INTERNATIONAL JOURNAL
OF
CONSERVATION SCIENCE

ISSN: 2067-533X Volume 12, Issue 3, July-September 2021: 1061-1076 www.ijcs.ro

WATER QUALITY OF THE HABITAT OF TWO ENDANGERED HIGH-ANDEAN FROGS USING AQUATIC MACROINVERTEBRATES AS BIOINDICATORS

Manuel SILVA-POMA[1,2,4*], Luis CASTILLO[3,4], Roberto ELIAS[3,5],
Jorge PERALTA-ARGOMEDA[1,6], Ana HUAMANTINCO-ARAUJO[1]

[1] Laboratorio de Invertebrados Acuáticos, Facultad de Ciencias Biológicas, Universidad Nacional Mayor de San Marcos, Lima, Perú.
[2] Programa de Maestría en Biodiversidad y Gestión en Ecosistemas, Facultad de Ciencias Biológicas, Universidad Nacional Mayor de San Marcos, Lima, Perú.
[3] Denver Zoological Foundation, Denver, Colorado, USA
[4] Asociación GRUPO RANA, Lima, Perú.
[5] Facultad de Medicina Veterinaria y Zootecnia, Universidad Peruana Cayetano Heredia, Lima, Perú.
[6] Departamento de Entomología, Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Perú.

Abstract

Telmatobius macrostomus and Telmatobius brachydactylus are two high Andean aquatic frogs categorized as "Endangered" by the Red List of the International Union for Conservation of Nature and by the Peruvian Legislation. With the aim of evaluating the water quality of their habitats, four bimonthly samplings in the field were carried out at 12 study sites located in the Junin and Pasco regions of Peru. Physicochemical, hydrological and microbiological variables were measured. Samples of aquatic macroinvertebrates were taken and the following water quality indices were applied: Andean Biotic Index (ABI) and Family Biotic Index (FBI). The results show that the environmental variables total dissolved solids (TDS) and total coliforms exceed the Environmental Quality Standards (ECA) for water according to Peruvian regulations. The aquatic macroinvertebrate community showed differences in its community structure between sampling stations and indicated moderate to good water quality according to the applied indices, except in the stations that would be being impacted by cattle farming and chuño production (ancestral activity whereby freeze-dried potatoes are fermented in cold, running stream water), both activities impact the water quality of the studied habitats and could influence the presence of frogs, because both species generally inhabit clean aquatic ecosystems.

***Keywords**: Biomonitoring; Bioindicator; Aquatic macroinvertebrates; Contamination; Telmatobius; Frogs; Central Andes*

Introduction

High Andean aquatic ecosystems have a great importance due to the physical, chemical and biological processes that take place in them, and the ecosystem services they provide, the most important are the availability of drinking water, protection against disturbances, irrigation and energy production (hydroelectric [1]). The high Andean rivers are particularly important because they have unique fauna adapted to extreme environmental conditions with fluctuations in temperature and fluctuations in oxygen concentration and saturation [2]. These ecosystems continue to be impacted mainly due to human activities, including livestock farming, mining,

---

[*] Corresponding author: msilvap2592@gmail.com

wastewater discharge, and others. These activities affect the biodiversity that inhabits the rivers and streams, as is the case of the high Andean aquatic species *Telmatobius macrostomus* [3] “Lake Junin giant frog” and *Telmatobius brachydactylus* [3] “Wanchas de Junin”, which are categorized as “Endangered” (EN) by the Red List of the International Union for the Conservation of Nature [4, 5] and by the Peruvian Legislation [6]. Both species belong to one of the genera with the highest number of threatened or near threatened species [7]. *Angulo* [8] considers that the main causes for the decrease of their populations are the reduction of their habitat range, hunting for human consumption, introduction of exotic species, deterioration of the quality of their habitats, among others. For this reason, it is important to know more about the condition of their habitats and generate good water quality information for adequate management of both species.

Telmatobius macrostomus fills an important role in the food chain, adult individuals feed on fish and macroinvertebrates [9, 10], with *Telmatobius brachydactylus* there is no information on the adult diet, however both species share their habitat with a wide range of diversity of aquatic benthic macroinvertebrates [9, 11] so it is assumed to be similar.

In several countries around the world, water quality monitoring increasingly includes aquatic macroinvertebrates as bioindicators in their methodology and to continue improvements on a global scale [12]. Based on the sensitivity to environmental changes of aquatic macroinvertebrates, various indices have been developed over the years [13], for example, indices such as Anden Biotic Index (ABI), Biological Monitoring Working Party Score (BMWP), Family Biotic Index (FBI) have been adapted for South America and are currently used in water quality assessment [14-16].

The objective of the present study was to evaluate the water quality of the habitats of *Telmatobius macrostomus* and *Telmatobius brachydactylus* and identify possible sources of contamination.

Materials and methods

Study area

The Junin National Reserve, Historic Sanctuary of Chacamarca and National Sanctuary of Huayllay, are protected areas located in the Junin and Pasco. They belong to the National System of Natural Protected Areas by the State (SINANPE) and are located in the Central Andes of Peru. These Protected Natural Areas are sites of great importance due their high species endemism, as is the case of the *Telmatobius macrostomus* and *Telmatobius brachydactylus* species.

The study area corresponds to a plain zone and is surrounded by the eastern and western cordillera of the central Andes. It is categorized as very humid Subalpine Tropical Paramo according to the Holdridge scale [17]. The climate is characterized by being cold and dry year-round, with temperatures that can drop below 0 ° C during winter months.

Based on the historical records on the presence of both species in some bodies of water [9, 18] eigth sampling stations and four additionally control stations were selected, which did not show significant impact. The stations were sampled in the months of August, October, December 2019 and February 2020 (Table 1 and Fig. 1).

Table 1. Location of the sampling stations in the Junin and Pasco regions

Station	Name	Province	S	W	Altitude (msnm)
E1	Purgación	Pasco	10°58'16.1112"	76°19'55.6224"	4167
E2	Control	Pasco	10°50'16.0152"	76°15'57.6227"	4197
E3	Ayac	Junin	11°04'49.6020"	76°09'10.4112"	4140
E4	Chacachimpa	Junin	11°04'50.6424"	76°09'10.3823"	4025
E5	Control	Junin	11°12'19.1844"	75°58'53.4720"	4136
E6	Huarmipuquio	Junin	11°09'05.3316"	76°02'09.6929"	4118
E7	Paccha	Junin	11°08'26.2284"	76°04'25.4664"	4106
E8	Control	Junin	11°08'29.1516"	76°04'27.2901"	4105
E9	Huayricucho	Junin	10°59'23.6292"	76°01'16.2171"	4093
E10	Río amarillo	Junin	10°55'29.1972"	76°05'05.4683"	4079
E11	Control	Junin	10°50'55.6044"	75°59'05.6173"	4346
E12	Uco	Junin	11°04'09.1884"	76°00'12.7396"	4108

