Anurans, the Group of Terrestrial Vertebrates Most Vulnerable to Climate Change: A Case Study with Acoustic Monitoring in the Iberian Peninsula

RAFAEL MÁRQUEZ[1], DIEGO LLUSIA[1], JUAN FRANCISCO BELTRÁN[2], JOSÉ PEDRO DO AMARAL[3] & RICHARD G. BOWKER[3]

[1] Fonoteca Zoológica, Dept. de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales (CSIC), José Gutiérrez Abascal 2, 28006 Madrid, Spain, E-mail: rmarquez@mncn.csic.es
[2] Departamento de Fisiología y Zoología, Facultad de Biología, Universidad de Sevilla, Avda. Reina Mercedes 6, 41012 Sevilla, Spain.
[3] Dept. of Biology. Western Kentucky University. 1906 College Heights Blvd. Bowling Green Kentucky, KY 42101. USA.

Abstract. We report preliminary analyses from an ongoing sound monitoring project that involves five species of anurans: two species of tree frogs in the genus *Hyla* (Hylidae) and three species of midwife toads in the genus *Alytes* (Discoglossidae) in the Iberian Peninsula. Each station was monitored with an automated recording system based on solid state recorders, coupled with programmable temperature and relative humidity probes. We present comparative data of vocal activity of two populations of *Alytes cisternasii* from thermal extremes of the species range using human detection and commercial automated sound recognition software. Parameters such as duration of reproductive season, preferred temperatures for calling activity and relation with relative humidity are discussed. We compare the performance of analysing the recordings between an automated system of detecting the presence of *Alytes cisternasii* calls and listening of the recordings by non-expert personnel.

The most recent reviews of world wide anuran species conservation indicate that anurans are among the most threatened groups, even moreso than mammals or birds (STUART ET AL., 2004). Among the many factors that are related to that threat, global climatic change appears to be related to the declining populations. Temperature influences the physiology, ecology and behavior of anurans. These ectothermal vertebrates can select their body temperature from a mosaic of temperatures in their environment. Furthermore, temperature indirectly determines the availability of surface water, an element essential for the development of their larvae and for the survival of the adults. Therefore, an increase in temperature related to global climatic change is likely to impact the biology and conservation of anurans.

The geographic location of the Iberian Peninsula makes it particularly vulnerable to these changes. The different predictions of climatic change indicate a general increase in temperatures as well as long term changes in water availability, resulting from a decrease in rainfall for a number of regions (MMA, 2005).

TEMPURA is the acronym for the project "Adaptations of anurans to climate change: comparative study of population in thermal extremes", funded by the Ministerio de Educación y Ciencia, Spain (Programa Nacional I+D+i), and involves researchers of 4 institutions: Western Kentucky University (USA), Universidade de Lisboa (Portugal), Universidad de Sevilla (Spain) and the Museo Nacional de Ciencias Naturales-CSIC, (Spain).

Within the project TEMPURA we investigate what will be the response of some anuran species to variations in the temperature and water availability of their habitats, among other parameters, we investigate the relationship between acoustical activity of anurans and weather variables in 10 populations of 5 species of anurans. Two sites were selected per species, one of them in the coldest area of their range, and another one in the hottest area of their range. The species selected for the study were three continental species of midwife toads in the genus *Alytes* (*A. obstetricans, A. cisternasii* & *A. dickhilleni*), and two species of treefrogs in the genus *Hyla* (*H. arborea* & *H. Meridionalis*; Fig. 1). The main goals of this project are 1) to determine the reproductive phenology of the species using male calling activity as the indicator; 2) compare the relationship between temperature and humidity and phenology between the populations occuring in warm (xeric) and the cold (mesic) habitat extremes (determining whether any between populations differences respond to phenotypic plasticity or to adaptation), and to complete the comparison comparing the results between species within genera, and between genera, to get an indication of the evolutionary history of the adaptations. In this paper we particularly consider the problem of the analysis of the arrays of recordings to detect acoustical activity of a focal species, and we compare the performance of an automated software-based system and listening of the recordings by non-expert personnel.

