmentation and the factors controlling population connectivity. There are many different studies suggesting the possible ways to protect and conserve amphibian populations, however, the best way to start preserving amphibian populations could be to identify important amphibian habitat characteristics and to prevent human-caused modification of these variables in the first place.

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Appendix A

The sampled breeding and non-breeding sites of *M. caucasica* in Turkey (TR) and Georgia (GEO) are listed.

Breeding sites: Kovanlık-Giresun (TR), Karapınar (two sampling sites)-Giresun (TR), Bayındır (two sampling sites)-Giresun (TR), Turnalık (three sampling sites)-Ordu (TR), Çambaşı-Ordu (TR), Sümela-Trabzon (TR), Coşandere (two sampling sites)-Trabzon (TR), Kiraz Yaylası-Trabzon (TR), Uzungöl (three sampling sites)-Trabzon (TR), Demirkapı (three sampling sites)-Trabzon (TR), Ayder (three sampling sites)-Rize (TR), Kavrun-Rize (TR), Çamlıhemşin-Rize (TR), Ülküköy-Rize (TR), Fındıklı-Rize (TR), Çarnali-Batumi (GEO), Batumi Botanical Garden-Batumi (GEO), Goderdzi Pass (four sampling sites)-Akhaltsikhe (GEO), Atskuri-Akhaltsikhe (GEO), Kekia-Borjomi (GEO), Savaniskhevi-Borjomi (GEO), Chitakhevi-Borjomi (GEO), Abastumani-Akhaltsikhe (GEO).

Non-breeding sites: Karagöl (two sampling sites)-Artvin (TR), Goderdzi Pass-Akhaltsikhe (GEO), Abastumani-Akhaltsikhe (GEO), Kekia-Borjomi (GEO), Nedzvi (three sampling sites)-Borjomi (GEO).

References

[1] P. Dazsak, A.A. Cunningham, A.D. Hyatt, Infectious disease and amphibian population declines, Diversity and Distributions 9 (2003) 141–150.

[2] K.R. Lips, Mass mortality and population declines of anurans at an upland site in Western Panama, Conservation Biology 13 (1999) 117–125.

[3] V. Morell, Are pathogens felling frogs? Science 284 (1999) 728–731.

[4] S.K. Sessions, S.B. Ruth, Explanations for naturally occurring supernumerary limbs in amphibians, Journal of Experimental Zoology 254 (1990) 38–47.

[5] P.T.J. Johnson, K.B. Lunde, E.G. Ritchie, A.E. Launer, The effect of trematode infection on amphibian limb development and survivorship, Science 284 (1999) 802–804.

[6] A.R. Blaustein, D.B. Wake, W.P. Sousa, Amphibian declines: Judging stability, persistence, and susceptibility of populations to local and global extinctions, Conservation Biology 8 (1994) 60–71.

[7] J. Bonin, J. DesGrange, M. Rodrigued, M. Ouellet, Anuran species richness in agricultural landscapes of Quebec: foreseeing long-term results of road call surveys, in: D.M. Green (Ed.), Amphibians in Decline: Canadian Studies of a Global Problem, Society for the Study of Amphibians and Reptiles, St. Louis, Missouri, USA, 1997, pp. 141–149.

[8] T. Hayes, K. Haston, M. Tsui, A. Hoang, C. Haeffele, A. Vonk, Herbicides: Feminization of male frogs in the wild, Nature 419 (2002) 895–896.

[9] D.F. Bradford, F. Tabatabai, D.M. Graber, Isolation of remaining populations of the native frog, *Rana muscosa*, by introduced fishes in Sequoia and Kings Canyon National Parks, California, Conservation Biology 7 (1993) 882–888.

[10] L.A. Morgan, W.A. Buttemer, Predation by the non-native fish *Gambusia holbrooki* on small *Litoria aurea* and *L. dentata* tadpoles, Australian Zoologist 30 (2) (1996) 143–149.

[11] J.A. Pounds, M.P.L. Fogden, J.H. Campbell, Biological response to climate change on a tropical mountain, Nature 398 (1999) 611–615.

[12] P.S. Corn, Amphibian declines: review of some current hypotheses, in: D.W. Sparling, G. Linder, C.A. Bishop (Eds.), Ecotoxicology of Amphibians and Reptiles, U.S. Geological Survey, Midwest Science Center, Columbia, MO, 2000, pp. 663–696.

[13] D.M. Green, Amphibians in Decline: Canadian Studies of a Global Problem, Society for the Study of Amphibians and Reptiles, St. Louis, Missouri, USA, 1997.

[14] W.E. Duellman, L. Trueb, Biology of Amphibians, The John Hopkins Press Ltd., London, 1994.

[15] F. Ensabella, S. Loriga, P. Formichetti, R. Isotti, A. Sorace, Breeding site selection of *Bufo viridis* in the city of Rome (Italy), Amphibia-Reptilia 24 (2003) 396–400.

[16] K.L. Griffis-Kyle, Sublethal effects of nitrite on Eastern Tiger Salamander (*Ambystoma tigrinum tigrinum*) and wood frog (*Rana sylvatica*) embryos and larvae: Implications for field populations, Aquatic Ecology 41 (1) (2007) 119–127.

[17] M.B. Araújo, P.H. Wiliam, R.J. Fuller, Dynamics of extinction and the selection of nature reserves, Proc. R. Soc. Biol. Sci. B 269 (2002) 1971–1980.