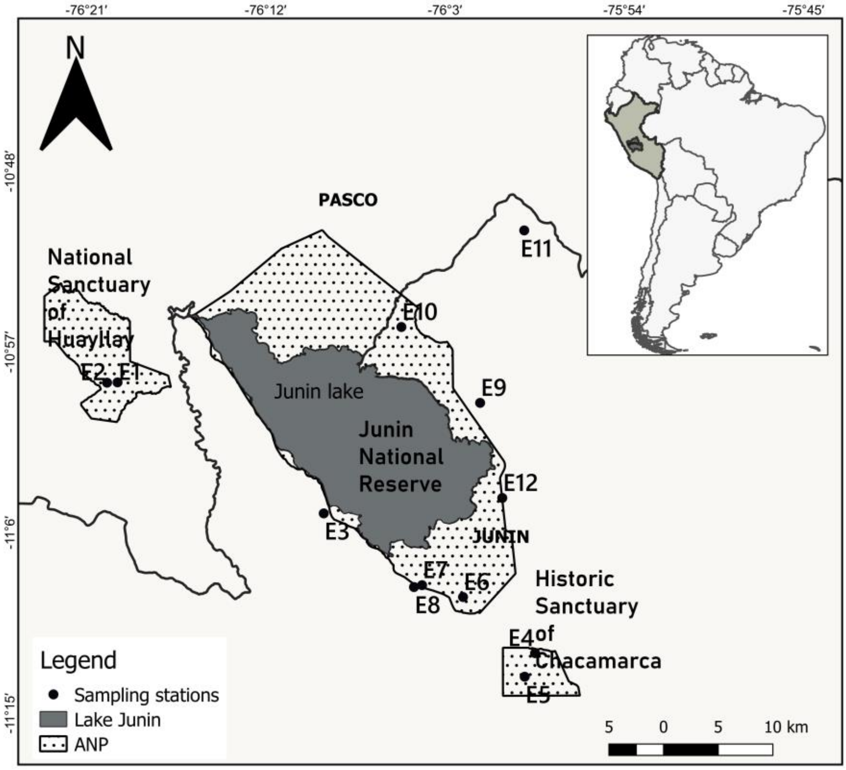

Fig. 1. Location of the sampling stations in the Junin and Pasco regions

Environmental variables

In each field outing, the environmental variables dissolved oxygen (DO), water temperature (°C), electrical conductivity, total dissolved solids (TDS), pH, turbidity, nitrates, phosphates, total coliforms, width, depth and speed were measured. These variables were measured in the field except for total nitrates, phosphates and coliforms; for which water samples were taken and analyzed off site, according to the standards of the ISO/IEC 17025 standard for laboratory tests.

Aquatic macroinvertebrates

The collection of aquatic macroinvertebrates was carried out using a type D net (500µm mesh aperture). In each sampling campaign, three replicas were taken in each sampling stations; the samples were placed in plastic bags and preserved with 96% alcohol and were transferred to the Laboratorio de Invertebrados Acuáticos of the Universidad Nacional Mayor de San Marcos for processing and identification with the help of the keys proposed by *Domínguez & Fernández* [19] and *Prat et al* [20].

Analysis of data

To show differences in the abundance and richness values between sampling stations, the Kruskall-Wallis test was performed and the error bars of the mean were plotted; to show spatial patterns between the sampling stations and the environmental variables, the principal component analysis (PCA) was carried out. All these procedures were carried out with the RStudio [21] software with the ggplot2 and vegan packages. To demonstrate the ordering of the sampling stations based on their community structure, the non-metric multidimensional scaling analysis (NMDS) was performed, prior to that the abundance data were transformed to log (x + 1) and the similarity index of Bray-Curtis [22]. To show differences in the composition of aquatic macroinvertebrates, at the spatial and temporal level, the ANOSIM routine (“analysis of similarities”) was used, starting from the null hypothesis that there are no differences between the composition of the sampling stations. To identify the species responsible for the differentiation of the composition between sampling stations and their contribution, the SIMPER method (“similarity percentages”) was used; all procedures were performed in the PRIMER v6 software [23].

To determine the water quality of the sampling stations, the ABI and FBI indices were applied; to calculate the ABI, the tolerance values of the families present in each station were added [24], the FBI calculation was carried out by adding the products of the tolerance values of each family with their respective abundance [14]. In a complementary manner, the River Habitat Index (IHF) was calculated to evaluate habitat conditions [25] and the main sources of obvious contamination were described.

Results and Discussion

Environmental variables

Regarding the physicochemical variables (Table 2), the water temperature showed values between 9.4 and 18.6°C, the dissolved oxygen presented its minimum value (5.91mg/L) in E11 in August, while the maximum value (7.65mg/L) was recorded at E9 in December. The pH values presented slightly alkaline values and showed values between 7.58 and 9.1. These values are similar to those reported by other studies in areas at a similar altitude [2, 26], with low temperatures, low DO and pH with a tendency to neutrality.

According to peruvian legislation [27], the electrical conductivity did not exceed the Environmental Quality Standards (ECA) of the water and presented notable fluctuations between stations and sampling fieldworks, finding its minimum value (70µS/cm) in E1 in October and its maximum value (830µS/cm) in E4 in February, the TDS showed its minimum (124mg/L) and maximum value (450mg/L) in E4 in August and February respectively, exceeding ECA from water (>100mg/L). Turbidity did not exceed the ECA of the water and showed its minimum value (1.47 NTU) in E8 in August, while its maximum values were registered in February in stations E10 (62.5 NTU) and in E1 (62.6 NTU).

Nitrates and phosphates mostly did not exceed the detection limits for each test (0.044 and 0.006mg/L respectively), registering the maximum value of nitrates in E3 in February (3.69mg/L), phosphates are related with agriculture through the use of fertilizers, however, this parameter mostly presented low values, the maximum being 0.476mg/L indicating average water quality according to *Lavie et al* [28] for phosphates.

