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J F S R JOURNAL OF FORENSIC SCIENCE AND RESEARCH
ISSN 2575-0186

Research Article

Diatom Analysis and Postmortem Changes in a Rabbit Model: A Comparative Study of Marine and Freshwater Drowning

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Abstract

The diatom test is a valuable forensic tool for diagnosing drowning, but its reliability can be influenced by the drowning medium. Furthermore, differential postmortem changes in marine versus freshwater environments are not fully characterized, potentially affecting the interpretation of evidence. This study aimed to compare diatom recoverability from organs and postmortem changes in rabbits drowned in marine versus freshwater ecosystems. Based on water sample analysis, the drowning mediums exhibited significant variation, with marine sites Pirate and Malindi beaches having the highest diatom concentration (2000±231 and 2000±195 cells/mL, respectively), followed by Kilifi bay (1500±147 cells/mL) and the freshwater control having the lowest (800±102 cells/mL). In rabbit drowning models, a significantly higher concentration of diatoms was recovered from the lungs of marine-drowned rabbits compared to freshwater, with Kilifi bay yielding the highest diatom count (3000±600 cells/mL). Furthermore, post-mortem body weight gain was significantly greater in fresh water drowning (22.64%) than in marine water (up to 13.61%), and distinct patterns of temperature decrease and external decomposition were observed between the two mediums.

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Submitted: October 30, 2025
Approved: November 10, 2025
Published: November 11, 2025

How to cite this article: Ogare J, Chimbevo LM. Diatom Analysis and Postmortem Changes in a Rabbit Model: A Comparative Study of Marine and Freshwater Drowning. J Forensic Sci Res. 2025; 9(2): 216-220. Available from: https://dx.doi.org/10.29328/journal.jfsr.1001106

Keywords: Forensic pathology; Diatom test; Drowning; Postmortem changes; Marine; Forensics; Algor mortis; Rigor mortis; Kenya

Introduction

Postmortem changes occur naturally in sequence after death [1]. Although they proceed in an orderly manner, several factors affect them externally and intrinsically [2]. Understandably, the common Postmortem changes allow forensic pathologists to more accurately estimate the Postmortem interval [3]. Cases that involve retrieved bodies from water bodies require proper knowledge in forensic pathology. Determination of the cause of death for such human remains usually involves conducting an autopsy and collecting tissues for analysis, and also screening of the available piece of evidence that relies on inference to connect them to a conclusion of facts, such as fingerprints, semen at etc. Marine waters are salty with thousands to millions of salts [4]. These salts have been proven to have an impact on the mummification of the carrion [5]. However, the effects of salts on the integrity of the circumstantial evidence and postmortem changes are yet to be realized. The study was designed to investigate whether a relationship exists between the saltiness of the water body and the integrity of evidence, together with the observable post-mortem changes.

Methodology

Study area and sample collection

The study was conducted using water samples collected from three public beaches along the Kenyan coast: Jomo Kenyatta (Pirates) in Mombasa County, and Kilifi Bay and Malindi beaches in Kilifi County. Twenty litres of seawater were collected from each site in sterile containers and transported to the laboratory at the Technical University of Mombasa for analysis and drowning experiments.

Animal model and housing

Domestic rabbits (*Oryctolagus cuniculus*) were obtained from the Technical University of Mombasa's Animal House Unit. The animals were housed under standard laboratory conditions (26 °C, 60% humidity, natural light-dark cycle) and provided with standard rabbit pellets and water *ad libitum* before experimentation.

Experimental design and drowning procedure

An experimental design was employed with 30 rabbits randomly assigned to five groups (n=6 per group). The groups were as follows: Group I (drowned in seawater); Group II (sacrificed before submersion in seawater); Group III (drowned in tap water); Group IV (sacrificed before submersion in tap water); and Group V (sacrificed control, not submerged). Rabbits in control groups (II and IV) were euthanized via pentobarbitone sodium (75 mg/kg) injection and cervical dislocation before submersion. All submersion procedures were conducted for 30 minutes at room temperature with intermittent water movement to ensure contact with diatoms.