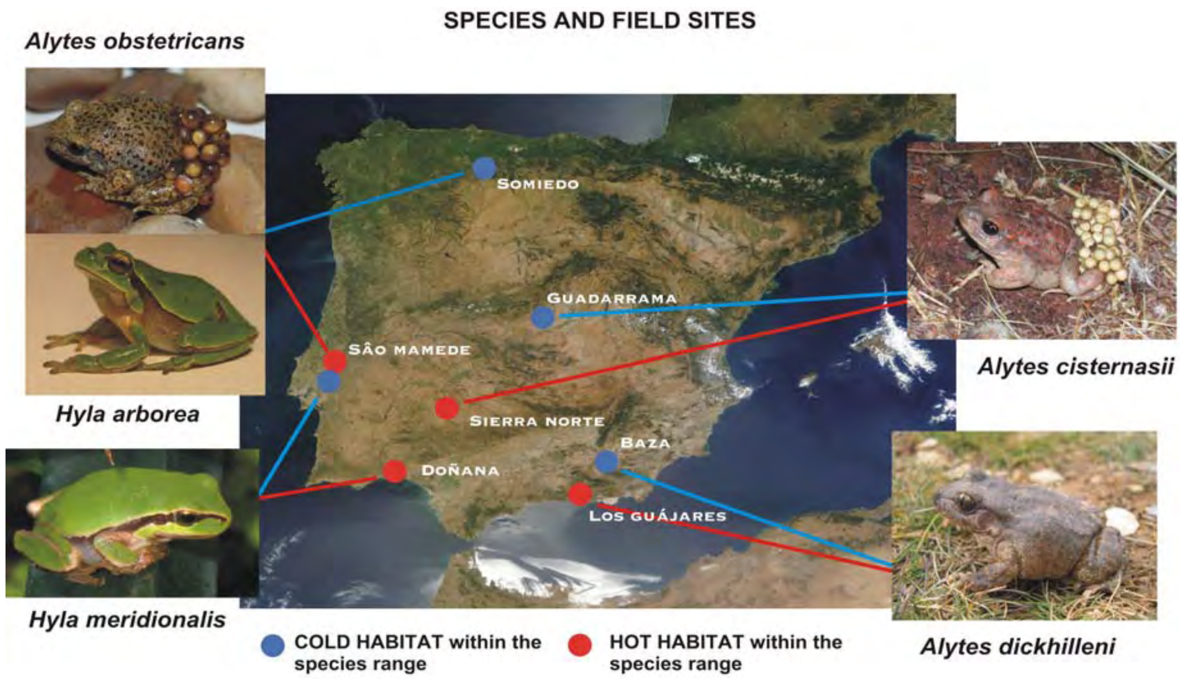

Figure 1: Location of monitoring stations.

Call Characteristics

The spectral and temporal characteristics of the species involved in this study are shown in Fig. 2, (sounds from MÁRQUEZ & MATHEU, 2004). While the two hylid calls are mainly pulsed sounds with wide frequency spectra, the three discoglossids have short and tonal sounds with no frequency modulation and extremely simple harmonic structure (pure tones, MÁRQUEZ & BOSCH, 1995). Consequently the choice of genera allows interspecific and intergeneric comparisons, it also allows exploration of environmental effects on very different sound types.