Table 2. Physicochemical variables in each month of sampling

Station	Month	T°	OD (mg/L)	pH	Conductivity (µS/cm)	TDS (mg/L)	Turbidity (NTU)	Nitrates (mg/L)	Phosphates (mg/L)
E1	August	10.7	6.02	8.38	102	135	11.21	1.380	0.015
	October	10.1	6.60	7.79	70	200	10.42	1.280	<0.006
	December	11.2	6.26	7.92	80	200	8.00	0.066	<0.006
	February	11.3	6.51	7.58	120	150	62.60	0.088	0.089
E2	August	11.8	6.21	8.25	269	182	12.24	1.150	<0.006
	October	10.4	6.87	7.89	140	200	14.35	1.180	<0.006
	December	13.2	5.96	7.60	200	215	12.10	0.101	<0.006
	February	11.8	6.38	7.71	130	150	39.70	<0.044	0.095
E3	August	13.5	6.32	8.40	657	438	31.7	2.550	0.010
	October	14.8	6.40	8.26	360	180	18.1	1.290	0.108
	December	9.8	6.12	8.41	601	360	23.2	0.124	<0.006
	February	13.4	6.14	8.64	410	220	49.1	3.690	0.381
E4	August	11.7	6.86	8.65	194	124	13.74	0.366	0.017
	October	9.4	6.67	8.00	650	330	29.20	1.710	<0.006
	December	16.7	6.35	8.32	630	350	24.40	0.207	<0.006
	February	11.5	6.6	8.26	830	450	41.30	1.150	0.327
E5	August	10.8	7.15	8.44	432	286	7.11	1.100	<0.006
	October	10.1	6.98	7.95	350	180	28.10	2.000	<0.006
	December	15.5	6.07	7.93	360	200	44.50	0.202	<0.006
	February	10.8	6.3	8.08	370	200	41.50	0.249	0.416
E6	August	14.4	6.90	8.49	641	426	17.54	0.217	<0.006
	October	11.2	6.98	8.57	350	180	39.20	0.050	<0.006
	December	16.4	6.45	8.41	580	320	15.00	<0.044	<0.006
	February	13.3	6.63	8.34	500	280	34.40	<0.044	0.034
E7	August	14.8	6.29	8.70	579	386	5.02	0.689	0.007
	October	16.4	6.62	8.03	380	390	11.65	<0.044	<0.006
	December	12.3	6.51	8.28	420	340	7.65	0.113	<0.006
	February	17.2	6.3	8.52	430	270	43.70	0.129	0.074
E8	August	15.1	6.91	8.60	566	380	1.47	0.678	0.019
	October	17.3	6.35	7.75	380	190	22.00	<0.044	<0.006
	December	13.4	6.18	8.16	412	321	18.00	0.171	<0.006
	February	17.4	6.15	8.35	510	270	36.40	0.338	0.149
E9	August	9.7	6.40	7.85	532	348	14.23	0.092	1.670
	October	13.6	6.42	7.58	400	200	30.00	<0.044	<0.006
	December	18.6	7.65	8.33	460	250	28.00	0.056	<0.006
	February	15.2	6.50	8.22	650	360	35.90	3.230	<0.006
E10	August	13.2	6.50	8.66	548	350	8.34	<0.044	0.026
	October	18.2	6.90	8.56	280	140	9.44	<0.044	<0.006
	December	12.6	6.50	8.57	380	210	5.93	<0.044	<0.006
	February	11.7	6.25	8.41	300	170	62.50	<0.044	0.037
E11	August	13.1	5.91	8.78	626	416	16.93	<0.044	<0.006
	October	15.2	6.44	7.90	260	130	10.42	2.070	<0.006
	December	12.7	6.66	8.34	360	160	8.00	0.044	<0.006
	February	15.6	7.01	8.7	650	360	57.30	<0.044	<0.006
E12	August	14.1	7.03	8.42	641	428	16.31	<0.044	<0.006
	October	18.5	6.47	7.73	400	210	14.35	<0.044	<0.006
	December	13.5	6.98	8.95	440	360	17.02	<0.044	<0.006
	February	17.5	7.13	9.10	650	240	42.50	<0.044	0.476

Regarding the hydrological variables (Table 3), the width presented the lowest values in E2, registering its minimum value in October (0.33m), while the highest values were registered in E12, registering its maximum value in February (12.3m). The depth presented its lowest values in E2, E3 and E10, registering its minimum value in E10 in August (0.13m), while the highest values were registered in E5, registering its maximum value in E5 in February (1.57m).

Table 3. Hydrological and microbiological variables in each month of sampling

Station	Month	Width(m)	Depth (m)	Current velocity (m/s)	Total coliforms (NMP/100 mL)
E1	August	0.64	0.29	0.26	7.8
	October	0.63	0.26	0.37	1300
	December	0.71	0.37	0.30	490
	February	1.09	0.28	1.11	17
E2	August	0.36	0.23	0.30	11
	October	0.33	0.16	1.09	4.5
	December	0.45	0.21	1.21	230
	February	0.50	0.25	1.35	<1.8
E3	August	0.72	0.17	0.23	78
	October	0.74	0.22	0.16	110
	December	0.76	0.33	0.43	45
	February	0.77	0.38	0.83	110
E4	August	4.56	0.45	0.77	230
	October	4.52	0.44	0.47	170
	December	4.56	0.66	0.39	450
	February	5.26	0.79	0.51	93
E5	August	9.30	1.06	0.10	6.8
	October	10.00	1.11	0.11	23
	December	10.31	1.21	0.15	130
	February	10.50	1.57	0.32	93
E6	August	2.26	0.71	0.16	20
	October	2.56	0.73	0.09	11
	December	2.61	0.85	0.15	790
	February	2.85	0.89	0.17	780
E7	August	1.25	0.26	0.49	490
	October	2.80	0.23	0.39	130000
	December	3.03	0.33	0.50	2200
	February	3.60	0.37	0.91	230
E8	August	1.70	0.49	0.08	170
	October	2.09	0.51	0.50	79000
	December	2.51	0.68	0.54	7000
	February	3.28	0.83	0.79	780
E9	August	1.97	0.35	0.37	330
	October	1.54	0.21	0.64	2000
	December	1.99	0.26	0.75	20
	February	1.92	0.27	0.77	23
E10	August	3.15	0.13	0.12	20
	October	3.77	0.20	0.10	200
	December	3.66	0.17	0.09	1300
	February	4.30	0.55	0.50	2100
E11	August	11.93	0.78	0.01	2
	October	10.63	0.70	0.69	78
	December	11.97	0.81	0.66	6.8
	February	12.30	0.95	0.68	230
E12	August	3.95	0.50	0.12	2
	October	6.96	0.60	0.08	20
	December	8.88	0.66	0.50	20
	February	8.41	0.70	0.75	3300

Regarding the current speed, this presented its minimum value (0.01m/s) in E11 in August, while its maximum value (1.35m/s) was found in E2 in February. In general, these values reflect normal trends in which the increase in hydrological variables is evidenced due to increased rainfall [29, 30].

About total coliforms (Table 3), they mostly exceeded the ECA of water, their minimum value (<1.8NMP/100mL) was recorded in E2 in February, while their maximum value (130,000 NMP/100mL) was recorded in E7 in October. These high values, depending on the use of water, could cause damage to human and aquatic organisms [31], because no domestic or industrial wastewater was observed in the areas with the highest concentration of coliforms, the high number could be from organic fecal matter of animal origin since in these areas' livestock is present throughout the year [32].

