ARAŞTIRMA MAKALESİ

ADLİ TIP DERGİSİ
TURKISH JOURNAL OF FORENSIC MEDICINE

Geliş: 24.07.2025
Kabul: 19.12.2025

Evaluation of Postmortem Decay Processes in the Underwater Environment of the Black Sea Using A Rat Model: Seasonal, Biological and Microbial Dynamics

Karadeniz'in Sualtı Ortamında Ölüm Sonrası Bozunma Süreçlerinin bir Sıçan Modeli Kullanılarak Değerlendirilmesi: Mevsimsel, Biyolojik ve Mikrobiyal Dinamikler

Kürşat Mendi[1]

[1] Sinop Provincial Police Department, Sinop, Türkiye

Öz

Amaç: Bu araştırmanın amacı, Karadeniz ekosistemine özgü nekrofaj fauna çeşitliliği ile bu organizmaların postmortem çürüme süreçlerine etkisini mevsimsel açıdan değerlendirmektir. Türkiye'nin Karadeniz kıyısında, Sinop ili açıklarında gerçekleştirilen deneysel çalışmada, insan kadavralarını temsilen sıçan örnekleri kullanılmıştır. Bahar (Nisan-Mayıs 2024) ve kış (Aralık 2024–Ocak 2025) dönemlerini kapsayan çalışmada, denekler 13 metre derinlikteki deniz tabanına sabitlenerek 92 gün süresince günlük dalış gözlemleriyle takip edilmiştir. Süreç boyunca su sıcaklığı, pH, tuzluluk ve biyotik etkileşimler gibi çevresel değişkenler düzenli olarak kaydedilmiştir.

Yöntem: Çürüme süreci, hem makroskobik deformasyon hem de mikrobiyal düzeyde analiz edilmiştir. Deformasyonun mekânsal yayılımı, vücut bölgeleri bazında sınıflandırılarak ayrıntılı şekilde raporlanmıştır. Ayrıca, Shore A tipi durometre ile su altı koşullarında doku sertliği ölçümleri gerçekleştirilmiş ve zamansal değişim nicel verilerle izlenmiştir.

Bulgular: Nekrofaj topluluklara ilişkin analizlerde, Karadeniz'e özgü leş yiyici türlerin dağılımı detaylı biçimde sıralanmış; kış döneminde tür zenginliği ve biyolojik etkileşimlerin daha yüksek olduğu belirlenmiştir.

Sonuç: Metagenomik dizileme verileri, özellikle anaerobik ortamlarda etkili olan Sulfurovum gibi sülfat indirgen bakterilerin baskın olduğunu, Arkea domainine ait Aenigmarchaeota şubesinin ise düşük çeşitlilik gösterdiğini ortaya koymuştur. Sonuçlar, sualtı adli vakalarda biyolojik ve çevresel göstergelerin önemini vurgulamaktadır.

Anahtar Kelimeler: Adli tafonomi, Nekrofag fauna, Ayrışma dinamikleri, Karadeniz'in sualtı leşçi türleri, Sualtı cesetleri, Sualtı postmortem dönüşüm süreci

Abstract

Aim: The aim of this research is to evaluate the diversity of necrophagous fauna specific to the Black Sea ecosystem and the effect of these organisms on postmortem decay processes from a seasonal perspective. In the experimental study carried out off the coast of Sinop, on the Black Sea coast of Türkiye, rat samples were used to represent human cadavers. In the study covering the spring (April-May 2024) and winter (December 2024–January 2025) periods, the subjects were fixed to the seabed at a depth of 13 meters and followed with daily dive observations for 92 days. Environmental variables such as water temperature, pH, salinity and biotic interactions were regularly recorded throughout the process.

