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ORIGINAL ARTICLE

Protocol to classify the stages of carcass decomposition and estimate the time of death in small-size raptors

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Abstract

One of the most common wildlife crimes involving birds worldwide is malicious poisoning. Post-mortem examination and toxicological analysis are essential for a proper diagnosis of the cause of the poisoning. However, investigators often require an estimate of the time of death, which is best determined by identifying the stage of carcass decomposition. The aim of this article is to propose a scoring method to classify the stages of carcass decomposition and thus provide an estimate of the time of death in small-size raptors. This protocol can be used by practitioners, forensic veterinarians, researchers, authorities and personnel collecting carcasses in order to standardize methods and minimize subjectivity. For this purpose, 12 carcasses of Common kestrel (*Falco tinnunculus*) were exposed to external weather conditions (in the period 4–19 July 2019) in Murcia, Southeastern Spain. The ambient temperature and relative humidity, body core temperatures and carcass weights were measured at intervals over the study period. Necropsies were performed (2 birds at each interval) at 1–2 h, 24 h, 72 h, 96 h, 7 days and 15 days after death. The necropsy of a previously frozen bird was performed to act as a comparison with non-frozen fresh individuals. Six stages of the post-mortem autolytic process were selected: fresh carcass, moderate decomposition, advanced decomposition, very advanced decomposition, initial skeletal reduction and complete skeletal reduction. To classify the carcasses according to these categories, a scoring method is proposed considering 5 parameters: state of the eyeballs, tongue/oral cavity, pectoral muscle, internal organs and other features. Several parameters affecting the process of the decomposition are discussed.

Keywords Autolysis · Carcass · Decomposition · *Falco tinnunculus* · Necropsy · Time of death · Forensic

Introduction

One of the most common wildlife crimes worldwide is poisoning, being a challenge not only for wildlife managers, enforcement authorities and veterinarians but also for toxicology and forensic science laboratories. In Spain alone, between 1992 and 2013, 18,503 animals were identified as having been poisoned, including kites, vultures, eagles, wolves and bears (Cano et al. 2016). This implies a significant threat to European wildlife biodiversity. As an example, more than 90% of European populations of Cinereous vulture (*Aegypius monachus*), Common vulture (*Gyps fulvus*) and Egyptian vulture (*Neophron percnopterus*) are breeding in this country. For that reason, current efforts within the research community are also focused on supporting the fight against wildlife crime.

Post-mortem examination and toxicological analysis are essential for the accurate diagnosis of wildlife poisoning cases (Brown et al. 2005). Equally, determining the time of death is important to help identify the circumstances surrounding the event within a time frame and to support investigations into the identity of those responsible. This is a very important issue in judicial processes, and particularly in criminal offences involving wildlife. Frequently, forensic toxicology laboratories receive a wide variety of biological matrices or full carcasses from wild mammals and birds in different decomposition stages. However, few information is available on the process

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of decomposition and the possibility to assess the time of death in avian species (Brooks 2016; Jarmusz and Bajerlein 2019; Oates et al. 1984). There is also a lack of appropriate and standardized protocols for the correct classification of the carcass decomposition in wild birds. Therefore, appropriate and easy-to-follow protocols are needed to classify the stages of carcass decomposition and estimate the time of death in wild birds, which are validated and may be used to support the investigation of wildlife poisoning crime scenes, the prosecution of those responsible and better case resolution.

The aim of this article is to propose a scoring method for carcass classification according to the degree of decomposition and estimation of time of death in small-sized raptors. For this purpose, a decomposition experiment was carried out in Common kestrels (*Falco tinnunculus*). This species was selected since there were a sufficient number of wild bird carcasses (of similar morphology) available in the Wildlife Recovery Centre (WRC). This protocol can be used by practitioners, forensic veterinarians or researchers in order to standardize methodologies and estimate the time of death in small-sized raptors under similar weather conditions. The protocol is also intended to provide a resource for official authorities or personnel in charge of carcass collection in the environment. We give details so that non-specialists can also follow the protocol, classify the carcass decomposition stage trying to minimize subjectivity and estimate the time of death. This may help forensic toxicology laboratories to improve the wildlife poisoning diagnosis as the data will have been gathered from the crime scene without being lost due to delays.

Materials and methods

The experiment was conducted using 13 carcasses of Common kestrel (test birds). The individuals selected came from the "Santa Faz" WRC, Alicante, Southeastern Spain. Birds used in the decomposition experiment were euthanized because of an unfavourable prognosis to be released to the wild due to flight impairment caused by traumatic wing injuries. The individuals were kept for at least 1 month under the same management conditions at the WRC to ensure the homogeneity of the population. Detailed information of the individuals is described in Table S1 (Supplementary Material). All procedures performed were in accordance with the ethical standards of the *Comité Ético de Experimentación Animal* (CEEA)—University of Murcia (CEEA 549/2019), and all applicable institutional, local, and national guidelines and laws were followed. Euthanasia was performed by intravenous administration of a lethal dose of sodium pentobarbital. The investigation took place at the outdoor facilities of the Toxicology and Forensic Veterinary Service at the University of Murcia, southeast of Spain. Immediately after euthanasia the carcasses were placed in sternal recumbency on a dry gravel substrate. They were left exposed continuously to outside weather conditions (see Table S2 for additional details) and protected from predation with a wire mesh cage (Fig. 1). Twelve carcasses were used to carry out the decomposition experiment, and, additionally, one carcass was frozen at − 20 °C 6 h after euthanasia to assess the effect of freezing, since the freezing process is known to cause histological changes and gross appearance of carcasses (Cooper 2013).

The autolytic process study was carried out during the period from 4 July (8:30 p.m.) to 19 July (11:00 a.m.) 2019. The ambient relative humidity, ambient air temperature and internal temperature of the carcasses were continuously measured using probes. The mean ± SD (min-max) ambient air temperature (°C), humidity (%), day duration (hours) and wind speed (km/h) recorded were 30 ± 2 (24–33) °C, 54 ± 8 (45–70)%, 14:33:45 ± 0:05:05 (14:25:00–14:41:00) hours and 9.16 ± 1.17 (6.90–11.30) km/h, respectively (Table 1; Fig. 2; Table S2). All carcasses (except for the 2 individuals necropsied on day 0 and the frozen carcass) were weighed daily at the same time every day (Fig. 3).

