

# The estimation of *post-mortem* interval (PMI) in reptiles and amphibians: Current knowledge and needs

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Studies on *post-mortem* interval (PMI or time since death estimations) in reptiles and amphibians have been sparse. Some limited information exists but this has usually emanated from the work of veterinarians and biologists and is often restricted to individual case reports or small samples. As a result, there is little that can be used reliably in forensic cases and investigators often have to depend on data from mammalian and avian studies. Work on fish, mainly directed towards promoting food hygiene, may or may not be relevant. Reptiles and amphibians present a range of challenges in terms of accurately assessing changes due to autolysis and decomposition, owing to their high variability in morphology and lifestyle. In particular, there are effects due to ectothermy, the different anatomical features of these two groups of vertebrates, the marked variation in body size of diverse taxa of reptiles and amphibians, and seasonal fluctuations in subcutaneous and internal fat content. Eggs, embryos and fetuses present particular challenges and the presence of a larval stage in amphibians is a further complication. Many basic questions remain unanswered. Specific research is needed, and this should involve both amateur and professional herpetologists. Ways in which useful information may be collated include the keeping of properly recorded accounts of changes in dead reptiles and amphibians, especially those kept in captivity or which can be carefully monitored, such as road-kills. Experimental studies are also needed, carried out in collaboration with for example pathologists.

*Key words:* autolysis, decomposition, *post-mortem* interval (PMI), reptiles and amphibians, time since death.

## INTRODUCTION

The *post-mortem* interval (PMI - the time that has elapsed since death) can be very important in forensic investigations. There has been extensive work on PMI in humans (Knight, 2002; Di Maio & Di Maio, 2001), but remarkably little specific research appears to have been carried out on PMI in animals. Exceptions include the scholarly study on *post-mortem* histological changes in laboratory rats by Seaman (1987) and the findings yielded by domestic pigs to provide baseline data for human forensic studies (Archer, 2004; Schoenly et al., 2007). Erlandsson and Munro (2007) reported studies in domesticated (beagle) dogs and even in this context lamented that “in the veterinary medical field there is a paucity of published data...”, a sentiment that is undoubtedly true in other areas of animal, especially wildlife, forensics. Publications on animal forensics include ectothermic vertebrates, and also invertebrates (Cooper & Cooper, 2007). The seminal paper on medico-legal necropsy by Wobeser (1996) refers to “wildlife” but in fact deals predominantly with mammals.

In this paper, the need for research on PMI in reptiles and amphibians is discussed. Throughout, the term “decomposition” is considered to be synonymous with “*post-mortem* change” and this is assumed to involve a combination of autolysis (the breakdown of tissues as a result of the chemical action of intracellular enzymes) - and putrefaction, the breakdown of tissues by bacteria and sometimes other organisms. Mummification is considered a form of decomposition, characterized by desiccation

of tissues, which is especially likely to be encountered under conditions of high temperature and low relative humidity (Figs. 1 and 2). The term “taphonomy” is used in a broader sense here, comprising the study of *post-mortem* transport, burial, decomposition and compaction that occur in a carcass after death and which may lead, ultimately, to fossilization.

### Considerations in reptiles and amphibians

The features of the classes Reptilia and Amphibia that are relevant to both the progression and the evaluation of PMI are listed in Table 1. In particular, information on environmental parameters, both before and after death, is important. In the case of captive reptiles and amphibians, knowledge of the temperature at which the animals have been kept and of any treatment given is vital in forensic investigations, including exact location of death (e.g. under heat lamp). When working with free-living reptiles and amphibians, meteorological advice concerning temperature and humidity may be critical to accurate assessment of PMI.

Clearly, the forensic investigator needs to be familiar with the biology of the species. It seems likely that those studying PMI in reptiles and amphibians need to have knowledge of histology of these taxa and not rely on data from mammals.