Methods;The decay process was analyzed at both macroscopic deformation and microbial level. The spatial distribution of deformation was reported in detail, classified on the basis of body regions. Additionally, tissue hardness measurements were performed under underwater conditions with a Shore A type durometer and temporal changes were monitored with quantitative data. In the analyses of necrophagous communities, the distribution of scavenging species specific to the Black Sea was listed in detail, and it was determined that species richness and biological interactions were higher in the winter period.

Results and Conclusion; Metagenomic sequencing data revealed that sulfate-reducing bacteria such as Sulfurovum, which are particularly effective in anaerobic environments, are dominant, while the Aenigmarchaeota phylum of the Archaea domain shows low diversity. The results highlight the importance of biological and environmental indicators in underwater forensic cases.

Keywords: Forensic taphonomy, Necrophagous fauna, Dynamics of decomposition, Underwater scavenging species of the Black sea, Underwater corpses, underwater postmortem transformation process

Nasıl Atıf Yapmalı: Mendi K. Evaluation of Postmortem Decay Processes in the Underwater Environment of the Black Sea Using A Rat Model: Seasonal, Biological and Microbial Dynamics. Adli Tıp Dergisi 2025;39(3):(269-286) https://doi.org/10.61970/adlitip.1748857

Sorumlu Yazar: Kürşat Mendi, Sinop Provincial Police Department, Sinop, Türkiye .
E-posta: kursat128@gmail.com

INTRODUCTION

In forensic medical evaluations of aquatic deaths, the effects of environmental conditions on decay processes play an important role. Due to low temperature, limited oxygen and microbial differences, underwater decay tends to proceed more slowly compared to terrestrial environments. However, the rapid biological destruction of soft tissues by necrophagous fauna may cause postmortem changes to be confused with traumatic lesions, making forensic evaluation difficult (1).

In this context, the Black Sea attracts attention with its unique chemical features such as suboxic zones and hydrogen sulfide accumulation in deep water layers (2). This environment allows for the extraordinary preservation of organic material and also prepares the ground for microbial activity and differentiation of chemical cycles (3).

However, considering this unique environmental structure, the lack of scientific data on underwater decay processes specific to the Black Sea is striking. This gap necessitates the development of forensic research in the region. The main objective of this study is to investigate the structural and temporal characteristics of the underwater decay process and accompanying textural deformations through an experimental model that mimics the environmental conditions specific to the Black Sea. The need for original data on postmortem decomposition, especially in oxygen-limited marine environments, provides scientific justification for this research (4).

One of the methodologically original aspects of the study is that the Shore A type durometer was used for the first time to measure biological tissue hardness under underwater conditions (1). The quantitative data obtained with this device allowed the objective assessment of the change in hardness loss in soft tissues over time. The absence of a similar application in the literature in this context puts the study in a pioneering position methodologically.

The research also aims to support the monitoring of postmortem stages in bodies that do not float to the surface of the water, to facilitate the differentiation of deformations due to environmental conditions from possible traumatic injuries, and to strengthen the scientific basis for the evaluation of forensic findings. It is anticipated that the findings obtained will contribute to the interpretation of postmortem changes in forensic autopsy processes (1,5).

MATERIALS AND METHODS

Study Area and Experimental Timhandine

emaln Within the scope of this research, a ship pier with a salinity of approximately 1,8 % and a depth of 13 meters off the coast of Sinop, on the Turkish Black Sea coast, was determined as the experimental field (2). Experimental applications were carried out in the spring (April–May 2024) and winter (December 2024–January 2025) periods to represent the seasonal variability of marine biological activity. Two separate experimental cycles were planned, taking into account the effects of seasonal differences on necrophagous populations.

During the monitoring period, which lasted 92 days in total, environmental parameters and video and photo documentation were analyzed according to a structured observation protocol. Monitoring periods were determined based on literature findings on the time it takes for bodies to reach buoyancy in aquatic environments (6). The experimental period was extended beyond the planned period in order to ensure that all underwater decomposition stages could be monitored, as only one specimen was observed to surface.