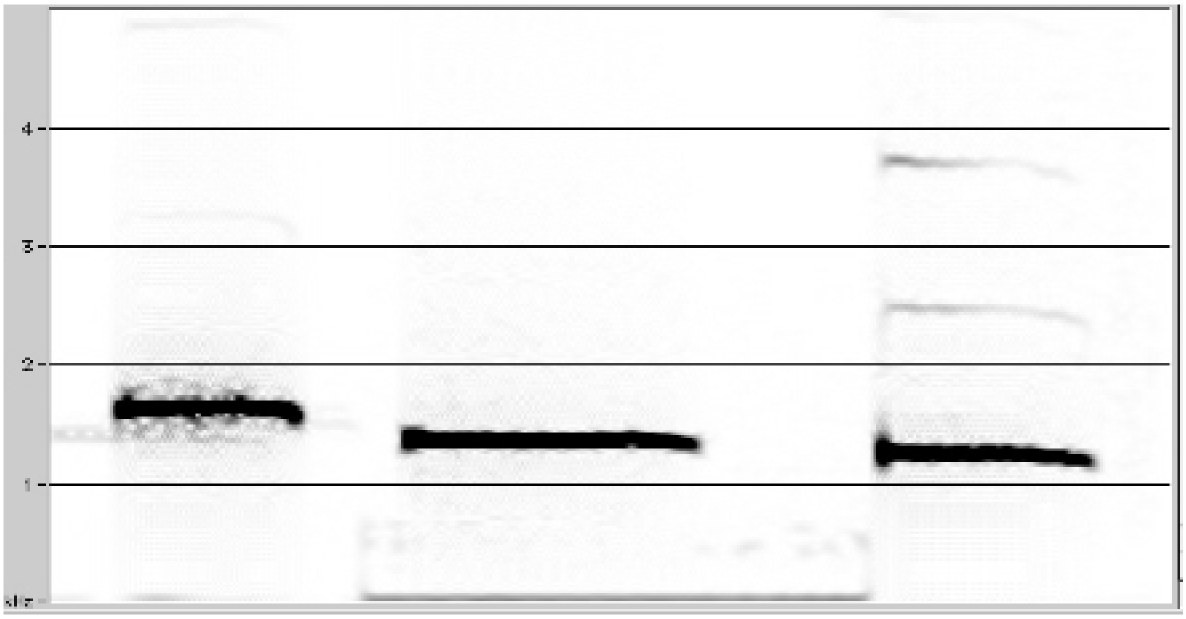

Figure 2A: Audiospectrogram of one advertisement call of *A. cisternasii*, one of *A. dickhilleni*, and one of *A. obstetricans* (FFT window size, 1024 points; total duration 900 milliseconds).

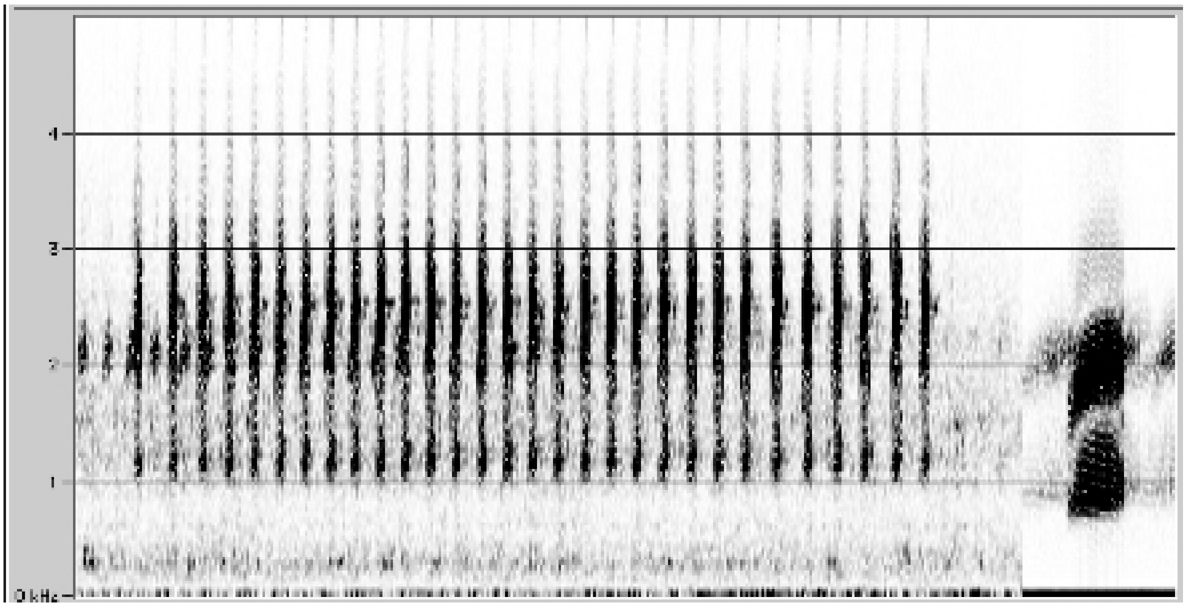

Figure 2B: Audiospectrogram of one advertisement call of *H. arborea* and one of *H. meridionalis* (FFT window size, 1024 points; total duration 9.9 secs).

Methods: Automated Monitoring System

The recording system includes four main elements (Fig. 3): 1) An omnidirectional condenser microphone Fonestar FCM-62 (powered by a AA alkaline battery); 2) a digital recorder Marantz MPD-660 controlled by; 3) an "Amphibulator", a custom made programmable timer developed by the Department of Biology and the Department of Engineering of Western Kentucky University, and 4) a 12v battery (40 Ah or more). The recording protocol was record 3 min per hour, 24 hours per day.