### Literature review

Few specific studies on reptile and amphibian PMI have been reported. This dearth of information was recognized by Frye (1999), who published a short, but very important,



**Fig. 1.** A “dhub”, *Uromastyx* species, that has been dead under desert conditions for several weeks. Much of the body is mummified but areas of skeleton are exposed as a result of invertebrate scavenging.

paper on the establishment of time of death in reptiles and amphibians. Frye (1999) outlined key “*post-mortem* indicators” including presence or absence of blood flow or myocardial contractions (using ultrasonic Doppler devices and electrocardiography), the ability of skeletal muscle to respond to galvanic stimulation, intra-ocular pressure and deep-core body temperatures. He also lists other changes that could be used in the assessment, such as rigor mortis, lividity (hypostasis), bile-staining, chromatophore relaxation and expansion, and the development of a characteristic odour. Frye’s 1999 paper and a sequel (Frye, 2008) remain a crucial guide to the criteria that can be of value in assessing PMI in reptiles and amphibians, but neither provides any indication of the time it takes for such changes to occur.

It would be wrong to assume that nothing has been published on *post-mortem* change in reptiles and amphibians (Sis & Landry, 1992; Cooper, 2008). However, the literature is scattered in a variety of diverse journals and reports, and has usually been incidental to other studies. One notable exception was the research on deterioration of green sea turtle (*Chelonia mydas*) eggs after known embryo mortality (Phillott & Parmenter, 2007). Often entomologists have played the leading role in providing the few data that we have. Recently, for example, Nelder et al. (2009) studied succession patterns of blowflies (Diptera: Calliphoridae) using specimens of freshly-frozen American alligators, *Alligator mississippiensis*, mirroring to a certain extent earlier work employing the same model by Watson & Carlton (2005). In the same vein, Cornaby (1974) used lizards (*Iguana iguana* and *Ctenosaura similis*) and the cane toad (*Bufo marinus*) to investigate the arthropods that visited these carrion baits. Kneidel (1984) studied frogs, toads and snakes, amongst other vertebrates and invertebrates, in studies on carrion-breeding Diptera and specifically drew attention to the need for more work on small animals in contrast to the more numerous research projects on large mammals.

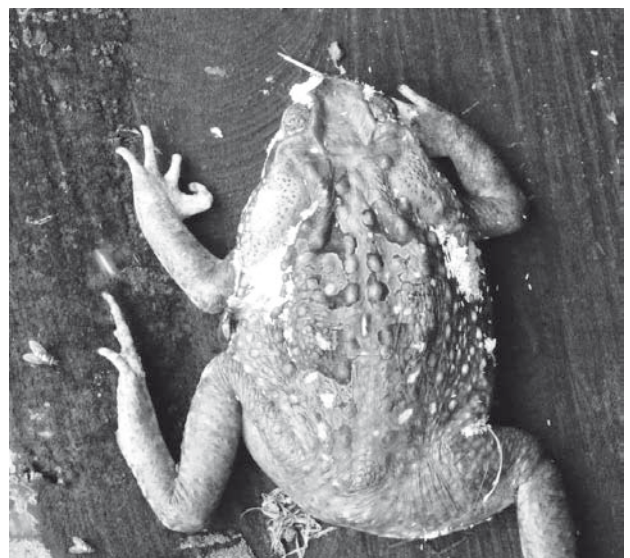
Fretey & Babin (1998) attempted to link arthropod succession in dead leatherback turtles (*Dermochelys coriacea*) with determination of PMI. However, some invertebrate species have been reported to show a “host shift,” moving from dead to live animals for food, a

strategy that can easily complicate forensic studies. An example is the burrowing beetle (*Nicrophorus pustulatus*), which will devour the eggs of snakes (Smith et al., 2007). Certain signal flies (Diptera: Platystomatidae) usually scavenge dead sea turtle eggs but may on occasion devour live embryos (Hall & Parmenter, 2006). The scuttle fly (*Megaselia scalaris* Diptera: Phoridae) is increasingly recognized as a species with broad feeding habitats (Zwart, 2005), and is known to attack live, as well as dead, animals including frogs (Villa & Townsend, 1983). It is recognized for its importance in human forensic medicine (Dewaele et al., 2000). These and other species could thwart assessment of PMI in reptiles and amphibians as it would not necessarily be clear as to whether they initially attacked a live or a dead host.

Other information that can help in assessing PMI in reptiles and amphibians can be found in ecological studies. To investigate the fate of dead chelonians (Dodd, 1995), a key was developed for evaluating time since death of desert tortoise osteological remains (Averill-Murray, 2000).

