THE USE OF CLAY MODELS IN AMPHIBIAN FIELD STUDIES: A SHORT REVIEW

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ABSTRACT

Clay models are realistic replicas of live animals that are frequently used in ecological and ethological field studies. These kind of models, usually made from plasticine, are malleable, easy to shape, colour and relative inexpensive. In addition, plasticine models retain marks on their surface allowing the identification of the predator and of the body part of the prey that was attacked. In this short review we retrieved and analysed a preliminary list of studies published until December 2017, that used clay replicas of amphibians in ecological field studies. Overall 25 publications were analysed. The first scientific paper using amphibian clay models was published in 1994, but only after the year 2005 the use of clay replicas became frequent in herpetological field researches. The majority of studies were performed in tropical or subtropical ecosystems of Central and South America, and only a relative small number of studies were executed in temperate forests of North America and Europe. The most studied family was Dendrobatidae with nine species. In Urodela the Plethodontidae, with four species, was the most studied family. After the analysis of the main features concerning technical aspects, geographic distribution and temporal trend of these kind of studies, the pros and cons of the use of amphibian clay models are synthetically discussed.

KEY WORDS: aposematism, colour polymorphism, crypsis, plasticine, predation.

INTRODUCTION

Predation events on amphibians can be observed directly in the field or assessed indirectly by analysing stomach or faecal contents (Wells, 2007) or by stable isotope analyses (Remon et al., 2016; Willson et al., 2010) of predators. Indeed, many vertebrate and invertebrate animals are known to feed upon adult amphibians in the wild (for a review see Chapter 14 in Wells, 2007). However, most amphibian species are small, cryptic and nocturnal and, therefore, direct observation of their interactions with predators is problematic and often practically impossible. For these reasons, ecologist have used some kind of animal replicas, usually made from modelling clay, to obtain information about the potential predators that attack or feed on their focal species (Bateman et al., 2017).

Here we briefly recall that modelling clay, known also as “plasticine” (in fact, a brand name that is now commonly used to indicate a material), is obtained by mixing clay, wax and paraffin oil. This mixture produces a material that is soft, non-toxic, malleable, (i.e.it retains marks and impressions on its surface), easy to shape, colour and, finally, inexpensive. Interestingly, the analysis of the size and shape of the marks sometimes allows the identification of the predator giving important ecological information (e.g., Kuchta, 2005 and Salvidio et al., 2017), Usually, clay models are shaped to a realistic position imitating the appearance and size of the study species and then coloured with acrylic paint. In this way, the odour of the raw material is masked by the odourless paint reducing the influence of the modelling material on potential predators. However, being odourless introduces some other kind of bias, especially in those cases in which potential predators are attracted by smell rather than vision during prey search and detection (see also Rojas, 2016).

The different aspects discussed above are relevant in these kind of studies, in particular the one related to the costs of producing the models, because hundreds or even thousands of animal replicas made from modelling clay can be easily produced and displayed in the wild to test different ecological hypothesis (e.g., Mcelroy, 2016). In this way a high statistical power is achieved with a reduced field effort and usually with low financial budgets.

Concerning the use of animal clay models in evolutionary, ecological and ethological studies, a general evaluation has been recently carried out by Bateman et al. (2017). In general, clay or paraffin models have been used to mimic amphibians, reptiles, bird eggs and also small mammals (Bateman et al., 2017). The use of this kind of models is not restricted by the shape or colour of the focal species but mainly by its total size, this because models of large species will require a large amount of plasticine and will be difficult to transport and displace in the field.

In this review we analysed publications that used amphibian clay models to test different ecological hypothesis in field experiments, such as prey-predator interactions, intraspecific communication or habitat and microhabitat use (Rojas, 2016). Our aim was not to produce an exhaustive list of references on this specific subject, but to analyse temporal trend of these kind of studies, give some indication about the their general features and objectives and highlight the taxa involved and the geographic areas or ecosystems in which these studies were performed. Finally, we also introduced some of the main critical issues that may arise when using amphibian clay models in field experiments.

MATERIALS AND METHODS

Articles present in Scopus database and published up to December 2017, were retrieved by searching the following keywords in all article sections: “clay model”, or “paraffin model”, or “clay replica”, or “paraffin replica” in association with one of the following words: “amphibians”, “caecilian”, “frog”, “newt”, “salamander” and “toad”. In addition, references found in these papers were also checked. From each selected article the following information were obtained: author(s), Journal and year of publication, focal species and its family, material used in building the model (i.e. clay or paraffin), country and habitat in which the study was performed and if instrumental measurements of the colour of both the live species and their replicas were made. The existence of a temporal trend was assessed statistically by means of Mann-Kendall test (Gilbert, 1987).