Necropsies were performed at the following times after euthanasia: Day 0 (1–2 h), Day 1 (24 h), Day 3 (72 h), Day 4 (96 h), Day 7 and Day 15 (2 individuals per stage). The necropsy of the (defrosted to room temperature) frozen individual was performed after 1 week of freezing for comparison with the non-frozen fresh individuals (Day 0). During the necropsy, photographs were taken and detailed descriptions of the degree of external and internal decomposition of the carcasses and the presence of insect fauna were recorded. *Rigor mortis* of the carcass was also estimated. This information was collated and examined in order to select the carcass parameters, which showed identifiable signs of visible degradation that could be easily and clearly scored. Once identified, each parameter was scored with an increasing numerical value according to the decomposition degree, together with a detailed description and picture for each numerical score. The scores obtained in the different parameters were summed and a classification system in different degradation categories developed, where each stage or category is assigned a range of scores.

Results and discussion

Carcass decomposition was categorized according to the scoring system in six categories (Table 11). After a thorough examination of the photographs taken and the detailed descriptions registered on the degree of external and internal decomposition of the carcasses during the experiment, 5 different parameters were selected to be scored since they showed clear visible degradation during the decomposition process: (1) eyeballs, (2) tongue/oral cavity, (3) pectoral (breast) muscle, (4) internal organs (mainly the liver as a reference organ) and (5) other features (blood colour and feathers status). A score

Fig. 1 Carcasses of Common kestrel (*Falco tinnunculus*) in sternal recumbency with temperature/humidity probes inside the protective cage

system, ranging from 0 to 3, is assigned to each of these parameters according to the description and photographs presented in Table 22. After that, the scores obtained for the 5 parameters evaluated are summed in order to classify the stage of carcass decomposition into the following six categories, which were considered easily distinguishable: fresh carcass (0–2 points), moderate decomposition (3–6 points), advanced decomposition (7–11 points), very advanced decomposition (12–15 points) and initial skeletal reduction (16 points) (Table 11). Complete skeletal reduction is described but it was not scored, because the study arrives until day 15.

In cases of incomplete carcasses (e.g. due to predation) the main information should be reported from the liver and other internal organs. If one of the parameters is missing, an estimation could be done considering the most frequent score for other parameters. However, this protocol cannot be applied to carcasses with more than two parameters missed.

Different categories have been used to classify the phases of decomposition. However, they are generally referred to human bodies and/or mammal carcasses (Brooks 2016).

Carcass decomposition categories

The days elapsed after death in this experiment, the environmental conditions, the carcass decomposition stages and corresponding scoring, and the cadaveric fauna observed were compiled in Table 11. All the information that may be useful when estimating the carcass decomposition stage and time of death in future studies is also shown (Table 22).

The *fresh carcass* (1–2 h after death) has eyeballs with convex shape in lateral view which appear bright, the non-pigmented oral mucous membrane and the tongue remain pink and turgid, the pectoral muscle masses are red in colour and have a turgid consistency and it is easy to separate the skin from the muscle during the necropsy, the internal organs (especially the liver as a reference organ, since not all organs decay at the same rate) maintain their turgid structure, consistency and natural colour and the blood is red. The smell of the body is like fresh blood or has no smell (this characteristic should be noted; however, it is subjective). The feathers are in good condition and do not easily detach from the body. The *rigor mortis* process begins.

The carcass with *moderate decomposition* (1 day after death) has eyeballs which become opaque and lose their turgor with a collapsed appearance, the oral cavity and tongue are pale and have a dry appearance and the pectoral muscle also becomes red pale but is still easy to detach from the skin. The internal organs have a slightly dehydrated and dull structure and consistency (surface a little "wrinkled"), and the colour is darker compared with the natural colour, being generally reddish and homogeneous between the organs. The smell of decomposition begins to manifest itself, and the blood becomes red dark. The *rigor mortis* still present in beak and legs.

Table 1 Time of death estimation and carcass decomposition categories. Days after death, weather conditions, carcass decomposition stages, *rigor mortis*, and cadaveric fauna found in Common kestrel (*Falco tinnunculus*) during the experiment (4–19 July 2019).

Days (hours) after death*	Internal temperature (°C)/ambient (°C)/Relative humidity (%)[a]	Carcass decomposition category[b]	Scoring[c]	*Rigor mortis*[d]	Cadaveric fauna[e]
Day 0 (1–2)	29.91/26.75/65.35**	Fresh	0-2	30 min: in neck and legs 60 min: more intense (almost complete) in neck and legs (less appreciable in digits and beak) 90 min: complete in neck and legs and intense in wing and beak 150 min: almost complete 390 min: complete	Absent
Day 1 (24)	32.87/30.17/58.31	Moderate decomposition	3-6	After 24 h: slightly decreased in neck and digits (still present in beak and legs) 48 h: more noticeable decrease	Eggs and larvae (3 mm) in oral cavity, adult flies
Day 3–4 (72–96)	32.74/33.17/44.63 28.67/27.97/60.33	Advanced decomposition	7-11	72 h: disappeared completely	Larvae (up to 6 mm) within the carcass, coleoptera and grey fly adults
Day 7	32.81/30.10/50.65	Very advanced decomposition	12-15	-	Coleoptera and smaller number of larvae
Day 15	33.90/30.09/NM	Initial skeletal reduction	16	-	Coleoptera and ants
> 15 days Not studied		Complete skeletal reduction	-	-	Not studied

*It should be considered that the evolution of the decomposition, cadaveric fauna, and weight loss vary considerably depending on the environmental conditions (see Fig. 2), the animal characteristics, and death circumstances, so the time required to reach the different carcass decomposition categories must be adjusted according to each situation

**Data from 8.30 p.m.

[a] The mean value for all individuals per day is presented. NM: Not measured

[b] Based on the criteria presented on Table 2

[c] Total scoring = eyeball score (0–3) + tongue/oral cavity score (0–3) + pectoral (breast) muscle score (0–3) + internal organs score (liver, 0–3) + others score (feathers/blood, 0–3). See the description and pictures to score the different parameters in Table 2. Initial skeletal reduction stage is easily recognizable; it receives the total scoring of 16

[d] The time to reach the rigor mortis can vary depending on the species, size of the individual, and the circumstances surrounding death