Surprisingly little research on PMI in reptiles and amphibians seems to have been performed to interpret archaeological and palaeontological findings (Tyler et al., 1994; Mackness & Sutton, 2000; Rothschild, 2009). This is in striking contrast to studies on fossils of birds, where research has included studies on bioerosion of avian bones (Davis, 1997) and the monitoring of carcasses; the results of the latter were used to interpret findings in Jurassic and Eocene fossils (Davis, 1992, 1996; Davis & Briggs, 1998).

More work has been carried out on the decomposition of fish than that of reptiles and amphibians on account of food hygiene measures. There is much published information on this topic: a useful review is to be found on the Food and Agricultural Organization (FAO) website (<http://www.fao.org/docrep/v7180e06.htm>). Even in fish,



**Fig. 2.** *Post-mortem* decomposition can be very rapid in hot, humid environments, as illustrated by this toad (*Bufo marinus*). Within 24 hours there are clusters of fly eggs (white), including some adjacent to the animal's left parotoid gland.

**Table 1.** Factors that may influence *post-mortem* change (autolysis) in reptiles and amphibians.

Species	Factor (s)	Possible effects	Questions
Reptiles and amphibians	Ectothermy May retard and/or complicate <i>post-mortem</i> change: for example, bleeding may occur after clinical death. Temperature plays a key part in the biology of living reptiles (see Lance, 2003), and the ectothermic nature of both them and amphibians may still be significant after death.	Tissues may be resistant to hypoxia: for example, the animal's heart may continue to beat long after clinical death (Cooper et al., 1989; Mader, 2005)	What is the relevance of ectothermy, especially the resistance of the tissues of some species to hypoxia and other changes?
	Marked variation in body size. Depending upon other factors (usually environmental), rates of <i>post-mortem</i> change may differ markedly between species.	Small animals cool and warm more rapidly than those that are large.	Can differences in PMI on account of size be reliably predicted and measured?
	Seasonal variation in subcutaneous and internal fat content	Changes in rate of autolysis of organs and tissues depending upon the time of year	How significant are cyclical changes in amount of subcutaneous fat and size of fat bodies?
	Different appearance from mammals in terms of some tissues (e.g. nucleated red blood cells and melanin deposits in the liver)	Can complicate interpretation of changes (e.g. nuclei of red blood cells may resemble pyknotic or degenerating cells)	Do these differences from mammals influence the speed and mechanisms of <i>post-mortem</i> decomposition or only hamper interpretation?
	Habitat in the wild: terrestrial, arboreal, fossorial, amphibious, aquatic	Fluctuating temperatures and rainfall may influence decomposition, as in mammals (Archer, 2004). In hot dry locations, carcasses may become mummified rather than decompose (Fig. 1). Alternating exposure to different conditions can easily complicate assessment of PMI (Fig. 2)	Can differences in habitat and environment be reliably predicted and measured?
	Management in captivity, especially the provision of artificial heat and treatment	May accelerate or retard <i>post-mortem</i> decomposition or complicate clinical tests (Frye, 1999)	As above; can the effects be predicted and measured?
Reptiles	Presence, to a greater or lesser extent, of a well-keratinised layer over the skin. Apparently, not yet studied in detail	The integument can be relatively impervious to attack by invertebrates, and may protect underlying viscera not only from them but also from some environmental factors such as rain. Most chelonians and crocodylians and many lizards have thick external keratin and a few reptiles have robust osteoderms. These structures may accelerate or retard the rate of decomposition, depending upon the circumstances. The periodic sloughing of skin, especially by snakes and lizards, may affect autolysis	How does the well-keratinized skin of a reptile, as opposed to the mucous skin of an amphibian, influence <i>post-mortem</i> change?
	Immature stages (fetuses/eggs). Fetuses and fetal material generally rapidly deteriorate	<i>Post-mortem</i> change is affected by the extent to which the fetus is protected by an eggshell (oviparous species) or is enclosed within the female tract (viviparous species), as well as by incubation temperatures and humidity	Are there significantly different features in terms of <i>post-mortem</i> change between embryonated and non-embryonated spawn and eggs?