Necropsy and tissue collection

Autopsy was performed on all rabbit cadavers 24 hours post-procedure. To prevent cross-contamination, separate sterile instruments were used for each animal. Samples of the liver, kidneys, spleen, lungs, brain, and femur were collected. Each tissue was divided; one portion was frozen at -21°C for diatom analysis, and another was fixed in 10% neutral buffered formalin for histopathological examination.

Postmortem and histopathological analysis

Postmortem changes, including algor mortis and rigor mortis, were recorded. For histological evaluation, formalin-fixed tissues were processed routinely, embedded in paraffin, sectioned at 6 µm, and stained with Haematoxylin and Eosin (H&E). Slides were examined under bright-field microscopy at various magnifications (10x, 40x, 100x).

Diatom analysis

Water and tissue samples were digested using a modified nitric acid method. Briefly, approximately 2-4 g of tissue was digested with 65% nitric acid (HNO_3), decalcified with Hydrochloric acid (HCl), and oxidized with 30% Hydrogen Peroxide (H_2O_2). The digested samples were centrifuged and washed repeatedly with deionized water. The resulting pellets were mounted on slides and examined under an optical microscope. Diatoms were quantified at 40x magnification and identified at 100x magnification by comparison with established literature.

Results

Following the outlined methodology, the results about diatom concentration and postmortem tissue changes in the rabbit models are presented. Diatom concentration in seawater (1500-2000 cells/mL) was significantly higher than in tap water (800 cells/mL). Drowning groups showed significantly higher diatom counts in organs than post-mortem submersion groups ($p \leq 0.05$). The lungs consistently had the highest diatom load. Rabbits drowned in freshwater showed a significantly greater percentage increase in total body weight (22.64%) compared to those in seawater (13.61%). Distinct visual postmortem changes were noted, including blowfly attraction to seawater carcasses versus flesh flies for freshwater.

Diatom concentration and physico-chemistry of drowning medium

The diatom concentration was significantly higher ($p \leq 0.05$) in seawater from Pirates and Malindi beaches (2000 ± 231 and 2000 ± 195 cells/mL, respectively) compared to Kilifi Bay (1500 ± 147 cells/mL) and tap water (800 ± 102 cells/mL). The seawater sites had higher pH and TDS levels than the freshwater control (Table 1).

RecoVerifiability of diatoms from organs

Antemortem drowning (Groups I & III) resulted in significantly higher ($p \leq 0.05$) diatom counts in all organs compared to post-mortem submersion (Groups II & IV). The lungs consistently showed the highest diatom concentration, followed by the heart, brain, and liver (Table 2). The control group (V) showed no diatoms. Drowning in seawater led to a higher organ diatom load than drowning in freshwater.

Postmortem changes

Rabbits subjected to antemortem drowning showed a significant increase in total body weight. The percentage weight gain was markedly higher in all freshwater drowning groups (22.64%) compared to all seawater drowning groups (the maximum being 13.61% for Group I). A significant decrease in body temperature (algor mortis) was observed in all submerged groups, with no significant difference between water types.

The average weight internal organs of the rabbits was measured and is represented in Table 4. Generally, in both the drowned (Group I and Group III) and the killed and submerged (Group II and Group IV) groups of rabbits, an increase in body organ weight was observed. However, the organs for the group drowned in seawater (Group I) had the greatest gain in organ weight as compared to the rabbit group drowned in freshwater (Group III). In both the drowned groups (Group I and Group III), lungs had the greatest gain in weight, followed by liver, kidneys, and spleen. The heart and the brain showed the least change in organ weight after drowning.

Table 1: Physicochemical properties and diatom concentration of the drowning media.

Drowning Medium	pH	Temperature (°C)	TDS (mg/L)	Diatom Concentration (cells/mL)
Pirates Beach	8.1	26.0	130	2000 ± 231[a]
Kilifi Bay	7.9	25.9	142	1500 ± 147[b]
Malindi Beach	8.3	26.1	133	2000 ± 195[a]
Fresh Water	7.6	26.0	79	800 ± 102[c]

Means with different superscript letters in the same column are significantly different ($p \leq 0.05$).

