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Anomalies in natural populations of amphibians: Methodology for field studies

A. Ohler and A. Dubois

Institut de Systématique, Evolution, Biodiversité, ISYEB - UMR 7205 - CNRS, MNHN, UPMC, EPHE, Muséum national d'Histoire naturelle, Sorbonne Universités, Paris, France

Abstract

To be really efficient and conclusive, studies on the anomalies in natural populations of amphibians must be carried out in a perspective clearly centered on this topic rather than being a side product of works dealing with other questions. Recommendations are offered here on the methodology for such studies.

Keywords: anomalies, methodology, amphibians, field studies.

Corresponding Author:
A. Ohler
annemarie.ohler@mnhn.fr

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1. Introduction

What is an "anomaly"?

The term 'anomaly' is derived from the Greek term ἄνομος (*anomos*), meaning 'lawless, wicked'. It designates any deviation of the phenotype (morphological or non-morphological) outside the range of variation of the phenotype considered to be 'normal' in a species, irrespective of its cause. This term conveys a wide variety of meanings.

A rich but also confusing terminology has been used in the literature to designate abnormal phenotypes. The terms 'monstrosities' and 'monsters' carry the teleological notion of 'mistake of nature'. That of 'deformities' (deformed specimens) carries the notion of 'form': it is therefore restrictive as it does cover anomalies that do not concern form (e.g., coloration anomalies). The terms 'mutation' and 'mutant' concern only anomalies with a genetic transmission. The term 'anomaly' is preferable to the preceding ones, as it has both a more general and more neutral meaning, referring just to 'normality', to 'abnormal' or 'deviant' specimens, without limiting this to some aspects of the phenotype or to some kinds of causes.

2. Studies of anomalies in natural amphibian populations: what are our aims?

Studies of anomalies in natural amphibian populations have several aims: (1) to evaluate the 'health' of these populations through the study of anomalies; (2) to establish the causes of the anomalies observed; and (3) to make predictions and recommendations. But in such studies the aim is not: (1) to create anomalies in the laboratory that would 'resemble' those found in the natural populations; and (2) to study the anomalies 'for themselves', for embryological, morphogenetic, teratogenetic or genetic purposes.

These distinctions have methodological consequences. Such studies should concentrate on the natural populations themselves and on animals collected in these populations, not on 'model organisms' from laboratory stocks. They should therefore be composed of two main steps: (1) field studies of the populations (including their environment) and (2) laboratory studies of specimens coming from these populations.

3. Study of anomalies in natural amphibian populations: methodological recommendations and warnings for field work

3.1. General recommendations

For the study of the causes of anomalies, morpho-anatomical descriptions (even very detailed ones) of isolated cases are of very limited, often merely 'anecdotal', interest. To be fully informative, the data should concern high numbers of animals (hundreds, preferably thousands or tens of thousands). Before starting anything, care should be taken to obtain all the necessary permits and legal documents allowing one access to the study sites and the right to handle large quantities of animals.

The coverage of a field survey for the study of anomalies should be well defined from the start. In a given area, the study should concern as many localities as possible (i.e., not only those where abnormals were found) to ascertain the distribution of the anomalies studied. It should cover all amphibian species in each locality (not only the 'target' species) in order to ascertain the presence or absence of taxonomic specificity in the anomalies studied.

3.2. Species specificity

As a matter of fact, many anomalies show species specificity. Of course, anomalies due to 'natural', random mutations are species-specific and often population-specific. But this is also the case for some 'phenotypic' anomalies like the anomaly *P* of *Pelophylax* (European green frogs). In contrast, anomalies due to severe environmental aggressions or disruptions (radiation, chemicals, pathogens, parasites) may concern several sympatric species and, depending on the factor involved, may occur in a single locality or over a larger area. This requires that we take into account all the amphibian species of a site, and not only those in which anomalies may have been discovered.

3.3. Study site

Any field survey must include a precise localisation of the site, with its coordinates, the 'official' names (as they appear in maps and other published documents) and their 'local' names, and a precise description of the site and means of access (roads, paths). This description should cover the various environments found on the site (lentic and lotic aquatic habitats, forested and agricultural lands, roads, human settlements, topography, elevation), and not only the habitats where amphibians were found. The distribution of all amphibian species on the site at the time of the study should be noted, as well as those of other organisms likely to interfere with amphibians, such as predators, parasites, vectors of pathologies and competitors.