[e] See Fig. 5

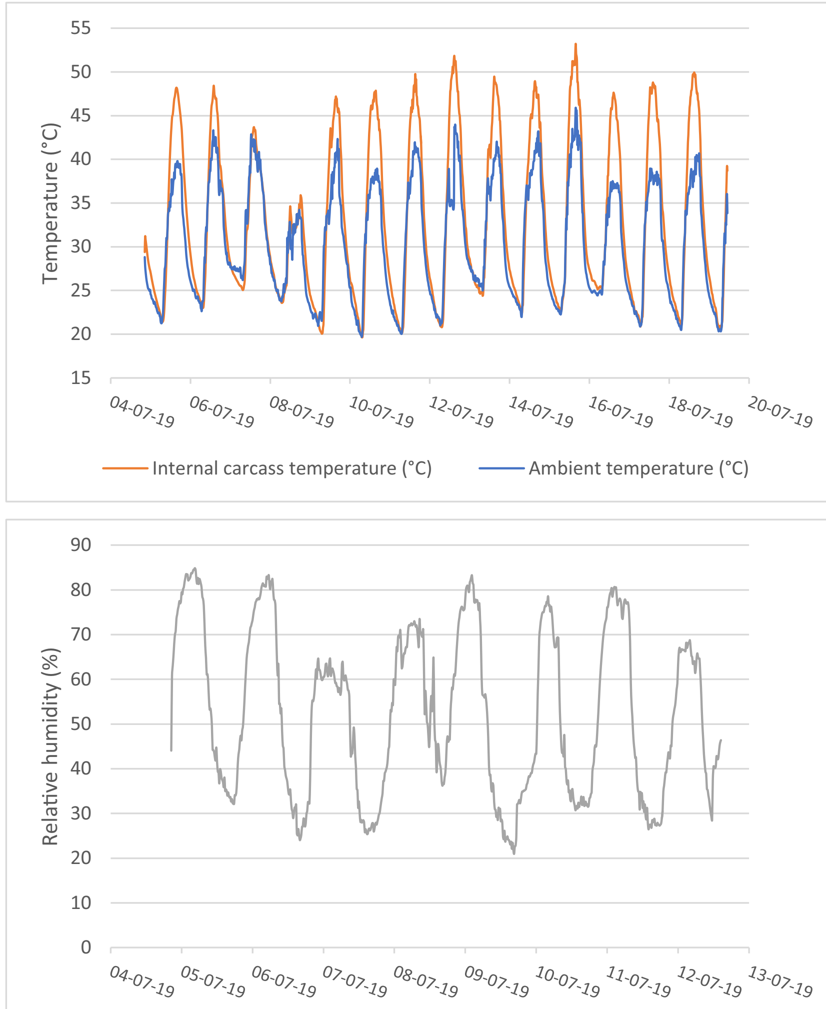

Fig. 2 Values for ambient/internal temperature and relative humidity during the test period (4–19 July 2019, Murcia, Spain). Note that due to problems with the probe, the relative humidity has only been measured until 12 July

The carcass with *advanced decomposition* (3–4 days after death) has completely dehydrated eyeballs; the oral cavity and tongue are dehydrated, wrinkled and obscured. The pectoral muscle is dark brown and has medium dehydration, making it difficult to separate the skin from the muscle during necropsy. The internal organs lose their structure, but they remain easily identifiable, their consistency is soft and friable and their colour is dark brown with a mixture of colours within each organ. The blood becomes brownish-black or is absent. The *rigor mortis* process is finished.

The carcass with *very advanced decomposition* (7 days after death) has eyeballs which have lost their structure completely; the tongue is stiff, showing a parchment-like appearance; and there is a dark colour to the oral cavity. Sometimes, detachment of the horny layer of the beak can be observed. The pectoral muscles are completely dehydrated, and the keel is clearly visualized; it is impossible to separate the skin from the muscle. The internal organs lose their structure, so it is difficult to identify them; however, some of them are guessed and others disappear, their consistency is dry or very friable and the colour is brownish, dark and uniformly homogeneous. The feathers easily detach from the skin.

The carcass with *initial skeletal reduction* (15 days after death) is characterized by a complete dehydration of the entire body, acquiring a dark and homogeneous colouration of dry, stiff and parchment-like appearance.

The carcass with *complete skeletal reduction* consists of bones and feathers, the soft tissues having disappeared. This last phase may take months to complete, depending on the environmental conditions, and in this study was not evaluated.

Within the category of the "fresh" carcass decomposition, the differences between non-frozen individuals and the frozen individual were evaluated. The main differences were observed in the lungs and brain. In the non-frozen individual (Fig. 4a), the lungs have their natural pink colour and the brain has a well-defined vascularization and pale pink parenchyma. Nevertheless, the frozen individual (Fig. 4b) shows congested, red lungs and the brain has a blurred vascularization and a more intense pink colouration of the parenchyma.

Determining the *degree of carcass decomposition* and the time of death is of special interest to be able to frame a crime/accident at a given time. When studying the autolysis in a corpse, which allows to *estimate the time of death*, several