Maximizing the time of autonomous functioning of the recording system is crucial to diminish maintenance costs, and this is an important issue if you consider that the 10 stations are located in distant points of the Iberian Peninsula. This requirement justified the choice of MP3 compression in the sound recording format. With the largest Compact Flash cards available at the beginning of the project (2GB) MP3 compresses sound in MONO theoretically allows for more than 72 hours of recordings (more than 60 days, recording 3 minutes per hour every hour). The Marantz PMD-660, however, has a lower maximum recording limit, which is determined by the three digits that encode the recordings which limit the number of recorded tracks to 999 or 41 days.

Each recording station is also equipped with environmental dataloggers that monitor periodically the air temperature and RH (Onset Hobo H08) as well as the soil temperature at 5-10 cm depth, and water temperature (Onset Pendant). Data are stored every five minutes during the calling activity of the monitored species and every 30 minutes during the rest of the year.

Figure 3: Diagram of the automated recording system.

Protection of the recording stations. - In order to prevent the theft or destruction of the recording stations in sites with public access, the units were housed in iron boxes which were attached to solid elements of the landscape or inserted in a concrete bed. The microphones were housed in an opening on the box or were placed nearby (Fig. 4).

Figure 4: Examples of location and protection of recording systems.

Calibration. - The calibration of the recording system including the methodology for setting similar recording levels in all the units, determining the shape and extension of the area covered by the station, and ultimately determining what percentage of the total population and the corresponding number of adults have been recently described (MÁRQUEZ ET AL., 2008).

Results

The first results regarding two populations of *A. cisternasii* were recently published (TEJEDO ET AL., 2008; MARQUEZ ET AL., 2008,). During 2006 these populations, which are located 400 kms apart in the Iberian Peninsula showed a remarkable degree of synchrony in the beginning of their reproductive season. In both cases acoustical activity started in the second week of September, coinciding with the first rainfalls of fall after the summer drought (this observation is in agreement with previous reports, CRESPO, 1982; RODRÍGUEZ-JIMÉNEZ, 1984, 1988; MÁRQUEZ, 1992; GARCÍA-PARÍS ET AL., 2004). However, there are important differences in the temperature and relative humidity between the periods of acoustical activity of the two populations. In the first weeks of activity air temperature was between 13-20ºC in the warm population during acoustical activity, whereas in the northern population males called at air temperatures of 8-15ºC. Regarding relative humidity, the maximae were similar in both populations reaching 100%, contrasting with the minimae which showed substantial differences between populations reaching below 50% in the southern population while always being above 74% in the northern population (Fig. 5).

Even the soil temperatures measured 5 cm deep were significantly different (Fig. 6) (t-Student=-4.79; p=0.000; n=48).

These preliminary results suggest that the toads are active at different temperatures although, in order to discriminate to what extent the differences result from a process of adaptation or simply reflect phenotypic plasticity, further studies are necessary.