Environmental data recorded during the study revealed the effects of salinity, pH, water temperature and depth on

underwater decay processes. These parameters, which are generally ignored in the literature, were found to play a decisive role in shaping the deformation patterns observed in cadavers.

Sample Size Determination and Power Analysis

To ensure methodological rigor, a power analysis was carried out to estimate the minimum number of samples needed to detect statistically meaningful differences during advanced stages of tissue degradation. Due to the absence of directly comparable underwater studies, reference parameters—particularly for estimating variability and effect magnitude—were inferred from a pig cadaver decomposition study by Chin, Sulaiman, and Othman (2010).

The analysis targeted two primary biological factors: seasonal shifts in tissue stiffness and osmotic weakening caused by extended immersion. A two-sample t-test with a two-tailed distribution was applied, assuming a substantial effect size (Cohen's d = 1.2) based on ecological contrasts between spring and winter and the expected impact of submersion-driven decay. At α = 0.05 and 80% power, a minimum of seven subjects per group was identified (n = 7).

As illustrated in Figure 1, the achieved power level of 0.811 confirms that the sample size is adequate to capture both environmental and physiological effects influencing decomposition dynamics

Ethical Approval and Subject Shandection

Before conducting this study, the necessary ethical approval was obtained from the Ondokuz Mayıs University Animal Experiments Local Ethics Committee (OMÜ-HADYEK). The research titled «Investigation of the Deformation Process of Rat Carcasses Under Sea Water» was evaluated at the committee meeting on April 25, 2024, and approved as project number 2024/08, in accordance with the Animal Rights and Ethical Experimentation Principles (Ethics Approval No: E-68489742-604.01-2400079539). Following this approval, the experimental process began.

Wistar albino laboratory rats obtained from the Experimental Animal Production Unit of Ondokuz Mayıs University were used for experimental applications. A total

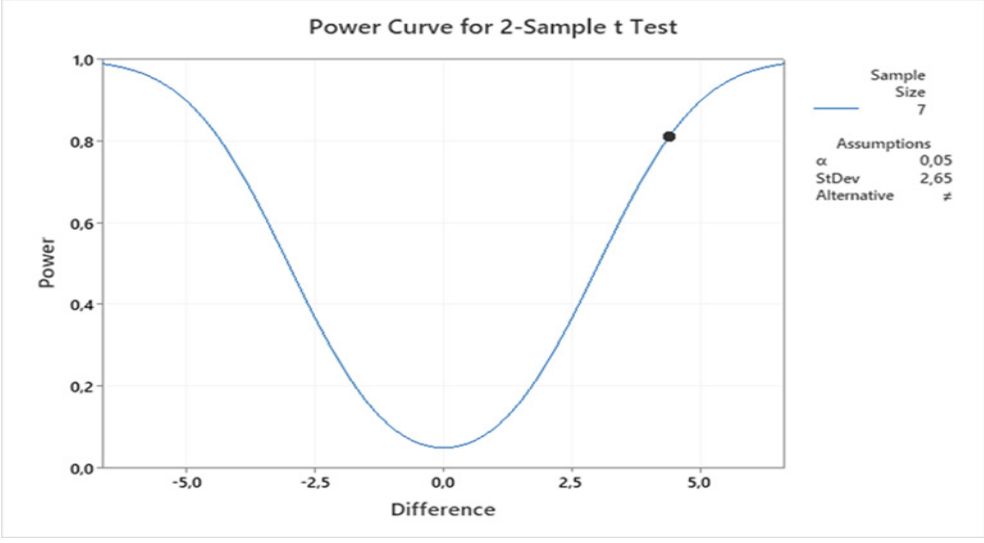

Figure 1. Power Curve for a Two-Sample t-Test.

of 14 adult individuals, each approximately five months old and with an average body weight of 200–420 grams, were included in the study to represent the spring and winter periods. Gender distribution was kept balanced in both periods.