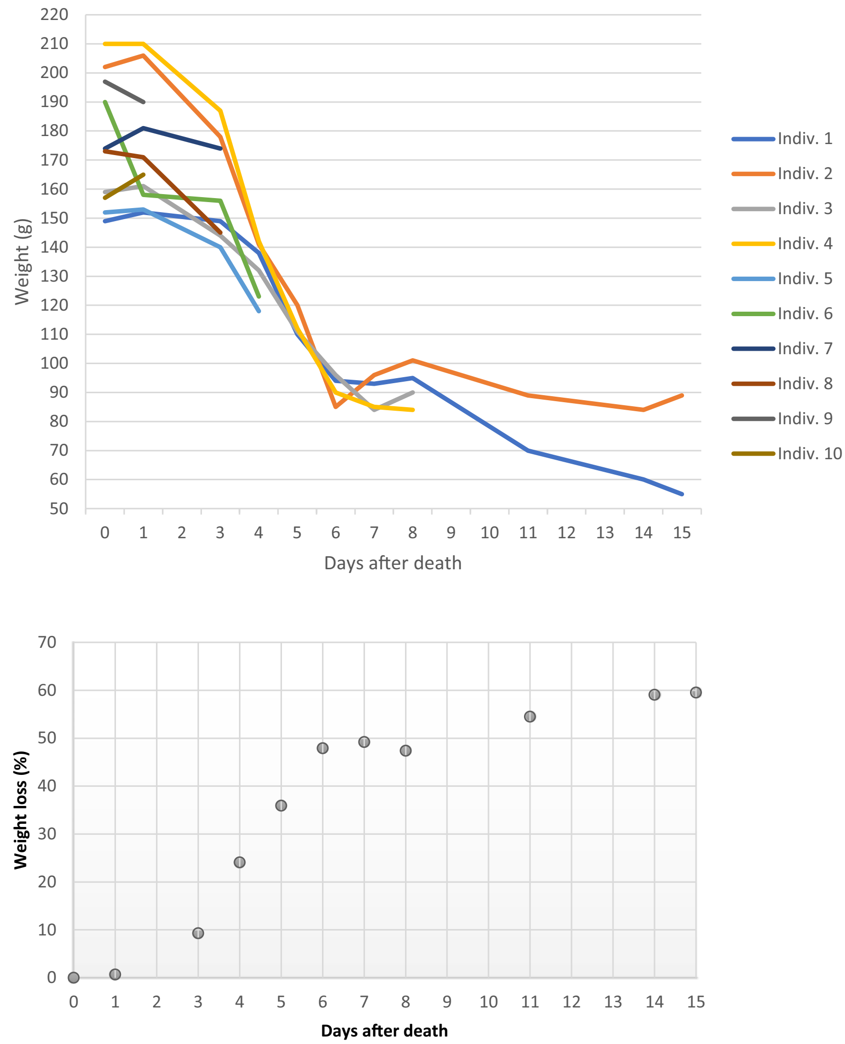

Fig. 3 Values for the weight (g) of 10 carcasses of Common kestrel (*Falco tinnunculus*) and average weight loss (%) in relation to the fresh carcasses (Day 0) during the test period (4–19 July 2019, Murcia, Spain)

parameters affecting the evolution of the decomposition process should be considered, i.e. the environmental conditions (temperature, humidity, rainfall), the species, the weight or size of the animal, its state of health or presence of wounds, the position and location of the corpse, the presence or absence of food in the gastrointestinal tract, the internal temperature of the corpse and the circumstances of death. For example, the autolysis process is expected to be faster in the presence of open wounds, or at high temperatures and humid environments (Brooks 2016; Cooper 2013; Oates et al. 1984). Therefore, it is essential to gather basic information regarding the body and the conditions that surround it. In addition, it is recommended to photograph the carcass in detail, including holes where cadaveric fauna can be observed, as well as the scene surrounding the carcass, in order to be able to consult the photographs if necessary. Document S1 (Supplementary material) includes printable field and lab documentation, i.e. a form compiling some basic information of interest to estimate the stage of carcass decomposition and the time of death (Document S1 A), and the scoring table with pictures (Document S1 B).

Furthermore, as explained before, certain external and internal characteristics of the carcass observable during the necropsy will help to classify the stage of decomposition, such as the colouration, structure and consistency of the organs and tissues, or the degree of dehydration. Due to the reaction of the hydrogen cyanide with the haemoglobin transformed into biliverdin by bacterial activity, a greenish colour stain appears in the surrounding tissues and skin, called "green spot." This spot is found in the abdominal area. The presence and extension of a green spot can also help to classify the degree of decomposition. The period in which it appears is called the chromatic period/stage, and it manifests and evolves in the early stages of decomposition. However, this spot is not always easy to identify, depending on the size of the animal (Gisbert Calabuig et al. 2004; Brooks 2016).

Table 2 Parameters, scoring and photos to classify the stages of carcass decomposition in small-size raptors

Parameter	Description	Points	Score
Eyeballs	They keep bright and with convex shape in lateral view.	0	
	Opacity, they lose their whole structure.	1	
	Completely dehydrated.	2	
	Absent.	3	
	Pink, turgid tongue.	0	
	Pale and dry.	1	
Tongue/Oral cavity	Dehydrated, dark and wrinkled.	2	
	Parchment-like appearance of the tongue and loss of natural color, it turns dark. Detachment of the horny layer of the beak.	3	
Pectoral (breast) muscle	Red color and turgid consistence. Easy to separate from de skin.	0	
	Red Pale. Easy to separate from de skin.	1	
	Dark brownish color, medium dehydrated. Difficult to separate from the skin.	2	
	Completely dehydrated, keel visualized. Impossible to separate from the skin.	3	
Internal organs	**(Liver as reference organ)**		
Structure	Turgid.		
Consistence	Bright.		
Color	Natural from each organ.	0	
Green spot	No spot or only dyes tissue in direct contact.		
Internal smell	Fresh blood/No smell.		
Structure	Slightly dehydrated (Surface a little “wrinkled”).		
Consistence	Slightly dehydrated and dull (Surface a little “wrinkled”).		
Color	Dark compared to the natural, homogeneous between the organs (reddish).	1	
Green spot	Just dyes the organs in direct contact.		
Internal smell	Decomposition smell starts.		
Structure	They lose it, but organs are well identified.		
Consistence	Softer or friable.	2	
Color	Dark-brownish and mix of colors inside the same organ.		
Green spot	Dyes all intestinal handles, dark green/blackish.		
Structure	Difficult to identify the organs, some have disappeared.		
Consistence	Dry or very friable.	3	
Color	Dark and homogeneous in all the organ (brownish).		
Green spot	Absent.		
	Feathers in good condition, they do not detach/red blood.	0	
Others	Red Dark blood.	1	
	Brownish-dark blood or blood missing.	2	
	Feathers detach.	3	
	TOTAL SCORE		

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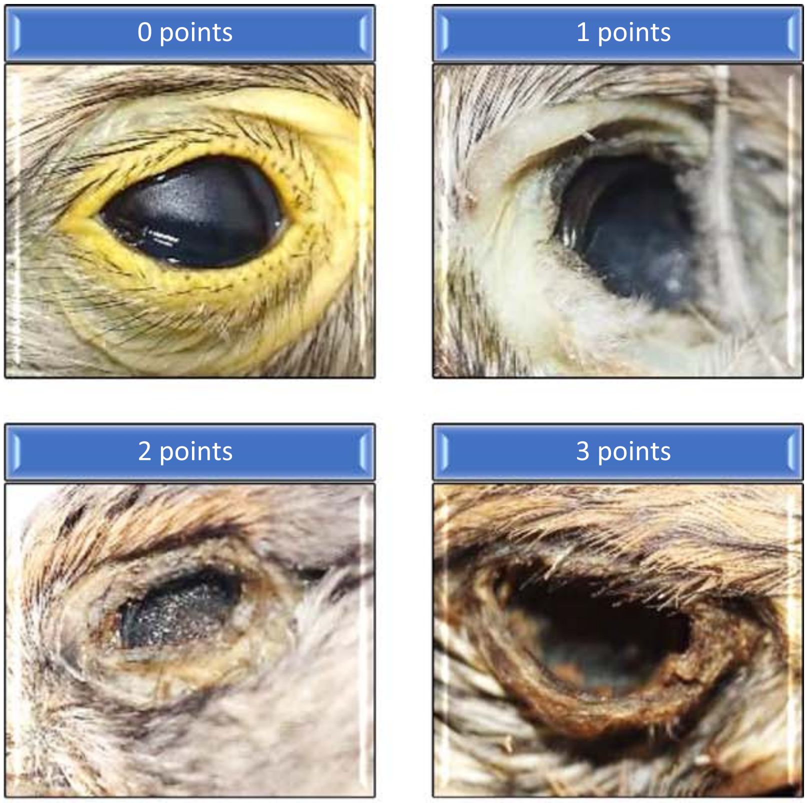
Time course of the *rigor mortis*