Amphibians	Generally a thin, poorly keratinized and mucous skin. Some exceptions: the dorsal skin of, for example, bufonids is thick, tuberculated and therefore relatively resilient to attack. Toxic secretions, such as bufotoxin, may or may not delay multiplication of bacteria and other organisms and deter certain predators.	The integument is susceptible to attack by invertebrates, fungi, and bacteria	How does the mucous skin of an amphibian, as opposed to well-keratinised skin of a reptile, influence <i>post-mortem</i> change?
Immature stages (spawn/tadpoles)	<i>Post-mortem</i> change is affected by the aquatic environment	Are there special forensic considerations when dealing with the larvae and spawn of amphibians, which are usually aquatic, and with the eggs of reptiles?	

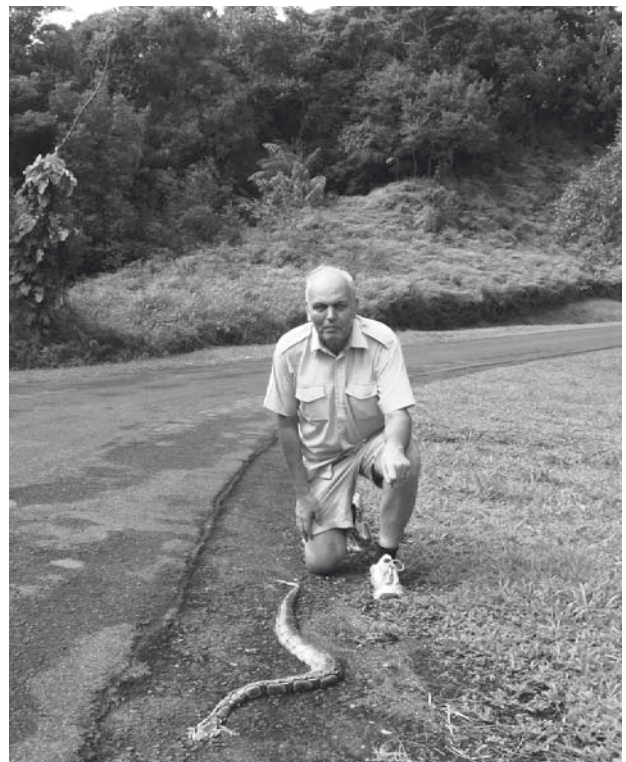
however, most of the research has focused on the main gross signs of *post-mortem* decomposition, such as the onset of rigor mortis, ocular changes, loss of scales and colour changes in skin, gills and internal organs, rather than on detailed study of the sequence of such features and the timing of their onset. Nevertheless, the experience obtained from studies of deterioration of fish may prove helpful in understanding PMI in reptiles and amphibians, perhaps especially the latter.

**The needs**

What is needed if we are to augment our present scant knowledge and understanding of PMI in reptiles and amphibians?

1. We need properly recorded accounts of the sequence of events and the time taken when given species of reptile or amphibian start to change (decompose/autolyse) after death. These data can readily be collected by amateurs such as field naturalists and those who keep captive animals. All it requires initially is for such persons to observe and record regularly what they see and smell as a frog, a salamander or a snake starts to decompose. Carefully monitored road-kills provide ideal material for such studies (Fig. 3), and may also, incidentally, provide data on the health of the animals (Wright et al., 2004). Even allowing for complications relating to the extent of injuries and the possible presence of predisposing infectious disease, this preliminary work could yield valuable basic information on the likely changes in different organs and provide initial guidance to those embarking upon more scientific investigation (below). Such observational studies are far from novel; however, in the past they have tended to concentrate on mammals (rabbits *Oryctolagus cuniculus*: Chapman & Sankey, 1955; pigs (*Sus scrofa*): Payne, 1963).
2. More studies could be initiated on individual species, making observations as above but looking at a number of dead animals kept under standard environmental conditions (temperature, relative humidity, with or without contact with carrion beetles, flies, etc.). Some guidance on method, especially how to contain such carcasses securely and how to trap insects that

- are attracted to the carrion, can be obtained from the aforementioned papers.
3. Specific research is called for on factors that are particularly relevant to these taxa. For example, the effects of *post-mortem* environmental factors on the morphology of reptile scales, similar to those already performed on mammalian hair (Quadros & Monteiro-Filho, 1998) should be undertaken.
4. Researchers must strengthen links with practitioners in other fields whose work might be relevant to a better understanding of PMI in reptiles and amphibians. Examples are veterinarians who deal with clinical cases of myiasis, where there can be a significant overlap of interest and where difficulties in interpretation can occur (Bolek & Coggins, 2002).