Within the scope of euthanasia procedure carried out in accordance with ethical rules and veterinary practice standards, Ketamine-Xylazine combination was administered intraperitoneally to rats (320 mg/kg). Pharmacological efficacy was evaluated after euthanasia and experimental process was started after death was confirmed.

Preparation and Transportation of Specimens

After the death of the euthanized rats for each experimental period was confirmed as a result of basic reflex checks, other procedures were continued.

The subjects› body surfaces were shaved to minimize external effects and to enable clear underwater observations. Descriptive identification procedures were completed by recording the gender and weight data of each individual.

Subjects were transferred from the laboratory to the experimental area in styrofoam boxes classified according to seasonal experimental groups and by providing cold chain conditions. After the transfer, the adaptation of the subjects to the environmental conditions was verified and the experimental process was started.

Underwater Positioning of the Specimens

In order to maintain the natural position of the subjects during the decomposition process, each individual was positioned on immobilizing ceramic plates placed on the seabed. In order to reduce the effects of currents and to balance the upward acceleration, the subjects were stabilized with flexible mooring systems. In order to observe the effects of the underwater necrophagous fauna without interruption, the individuals were placed at zero level on the bottom, and potential biological differences were observed using different anatomical positions and gender distributions.

Each individual was positioned at one-meter intervals and monitored to maintain the initial depth and position determined throughout the experiment. All observations were made in situ, without the subjects being brought to the surface. Observation periods were planned at daily intervals and each was carried out systematically within the framework of a pre-structured dive program.

During the research, the necessary legal permits were obtained from the relevant public institutions; diving activities were carried out by certified experts in accordance with safety protocols. Regular planning of dives throughout the experimental period ensured stability in terms of observation quality and continuity of environmental data.

Data Collection Instruments and Analytical Process

In order to monitor the hardness changes in soft tissues, a Shore A type durometer was used in this study, as seen in Figure 2. This method was applied for the first time in both field conditions and underwater environments, thus introducing a new method to the literature. In the current literature review, no example was found indicating that this device had been used in a forensic context to quantitatively assess biological soft tissue decay.

The durometer allowed the precise monitoring of the temporal progression of soft tissue deformation and provided objective, repeatable data on the decay process. Different devices of the same model were used in each experimental period in order to prevent oxidative effects that may occur as a result of the device being exposed to seawater during the measurement process.

All measurements were carried out in situ, without bringing the rat corpses to the surface, by diving only; thus,

data were obtained without external interference to the natural decay process.

Figure 2. Measurement of Muscle Tissue Hardness Using a Durometer in the Underwater Environment.

RESULTS

Among Many experimental studies have been conducted on postmortem processes in terrestrial environments, and thus important forensic findings have been obtained regarding the time, manner of death and stages of decay (1). On the other hand, research on death cases occurring in aquatic environments has been limited; in particular, the dynamics of decay processes have not been sufficiently elucidated.

In this context, the present study aims to systematically evaluate the deformation processes of underwater corpses under the unique environmental conditions of the Black Sea. The focus of the study is on the decay course of corpses that do not float to the surface, their potential to gain buoyancy, and the effects of environmental factors on this process. The data obtained contribute to the understanding of postmortem changes observed underwater; and enable the strengthening of forensic analyses in marine environments by evaluating the decay stages, the order of effects of necrophagous organisms, and environmental factors (7).