3.4. Sampling

It is not enough to collect and examine the abnormal specimens found during such a survey. Statistical data may be very useful for the interpretation of the observations, and the higher the number of amphibians examined, the better. Among about 100,000 specimens of *Bufo bufo* examined by our team in the Paris region from 1966 to 1975, several dozen anomalies were detected, but the 'background rate' of specimens showing anomalies due to genetic causes (mutations) was often below 1 or 0.1%, or even 0.01%. Therefore any higher rate should provoke attention, as it suggests the possibility of unusual causes of anomalies, but the estimate of this rate is meaningful only if based on more than 100 specimens, preferably more than 1,000. In many temperate species, such numbers are relatively easy to collect and examine during the breeding season.

During such surveys, sampling should always be random. Looking especially for abnormal specimens (e.g., albinotic or melanistic specimens, which are easily recognisable) would produce biased estimates of their frequency in the population. Random collection by hand or by net with small mesh is recommended. Trapping often introduces a collecting bias and should be avoided.

For an efficient field survey, appropriate equipment is needed, including boots, nets, headlamps, paper and pen, computer, recorder, camera, GPS, maps and containers. Also useful may be a magnifying glass, a chair and table, protection against rain, cold and/or sun, gloves, food and drink.

In order to study as many specimens possible (a minimum of 100 if possible, preferably above 1000), night surveys by teams of several researchers are more efficient, not only because amphibians are easier to collect when dazzled by electric light, but also because most species are active only or mainly at night. The best period for such studies is of course the breeding season, when thousands of specimens gather for reproduction.

3.5. Storage of specimens during study

Until the survey of a population is finished, the specimens should be provisionally stored in containers and not released immediately on the spot of collection: this prevents them from being examined and counted twice or more. Storage should allow specimens to move and be spacious enough to prevent overcrowding, along with the injuries and deaths that overcrowding causes.

During this storage period, if the study is made in the breeding season, males and females should be separated, and there should not be too many females in a container in order to prevent them from releasing their eggs. For such studies, it is not recommended to store the specimens, especially females, in plastic or tissue bags, as this also may induce the release of eggs. Large basins are appropriate containers. If it is technically possible to store them in a cold room (e.g. in a truck), this will contribute to the retention of the eggs by the females until they are released with the males in a habitat. All these precautions require that one maintain in a good condition the appropriate equipment for short-term stocking of living specimens.

3.6. Information acquisition and recording

Methodological recommendations are also useful concerning the acquisition and recording of information. Collection and examination of the specimens should be separated. It is much more efficient and ergonomic to catch and store the specimens first, and examine them altogether immediately after the end of collecting. Examination may also be conducted during the collecting, but this requires several teams of observers in different parts of the habitat. At any rate, examination of the specimens should not be postponed to a later date, as captive specimens may develop injuries, lay eggs or die.

For the examination of specimens, comfortable conditions should be preferred: a chair, table, and, if possible, protection from wind, rain, cold, etc. The best system consists of dividing the work between at least two persons: one examining the specimens and one noting the observations. For comparison purposes, it is better to examine all the specimens of one species first, then those of a second species, etc., and it is better if all the specimens in each of these categories are examined by the same person. For visual examination, the specimens should be held firmly in hand and the observations always made in the same order, e.g.: dorsal view of head and body, lateral views of each side of head (including eyes) and body, ventral view of head and body, dorsal and ventral views of each hand and foot. With some experience, the examination of a specimen without any external anomalies takes less than half a minute.

What information should be recorded during a field survey for anomalies? All individuals of each stage and sex collected should be carefully examined. Their taxonomic status, sex, stage or age, and all anomalies, should be recorded. All phenotypic anomalies, including 'tiny' anomalies and apparently 'accidental' ones (wounds), should be noted. Detailed descriptions, photographs and sketches should be made. This requires efficient ergonomics for information recording

To save time, it is also useful to have an ergonomic and standardised system for noting observations. Each specimen surveyed should be taxonomically identified, at least at some supraspecific level (genus, subgenus, species group). For brevity, the use of a three-letter code for each taxon is recommended, such as TEM for *Rana temporaria*, CRI for *Triturus cristatus*, PEL for *Pelophylax* sp., LIS for *Lissotriton* sp. or URO for Urodela sp. (this may be necessary for eggs or larvae).