Aquatic macroinvertebrates

In total, 34192 macroinvertebrates were collected (Table 4), of which 51% correspond to the insect group and 49% to other taxa (not insects). Within the insect group, 5 orders were found, within which the highest number of individual organisms observed were from the order Ephemeroptera with 5971 individuals, while the order with the highest richness was Diptera with 9 families and 18 genera. Within the group of non-insects, the most representative genus was *Hyalella* sp. with 6447 individuals. In general, the predominant families of insects were Baetidae at stations E1, E4, E5, E7 and E8, Elmidae at E6 and E12, Corixidae at E6, E11 and E12, Chironomidae at stations E3 and E10; within the group of non-insects, the predominant ones were Hyalellidae in E2, E4 and E5, Ostracoda in E9 and E10, and Oligochaeta in E9 (Fig. 2). In E9, the predominance of Oligochaeta would be related to the water quality of that season because oligochaetes are organisms with high tolerance to environmental contamination [33, 34]. The high abundance values of *Hyalella* and *Andesiops* in our study (Table 4) agree with reported in rivers of similar altitude in Peru, Ecuador and Bolivia, showing the predominance of these organisms in high altitude areas [29, 35].

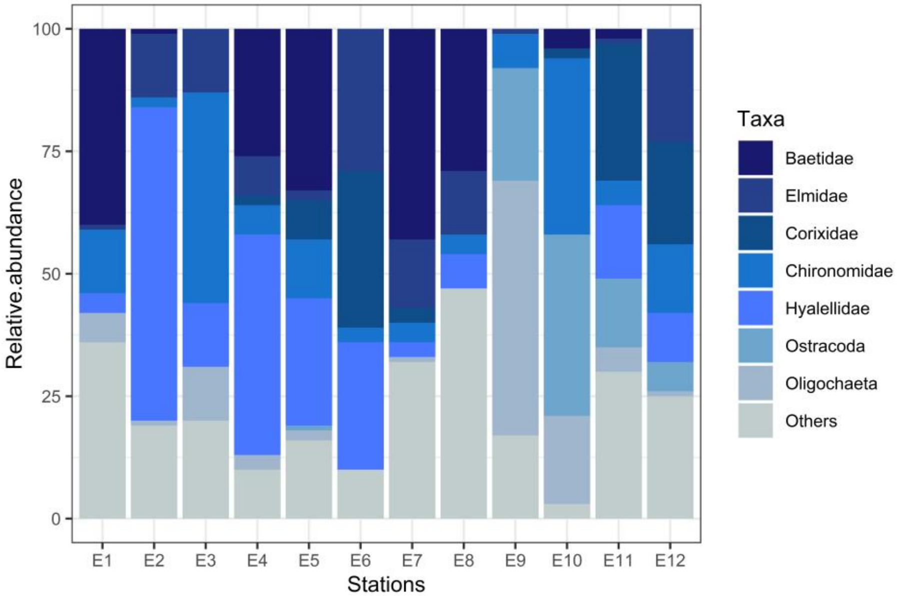

Fig. 2. Relative abundance (%) of each taxonomic group at the sampling stations

Table 4. Total taxonomic composition during all sampling months

Taxa			**Abundance**
EPHEMEROPTERA	Baetidae	*Andesiops* sp.	5951
	Leptophlebiidae	*Meridialaris* sp.	20
PLECOPTERA	Gripopterygidae	*Claudioperla* sp.	48
TRICHOPTERA	Hydroptilidae	*Metrichia* sp.	770
		Oxyethira sp.	167
	Hydrobiosidae	*Atopsyche* sp.	5
		Cailloma sp.	10
	Leptoceridae	*Nectopsyche* sp.	91
	Limnephilidae	*Anomalocosmoecus* sp.	194
COLEOPTERA	Elmidae	*Austrelmis sp.*	3919
	Hydrophilidae	*Tropisternus* sp.	16
	Dytiscidae	*Lancetes* sp.	30
		Hydrophorinae	54
	Scirtidae	Nd	72
HEMIPTERA	Notonectidae	*Notonecta* sp.	1
	Corixidae	*Ectemnostega* sp.	2412
ODONATA	Coenagrionidae	Nd	7
DIPTERA	Limoniidae	Nd	32
	Tabanidae	Nd	1
	Syrphidae	Nd	2
	Ephydridae	Nd	18
	Simuliidae	*Gigantodax* sp.	32
	Empididae	*Neoplasta* sp.	11
		Hemerodromiinae*	14
		Clinocerinae*	57
	Ceratopogonidae	Nd	2
	Chironomidae	*Alotanypus* sp.	137
		Pentaneura sp.	40
		Chironominae*	732
		Podonomus sp.	359
		Parochlus sp.	22
		Podonomopsis sp.	24
		Orthocladinae*	2080
		Corynoneura sp.	103
	Muscidae	Nd	9
OTROS	Nematoda	Nd	63
	Hydrozetidae	Nd	545
	Acari	Nd	71
	Poduromorpha	Nd	38
	Hyalellidae	*Hyalella* sp.	6447
	Ostracoda	Nd	1744
	Sphaeriidae	Nd	36
	Physidae	*Physa* sp.	1824
	Planorbidae	Nd	247
	Oligochaeta	Nd	1901
	Glossiphoniidae	Nd	188
	Tricladida	Nd	3646
	Total		34192

Nd: Taxonomic category not determined
* Taxonomic category of subfamily

Analysis of data

The Kruskall-Wallis tests for "k" independent samples for abundance found significant differences ($p<0.05$) between the sampling stations during all sampling campaigns, the highest abundance values were recorded in August, being the season E8, which presented the highest average abundance (1181), the lowest abundance values were recorded in February, finding its minimum value in the E1 station with 29 individuals (Fig. 3).

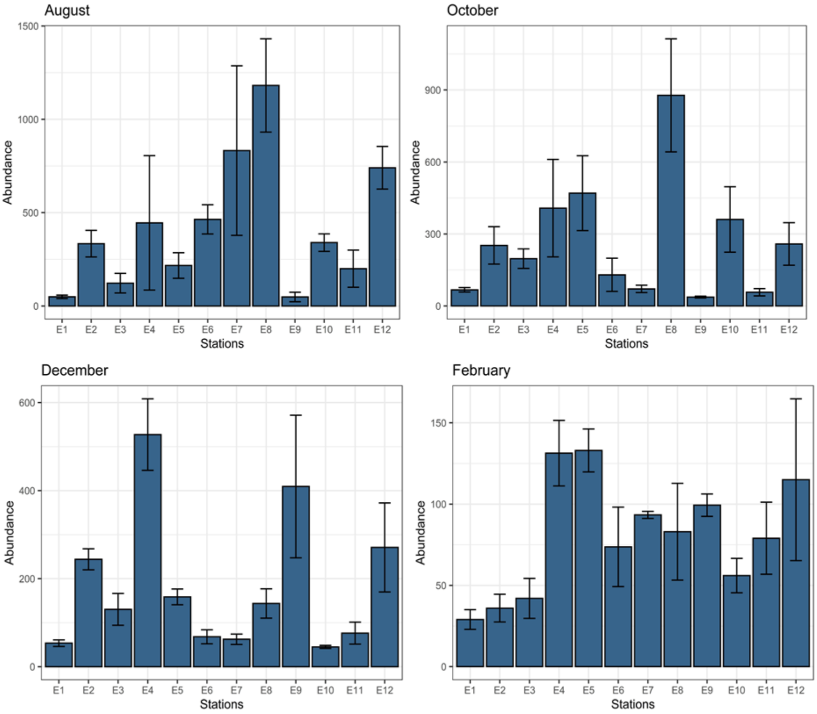

Fig. 3. Error bars of abundance between sampling stations during the sampling months.