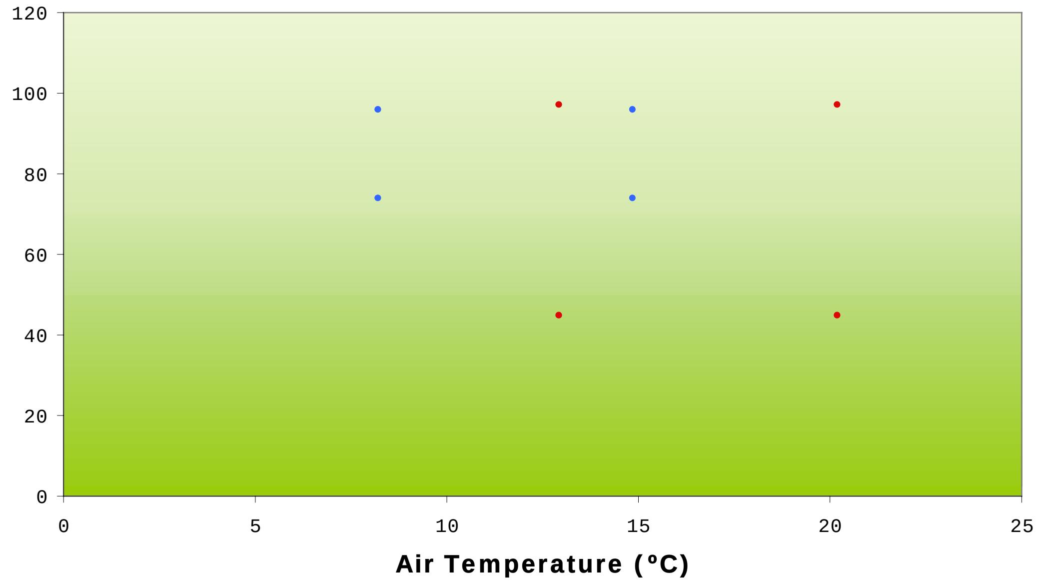

Figure 5: Air temperature and relative humidity during the hours of acoustical activity of the Iberian midwife toad (*Alytes cisternasii*) in the two study sites (fall 2006). Blue: Sierra de Guadarrama, Madrid (cold habitat). Red: Sierra Norte, Sevilla (warm habitat).

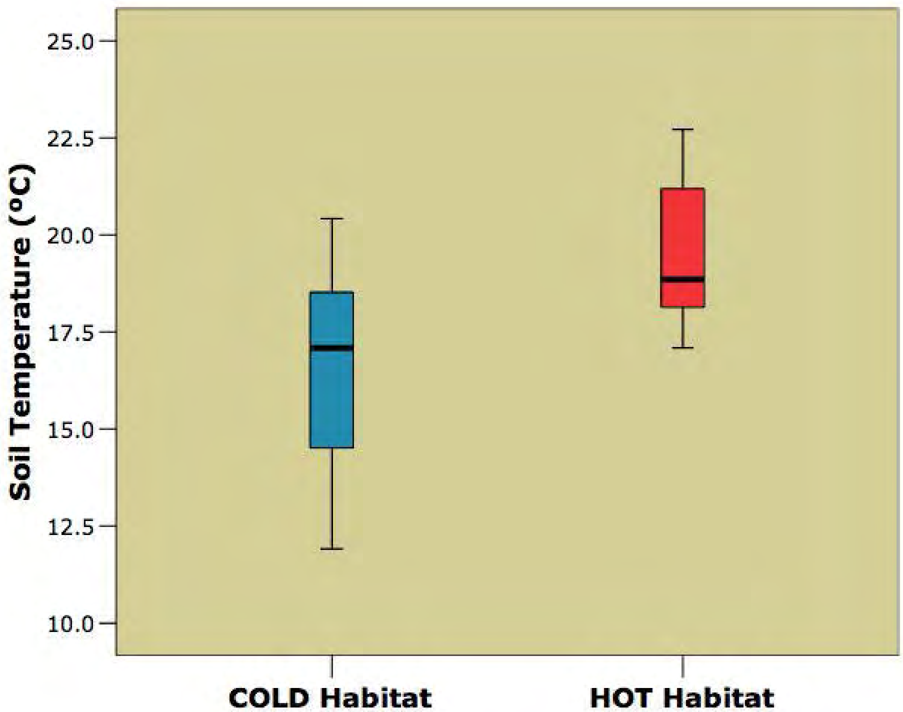

Figure 6: Mean soil temperature during the hours of acoustical activity of the Iberian midwife toad (*Alytes cisternasii*) in the two study sites (fall 2006). Blue: Sierra de Guadarrama, Madrid (cold habitat). Red: Sierra Norte, Sevilla (warm habitat).

Comparative Results of an Automated Sound Recognition System and Human Listening

We used the data from the first season of the two study populations of *Alytes cisternasii* to compare the effectiveness of an automated system when compared to the inspection of the files by students. The automated software used was Song Scope Bioacoustics Monitoring Software, a commercial program made by WildLife Acoustics. According to the developper of this software this program operates as follows “Song Scope builds Hidden Markov Models using algorithms specially designed to consider both the spectral/temporal components of individual syllables, but also how these syllables are arranged into songs for more complex avian vocalizations (frogs are generally single-syllable songs). A "recognizer" is really an HMM -- for the layman, a statistical model with "states" representing spectral fingerprints and probabilities of transitioning between states. Plus a little more...” (Ian Agranat, pers. com.). Again, the populations of *Alytes cisternasii* followed were: cold habitat (Sierra de Guadarrama, Madrid) and warm habitat (Sierra Norte, Sevilla).