Table 2: Average diatom counts per mL recovered in different organs (Lungs, heart, brain, and liver) in rabbits exposed to different treatments for water samples from Pirates beach, Kilifi bay beach, and Malindi beach.

Group	Pirates beach				Kilifi Bay Beach				Malindi Beach			
	Lungs	Heart	Brain	Liver	Lungs	Heart	Brain	Liver	Lungs	Heart	Brain	Liver
Group I	2500^{b}±500	90^{a}±18	100^{d}±20	10^{a}±2	3000^{b}±600	130^{e}±43	175^{a}±35	75^{b}±15	2250^{d}±450	100^{a}±20	150^{c}±30	40^{b}±8
Group II	55^{a}±7	10^{b}±3	140^{c}±28	0^{b}±0	80^{a}±16	25^{b}±5	160^{b}±32	0^{c}±0	65^{a}±13	20^{c}±4	130^{a}±26	0^{c}±0
Group III	1600^{c}±320	1000^{d}±200	5^{a}±1	30^{c}±6	1600^{c}±320	1000^{d}±200	5^{c}±1	30^{a}±6	1600^{b}±320	1000^{d}±200	5^{b}±1	30^{a}±6
Group IV	35^{d}±7	15^{bc}±3	5^{a}±1	0^{b}±0	35^{e}±7	15^{c}±3	5^{c}±1	0^{c}±0	35^{c}±7	15^{b}±3	5^{b}±1	0^{c}±0
Group V	0^{e}±0	0^{c}±0	0^{b}±0	0^{b}±0	0^{d}±0	0^{a}±0	0^{d}±0	0^{c}±0	0^{e}±0	0^{e}±0	0^{c}±0	0^{c}±0

Mean values (n=2) ± SEM. Group I: Killed by drowning in sea water; Group II: Killed and submerged in sea water; Group III: Killed by drowning in fresh water; Group IV: Killed before submerged in fresh water; Group V: Killed not submerged in sea water nor fresh water. Values appended by different superscript letters within the column are statistically and significantly different ($p \leq 0.05$).

Table 3: Average weight (kg) and percentage weight gain of rabbits before and after drowning or submerging in water samples collected in different public beaches.

Group	Pirates			Kilifi			Malindi		
	Before	After	%Gain	Before	After	%Gain	Before	After	% Gain
Group I	3.25^{b}±0.01	3.68^{d}±0.04	13.23^{a}	2.94^{e}±0.02	3.34^{c}±0.01	13.61^{a}	3.10^{b}±0.06	3.45^{d}±0.02	11.29^{a}
Group II	2.75^{a}±0.01	3.05^{b}±0.08	10.91^{d}	3.04^{b}±0.05	3.35^{c}±0.04	11.51^{d}	2.98^{b}±0.01	3.36^{e}±0.02	12.75^{d}
Group III	2.65^{a}±0.05	3.25^{c}±0.01	22.64^{b}	2.65^{a}±0.052	3.25^{c}±0.01	22.64^{b}	2.65^{a}±0.05	3.25^{c}±0.01	22.64^{b}
Group IV	2.64^{a}±0.03	3.05^{b}±0.02	15.53^{c}	2.64^{a}±0.03	3.05^{b}±0.02	15.53^{c}	2.64^{a}±0.03	3.05^{b}±0.02	15.53^{c}
Group v	3.05^{c}±0.02	3.03^{b}±0.01	-0.01^{e}	3.05^{b}±0.02	3.03^{b}±0.01	-0.01^{e}	3.05^{b}±0.02	3.03^{b}±0.01	-0.01^{e}

Mean values (n = 2) ± SEM. Group I: Killed by drowning in sea water; Group II: Killed and submerged in sea water; Group III: Killed by drowning in fresh water; Group IV: Killed before submerged in fresh water; Group V: Killed not submerged in sea water nor fresh water ($p = 0.0415$). Values appended by different superscript letters within a column and within a row are statistically and significantly different ($p \leq 0.05$).