The Kruskall-Wallis tests for “k” independent samples for richness found significant differences ($p<0.05$) between the sampling stations during all the sampling campaigns except August ($p = 0.08$), the highest values of Average wealth was observed in August, finding its maximum value in E8 with 18 species, while the lowest values of average wealth were observed during February, finding its minimum value in the E10 season with four species (Fig. 4). The high abundance and richness values reflect the natural tendency of these ecosystems, observing the greatest environmental stability in times of low rainfall, which correspond to August and October [29].

The Principal Component Analysis showed that the first component explained 25.49% of the variance while the second component explained 16.72% of the variance; In general, the graph shows a trend towards grouping by sampling fieldwork (Fig. 5), where the sampling stations corresponding to the February fieldwork are observed, grouped mainly on the basis of hydrological variables reflecting temporality [29, 30], however the two components together explain less than 50% of the accumulated variance, it is recommended for subsequent studies to take into account other variables that allow the model to be improved.

The results of the NMDS (stress of 0.21) showed the grouping of stations E9 and E10, E3 and E1, finally the rest of the stations that were far from these two groups were observed (Fig. 6). The grouping of E1 with E3 would be related to the morphology of these rivers, since both were of similar size, while in the case of E9 and E10 they would be grouped due to their low richness. At the temporal level, the ANOSIM routine did not show significant differences between the sampling months ($R = 0.009$), however, at the spatial level, the ANOSIM routine showed significant differences ($R = 0.738$) between sampling stations, which was complemented by the SIMPER analysis, finding dissimilarities between 34.98% and 79.52%, being the *Andesiops* and *Hyalella* genus that contributed the most to these dissimilarities. The differences in the community structure between sampling stations are mainly due to the great differences between the hydrological variables, substrates, presence of macrophytes, among others.

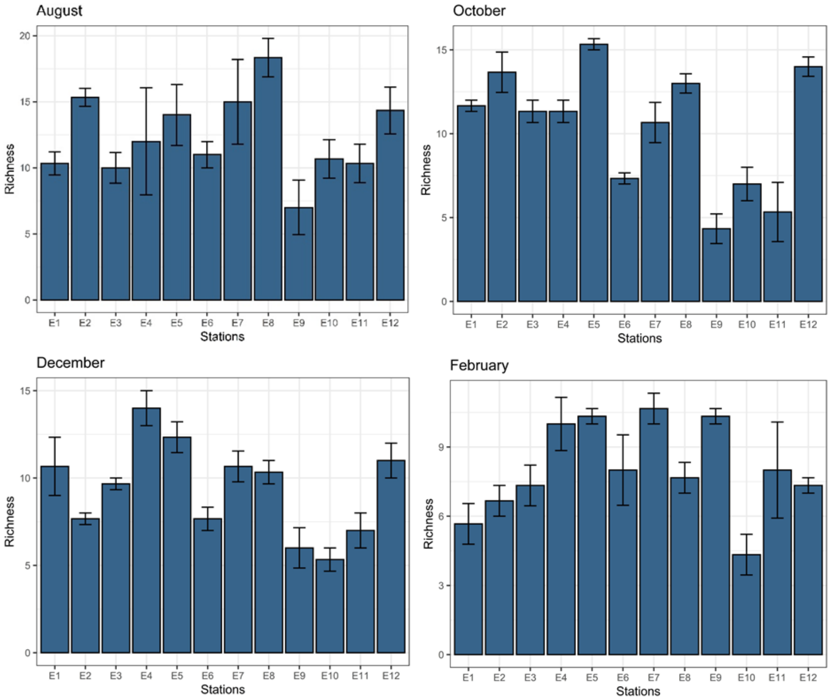

Fig. 4. Error bars of richness between sampling stations during the sampling months.

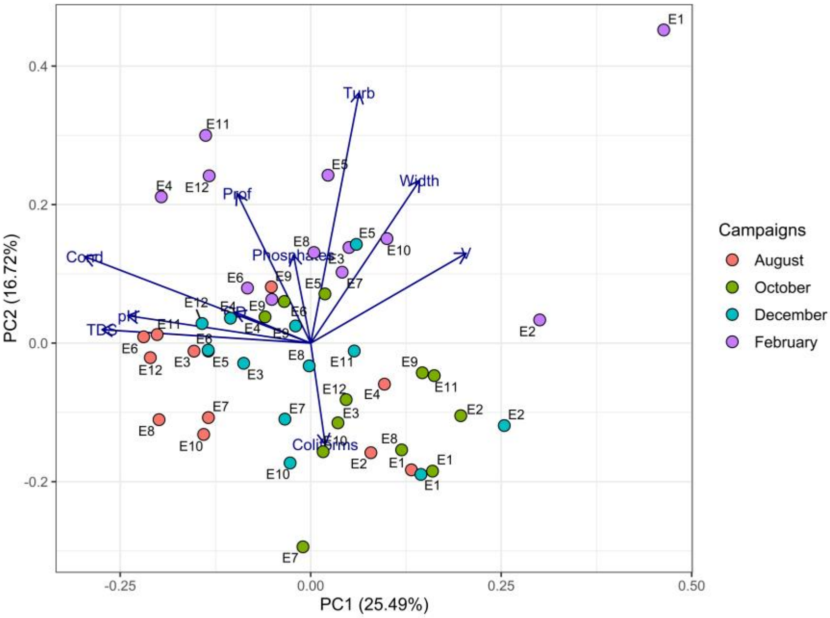

Fig. 5. Principal components analysis and proportion of the cumulative explained variance of the principal components

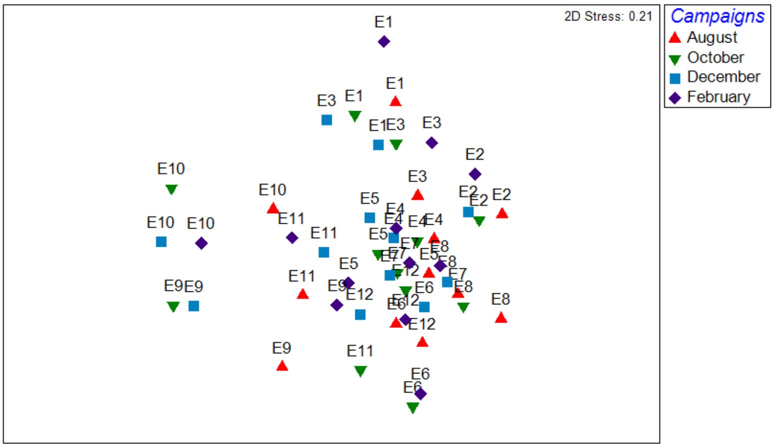

Fig. 6. Non-Metric Multidimensional Scaling Analysis of the sampling stations.