In this study, when Table 1 is examined, qualified data on underwater decay processes were obtained during the 92-day observation period covering the spring and winter periods with dives carried out at regular intervals. Measurements on soft tissue hardness could not be made as of the 11th day in the spring period and the 12th day in the winter period; this situation showed that the tissues became

Table 1. Daily variations in physical and microbiological parameters: A comparative analysis across different periods

Day	Date		Shore Grade		Buoyancy		pH		Water Temperature		Initial Microbial Activity Zones	
	Spring	Winter	Spring	Winter	Spring	Winter	Spring	Winter	Spring	Winter	Spring	Winter
1.	2.05.2024	4.12.2024	0,6	16	(7/7) -	(7/7) -	8,5	8,12	14	13	Submersion Day	
2.	3.05.2024	5.12.2024	0,5-0,4	10.Kas	(7/7) -	(7/7) -	8,75	8,2	15	13	-	-
3.	4.05.2024	6.12.2024	0,5-0,4	0,9-0,8	(7/7) -	(7/7) -	8,72	8,25	17	13	-	-
4.	5.05.2024	7.12.2024	0,4-0,3	0,9-0,6	(7/7) -	(7/7) -	8,76	8,21	17	13	-	-
5.	6.05.2024	8.12.2024	0,4-0,2	0,8-0,4	(7/7) -	(7/7) -	8,74	8,25	15	13	-	cervical, abdominal
6.	7.05.2024	9.12.2024	0,4-0,2	0,8-0,3	(7/7) -	(7/7) -	8,78	8,22	14	13	cervical, abdominal, gluteal	+
7.	8.05.2024	10.12.2024	0,4-0,2	0,6-0,3	(7/7) -	(7/7) -	8,77	8,19	15	13	thoracic	gluteal
8.	9.05.2024	11.12.2024	0,3-0,2	0,5-0,3	(7/7) -	(7/7) -	8,73	8,22	16	13	dorsal, cranial	cranial
9.	10.05.2024	12.12.2024	0,2-0,1	0,4-0,3	(7/7) -	(7/7) -	8,81	8,26	16	13	oral, nasal	thoracic
10.	11.05.2024	13.12.2024	0,1-0	0,3-0,2	(7/7) -	(7/7) -	8,75	8,25	15	13	femoral	dorsal