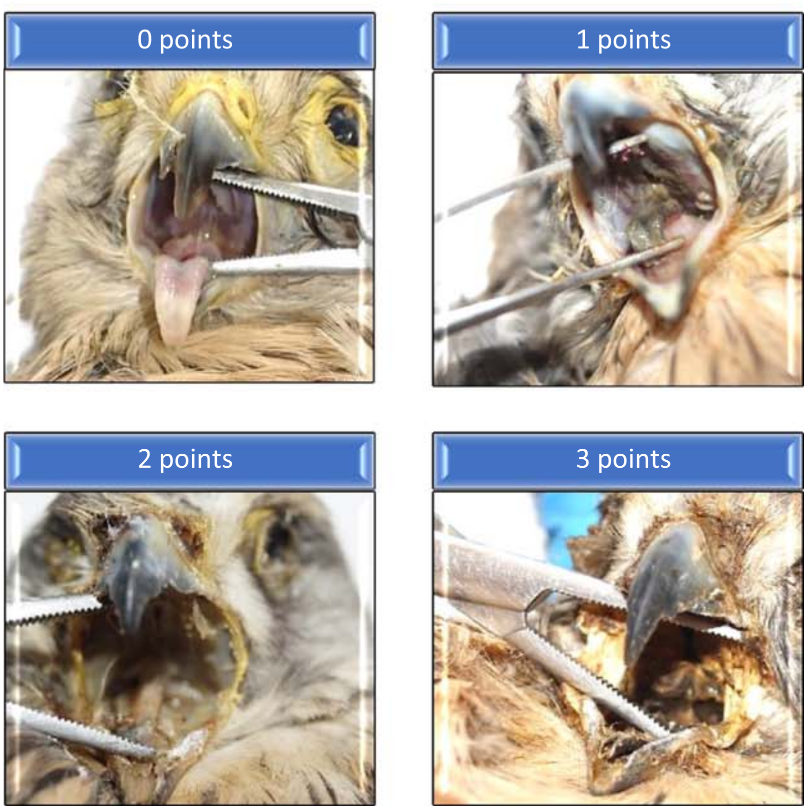
In addition to these criteria, the *rigor mortis* of the carcass will also help estimate the time of death in the early stages. According to a study in ducks, the peak of *rigor mortis* in muscles of the jaw, neck and legs is usually reached 1 h after death, while stiffness in the pectoral muscles appears after 1.5 h (Morrow and Glover 1970). In other study in mallards, total stiffness was described 1–2 h after death (Oates et al. 1984). In

Table 2 (continued)

Eyeballs



Tongue / Oral cavity



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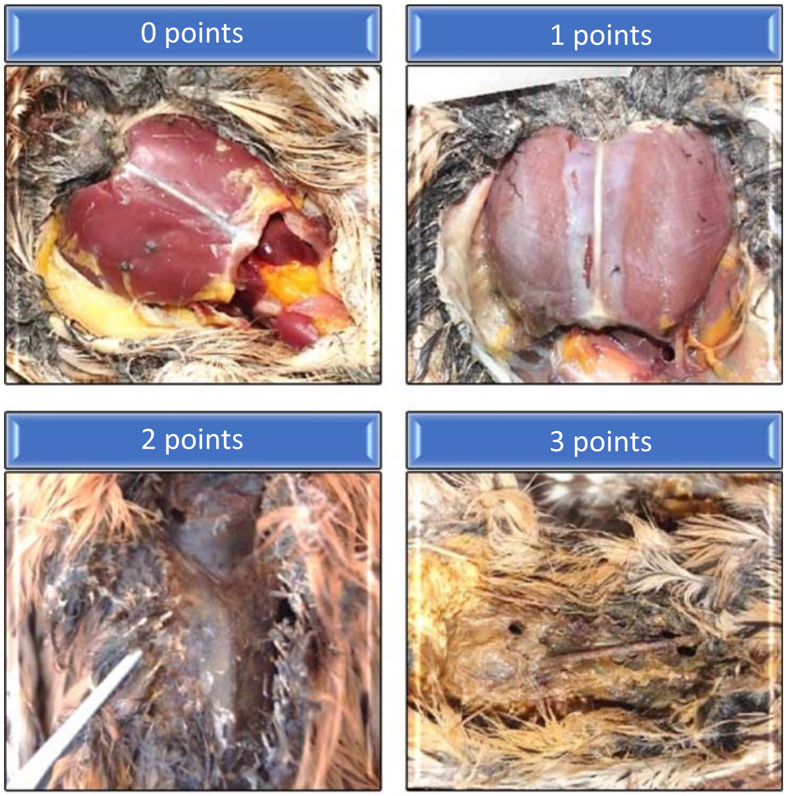
Pectoral (breast) muscle



Internal organs (1st part: general view)