Ergonomic data recording can also be facilitated through the use of standard symbols or abbreviations for sex and stage, such as: ♂, male; ♀, female; ad., adult; sub., subadult; juv., juvenile; im., imago (just metamorphosed, before first hibernation or another landmark event in development); tad., tadpole (0L, 0 leg; 2L, 2 legs; 4L, 4 legs); lar., larva; ω, egg.

The use of standard abbreviations and symbols facilitates the description of anomalies. They can be used to designate the limbs (LFL, left fore limb; RFL, right fore limb; LHL, left hind limb; RHL, right hind limb) and some frequent anomalies: e.g., ← for clinodactyly towards the body axis and → for clinodactyly towards the exterior of body. The numbers of digits can be abbreviated as follows: 5-5/4-4 (LHL-RHL/LFL-RRL). Figure 1 shows some digit anomalies and some possible abbreviations for them.

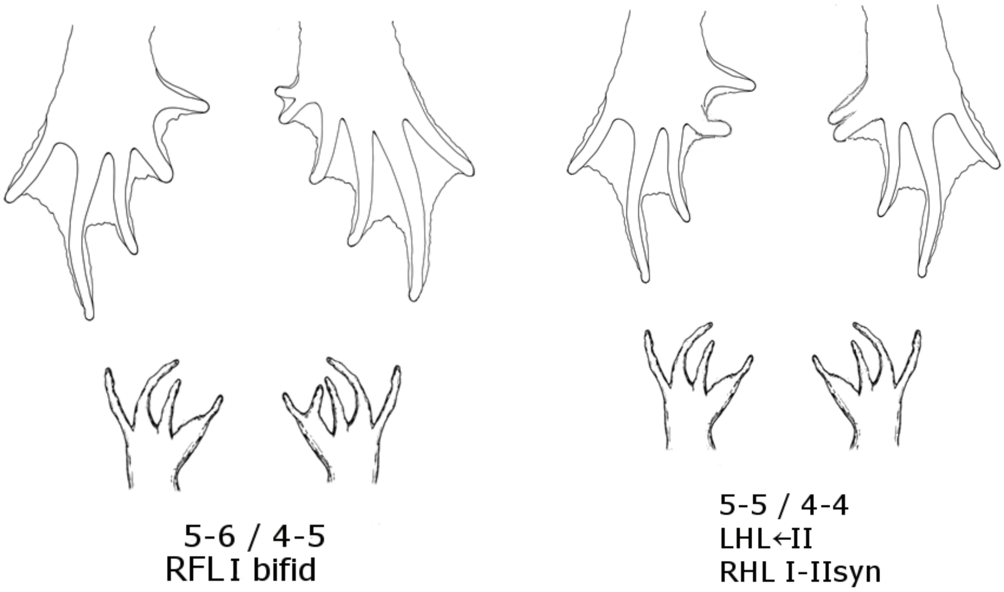

Figure 1: Examples of standard descriptions of anomalies using abbreviations and symbols.

On the other hand, in such studies it is not necessary to record details that would be useless for the purpose of the work. Thus, it is not useful to spend time describing all other particularities which do not belong to the domain of anomalies and wounds: e.g. size, coloration within the range of 'normal' variation, etc. Of course, this requires some knowledge of the 'normal' phenotypic variation of the species studied, which can be obtained only through practice and the examination of many specimens. This remark has a wider application: studies of anomalies require a 'good eye', which is often obtained after the examination of thousands of specimens over years.

Figures 2 and 3 show field notes taken with standard abbreviations during a night survey of an amphibian population in France and its subsequent 'transcription' in words in a more explicit document.

