INTRODUCTION

Research and casework focused on the decomposition process of human bodies in terrestrial environments are extensive [1-4]. These studies comprise decomposition models in urban and rural settings, their variation within and between seasons and their decomposition patterns, either linked to different body disposals, such as burial or exposure, and/or to the different environments where bodies are recovered [1-3]. Thanks to such a body of research, many forensic cases are successfully investigated. However, 71% of the planet is covered by water, in either natural or artificial locations that could potentially host a death scene [5]. In such cases, the investigation process can be extremely complex, requiring special equipment and highly trained personnel [5]. Aquatic environments can be classified as permanent/stable (e.g. rivers, lakes or the sea), or cyclical/seasonal (e.g. tidelands, beaches or floodplains) [6, 7]. Contemporary literature, especially case studies, mostly concerns human remains found in stable environments, whereas only a scant number of studies focus on cyclical/seasonal environments.

Studies in the field of aquatic forensics have been recently developed, of which several are dedicated to the post-mortem modifications of human remains in aquatic environments, with the associated fauna [8-15]. Submersion in fresh or salt water alters the decomposition process because water temperature, usually low, hinders the putrefaction process and prevents access to the typical necrophagous fauna, which facilitates the decomposition process [16]. Furthermore, aquatic environments do not rely on the typical medical parameters of PMI estimation [17, 18]. This is partly due to the formation of adipocere, a decomposition process alternative to putrefaction and affected by high individual and environmental variability [19-21]. As a consequence, the estimation of the post-mortem interval (PMI), the post-mortem submersion interval (PMSI), and possibly the floating interval (FI) of human remains found in water is still considered one of the most difficult dilemmas facing...
forensic experts [22]. In several cases, the PMI/PMSI/FI estimation can be facilitated by using aquatic, semi-aquatic and terrestrial micro and macro-organisms, feeding directly on the body or using the body as a shelter or surface of attachment [14, 15, 22-24]. However, estimations can be extremely difficult because the colonization of human remains in water can occur by a combination of such organisms reaching the body during the different sinking and floating phases of decomposition in water [1, 5].

To date, several experiments and casework have focused on the decomposition and the fauna associated to bodies displaced in open and stable water environments (e.g. rivers, lakes and the sea), as well as in delimited waters (e.g. tanks and wells) [5, 14, 25-27]. Nonetheless, no publications have been produced on bodies discovered in the mud, following a flooding event.

The present case describes the multidisciplinary forensic investigation on a moderately decomposed body found in a river floodplain in Italy. Pathological, anthropological and entomological findings allowed to obtain a full biological profile and reconstruct the events that happened before and after death.

CASE PRESENTATION

In mid-November 2010 a hunter walking on the Po river bank near Parma (Emilia-Romagna Region, in the north-east of Italy, Fig. 1) informed the authorities of the presence of human remains in the mud, which were spotted from a distance. The floodplain area, of roughly 65 hectares, had drained naturally in the past 10 days, after flooding due to recent heavy rain. The depth and the soft consistence of the mud prevented the use of vehicles to reach the death scene. The police, the forensic team and the recovery team eventually reached the scene by helicopter. The human body remained untouched until the arrival of the pathologist at the scene, who described it as lying prone in the mud, completely undressed and covered by a large number of “dark” spiders, which were also swarming in the surrounding environment. All the operations at the scene were highly impractical and dangerous due to the presence of mud; therefore, no insect material was collected, either from the body and/or from the environment surrounding it. At the completion of the CSI operations, the body was placed in a standard plastic body-bag, which was then placed in a wire metal basket and winched up to the helicopter, to be transported to the closest mortuary for post-mortem examination. While no trauma was evident on the body, the prosecutor’s office considered the case worthy of a full forensic investigation, to determine the identity of the deceased person, the cause, the manner and the time since death, and whether the floodplain was the primary death scene.

Environmental data, such as temperature and rainfall in the month before the body was recovered, were obtained from the closest weather station of the National agency for environmental protection, “ARPA” (13 Km). A set of forensic analyses was performed, such as obtaining prints, a Total-Body 3D CT-Scan, external examination and autopsy, an anthropological examination and toxicology, entomology and taphonomy assessments. The details of the forensic analyses are shown in Table 1. The fauna colonizing the body was collected during the autopsy, preserved accordingly to the forensic entomology guidelines [28], and identified at species level.

RESULTS

In the month prior to the discovery of the body the environmental temperatures varied between 5.5 and 17.0 °C, with an average of 8 °C (± 6.04 SD). A total of 192.6 mm of rain had caused a flooding event. Both findings are typical of the Autumn season in the north of Italy.