Table 4: Average weight changes (grams) of organs (Lungs, heart, brain, and liver) in rabbits exposed to different treatments for water samples from Pirates' Beach, Kilifi Bay Beach, and Malindi Beach.

Group	Pirates beach						Kilifi Bay Beach						Malindi Beach					
	Liver	Kidney	Spleen	Lungs	Brain	Heart	Liver	Kidney	Spleen	Lungs	Brain	Heart	Liver	Kidney	Spleen	Lung	Brain	Heart
Group I	4.20±0.13	13.33±0.67	1.56±0.07	25.27±1.67	8.38±0.87	18.28±1.12	4.11±0.27	13.00±0.74	1.75±0.05	23.47±2.11	8.13±0.77	18.00±1.15	4.01±0.07	10.67±1.20	1.50±0.03	24.10±1.18	8.40±0.77	18.32±1,22
Group II	3.90±1.01	11.98±0.27	0.99±0.04	22.67±1.33	8.13±1.11	18.28±1.01	3.99±0.67	11.15±0.75	1.01±0.02	22.87±1.27	8.34±1.01	17.98±1.18	4.25±0.13	12.01±1.24	1.03±0.06	22.97±1.40	8.21±1.12	18,43±1.37
Group III	3.85±0.97	12.76±0.67	1.20±0.06	25.23±1.47	8.71±0.77	18.31±1.02	3.87±0.57	12.30±1.01	1.25±0.01	24.27±1.98	8.38±0.96	18.30±1.66	3.42±1.12	12.54±1.12	1.35±0.01	26.00±1.67	8.35±0.67	18.21±1.45
Group IV	3.00±0.09	9.62±0.33	0.98±0.02	23.04±1.37	8.38±0.97	18.31±1.12	3,12±0.06	9.55±0.13	0.95±0.03	23.42±1.12	8.15±1.11	18.32±1.01	3.35±1.37	10.03±1.32	1.05±0.11	24.00±1.46	8.37±1.11	18.38±1,36
Group V	3.57±0.99	9.09±1.13	1.08±0,08	22.54±1.67	8.07±1.01	18.01±1.78	3.02±0.07	9.67±1.21	1.06±0.06	23.08±1.37	8.12±1.01	18.30±1.77	3.31±0.07	11.37±1.67	1.36±0.12	23,12±1.78	8.12±1.11	18.35±1,55

Mean values (n=2) ± SEM. Group I: Killed by drowning in sea water; Group II: Killed and submerged in sea water; Group III: Killed by drowning in fresh water; Group IV: Killed before submerged in fresh water; Group V: Killed not submerged in sea water nor fresh water.

A significant decrease in temperature was observed in all experimental grous of rabbits, as presented in Table 5. Freshwater drowning (Group III) led to a significant decrease in temperature ($p \leq 0.05$) from the initial temperature (Table 5) to the maximum percentage temperature decrease of 27.65% in Pirates Beach and Malindi Beach water samples. Further observation of the bodies of rabbits in the different experimental groups was done. In the bodies of rabbits drowned in marine water (Group I) and killed and submerged in marine water (Group II), there was blowfly visitation, haemorrhage in the right lungs, and significant marbling and pigmentation in the liver were observed within 24 hours. In the rabbit bodies drowned in fresh water (Group III) and those killed and submerged in fresh water (Group IV), visitation by flesh flies, absence of haemorrhage in both lungs, negligible marbling, and normal liver were observed within 24 hours. Marine water drowning (Group I) from different beaches did not have a significant influence on the percentage temperature decrease ($p \leq 0.05$). Similarly, killing and submerging the rabbits in marine water from different beaches (Group II) had no significant influence on the percentage decrease in temperature ($p \leq 0.05$). However, an observed significant percentage decrease in temperature ($p \leq 0.05$) was observed when marine water drowning (Group I) was compared to the freshwater drowning (Group II). Similarly, a significant difference in percentage decrease in temperature ($p \leq 0.05$) was observed when marine water submerging (Group II) was compared with fresh water submerging (Group IV).