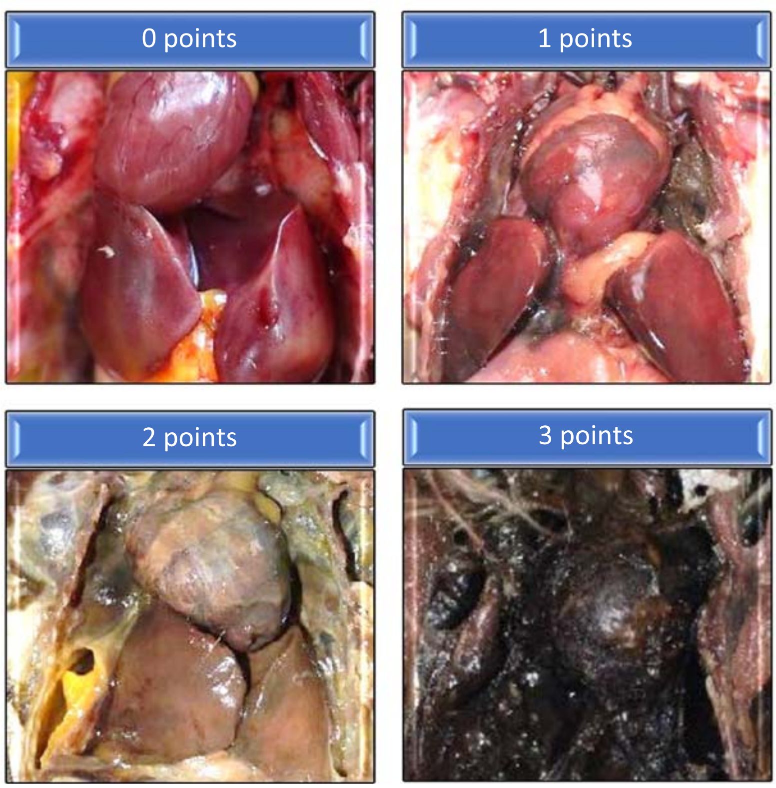
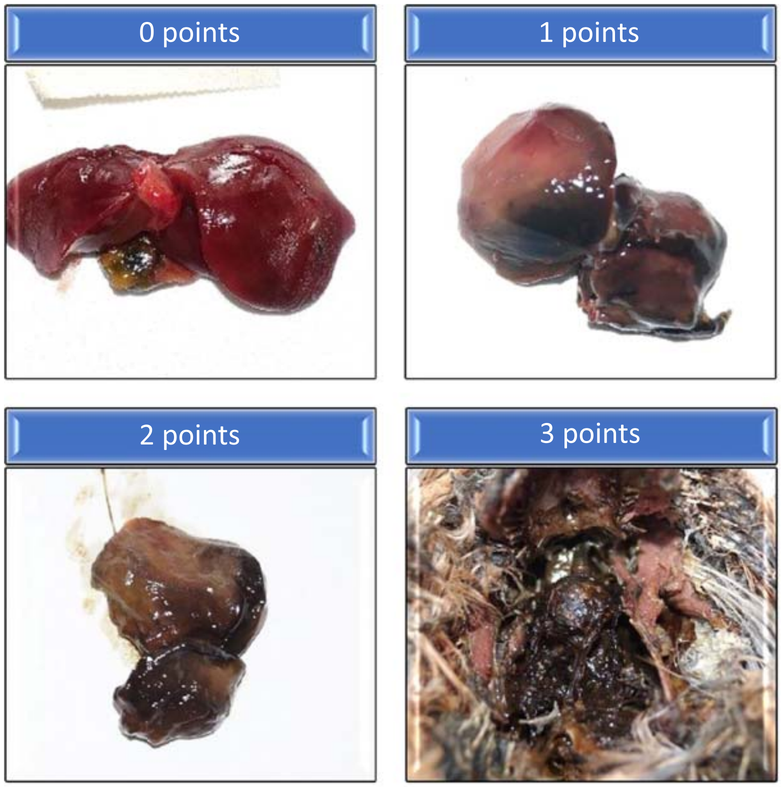
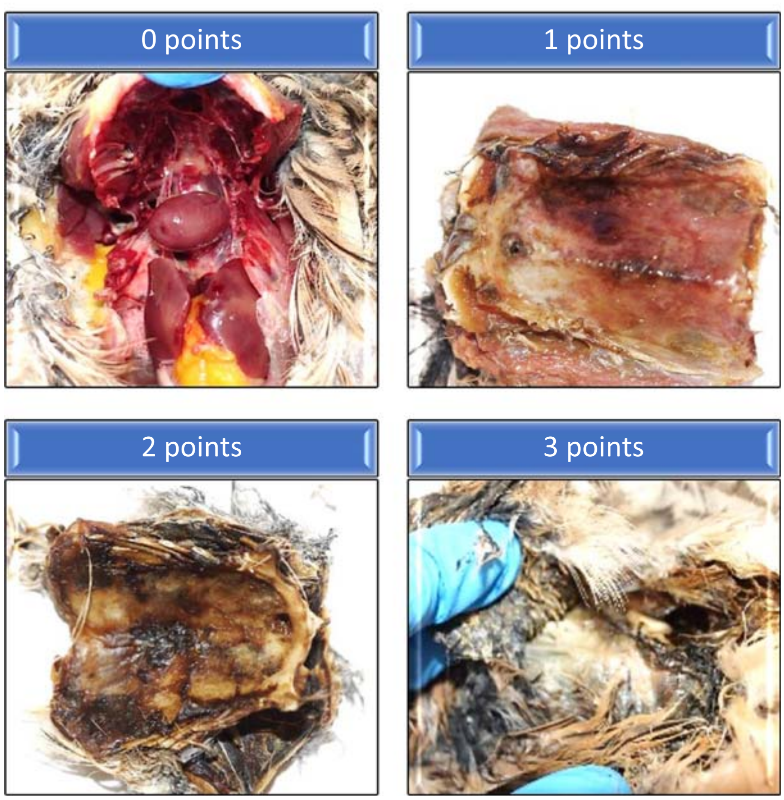


Table 2 (continued)

Internal organs (2nd part: liver as reference organ)