RESULTS

In total, 25 publications that used amphibian artificial replicas were retrieved (see Annex 1). According to Scopus database, the first article that used amphibian clay models was published in 1994 and no other study was available until 2004 (Figure 1). From then onwards several other studies were published giving a general increasing trend for the entire period 1994-

2017 (Mann-Kendall trend test: P = 0.0004) and also for the shorter period 2005-2017 (Mann-Kendall trend test: P = 0.024).

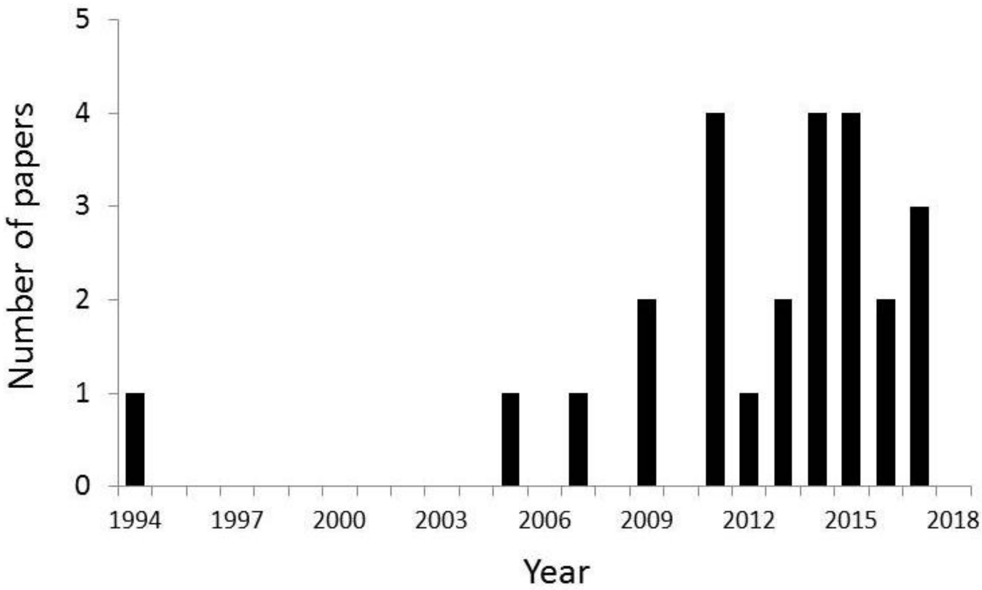

Fig. 1. Temporal trend of papers using amphibian models. The complete list is given in Table 2.

However, one of these articles was not an experimental field study, but rather a technical paper proposing an improved technique to produce simultaneously large numbers of frog models to be used in successive experiments (Yaeger et al., 2011). Therefore, this paper was not included in the following analyses that are based on the remaining 24 field studies.

The majority of studies used amphibian models made in plasticine (22/24 = 92%), while only two (8%) used models made with paraffin wax, a soft material made with a mixture of hydrocarbon molecules. The main difference between plasticine and paraffin wax is that at room temperature the former can by shaped by hand, while the latter has to be warmed to about 37°C and then melted into casting moulds.

Concerning the ecosystems in which the studies were performed, 17 out of 24 (71%) were tropical or subtropical forests of Central or Southern America (Brazil, Colombia, Costa Rica, French Guiana, Panama and Peru). Only 7 (29%) studies were performed in temperate forests of Europe (Italy and Spain) or the United States of America. All the studies were conducted in superficial terrestrial habitats, with the exception of the one by Salvidio et al. (2017) that compared the predation rate observed on models placed inside caves and in adjacent woodland habitats.

The complete list of the experimental species is reported in Annexe 1. The tropical family Dendrobatidae (that comprises almost two hundred tropical species, of which several conspicuously coloured species and known as "poison dart frogs") was the most studied (Table 1). This is not surprising, because many Dendrobatidae species are diurnal and brightly coloured also bearing aposematic patterns to advertise predators of the presence of toxic alkaloids in their skin (Saporito et al., 2007).

Table 1. Amphibian families and species studied by use of plasticine or paraffin models. Some studies involved more species simultaneously; *one study concerned two species belonging to two families, therefore this study was counted twice. Total numbers of species were obtained from the weabsite "Amphibian species of the world 6.0": http://research.amnh.org/vz/herpetology/amphibia/

Family	Number of studies	Number of study species	Studied species/total number in family
Dendrobatide	16	9	9/194 (5%)
Plethodontidae	5*	4	4/474 (1%)
Salamandridae	3*	2	2/120 (2%)
Bufonidae	1	1	1/604 (0%)

The aim(s) of the studies were diverse and in some cases multiple. Aposematism, mimicry and predator pressure exerted on different colour morphs of the same species found in different habitats or geographic regions were the most common research topics. Only in one study the Authors used clay models to test intraspecific communication between males of the Dendrobatid *Oophaga granuliferus*, showing that acoustic rather than visual stimuli elicited behaviour territorial responses in this species (van Wijngaarden and van Gool, 1984).