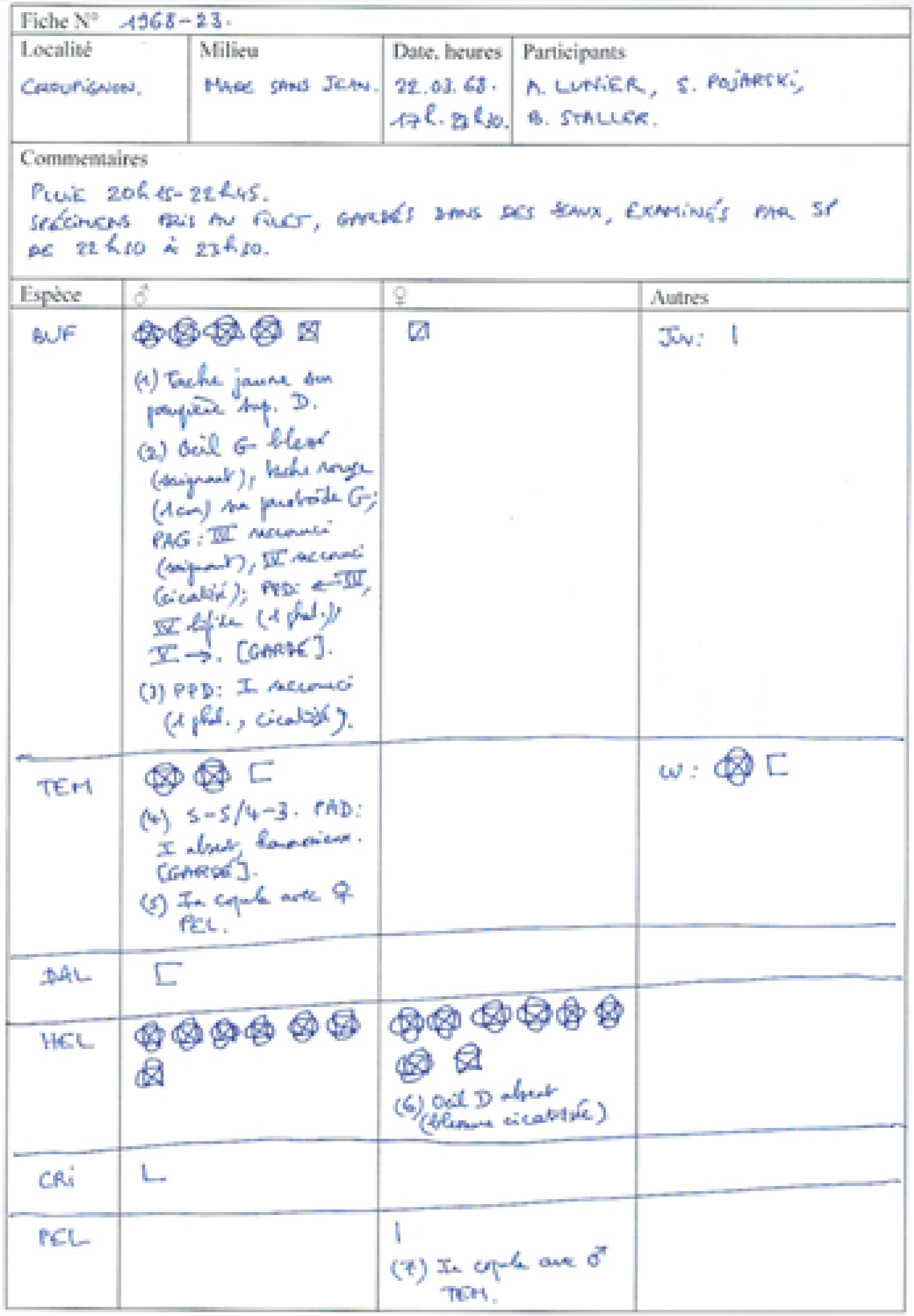

Figure 2: Example of field observation sheet on anomalies in amphibian populations.

Other potentially useful data should also be recorded. They include biological and ecological data on sympatric amphibian and non-amphibian species, which may be