Regarding the quality index, the Andean Biotic Index (ABI) showed low scores for E9 and E10, which corresponds to Bad ecological status, with its minimum score (16) being E10 in February; the rest of the stations showed Moderate to Good ecological status, with the highest scores in E2 (70) and E8 (73), which correspond to two control stations, in the months of October and August, respectively (Table 5).

Table 5. Ecological Status according to ABI in each month of sampling

Estaciones	ABI							
	Score	August	Score	October	Score	December	Score	February
E1	62	Good	54	Good	51	Good	37	Moderate
E2	62	Good	70	Good	42	Moderate	55	Good
E3	47	Good	46	Good	27	Moderate	46	Good
E4	49	Good	50	Good	47	Good	41	Moderate
E5	52	Good	47	Good	47	Good	49	Good
E6	42	Moderate	38	Moderate	37	Moderate	41	Moderate
E7	64	Good	52	Good	51	Good	44	Moderate
E8	73	Good	54	Good	41	Moderate	34	Moderate
E9	31	Moderate	20	Bad	24	Bad	39	Moderate
E10	41	Moderate	19	Bad	26	Bad	16	Bad
E11	45	Good	41	Good	38	Moderate	40	Moderate
E12	64	Good	60	Good	54	Good	32	Moderate

The Families Biotic Index (FBI) showed generally from Harmful to Good quality, the category of Very harmful quality was predominant in the E10 station during all sampling fieldworks, while in E9 it was only presented in October and December, and in E3 only in the month of December; The category of Very good quality was only registered in stations E2 and E11 in December and August, respectively (Table 6).

The River Habitat Index (IHF) shows that the sampling stations are suitable for the development of a diverse benthic community, since they present greater heterogeneity and diversity of substrates, with the exception of station E6, which showed limited capacity; among the sources of impact identified, the most frequent were livestock and the presence of solid waste (Table 7). In general, the FBI and ABI indices showed similarity in terms of their water quality values, especially in stations E9 and E10, which presented the lowest scores, being cataloged with terrible, bad and very harmful quality for each index respectively, these results differ from those reported by *Watson et al* [18] who found on average moderate water quality for *Telmatobius macrostomus* habitats, while we report good and regular quality in most stations. Regarding the controls, these presented good quality except for the months of higher rain, following the natural pattern of these ecosystems due to the seasonality, since the increase in rain decreases the diversity naturally [36].

Table 6. Water quality according to FBI in each month of sampling

Estaciones	FBI							
	Score	August	Score	October	Score	December	Score	February
E1	5.61	Regular	5.5	Regular	5.55	Regular	4.39	Good
E2	4.41	Good	4.48	Good	4.17	Very good	4.7	Good
E3	4.96	Good	6.84	Harmful	7.3	Very harmful	5.56	Regular
E4	5.33	Regular	4.93	Good	5.05	Regular	5.21	Regular
E5	5.13	Regular	6.06	Unhealthy	5.52	Regular	4.93	Good
E6	5.16	Regular	4.35	Good	4.92	Good	4.61	Good
E7	5.49	Regular	5.5	Regular	5.52	Regular	5.33	Regular
E8	5.6	Regular	5.52	Regular	5.59	Regular	5.29	Regular
E9	6.7	Harmful	8.46	Very harmful	8.33	Very harmful	6.83	Harmful
E10	7.66	Very harmful	7.37	Very harmful	8.21	Very harmful	8.47	Very harmful
E11	4.2	Very good	4.69	Buena	5.31	Regular	6.28	Unhealthy
E12	5.75	Regular	5.5	Regular	6.09	Unhealthy	5.71	Regular

Table 7. Scoring for the IHF index and evaluation of the main sources of contamination

Station	IHF	Capacity	Sources of contamination
E1	46	Appropriate	Remains of garbage, laundry.
E2	53	Appropriate	No obvious impact.
E3	53	Appropriate	Trash remains and little development of livestock.
E4	41	Appropriate	Sewage water and little development of livestock.
E5	41	Appropriate	No obvious impact.
E6	37	Limited	Little development of livestock and canal cleaning.
E7	47	Appropriate	Little development of livestock.
E8	43	Appropriate	Little development of livestock.
E9	44	Appropriate	Abundant remains of garbage, high development of livestock and production of chuño.
E10	50	Appropriate	Abundant remains of garbage, high development of livestock.
E11	42	Appropriate	No obvious impact.
E12	46	Appropriate	Remains of garbage and little development of agriculture.

The alteration in the water quality of stations E9 and E10 is mainly due to the effect of livestock, since it was the main activity observed in the sampling stations, it is also known that this activity affects air, water and soil, also altering the quality of riparian habitats [37-40], studies on the effect of grazing also show negative impact on the community of aquatic macroinvertebrates, decreasing their abundance [41], this could also indirectly affect frogs as they feed of aquatic macroinvertebrates. In E9, the production of chuño was also observed as an impact, since during the production process, glycoalkaloids are released into the river, which are toxic substances that protect the tuber against other organisms such as insects [42] and comes from the shell potato [43], some studies show that potato glycoalkaloids and other compounds disrupt sodium ion channels in frog skin and frog embryonic cell membranes, which could lead to embryotoxic effects [44, 45], and could be a cause for the limited presence of Telmatobius macrostomus and Telmatobius brachydactylus.

Conclusions

Our results show that the majority of *Telmatobius macrostomus* and *Telmatobius brachydactylus* habitats present Good to Moderate water quality in their habitats for the applied indices, except for stations E9 and E10 where the water quality was poor, this would be mainly related to livestock, solid waste and chuño production, which are the most important factors for the contamination of their habitats. For this reason, monitoring should continue to involve communities to raise awareness about the care of these ecosystems and the value of clean water for them, their livestock, their farms and the frogs.