11.	12.05.2024	14.12.2024	0	0,1-0	(7/7) -	(7/7) -	8,72	8,1	14	12	orbital, caudal	+
12.	13.05.2024	15.12.2024	0	0	(7/7) -	(7/7) -	8,73	8,25	15	12	+	oral, nasal, caudal
13.	14.05.2024	16.12.2024	0	0	(7/7) -	(7/7) -	8,72	8,31	14	13	brachial	brachial
14.	15.05.2024	17.12.2024	0	0	(7/7) -	(7/1) +	8,76	8,19	15	12	hand	+
15.	16.05.2024	18.12.2024	0	0	(7/7) -	(7/1) +	8,76	8,28	15	12	foot	+
16.	17.05.2024	19.12.2024	0	0	(7/7) -	(7/1) +	8,79	8,27	17	12	+	+
17.	18.05.2024	20.12.2024	0	0	(7/7) -	(7/1) +	8,76	8,26	16	12	+	hand
18.	19.05.2024	21.12.2024	0	0	(7/7) -	(7/7) -	8,76	8,24	16	12	+	foot
19.	20.05.2024	22.12.2024	0	0	(7/7) -	(7/7) -	8,72	8,2	17	12	+	+
20.	21.05.2024	23.12.2024	0	0	(7/7) -	(7/7) -	8,73	8,23	17	12	+	+
21.	22.05.2024	24.12.2024	0	0	(7/7) -	(7/7) -	8,75	8,24	17	12	+	+
22.	23.05.2024	25.12.2024	0	0	(7/7) -	(7/7) -	8,74	8,26	18	12	+	+
23.	24.05.2024	26.12.2024	0	0	(7/7) -	(7/7) -	8,65	8,22	15	12	+	+
24.	25.05.2024	27.12.2024	0	0	(7/7) -	(7/7) -	8,64	8,22	13	12	+	+
25.	26.05.2024	28.12.2024	0	0	(7/7) -	(7/7) -	8,74	8,27	16	12	+	+
26.	27.05.2024	29.12.2024	0	0	(7/7) -	(7/7) -	8,74	8,24	16	12	+	+
27.	28.05.2024	30.12.2024	0	0	(7/7) -	(7/7) -	8,75	8,2	16	12	+	+
28.	29.05.2024	31.12.2024	0	0	(7/7) -	(7/7) -	8,74	8,28	16	12	+	+
29.	30.05.2024	1.01.2025	0	0	(7/7) -	(7/7) -	8,78	8,27	18	11	+	+
30.	31.05.2024	2.01.2025	0	0	(7/7) -	(7/7) -	8,72	8,24	15	12	+	+
31.	1.06.2024	3.01.2025	0	0	(7/7) -	(7/7) -	8,76	8,27	16	12	+	+
32.	2.06.2024	4.01.2025	0	0	(7/7) -	(7/7) -	8,73	8,26	16	12	+	+
33.	3.06.2024	5.01.2025	0	0	(7/7) -	(7/7) -	8,76	8,26	17	12	+	+
34.	4.06.2024	6.01.2025	0	0	(7/7) -	(7/7) -	8,73	8,35	17	11	+	+
35.	5.06.2024	7.01.2025	0	0	(7/7) -	(7/7) -	8,73	8,28	16	12	+	+
36.	6.06.2024	8.01.2025	0	0	(7/7) -	(7/7) -	8,74	8,23	16	12	+	+
37.	7.06.2024	9.01.2025	0	0	(7/7) -	(7/7) -	8,73	8,3	18	11	+	+
38.	8.06.2024	10.01.2025	0	0	(7/7) -	(7/7) -	8,71	8,24	18	11	+	+
39.	9.06.2024	11.01.2025	0	0	(7/7) -	(7/7) -	8,73	8,24	19	11	+	+
40.	10.06.2024	12.01.2025	0	0	(7/7) -	(7/7) -	8,75	8,28	20	11	+	+
41.	11.06.2024	13.01.2025	0	0	(7/7) -	(7/7) -	8,68	8,2	19	12	+	+
42.	12.06.2024	14.01.2025	0	0	(7/7) -	(7/7) -	8,67	8,36	15	11	+	+
43.	13.06.2024	15.01.2025	0	0	(7/7) -	(7/7) -	8,7	8,33	17	11	+	+
44.	14.06.2024	16.01.2025	0	0	(7/7) -	(7/7) -	8,67	8,33	16	11	+	+
45.	15.06.2024	17.01.2025	0	0	(7/7) -	(7/7) -	8,76	8,3	13	11	+	+

completely open to the effects of necrophagous organisms as a result of osmotic softening (1). Especially in the winter period, a significant increase was observed in the diversity and number of species participating in deformation (8).

As seen in the «Buoyancy» column in Table 1, no subjects surfaced in the spring, while in the winter, only one subject out of 7 rats temporarily gained positive buoyancy for 4 days, including days 14–17. this finding demonstrates the influence of water temperature and decay pressure on buoyancy and suggests that mass gain from water intake in subjects placed in the ventral position should not be confused with gas accumulation.

As can be seen from the column titled « Initial Microbial Activity Zones « in Table 1, the colonization process began

on the 6th day in spring and the 5th day in winter, and gradually spread throughout the body over the following days. In both periods, microorganisms were found to first proliferate in the abdomen and neck regions, eventually reaching the feet and extremities. The higher colonization rate in spring was associated with increased microbial activity due to water temperature.

The research findings revealed that the interest of necrophagous organisms in rat corpses varied depending on the hardness level of the tissues and the stage of decay. Throughout the experimental period, no observable or measurable differences were found between male and female subjects in terms of tissue degradation patterns. The fact that the subjects were positioned in direct interaction with the underwater fauna allowed these observations to be obtained under natural conditions. In seasonal comparisons, a significant increase in both scavenger diversity and species interaction frequency was observed during the winter months.

When Table 2 is examined; the first colonizer species

Table 2. Daily observations of biotic interactions and anatomical deformation areas during the postmortem deformation process: A comparative analysis between periods.