Sheet number: 1968-23			
Locality: Croupignon	**Habitat:** Mare sans Jean	**Date & time:** 22 March 1968 17 h – 23 h 30	**Personnel:** Augustin Lunier, Saturnin Pojarski, Barbara Staller
Comments: Raining from 20 h 15 to 22 h 45 Specimens caught by net, kept in buckets, examined by SP from 22 h 30 to 23 h 30			
Species	♂	♀	**Other**
Bufo bufo	46 [1] Yellow spot on right eyelid [2] Left eye recently wounded (still bleeding), red spot of 1 cm in diameter on skin of left parotoid; left forelimb: finger III injured and shortened (still bleeding) and finger IV injured shortened (ancient injury, now scarred); right hindlimb: toe III with medial clinodactyly (directed towards inside of foot), toe IV bifid (last phalanx duplicated) and toe V with lateral clinodactyly (directed towards outside of foot). **Specimen kept alive:** 1968-23-2 [3] Right hindlimb: toe I shortened (1 phalanx, scarred)	5	1 juvenile
Rana temporaria	23 [4] Right forelimb with ectrodactyly: finger I missing, harmonious. **Specimen kept alive:** 1968-23-4 [5] Found in copula with ♀ [7] of *Pelophylax*	0	13 clutches
Rana dalmatina	3	0	0
Lissotriton helveticus	68	77 [6] Right eye missing (healed injury)	0
Triturus cristatus	2	0	0
Pelophylax sp. (*esculentus* ?)	0	1 [7] Found in copula with ♂ [5] of *Rana temporaria*	0

Figure 3: Example of transfer of the field observation sheet in Figure 2 to a more explicit document.

involved in predation, parasitism or competition or may be responsible for some non-genetic anomalies. Data on the biotic and abiotic environment should also be recorded, including descriptive data (particularly of 'unusual' characteristics of the habitat), a physico-chemical survey (especially if some data or observations suggest that they can be relevant for the causation of anomalies) and data on the history and changes in the habitat. One should always beware of 'official' information (from owners, authorities, etc.), which may be incomplete, biased, misleading or completely wrong. It is always preferable to count on one's own observations and enquiries than on second-hand data of unverified origin.

Whenever possible, the population size of the studied species should be estimated (e.g. by capture-recapture methods). This may prove important as small populations may have a higher inbreeding rate, which may have an impact on the rate of mutant specimens. Data on the history of the site, especially if there were changes in the habitat, may also be relevant, as recent populations or populations that may have suffered a recent demographic bottleneck may also have a higher inbreeding rate.

3.7. Specimens kept for laboratory study

At the end of the process of examination of specimens in the field, all specimens which appear 'interesting' for further study (growth, regeneration experiments, crossings, gynogenesis, etc.) should be kept alive and brought to the laboratory. This of course requires one to have all the necessary permits. All other specimens should be released on the spot of capture but only *after* having examined them *all* in order not to examine the same specimens several times.

The specimens that should be kept for laboratory work are those for which observations or experiments in captivity can bring additional information, notably on their causes. This includes, firstly, coloration anomalies due to the absence or unusual distribution of some pigments or pigmentary cells (albinism, melanism, blue or golden frogs, translucent belly skin, etc.): these are often caused by simple mutations, as can be ascertained in one generation by gynogenesis or in two generations by crosses. For some 'well-known' pigmentary anomalies (like albinism or black eyes), specimens should be kept even when the anomaly is unilateral or partial (concerning only some regions of the body). All bilateral (and preferably symmetrical and harmonious) anomalies of limbs and eyes are also likely to be caused by mutations and should be kept for study. This also applies to other 'harmonious' anomalies of legs and the head that suggest they are the result of a developmental process and not an accident having occurred after development (due to predation, disease, parasitism, etc.). A few 'normal' specimens of both sexes from the same population should also be kept for use as controls in experimental studies of the abnormal ones (e.g., for crossings or gynogenesis; see Dubois & Ohler, this volume).

4. Conclusion

In the recent years, many publications have been devoted to studies of anomalies in amphibian populations in various countries of the world. Unfortunately, an significant proportion of these works are disappointing, as the methodologies followed were unsatisfying and quite far from the recommendations presented above. One of the reasons for this situation is that few studies clearly centered on the study of anomalies were carried out. The data on anomalies were collected 'incidentally' as a side-result of studies centered on other questions (ecology, behaviour, conservation biology, mapping, etc.). However, as shown above and in other contributions to this volume, the study of anomalies in natural amphibian populations is a research domain of its own, which should follow its own methodology and use appropriate equipment. The quality of research on this matter will improve when the biologists undertaking them understand this need.

References

A list of references on these questions would occupy many pages and cannot be provided here. Such references are available in the volume on anomalies in natural populations of amphibians edited by K. Henle, A. Dubois and V. Vershinin and published in *Mertensiella*.