**Fig. 3.** A fresh road-kill presents opportunities to study *post-mortem* change under relatively controlled conditions. This boa constrictor (*Boa constrictor*) was killed overnight in Trinidad, conveniently on the edge of a forest track.

Important questions about the estimation of PMI in reptiles and amphibians that still need to be addressed are given in Table 1. Some other, more general, considerations are listed below:

- Is it reasonable to study reptiles and amphibians together, or are they too different in morphology to be considered similar? Many veterinary pathologists would argue that reptiles decay more slowly than amphibians and seem less susceptible to putrefaction.
- What effect does the presence or absence of ingesta in the gastro-intestinal tract of reptiles and amphibians have on *post-mortem* change? To what extent does this reflect the resident or transient flora? Does this mean that there are differences between herbivorous, omnivorous and carnivorous species?
- How significant is scavenging in altering the early taphonomic processes? Do species of reptiles and amphibians differ greatly in the extent to which they are attacked by vertebrate and invertebrate predators and in the sort of damage that these inflict? Does mutilation by scavengers affect insect succession on carcasses of reptiles and amphibians as it does some species of mammal (Ellison, 1990)? How different is insect and other arthropod activity on the carcasses of reptiles and amphibians from that which is well-documented in mammals?
- To what extent can we extrapolate published data from fish to amphibians to reptiles? Do those that are wholly (tadpoles, neotenus species), or partially aquatic have more in common with fish than do terrestrial species? Are recognized features of fish, such as their low muscle glycogen and correspondingly less *post-mortem* lactic acid in comparison with that of mammals (Gatica et al., 2008) true also of the other ectothermic vertebrates?
- Do methods used in mammal studies, such as assessment of muscle changes, quantification of gastric emptying and digestion, flow cytometry and vitreous fluid potassium measurements, have any relevance to reptiles and amphibians? Can we automatically assume that temperature-based estimation of PMI is not applicable to ectothermic animals or can the method be modified for use in these species?
- To what extent do *post-mortem* changes in dead reptiles and amphibians mirror those that are well-recognized in mammals? For example, is the sequence of autolysis similar, in that the liver and pancreas undergo *post-mortem* alteration rapidly, while muscle remains relatively unaffected. The broad-based classification of stages of decomposition defined so long ago by Bournemissza (1957) as “fresh, bloated, active decay, advanced decay and putrid/dry remains” appear to be applicable to large reptiles such as crocodylians (Watson, 2004; Watson & Carlton, 2005). Consider, however, that these gross observations may not reflect the changes at organ and tissue levels.
- How important in the tissues of reptiles and amphibians are *ante-mortem* factors in determining

*post-mortem* changes and the speed with which they occur? This is a well-recognized feature in other taxa, including non-mammalian species. In both ducks (Liao et al., 2009) and fish (Gatica et al., 2008; Thomas et al., 1999), struggling before death reduces “carcass quality.” What is the relevance of the health status of the animal and whether captive specimens have been treated before death with antibiotics that may have inhibited bacterial fermentation and putrefaction?

- Is the presence of an odour helpful in assessing PMI in reptiles and amphibians or, as Frye (1999) pointed out, is it better considered a sign of impending death and therefore, possibly, complicates analysis?
- Are data on PMI in reptiles and amphibians available in languages other than English? Such literature is often overlooked in studies, to the detriment of the research (Cooper, 2004).

### Conclusions

There would appear to be a pressing need for focused research on PMI in reptiles and amphibians. Such studies are complicated, sometimes hampered, by the large number of species involved and by their great variability in morphology. Nevertheless, significant contributions could be made if the quality of forensic evidence is to be raised. Both professional and amateur herpetologists have an important role to play.

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