Others



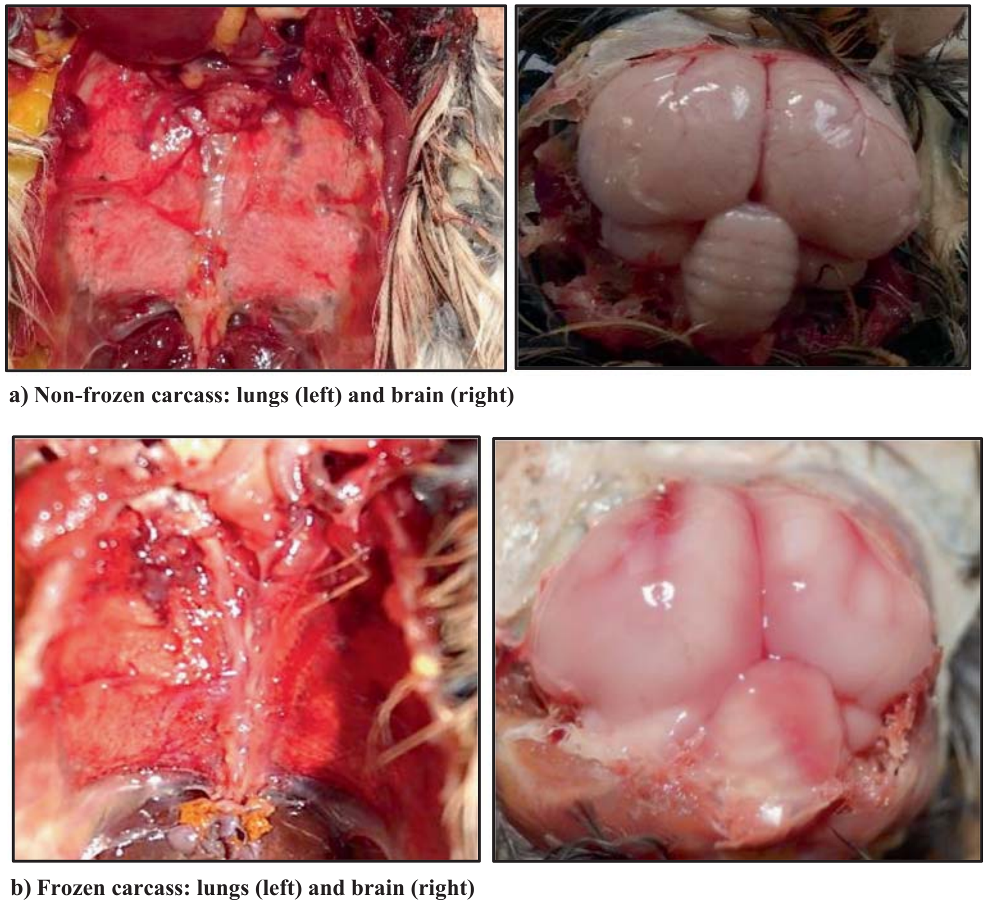


Fig. 4 Lungs and brain of fresh carcasses of Common kestrel (*Falco tinnunculus*): **a** Non-frozen carcass. Lungs show their natural pink colour and brain has well-defined vascularization and pale pink parenchyma. **b** Frozen carcass. Lungs are congestive and show red colour, and brain has blurred vascularization and deep pink parenchyma

this study, *rigor mortis* gradually appeared after death as follows: *rigor* in neck and legs after 30 min, more intense *rigor* (almost complete) in neck and legs (less appreciable in digits and beak) after 60 min, complete *rigor mortis* in neck and legs and intense *rigor* in wing and beak after 90 min, almost complete *rigor mortis* after 150 min and complete *rigor mortis* after 390 min. The *rigor mortis* slightly decreased in neck and digits (still present in beak and legs) after 24 h, with a more noticeable decrease after 48 h and finally, *rigor mortis* disappeared completely after 72 h. It should be taken into account that the time to reach the *rigor mortis* can vary depending on the species, size of the individual and the circumstances surrounding death (vigorous muscle exertion may accelerate the onset and degree of *rigor mortis*). In the protocol, *rigor mortis* is useful to distinguish between *moderate decomposition* or *advanced decomposition* stages when the difference is not clear. The *rigor mortis* will appear in the last stage of *moderate decomposition* and in the first stage of *advanced decomposition*. Therefore, after scoring the carcasses according to the criteria shown in Tables 1 and 2, the presence or absence of *rigor mortis* will help to differentiate between both stages if they are not clear.

Forensic entomology

The *estimation of the time of death* is easier within the first 72 h; however, the carcass provides less information after that, and *forensic entomology* can be very useful for providing estimates after days, weeks and even months after death (Barnes 2013). An expert in entomology is needed for a proper identification and interpretation of cadaveric fauna found in a carcass, and the insects may be highly variable depending on the geographical area, season and circumstances. For this reason, this parameter is not considered in the scoring method proposed in this article, although some basic data gathered during necropsies is provided.

The identification and analysis of the insects found in the body do not indicate the exact date of death but allows us to estimate the minimum post-mortem interval. There are several studies investigating the temporal pattern of insects during the decomposition sequence in different animals (see review by Barnes 2013). There are mainly three methods to carry out this estimate: by ageing blowfly larvae in the corpse, through the succession of insects or by seasonality of their activity (Barnes 2013). The methodology used will depend on the cadaveric fauna available. Barnes (2013) provides a detailed description of these methods. Nonetheless, it should be considered that the size, smell, condition and position of the corpse affect the activity of the insects, as well as the geographical area, season and environmental factors. As for the first method, in general, the flies are the initial colonizers in a carcass, being quickly attracted to the body and depositing eggs in dark and damp areas such as eyeballs, nostrils, oral cavity, anus/cloaca, genital region and wounds. The eggs hatch at larvae at a rate that will depend on the environmental conditions and the species of insect involved. The larvae, after three phases of growth, pass to pupa, which will harden and darken to form an adult individual (Barnes 2013). Therefore, the minimum post-mortem interval can be estimated by determining the time elapsed since the egg laying, calculated using local meteorological data and the identification of the species and their developmental phase (Barnes 2013). Regarding the insect succession methodology, different species will be attracted to the body depending on the degree of cadaveric decomposition and the odour emitted. In general, fly species dominate the initial stages, while coleoptera (beetles) dominate later stages (Barnes 2013). Finally, the activity of insects and their development depends on the environmental conditions and, therefore, on the season, so that knowledge of different insect species and their activity pattern can help frame a crime in a certain time of the year (Barnes 2013). The presence of cadaveric fauna can complicate the identification of the organs, because of their necrophagous activity (Viero et al. 2019).

The different types of cadaveric fauna observed in our experiment were ants, arthropods, coleoptera and dipterans, in eggs, larvae and adult stages. In individuals necropsied at 24 h after death, eggs and 3-mm larvae were observed only in the oral cavity, as well as flies around the corpse, eggs being found even within a few hours after death; at 72 h after death, adult individuals of grey fly (*Sarcophagidae*), larvae and some coleoptera were found; at 96 h after death a large number of larvae of different sizes (maximum 6 mm) were observed throughout the interior of the body (including the brain and behind the pleura), as well as some beetles and adult flies. After 7 days, the quantity of larvae is lower and more beetles were found, and after 15 days, only ants and beetles were observed (Fig. 5).