Very few studies (2/24) tested with field experiments the technical designs of amphibian artificial models; for example, the influence of motion and of spot size on the predation rate of the models were tested by Paluh et al. (2014, 2015).

Finally since the year 2011, the measurement by means of field instruments such as spectrometers of the spectral colour characteristic (e.g., hue, saturation and brightness) of both the focal amphibian species and their clay replicas is often performed. Overall these measurements were made in almost half of the analysed papers (12/24 = 48%; Annex 1), but in recent publications instrumental colour measurement is becoming the standard procedure to select a model's colouration matching as close as possible the one of the living focal animal (e.g. Dreher et al. 2015).

DISCUSSION

This short review clearly indicates that, in amphibian ecological studies, the use of clay models is a relatively recent but steadily increasing experimental practice. This because the technique is simple and inexpensive, permitting the preparation of hundreds or even thousands of amphibian replicas (see Table 2). Displaying in the field large number of models enables researchers to achieve high statistical power and allows testing rare events that are difficult or even impossible to perform in the lab with experimental animals. In addition, the use of artificial models testing prey-predator interactions, anti-predator behaviours or habitat adaptations may not require ethical or capture authorizations that will be needed for this kind of experiments involving live animals. Therefore, the use of artificial models is very advantageous and practical when planning experiments that imply possible predation events.

Table 2. Articles, listed in chronological order, published up to December 2017 using amphibian models. N.S = not stated.

Author(s), Year	**Family and species**	**Country**	**Habitat**	**Model type and number**	**Colour analysis method**	**Journal**
van Wijngaarden and van Gool, 1994	Dendrobatidae - *Oophaga granuliferus*	Costa Rica	Premontane wet forest	Clay 16	No analysis	Amphibia-Reptilia 15: 171-181
Kuchta, 2005	Plethodontidae - *Ensatina eschscholtzii*	USA	Temperate forest	Clay 454	No analysis	Copeia 2005: 267-271
Saporito et al., 2007	Dendrobatidae - *Oophaga pumilio*	Costa Rica	Tropical forest	Clay 800	No analysis	Copeia 2007: 1006-1011
Fitzpatrick et al., 2009	Plethodontidae - *Plethodon serratus*	USA	Temperate forest	Clay 700	No analysis	BMC Ecology 9:12
Noonan and Comeault, 2009	Dendrobatidae - *Dendrobates tinctorius*	French Guiana	Forest leaf litter	Clay 1260	No analysis	Biol. Lett. 5: 51-54
Chouteau and Angers, 2011	Dendrobatidae - *Ranitomeya imitator*	Peru	Lowland tropical forest	Clay 1800	No analysis	Am. Nat. 2011 178: 810-817
Hegna et al., 2011	Dendrobatidae - *Oophaga pumilio*	Panama	Rainforest	Clay 840	Spectro-photometer	Ann. Zool. Fennici 48: 29-38
Velo-Anton and Costa-Rivera, 2011	Salamadridae - *Salamandra slamandra*	Spain	Eucalyptus plantation	Clay 80	No analysis	Herpetol. Notes 4: 299-301.
Yaeger et al., 2011	Technical paper			Clay		Herpetol. Rev., 42: 357-359.
Stuart et al., 2012	Dendrobatidae - *Oophaga pumilio*	Costa Rica	Tropical forest	Clay 2400	Spectro-meter	PLoS ONE 7: e48497
Hegna et al., 2013	Dendrobatidae - *Oophaga pumilio*	Costa Rica and Panama	Rainforest	Clay 1218	Spectroradiometer	Evol. Ecol. 27: 831-845
Richards-Zawacki, 2013	Dendrobatidae - *Oophaga pumilio*	Costa Rica	Tropical forest	Clay 1600	Spectrometer	Evol. Ecol. 27: 783-795
Kraemer and Adams, 2014	Plethodontidae, Salamandridae - *Plethodon cinereus, Notophthalmus viridescens*	USA	Temperate forest	Clay N.S.	Spectrometer	Evolution 68: 1197-1206
Paluh et al., 2014	Dendrobatidae - *Oophaga pumilio*	Costa Rica	Tropical forest	Clay 600	No analysis	J. Herpetol. 48: 249-254
Rojas et al., 2014	Dendrobatidae - *Dendrobates tinctorius*	French Guiana	Tropical forest	Paraffin 900	No analysis	Behav. Processes 109: 164-172