The human remains belonged to a Caucasian female, approximately cm 167 tall, overweight, aging between 40 and 50 years old but looking consistently older due to overall poor health condition. The body was found completely naked, with no associated personal objects, such as rings, watch, jewelry or dentures that could enhance a personal identification. The body was strewn with mud, leaf litter, small gravel, broken twigs, and colonized by immature insects and spiders which had not caused any visible damage (Fig. 2). The mouth showed a completely edentulous inferior dental arch, with a few teeth in the superior dental arch. All teeth were in poor hygiene and health condition. A monoradicular tooth was collected to be analysed by the Lamendin method, in order to estimate the deceased’s age.
The few other teeth were removed because they were unsteady in their maxillary bone sockets, or fractured at the dental neck, with hemorrhagic infiltrations most likely attributable to perimortem trauma and during the stages of a subsequent tumultuous fluvial transport [30].

Figure 1 Geographical location of the recovery, one of the many floodplain areas of the Po river, in north-east Italy (44°54'13.6"N 10°29'12.8"E). Source: Google Maps and Google Earth.

Table 1 Details of the forensic analyses performed, and the results obtained.

| Analysis        | Details          | Notes                                                                 |
|-----------------|------------------|----------------------------------------------------------------------|
| Prints          | Prints           | The maceration of the epidermis on the finger pads had erased all dermatoglyphics, so only the palmar prints were detectable. |
| Imaging         | Total-body 3D CT-Scan | General appearance.  
| External examination | Sex identification.  
|                  | Dental health and hygiene.  
|                  | Assessment of trauma.  
|                  | Objects, jewels and dentures.  |                                                        |
| Autopsy         | Pathology of the soft tissues and organs.  
|                  | Assessment of trauma.     |
Anthropology  
Age estimation (Lamendin method).  
Pathology of bone and joints. [29, 31, 32]

Toxicology  
Qualitative analysis by a screening immunochemical assay on samples of liver and abdominal putrefactive fluid.

Taphonomy  
Assessment of skin and muscle lacerations, and of the morphology of their margins.  
Presence of hemorrhagic infiltrations.

Entomology  
Presence of fungi and/or moulds.  
Assessment of transformative processes, such as putrefaction and adipocere.

*Calliphora vomitoria* (L.)  
(Diptera: Calliphoridae)  
*Dolomedes fimbriatus*  
(Clerck) (Araneae: Pisauridae)

The reliability of the toxicological analysis was limited by decomposition, since chemical processes occurring during decomposition are known to interfere with the detection of xenobiotic parent drugs or metabolites [44, 45].

Figure 2 The body strewn with mud, leaf litter, small gravel and broken twigs (A), with particular of the colonization by raft spiders (B).
Lytic lesions in the cranial vault, initially observed by CT-Scan and later confirmed at the autopsy, suggested a blood pathology, possibly malignant like lymphoma or multiple myeloma, or, less likely, an infectious disease, such as tuberculosis or syphilis [31, 32]. Osteoarthritic alterations to both radial and femoral heads, as well as the dorsal hyperkphosis, were only slight, indicating a younger age than the apparent one.

Besides the absence of traumatic findings (except for a few teeth), the external examination disclosed a light skin colour, pierced ear lobes and possibly light irises. The autopsy findings, such as diffused hearth valvular sclerosis, hypotrophy of the papillary muscles of the heart valves and moderate arteriosclerosis, were consistent with high blood pressure (hypertension) in life.

Qualitative toxicological analysis was performed with a commercial immunochemical kit (Ram®-Medisoft Group) and gas-chromatography (Dani Instruments SpA); results revealed the presence of psychoactive drugs (benzodiazepines and tricyclic antidepressants) in the liver parenchyma and in the abdominal putrefactive fluid. The cause of death remained undetermined, but the set of findings suggested suicide by drowning.

The PMI estimation was related to the PMSI. Different taphonomic findings indicated a freshwater submersion consistent with the time of the last fluvial flood, which occurred ten days before the recovery of the body. This estimation was based on findings such as localized adipocere in a very early stage, intact fingers and toes, disappearance of dermatoglyphics due to skin maceration and scarce macrofaunal lesions. Most likely, after flooding the floodplain, water drained progressively out and the body was left in a cold, humid but dry environment for a few days, thus allowing the growth of molds patches on the skin.

The fauna colonizing the body consisted of a small number of first and second instar of Calliphora vomitoria (L.) (Diptera: Calliphoridae) and several raft spiders, Dolomedes fimbriatus (Clerck) (Araneae: Pisauridae). The identification of the arthropods was confirmed by a taxonomist [33, 34].