The software used required first the construction of models of recognition. Three different sound files were analyzed to build a recognizer, one of them included high quality recordings from a sound guide (MÁRQUEZ & MATHEU, 2004) whereas the other two files were recordings from the automated recording system where *Alytes* sounds were confirmed to exist. More than 8000 recordings were scanned corresponding to daytime and night recordings from fall 2006 to early summer 2007. SongScope yielded 200 positive recordings (presence of at least one call) with the sensitivity level that was selected (9 in a scale of 10).

Also, a subsample of the recordings were listened by two students with no prior experience in call detection, the subsample selected was the first three hours after dusk during the months of the reproductive season (the recordings made at the times with a maximum probability of midwife toads calls). More than 1000 3-min recordings were listened by the students and 96 of the recordings scored positive (presence of at least one call).

All the files that scored positive were listened again by experienced researchers to confirm the presence of the species call (Table 1). Only 3.6 % y 7.1% of the recordings that scored positives in the human listening process were confirmed to have *Alytes* calls in them. Song Scope was more effective since 15.3 % and 19.7% of the positive files were confirmed by subsequent expert screening of the recordings. However, the large percentage of false positives (Type II error) with both methodologies is remarkable, and only partially explainable by the simplicity of the call. The presence of “acoustical mirages” or of “oversensitive acoustical search image” have been noted previously by the authors as a psycho-acoustic phenomenon which is triggered in sound environments which are far away from the midwife toads breeding grounds and results in the sensation of detecting midwife toad calls erroneously, presumably when a burst of energy occurs within the frequency range of these species.

Another important measure of the efficiency of the sound recognition system is the number of recordings that included midwife toad calls that did not score positively in the automated test (Type I error, Table 2). In order to determine the importance of that type of error we had expert ears listen to 86 tracks selected from the dates and hours of maximum probability of calling. The software used fail to detect 35% of the files that actually contained *A. cisternasii* calls (24% in the hot habitat and 50% in the cold habitat). These figures are somewhat disappointing and may be related to the limit of 10 dB sensitivity (sound/noise) of the software or to the variability of the frequency of the call within the population that may exceed the range established for the software scanning (if this is the case, the problem would probably be solved by further improving the recognizer model).

Table 1: Type II error (call detected in track not containing the call). Comparative results between non-expert human listener and automatic detection system (Song Scope) for mating calls of *Alytes cisternasii*. Pooled recordings of hot and cold populations.

Detection system	Population	Nº tracks analyzed	Total tracks with positive scores	Confirmed positive	False positive	% Type II Error	% Accurate
Non-expert human listener	Hot	563	42	3	39	92.9	7.1
Automatic	Hot	2364	124	19	105	84.7	15.3
Non-expert human listener	Cold	532	55	2	53	96.4	3.6
Automatic	Cold	6597	76	15	61	80.3	19.7

Table 2: Type I error (call not detected in a track that included a call). Results of automatic detection system (Song Scope) for mating calls of *Alytes cisternasii*. Pooled recordings of hot and cold populations.

Detection system	Population	Nº tracks analyzed	Total tracks with positive scores	Tracks with calls detected	Tracks with calls not detected	% Type I Error	% Accurate
Automatic	Cold	34	34	8	26	50.0	50.0
Automatic	Hot	52	52	16	36	23.8	76.2
Automatic	Total	86	86	24	62	35.1	64.9

Conclusions

Of all of the species studied in TEMPURA, *A. cisternasii* is the species that occurs throughout a more homogeneous habitat and has the least fragmented distribution. It was therefore, a priori, the species that was less likely to show substantial thermal differences between populations at thermal extremes of its distribution. However, the siginificant differences of activity temperatures observed in this preliminary study, together with the old evolutionary age of the lineage (the differenciation of *A. obstetricans* and *A. cisternasii* has been estimated to have occurred 16 m. y. ago with the formation of the Betic Strait, Arntzen & García-París, 1996; Martínez-Solano et al. 2004) indicate that there may have been a substantial selective pressure related to thermal conditions, and ample opportunity for adaptations to evolve. Further insight on these processes may be produced by ulterior progress in TEMPURA research.

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