Discussion

Diatom concentration at several public beaches along the coastal region

To establish the concentration of diatoms in the beaches along the Kenyan Coastal strip, the water samples were digested and processed for the diatom test using the modified

Table 5: Average temperature changes (°C) and percentage decrease in temperature of rabbits before and after drowning or submerging in water samples collected in different public beaches.

Group	Pirates			Kilifi			Malindi		
	Initial	After 24 hrs	%Decrease	Initial	After 24 hrs	%Decrease	Initial	After 24 hrs	%Decrease
Group I	$38.50^{b}\pm1.46$	$26.00^{cd}\pm1.13$	32.47^{a}	$38.40^{b}\pm1.00$	$25.85^{c}\pm1.37$	32.68^{a}	$38.25^{b}\pm1.13$	$25.40^{c}\pm1.01$	33.59^{a}
Group II	$38.25^{b}\pm1.13$	$28.21^{d}\pm1.01$	26.25^{b}	$38.20^{b}\pm1.17$	$28.25^{d}\pm0.94$	26.05^{b}	$38.15^{b}\pm0.67$	$27.95^{d}\pm0.77$	26.74^{b}
Group III	$38.70^{b}\pm1.27$	$28.00^{d}\pm1.11$	27.65^{b}	$38.35^{b}\pm1.08$	$28.10^{d}\pm0.67$	26.73^{b}	$38.30^{b}\pm1.27$	$27.71^{d}\pm1.12$	27.65^{b}
Group IV	$38.25^{b}\pm1.12$	$28.65^{d}\pm1.37$	25.10^{d}	$38.20^{b}\pm1.12$	$28.65^{d}\pm1.13$	25.00^{d}	$38.15^{b}\pm1.11$	$28.40^{d}\pm1.00$	25.56^{d}
Group V	$38.05^{b}\pm1.02$	$25.65^{cd}\pm1.31$	32.59^{a}	$38.05^{b}\pm1.02$	$25.65^{c}\pm1.31$	32.59^{a}	$38.05^{b}\pm1.02$	$25.65^{c}\pm1.31$	32.59^{a}

Mean values (n = 2) ± SEM. Group I: Killed by drowning in seawater; Group II: Killed and submerged in seawater; Group III: Killed by drowning in freshwater; Group IV: Killed before submerged in freshwater; Group V: Killed not submerged in seawater nor freshwater. Values appended by different superscript letters within a column and within a row are statistically and significantly different ($p \leq 0.05$).

nitric acid method as described by [6]. Kilifi Bay had the lowest concentration of diatoms, with a figure of 300 cells/mL of the sample, while Malindi and Pirates Beach had equal concentrations of 400 cells/mL. This might have been attributed to by the pollution of sea water in Kilifi county which has greatly increased by 4% due to industrial sewage disposal as had been recorded in previous literature [7] thereby negatively affecting the survival of the diatoms in that location as diatoms may be affected morphologically and demographically by different sources of stress, producing teratologies [8] and their assemblage responds to the stressors by shifting on their composition and relative abundance

Recoverability of diatoms from organ samples collected from drowning rabbits

When drowning occurs, water enters the lungs, carrying along with it microscopic content, which includes the diatoms. The whole content enters the bloodstream through the rapture in the peripheral alveoli before being carried to the other organs, such as the liver and the heart. Naturally, the microscopic contents (diatoms) pass into the blood as well [9]. To establish the number of diatoms recoverable from specific organs in the drowned carrion Autopsy was conducted on all rabbit cadavers after 24 hours of retrieval and confirmation of death, and internal organs were collected. The organs were digested and processed for a diatom test using the modified nitric acid method as described by [6]. From this study, it was established that the lungs had the highest number of diatom cells, followed by the heart and the liver. During drowning as an asphyxial activity, the lungs interact in the first phase with the water containing the diatoms, as already explained. When the diatoms enter the bloodstream through rupture in the peripheral alveoli, as explained, their concentration in the heart gets elevated as a result of the normal circulation.