It is observed that the first phases of decomposition are rapidly reached, while the last ones (very advanced decomposition, initial and complete skeletal reduction) require more time. The loss of body weight (represented as a percentage respect to the fresh body, see Fig. 3 and Table S3 in Supplementary Material) and the cadaveric fauna found are also presented. Some weak points of this study that should be considered are as follows: (i) the limited number of

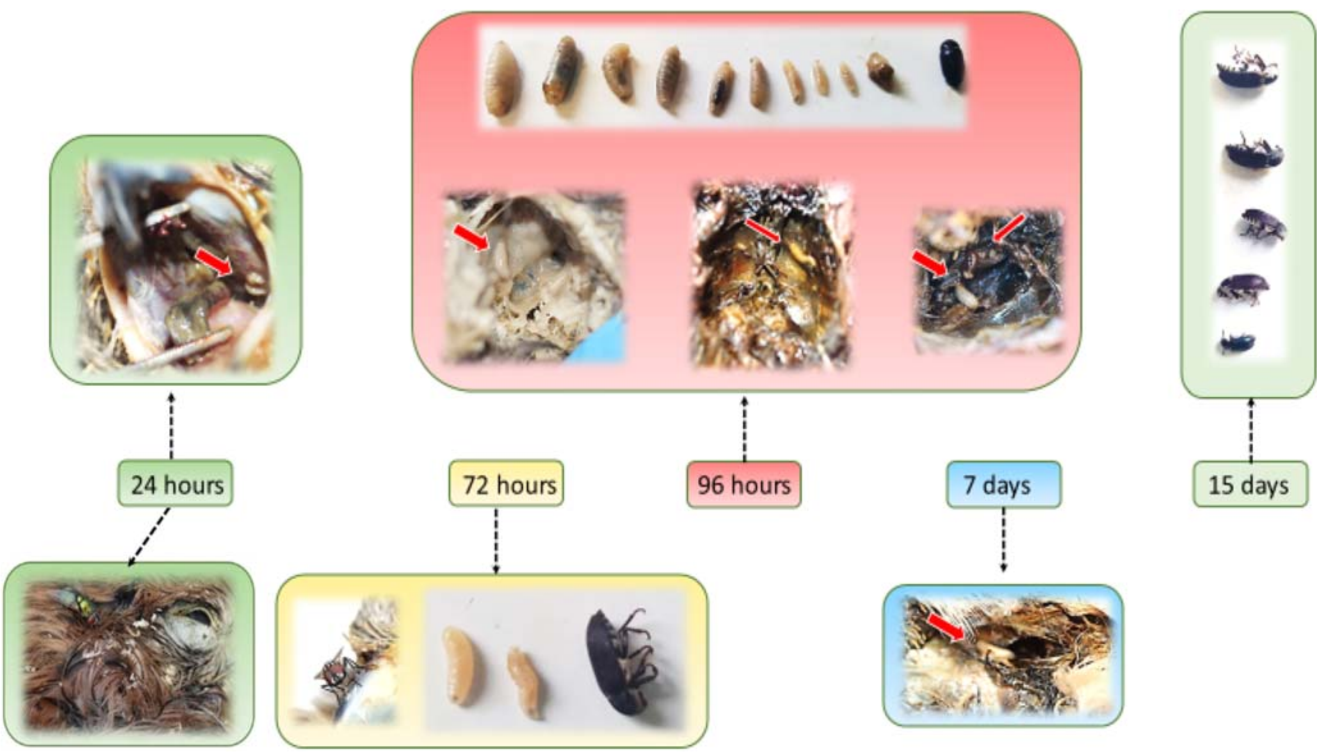


Fig. 5 Cadaveric fauna found during the experiment in Common kestrel (*Falco tinnunculus*). A few hours after death: fly eggs can be found in the oral cavity. At 24 h after death, eggs and 3-mm larvae are observed only in the oral cavity, as well as mobile adult flies. At 72 h adult individuals of grey fly (*Sarcophagidae*), larvae, and some beetles were found. At 96 h a large number of larvae of different sizes (up to 6 mm) were found throughout the interior of the body (including the brain and behind the pleura); some beetles and adult flies were found. At 7 days plus, the quantity of larvae is smaller and more beetles were found. At 15 days, only beetles and ants were found

individuals available for the study to make strong generalizations and interpretations; (ii) the daily handling of the carcasses to record body weights in this experiment may also affect the rate of decomposition, and it may alter oxygenation and colonization by bacteria and insects; however, it was necessary to gather the body weight and assess changes in weight over time; (iii) as previously mentioned, the process of these parameters varies considerably depending on various factors (environmental conditions, characteristics of the animal, circumstances of death, etc.), so the estimation of the time of death must be adjusted according to the conditions of each case, and (iv) the barbiturate used for euthanasia could have an effect on the decomposition and/or cadaveric fauna activity; however, no studies evaluating this potential effect have been found in the literature and further studies would be needed to better understand this issue. In spite of this, the present study provides valuable information, considering that these types of studies in wild birds are scarce. This protocol will help to standardize methodologies and minimize subjectivity. The new scoring method for carcass classification according to the degree of decomposition and estimation of time of death in small-sized raptors can be used by practitioners, forensic veterinarians, researchers, official authorities or personnel in charge of carcass collection in the environment.

Conclusions

When determining the degree of carcass autolysis and estimating the time of death, the most relevant parameters (i.e. environmental conditions, characteristics of the carcass and circumstances of the death) must be considered. This protocol proposes a scoring method that will aid the classification of the stage of carcass decomposition and estimation of the time of death in birds. Our investigation was conducted under a limited number of environmental conditions and using small-sized raptors, which had been euthanized as a reference. The principal decomposition changes were observed during the first 7 days. In 15 days, the initial skeletal reduction was reached which was progressing beyond the investigation period, and it was obvious that more time was needed to reach the stage of complete skeletal reduction. Furthermore, changes in colour and vascularization definition in the frozen carcass compared with fresh carcasses were observed, confirming that potential histological changes affecting the appearance of some organs should be considered when the necropsy of a frozen carcass is carried out. It is recognized that this investigation has some inevitable limitations such as the small sample size studied, the daily handling of the carcasses (for weighing and examination), and the specific weather conditions prevailing and the special fauna (bacteria, insects, etc.) present in the geographical area selected. It is also noted that although the importance of using additional descriptors such as smell and colour descriptions is obvious, it may not be possible to include them in an objective scoring protocol such as the one proposed here. The investigation reported here is intended to be a starting point from which data may be collected and validated. Further studies with other avian species and different weather conditions would help to better classify carcass decomposition and estimate time of death.

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Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interest.

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