Acknowledgements

We thank the Denver Zoological Foundation and the National Geographic Society for the funding granted for the development of this study. To the Junin National Reserve, the Historic Sanctuary of Chacamarca, the Huayllay National Sanctuary, GRUPO RANA, Henry Tinoco and the local herding communities for their collaboration and support during the sampling campaigns. We thank Jhoany Mallqui and Ronald Fernández for their support in sample triage. To the Vice-Rector for Research and Postgraduate VRIP of the Universidad Nacional Mayor de San Marcos for funding for Research Groups: B19100531. We thank Tracy Stetzinger for support in helping with the translation of the manuscript.

References

[1] S. Sabater, A. Elosegi, *Presentación: importancia de los ríos*, **Conceptos y técnicas en ecología fluvial**, Fundacion BBVA, 2009, pp. 15–21.

[2] D. Jacobsen, R. Marín, *Bolivian Altiplano streams with low richness of macroinvertebrates and large diel fluctuations in temperature and dissolved oxygen*, **Aquatic Ecology, 42**(4), 2008, 643–656. https://doi.org/10.1007/s10452-007-9127-x

[3] W. Peters, **Über die von Dr. J. J. v. Tschudi beschriebenen Batrachier aus Peru,** Monatsbericht der königlich preussischen Akademie der Wissenschaften zu Berlin 1873, pp. 622-624.

[4] * * *, IUCN SSC Amphibian Specialist Group, *Telmatobius brachydactylus*, The IUCN Red List of Threatened Species 2018: e.T56329A89204767. http://dx.doi.org/10.2305/IUCN.UK.2018-1.RLTS.T56329A89204767. [Accessed on 25 November 2019].

[5] * * *, IUCN SSC Amphibian Specialist Group, *Telmatobius macrostomus,* The IUCN Red List of Threatened Species 2018: e.T2645A89195689. http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T2645A89195689. [Accssed on 25 November 2019].

[6] * * *, **Libro Rojo de la Fauna Silvestre Amenazada del Perú** (Primera edición), SERFOR, Lima, Perú, 2018, pp. 1- 532.

[7] C. Aguilar, C. Ramírez, D. Rivera, K. Siu-Ting, J. Suarez, C. Torres, *Anfibios andinos del Perú fuera de Áreas Naturales Protegidas: amenazas y estado de conservación*, **Revista Peruana de Biología, 17**(1), 2010, pp. 5–28. https://doi.org/10.15381/rpb.v17i1.46

[8] A. Angulo, *Conservation needs of batrachophrynus and telmatobius frogs of the andes of Peru*, **Conservation and Society, 6**(4), 2010, pp. 328–333. https://doi.org/10.4103/0972-4923.49196

[9] A.S. Watson, A.L. Fitzgerald, O.J.D. Baldeón, *Diet composition and prey selection of Telmatobius macrostomus, the Junín giant frog*, **Endangered Species Research, 32**(1), 2017, pp.117–121. https://doi.org/10.3354/esr00785

[10] H. De Macedo, *Anotaciones para el conocimiento zoológico del género Batrachophrynus (Amphibia: Salientia),* **Tesis universitaria para optar el título de Bachiller,** Universidad Nacional Mayor de San Marcos, Lima, Perú, 1950 p. 27.

[11] D. Álvarez, D. Soley, A. Watson, *Aquatic Entomology Group in Bioblitz*, **Bioblitz in the Peruvian Andes (2016-2017)**, Nathan Soley (Editor). Primera edición, 2018, p. 42.

[12] D.F. Buss, D.M. Carlisle, T.S. Chon, J. Culp, J.S. Harding, H.E. Keizer-Vlek, H.E., ..., R.M. Hughes, *Stream biomonitoring using macroinvertebrates around the globe: A*

comparison of large-scale programs, **Environmental Monitoring and Assessment, 187**(1), 2014, Article Number: 4132.

[13] G. Roldán, *Los macroinvertebrados como bioindicadores de la calidad del agua: cuatro décadas de desarrollo en Colombia y Latinoamerica*, **Revista de La Academia Colombiana de Ciencias Exactas, Físicas y Naturales, 40**(155), 2016, pp. 254-274. https://doi.org/10.18257/raccefyn.335

[14] W.L. Hilsenhoff, *Rapid Field Assessment of Organic Pollution with a Family-Level Biotic Index*, **Journal of the North American Benthological Society, 7**(1), 1988, pp. 65–68. https://doi.org/10.2307/1467832

[15] M. Sánchez, *El índice biológico BMWP (Biological Monitoring Working Party score), modificado y adaptado al cauce principal del río Pamplonita Norte de Santander*, Bistua: **Revista de La Facultad de Ciencias Básicas, 3**(2), 2005, pp. 54–67.

[16] B. Ríos-Touma, R. Acosta, N. Prat, *The Andean biotic index (ABI): Revised tolerance to pollution values for macroinvertebrate families and index performance evaluation*, **Revista de Biologia Tropical, 62,** 2014, pp. 249–273. https://doi.org/10.15517/rbt.v62i0.15791

[17] L.R. Holdridge, **Life Zone Ecology**, Tropical Science Center, San José, Costa Rica, 1967, p. 417.

[18] A. Watson, A. Fitzgerald, O. Damian, R.K. Elias, *Habitat characterization, occupancy and detection probability of the Endangered and endemic Junín giant frog Telmatobius macrostomus*, **Endangered Species Research 32**(1), 2017, pp. 429–436. http://doi.org/10.3354/esr00821

[19] E. Domınguez, H.R. Fernández, *Macroinvertebrados bentónicos sudamericanos*, **Sistemática y biologıa**. Fundación Miguel Lillo,Tucumán, Argentina, 2009. https://doi.org/978-950-668-015-2

[20] N. Prat, R. Acosta, C. Villamarín, M. Rieradevall, **Guía para el reconocimiento de las larvas de Chironomidae (Diptera) de los Ríos Altoandinos de Ecuador y Perú**, Clave para la determinación de los principales morfotipos larvarios, Grupo de Investigación F.E.M., Departamento de Ecología, Universidad de Barcelona. 2018, http://www.ub.edu/riosandes/docs/CLAVE%20MACROMORFOLOGIA%20LARVAS%20V10.pdf

[21] * * *, RStudio Team, RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, 2019, URL http://www.rstudio.com/

[22] J.R. Bray, J.T. Curtis, *An Ordination of the Upland Forest Communities of Southern Wisconsin*, **Ecological Monographs, 27**(4), 1957, pp. 325–349. https://doi.org/10.2307/1942268

[23] K.R. Clarke, R. Gorley, P.J. Sommerfield, R.M. Warwick, **Change in Marine Communities an Approach to Statistical Analysis and Interpretation**, Third edition, United Kingdom, 2014, p. 52.