Post-mortem changes in marine and freshwater ecology using drowning rabbits

Body weight changes: Post-mortem changes are the changes that occur in carrion naturally in sequence after death. These changes proceed in a relatively orderly fashion, but they are affected by external factors and intrinsic characteristics [10] . Decomposition is greatly influenced by the sediment type of the sea floor [11] and, therefore, the type of water ecology influences the pattern of post-mortem changes observed in carrion. The weight of the rabbits was measured before and after drowning. The overall weight of the drowned rabbits was higher than their weight before drowning. There was no significant difference between the weight of the post-mortem drowned rabbits recorded before and after drowning in the previous literature. Drowning resulted in elevated mean organ weights for the lungs, liver, kidney, and spleen; only the mean heart and brain weights remain constant [12]. From this study, it was established that the average weight of liver, lungs, and spleen for drowned cases was higher than for the control; however, there was no change in the weight of the brain and heart.

Observation of the bodies drowned in fresh water versus in the sea.

As recorded in previous literature, Pre-autopsy information of investigative importance is necessary for interpretive context in water-related deaths and may be sparse or non-existent altogether, and all efforts to obtain any available information are necessary [13] physical changes occurring on the bodies of the rabbits drowned in seawater and in fresh water were observed to have significant differences. The carrion in fresh water were visited by flesh flies within the time period of 24 hours, while the carrion in sea water were visited by blowflies within the same period. This explains the rate of decomposition in the two carrion; the rabbits drowned in seawater decomposed at a higher rate compared to the rabbits drowned in fresh water.

Temperature changes

After death and cessation of circulation, the convectional transference of heat inside the body comes to a halt. Since no heat is being produced within the cadaver, the body starts losing heat due to the temperature difference between the body and the surroundings. The heat loss due to radiation is substantial at first, but later ebbs down. Most of the heat loss is attributable to conduction and convection of heat. This decrease in body temperature after death is termed algor mortis [1]. In this study, the temperature readings of the rabbits taken before and after drowning, in both water ecologies, were observed to decrease respectively. The temperature readings in the rabbits used for control experiments remained constant.

Conclusion and future work

In conclusion, the study was conducted as a state-of-the-art within the field of forensics dealing with the comparison of Post-mortem changes in marine and freshwater ecology using drowning rabbits, the recoverability of diatoms from organ samples collected from drowning rabbits, and the establishment of diatom concentration at public beaches along the Kenyan coastal region. The study was necessary in equipping the forensic investigation responsible for homicide with the knowledge of analyzing deeply the circumstantial evidence collected from carrion for investigation. The study seeks to form changes in the investigation of homicide cases by the Kenyan Directorate of Criminal Investigations. To fully appreciate this area, a look at differential changes in marine and freshwater ecology, the recoverability of diatoms from organs, and diatom concentration is important. Additionally, the present study revealed that marine salts have been proven to have an impact on the mummification of the carrion. This study established that Post-mortem changes in marine water ecology are significantly different from the changes observed in freshwater ecology. The rate of internal decomposition is higher for carrion in marine water as compared to in freshwater ecology. It is evident, therefore, that the saltiness of water bodies affects the post-mortem changes occurring in the bodies of the carrion. Additionally, the recoverability of diatoms in the organ samples of drowned rabbits has been established positively, with the highest concentrations being in the lungs and the heart. A limitation of this study is its small sample size (n=6 per group), which may affect the statistical power and generalizability of our findings. Despite this, the observed differences were large and consistent, suggesting a real effect. Further studies with larger cohorts are recommended to solidify these findings. Additionally, diatom species identification was not the focus of this paper, but represents a valuable avenue for future research to link a victim to a specific drowning site.

Ethical approval

The study was approved by the Technical University of Mombasa Ethical Review Committee (ERC/TUM003/21). All procedures adhered to international guidelines on animal use for scientific purposes.

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