[18] A. Egea-Serrano, F.J. Olivia-Paterna, M. Tejedo, M. Torralva, Breeding habitat selection of an endangered species in an arid-zone: the case of *Alytes dickhilleni* Arntzen & Garcia-Paris, 1995, Acta Herpetologica 1 (2) (2006) 81–94.

[19] A. Egea-Serrano, F.J. Olivia-Paterna, M. Torralva, Breeding habitat selection of *Salamandra salamandra* in the most arid zone of its European distribution range: application to conservation management, Hidrobiologia 560 (2006) 363–371.

[20] J. Glos, F. Wegner, K.H. Dausmann, K.E. Linsenmair, Oviposition-site selection in an endangered Madagascan frog: Experimental evaluation of a habitat model and its implications for conservation, Biotropica 40 (5) (2008) 646–652.

[21] IUCN 2008, 2008 IUCN Red List of Threatened Species, www.iucnredlist.org.

[22] D.N. Tarkhnishvili, R.K. Gokhelashvili, The amphibians of the Caucasus, Advances in Amphibian Research in the Former Soviet Union 4 (1999).

[23] D.N. Tarkhnishvili, I.A. Serbinova, The ecology of the Caucasian salamander in a local population, Asiatic Herpetol. Res. 5 (1993) 147–165.

[24] D. Hosmer, S. Lemeshow, Applied Logistic Regression, Wiley and Sons, New York, USA, 1989.

[25] S.A. Gagné, L. Fahrig, Effect of landscape context on anuran communities in breeding ponds in the National Capital Region, Canada, Landscape Ecology 22 (2007) 205–215.

[26] K.R. Lips, J.K. Reaser, B.E. Young, R. Ibáñez, Amphibian monitoring in Latin America: a protocol manual, Herpetological Circulars 30 (2001) 1–116.

[27] J. Loman, B. Lardner, Does pond quality limit frogs *Rana arvalis* and *Rana temporaria* in agricultural landscapes? A field experiment, Journal of Applied Ecology 43 (4) (2006) 690–700.

[28] I. Pavignano, C. Giacoma, S. A Castelano, Multivariate analysis of amphibian habitat determinants in North western Italy, Amphibia-Reptilia 11 (1990) 311–324.

[29] J.K. Skei, D. Dolmen, L. Rønning, T.H. Ringsby, Habitat use during the aquatic phase of the newts *Triturus vulgaris* (L.) and *T. cristatus* (Laurenti) in central Norway: proposition for a conservation and monitoring area, Amphibia-Reptilia 27 (3) (2006) 309–324.

[30] C.C. Vos, A.H.P. Stumpel, Comparison of habitat-isolation parameters in relation to fragmented distribution patterns in the tree frog (*Hyla arborea*), Landscape Ecology 11 (1996) 203–214.

[31] E.E. Werner, D.K. Skelly, R.A. Relyea, K.L. Yurewicz, Amphibian species richness across environmental gradients, Oikos 116 (10) (2007) 1697–1712.

[32] M. Evans, C. Yaber, J.M. Hero, Factors influencing choice of breeding site by *Bufo marinus* in its natural habitat, Copeia (1996) 904–912.

[33] C. Gascon, Population- and community-level analyses of species occurrences of central amazonian rainforest tadpoles, Ecology 72 (1991) 1731–1746.

[34] M. Spieler, K.E. Linsenmair, Choice of optimal oviposition sites by *Hoplobatrachus occipitalis* (Anura: Ranidae) in an unpredictable and patchy environment, Oecologia 109 (1997) 184–199.

[35] E.E. Werner, K.S. Glennemeier, Influence of forest canopy cover on the breeding pond distributions of several amphibian species, Copeia (1999) 1–12.

[36] B.S. Cassidy, One frog, two fish, red leg, few fish: Stress in the aquatic animal's ecosystem, Animal Lab News (Jan/Feb 2006).

[37] S.J. Hecnar, Acute and chronic toxicity of ammonium nitrate fertilizer to amphibians from southern Ontario, Environ. Toxicol. Chem. 14 (1995) 2131–2137.

[38] S.J. Hecnar, R.T. M'Closkey, Amphibian species richness and distribution in relation to pond water chemistry in South-western Ontario, Canada, Freshwater Biol. 36 (1996) 7–15.

[39] A. Marco, C. Quilchano, A.R. Blaustein, Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific Northwest, USA, Environ. Toxicol. Chem. 18 (1999) 2836–2839.
[40] L.Y. Jan, Elusive chloride channel coralled in salamander eggs, HHMI Research News (September 19, 2008).
[41] S.S. Kaushal, P.M. Groffman, G.E. Likens, K.T. Belt, W.P. Stack, V.R. Kelly, L.E. Band, G.T. Fisher, Increased salinization of fresh water in the northeastern United States, Proceedings of the National Academy of Sciences USA 102 (2005) 13517–13520.
[42] B.T. Hart, P. Bailey, R. Edwards, K. Hortle, K. James, A. McMahon, C. Meredith, K. Swadling, A review of the salt sensitivity of the Australian freshwater biota, Hydrobiologica 210 (1991) 105–144.
[43] Environment Canada and Health, Priority Substances List Assessment Report for Road Salts, ISBN 0-662-31018-7; Cat. No. En40-215/63E, 2001.
[44] Office of Water, Regulations, and Standards, Criteria and Standards Division, Ambient Water Quality Criteria for Chloride (Environmental Protection Agency, Washington, DC), EPA Pub. No. 440588001, 1988.
[45] S.A. Cushman, Implications of habitat loss and fragmentation for the conservation of pond breeding amphibians: A review and prospectus, Biological Conservation 128 (2006) 231–240.