[24] R. Acosta, B. Ríos, M. Rieradevall, N. Prat, *Propuesta de un protocolo de evaluación de la calidad ecológica de ríos andinos (CERA) y su aplicación a dos cuencas en Ecuador y Perú*, **Limnetica, 28**(1), 2009, pp. 35–64. https://doi.org/10.23818/limn.28.04

[25] I. Pardo, M. Álvarez, J. Casas, J.L. Moreno, S. Vivas, N. Bonada, … M.R. Vidal-Abarca, *El hábitat de los ríos mediterráneos. Diseño de un índice de diversidad de hábitat*, **Limnetica, 21**(3–4), 2002, pp.115–133.

[26] D. Jacobsen, *Contrasting patterns in local and zonal family richness of stream invertebrates along an Andean altitudinal gradient*, **Freshwater Biology, 49**(10), 2004 pp. 1293–1305. https://doi.org/10.1111/j.1365-2427.2004.01274.x

[27] * * *, **Estándares de Calidad Ambiental para Agua (ECA),** MINAM, El Peruano, 2017 pp. 6–9. Recuperado de http://www.minam.gob.pe/wp-content/uploads/2017/06/DS-004-2017-MINAM.pdf

[28] E. Lavie, J.A Morábito, S.E Salatino, A. Bermejillo, M. F. Filippini, *Contaminación por fosfatos en el oasis bajo riego del río Mendoza*, **Revista de La Facultad de Ciencias Agrarias, 42**(1), 2010, pp. 169–184.

[29] C. Molina, F. Gibon, J. Pinto, C. Rosales, *Estructura de Macroinvertebrados Acuáticos en un río altoandino de la Cordillera Real de Bolivia: variación anual y longitudinal en relación a factores ambientales*, **Ecología Aplicada, 7**(1-2), 2008 pp. 105-116.

[30] M.A. Silva Poma, **Caracterización ecológica de la fauna de macroinvertebrados acuáticos en dos quebradas altoandinas**, Ayacucho, Perú, 2018 p. 20 10.13140/RG.2.2.13166.64324

[31] N. Becerra-Tapia, A. Botello, *Bacterias coliformes totales, fecales y patógeneas en el sistema lagunar Chantuto-Panzacola, Chiapas, México*, **Hidrobiológica, 5**(1-2), 1995, pp. 87-94, https://hidrobiologica.izt.uam.mx/index.php/revHidro/article/view/584

[32] L.E. Gualdrón, *Evaluación de la calidad de agua de ríos de colombia usando parámetros físicoquímicos y biológicos*, **Dinamica Ambiental,** 1, 2018 pp. 83–102. https://doi.org/10.18041/2590-6704/ambiental.1.2016.4593

[33] R. Figueroa, C. Valdovinos, E. Araya, O. Parra, *Macroinvertebrados bentónicos como indicadores de calidad de agua de ríos del sur de Chile*, **Revista Chilena de Historia Natural, 76**(2), 2203, pp. 275–285. https://doi.org/10.4067/s0716-078x2003000200012

[34] R.T. Martins, N. Stephan, R.G. Alves, R.G, *Tubificidae (Annelida: Oligochaeta) as an indicator of water quality in an urban stream in southeast Brazil Tubificidae (Annelida: Oligochaeta) como indicador da qualidade da água de um córrego urbano do sudeste Brasileiro*, **Acta Limnol, Bras, 20**(3), 2008, pp. 221–226. Retrieved from http://ablimno.org.br/acta/pdf/acta20_vol3_05.pdf

[35] C. Villamarín, *Estructura y composición de las comunidades de macroinvertebrados acuáticos en ríos altoandinos del Ecuador y Perú*, **Diseño de un sistema de medida de la calidad del agua con índices multimétricos**, 2012 p. 29.

[36] D. Jacobsen, *The effect of organic pollution on the macroinvertebrate fauna of Ecuadorian highland streams*, **Archiv Fur Hydrobiologie, 143**(2), 1998, pp. 179–195. https://doi.org/10.1127/archiv-hydrobiol/143/1998/179

[37] T.R. Cumby, V.R. Phillips, *Environmental impacts of livestock production*, **BSAP Occasional Publication, 28**(28), 2001, pp. 13–22. https://doi.org/10.1017/s1463981500040930

[38] A. Jansen, A.I. Robertson, *Relationship between livestock management and the ecological condition of riparian habitats along an Australian floodplain river*, **Journal of Applied Ecology, 38**(1), 2001, pp. 63–75. https://doi.org/10.1046/j.1365-2664.2001.00557.x

[39] I.P. Ogbuewu, V.U. Odoemenam, A.A. Omede, C.S. Durunna, O.O. Emenalom, M.C. Uchegbu, … M.U. Iloeje, *Livestock waste and its impact on the environment*, Scientific **Journal of Review, 1**(2), 2012, pp. 17–32. http://sjournals.com/index.php/SJR/article/view/184

[40] M.A. Mora, L. Ríos, L. Ríos Ramos, J.L. Almario Charry, *Impacto de la actividad ganadera sobre el suelo en Colombia*, **Ingeniería y Región, 17**, 2017, pp. 1-12 https://doi.org/10.25054/22161325.1212

[41] I. Aganmwonyi, F. Iriabgonse, C. Emeribe, *Impact of Livestock Grazing on Abundance and Community Structure of Macroinvertebrate fauna of Ovia River, Edo State, Nigeria*, **Journal of Applied Sciences and Environmental Management, 19**(4), 2016, pp. 779-784 https://doi.org/10.4314/jasem.v19i4.27

[42] G.E. Nenaah, *Toxic and antifeedant activities of potato glycoalkaloids against Trogoderma granarium (Coleoptera: Dermestidae)*, **Journal of Stored Products Research, 47**(3), 2011, pp. 185–190. https://doi.org/10.1016/j.jspr.2010.11.003

[43] S. Eraso-Grisales, D. Mejía-España, A. Hurtado-Benavides, *Extracción de glicoalcaloides de papa nativa (Solanum phureja) variedad ratona morada con líquidos presurizados*,

Revista Colombiana de Ciencias Químico-Farmacéuticas, 48(1), 2019, pp. 181–197. https://doi.org/10.15446/rcciquifa.v48n1.80074

[44] J.T. Blankemeyer, B.K. Stringer, J.R. Rayburn, J.A. Bantle, M. Friedman, *Effect of Potato Glycoalkaloids, α-Chaconine and α-Solanine On Membrane Potential of Frog Embryos*, **Journal of Agricultural and Food Chemistry, 40**(10), 1992, pp. 2022–2025. https://doi.org/10.1021/jf00022a057

[45] J.T. Blankemeyer, R. Atherton, M. Friedman, *Effect of Potato Glycoalkaloids α-Chaconine and α-Solanine on Sodium Active Transport in Frog Skin*, **Journal of Agricultural and Food Chemistry, 43**(3), 1995, pp. 636–639. https://doi.org/10.1021/jf00051a015

Received: October 27, 2020
Accepted: August 1, 2021