Author(s), Year	**Family and species**	**Country**	**Habitat**	**Model type and number**	**Colour analysis method**	**Journal**
Author(s), Year	Family and species	Country	Habitat	Model type and number	Colour analysis method	Journal
Willink et al., 2014	Dendrobatidae - *Oophaga granuliferus*	Costa Rica	Tropical forest	Clay 3600	No analysis	Biol. J. Linn. Soc. 113: 580-589
Dreher et al., 2015	Dendrobatidae - *Oophaga pumilio*	Costa Rica and Panama	Rainforests	Clay 1600	Spectrometer	PLoS ONE 10: e0130571
Flores et al., 2015	Dendrobatidae - *Dendrobates auratus*	Panama	Organic coffee plantation	Clay 840	Spectrometer	Ecol. Evol. 5 : 4603-4616
Paluh et al., 2015	Dendrobatidae - *Oophaga pumilio*	Costa Rica	Tropical forest	Clay 800	No analysis	J. Herpetol. 48: 244-254
Rojas et al., 2015	Dendrobatidae - *Adelphobates galactonotus*	Brazil	Tropical forest	Paraffin 2016	Spectrometer	Behaviour 152: 1037-1057
Kraemer et al., 2016	Plethodontidae - *Plethodon cinereus*	USA	Temperate forest	Clay 900	Spectrometer	Biol. J. Linn. Soc. 118: 889-900
Mcelroy, 2016	Bufonidae - *Rhinella alata*	Panama	Forest leaf litter	Clay 2628	Photospectrometer	Biol. J. Linn. Soc. 117: 285-294
Preissler and Prohl, 2017	Dendrobatidae - *Oophaga pumilio*	Costa Rica	Tropical forest	Clay 2700	Spectrometer	Evol. Ecol. 31: 683-694.
Salvidio et al., 2017	Plethodontidae - *Speleomantes strinatii*	Italy	Temperate forests and caves	Clay 191	No analysis	Sci. Nat. 104: 20
Velo-Anton and Costa-Rivera, 2017	Salamadridae - *Salamandra slamandra*	Spain	Eucalyptus plantation and shrubland	Clay 105	No analysis	Acta Ethol. 20: 243-253

The majority of the analysed publications were executed in tropical or subtropical environments, in particular in rain forests where amphibian species are more diverse, diurnal and often characterised by bright colourations, in relation to some kind of aposematic defensive strategy (Rojas, 2016). However, some recent studies were also performed in temperate ecosystems mainly involving species belonging to the Plethodontidae and Salamandridae families (i.e., Kraemer and Adams, 2014; Kraemer et al., 2016; Velo-Anton and Cordero-Rivera, 2017). These data suggest that the use of clay models is rapidly expanding from tropical regions to less diverse but still understudied ecosystems in temperate areas.

However if, on one hand, this technique has many advantages, on the other it may have several challenging features that limit its use and may cause problems in a reliable interpretation of results. Therefore, many issues should be carefully evaluated before planning field experiments with clay models of amphibians (Rojas, 2016; Bateman et al., 2017). Firstly, the absence of motion may reduce the reliability of results obtained when using clay models, because many visual predators exploit movement to detect, select and attack their prey (Paluh et al., 2014). A second critical aspect is the absence of typical prey odours (Rojas, 2016). Indeed, experiments using odourless models are unable to correctly assess the importance of predators that do not rely on vision in prey selection and capture, as is the case of snakes and lizards that are known to prey upon amphibians, both in tropical and temperate ecosystems (Wells, 2007). A third criticism concerns the reliability of the replica colouration in comparison to the living focal species. This occurs because the experimenter eye may have a very different perception of light wavelengths in comparison to the many potential predators of amphibians. For example, many bird species possess a broader sensitivity in comparison to the human eye, in particular to ultraviolet wavelengths (Cronin et al., 2014). Therefore, without an instrumental measure of the spectral colour characteristics of the live animals and their plasticine models, it will be difficult to obtain reliable results on the relative proportion of attacks by different groups of predators, such as mammals, birds and reptiles that possess a different vision of colours. A fourth criticism addresses the continuous exposition of artificial models to potential predation in the wild. In fact, different species of predators and prey may display different daily patterns of activity. Therefore, it may become artificially possible that diurnal visual predators will unnaturally interact with clay models of nocturnal species or *vice versa*, this because models are available in the field continuously during 24 hours a day, producing paradoxical results with limited ecological relevance.

All these issues should be carefully addressed and resolved when planning experiments with clay or paraffin amphibian models, to reduce unreliable or spurious results, that could be difficult to interpret from a behavioural or ecological point of view.

In any case, the use of clay models remains a promising technique that, if used properly, could be helpful in describing the interactions between amphibians and their predators and that could contribute to better understanding the complex evolution of aposematism, crypsis, and the evolution of local colour morphs within conspecific populations of animals living in different habitats.

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