DISCUSSION

The investigation of a death scene in an aquatic or semi-aquatic setting is limited by the environmental conditions, the equipment and the training of the operators [35], whose safety is always of first importance. While standard operation procedures do exist to investigate such environments, the unique characteristics and circumstances of each case often challenge the experience of the investigative and forensic teams, which can make the difference in solving the case. Many body recoveries in water are wrongly performed because of lack of equipment. While the best practice suggests the use of special “water body bags”, ordinary plastic body bags, hooks and winches are often used. Water body bags are made of strong mesh with flush valves that allow the water inside them to escape without losing personal effects and body parts [35]. These water body bags are also inflatable, and can be attached to an air tank that can slowly inflate the bag, raising it to the surface [35]. In this case, the body was found undressed and with no personal belongings. Still, it is possible to infer that entomological evidence went missing during the recovery and the transportation of the body to the mortuary.

At the autopsy only young larvae of the blowfly C. vomitoria and raft spiders were found. This blowfly species is typical of rural areas and cold seasons in several countries, e.g. Europe and North America [36]. Considering the temperatures prior to the discovery of the body, they provided an estimation of a colonization time of approximately 4-5 days [36]. While the PMI was probably longer, only in the last 4-5 days adult female flies had access to the body to oviposit their eggs and take part to the decomposition process. The low temperature, the rain and the submersion of the body in water and mud covering the body’s orifices constituted a physical impediment in reaching the body before that time. In a freshwater or dump environment, fly eggs are able to survive even 66 hours underwater, but larvae drown after 3-4 hours [37, 38]. Therefore, the identification of alive larvae is a sign of the body being emerged from muddy waters 4-5 days before the recovery.
Raft spiders are large semi-aquatic spiders with an Eurosiberian distribution, associated with a variety of swampy areas, including flowing waters, alluvial forests and bog forests [39]. Due to the high variability of the body coloration and size, D. fimbriatus is often confused with the congeneric D. plantarius, although the latter is rarer and more stenoeious. The two species share similar ecological requirements and may be found in syntopy, therefore a correct identification is only possible by means of the examination of sexual characters under the stereomicroscope [40]. The main habitat of raft spiders are wet heathland, wet woodland, bogs and pools, sometimes water margins of ditches, ponds and slow flowing streams. Juveniles may be also found in much drier habitats [41]. Adults assume a hunting position on emergent water plants and prey on invertebrates trapped on the water surface. When threatened the spider retreats underwater [41]. Raft spiders have been observed swimming in water over a considerable distance, and they are able to remain submerged for up to 45 minutes, breathing air in bubbles captured by their hairs and/or in contact with their respiratory orifices [42]. The presence of raft spiders alone cannot be used to estimate either a PMI or a PMSI of a body, although in this specific case their massive colonization of both the body and its immediate surroundings was consistent with all the other circumstantial evidence, such as the draining of the flooding waters at least a few days before the recovery of the body. Furthermore, while raft spiders primarily feed on aquatic invertebrates such as pond skaters, dragonfly larvae, small fish and smaller aquatic spiders [41], the presence of adult flies and larvae active on remains may have opportunistically attracted their presence.

The PMI/PMSI estimation based on the entomological evidence provided by C. vomitoria was consistent with the other taphonomical findings. It is understood from the literature that adipocere starts appearing when a fresh body lies in cold water for a minimum of 2-3 weeks [19-21]. The limited localization and the very early stage of adipocere, the scarce macrofaunal lesions and the disappearance of dermatoglyphics on intact fingers can describe a PMI of such duration and consistent with the flood event happened 10 days before the recovery of the body, which location and position were secondary, that is linked to fluvial transport [1, 43].

The conclusions provided to the court were comprehensive of all the necessary information to lead to the deceased’s personal identification. Furthermore, a facial reconstruction was achieved and provided to the media. The cause and time of death, although ruled as undetermined for the lack of unquestionable evidence and by the challenging environment, were most likely a suicide by drowning, committed by a clinically ill, depressed and socially marginalised mature woman around the time of the latest flood (10 days before the body discovery).

In this case, the close cooperation between the forensic pathologist, the anthropologist, the entomologist and other ancillary experts, allowed to answer all the essential questions in the judicial investigation. Despite the combined effort between all the experts involved and the media coverage of the case, to this day, neither the set of information obtained by forensic analyses, the research of palmar prints on the police databases, or the facial reconstruction have led to any personal identification.

**Conflicts of Interest**
Authors declare none.

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**Author contribution statement**
E.E.G. performed the medico legal investigation, in collaboration with P.A.M. E.E.G. wrote the manuscript in consultation with P.A.M.

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