Day	Date		Scavenging organisms		Anatomical site of deformation		Predator species localized in the region	
	Spring	Winter	Spring	Winter	Spring	Winter	Spring	Winter
1.	2.05.2024	4.12.2024	Submersion Day					
2.	3.05.2024	5.12.2024	Turrithandla communis	Turrithandla communis	Diffuse contact	Diffuse contact		
3.	4.05.2024	6.12.2024	Turrithandla communis	Eriphia verrucosa, Clibanarius erythropus, Turrithandla communis	Diffuse contact	Nasal, Auricular, Foot		
4.	5.05.2024	7.12.2024	Turrithandla communis	Eriphia verrucosa, Turrithandla communis	Diffuse contact	Foot	Gobius cruentatus	Scorpaena porcus
5.	6.05.2024	8.12.2024	Turrithandla communis	Eriphia verrucosa, Turrithandla communis	Diffuse contact	Nasal, Foot, Caudal		
6.	7.05.2024	9.12.2024	Rapana thomasiana Crosse	Eriphia verrucosa, Turrithandla communis	Cranial	Caudal		Scorpaena porcus
7.	8.05.2024	10.12.2024	Turrithandla communis, Rapana thomasiana Crosse	Palaemon adspersus, Eriphia verrucosa, Turrithandla communis	Diffuse contact, Auricular	Cranial, Caudal		
8.	9.05.2024	11.12.2024	Turrithandla communis	Eriphia verrucosa, Turrithandla communis	Diffuse contact	Caudal		Scorpaena porcus, Gobius cruentatus
9.	10.05.2024	12.12.2024	Turrithandla communis	Turrithandla communis	Diffuse contact	Diffuse contact		
10.	11.05.2024	13.12.2024	Turrithandla communis	Eriphia verrucosa, Clibanarius erythropus, Turrithandla communis	Diffuse contact	Foot, Caudal		Gaidropsarus mediterraneus
11.	12.05.2024	14.12.2024	Turrithandla communis, Rapana	Eriphia verrucosa, Clibanarius erythropus, Turrithandla communis	Diffuse contact, Hand	Foot, Caudal, Cranial		Gobius cruentatus

			thomasiana Crosse				
12.	13.05.2024	15.12.2024	Turrithandla communis, Rapana	Clibanarius erythropus, Eriphia verrucosa, Turrithandla communis	Diffuse contact, Hand	Foot	
			thomasiana Crosse				
13.	14.05.2024	16.12.2024	Turrithandla communis	Clibanarius erythropus, Eriphia verrucosa	Diffuse contact	Foot, Thoracic, Cranial, Caudal	Scorpaena porcus
14.	15.05.2024	17.12.2024	Turrithandla communis	Clibanarius erythropus, Eriphia verrucosa, Parablennius tentacularis	Diffuse contact	Caudal, Thoracic, Foot, Cranial	NeoNeogobius mhandanostomusus mhandanostomus
15.	16.05.2024	18.12.2024	Turrithandla communis	Clibanarius erythropus, Eriphia verrucosa, Amphipoda	Diffuse contact	Dorsal, Cranial, Caudal, Foot, Abdominal	Scorpaena porcus, Gaidropsarus mediterraneus
16.	17.05.2024	19.12.2024	Turrithandla communis	Clibanarius erythropus, Palaemon adspersus, Eriphia verrucosa	Diffuse contact	Dorsal, Foot, Caudal	Scorpaena porcus
17.	18.05.2024	20.12.2024	Turrithandla communis	Eriphia verrucosa, Clibanarius erythropus, Palaemon adspersus	Diffuse contact	Caudal, Cranial, Dorsal	Gobius cruentatus
18.	19.05.2024	21.12.2024		Clibanarius erythropus		Dorsal, Foot	Gobius cruentatus
19.	20.05.2024	22.12.2024		Amphipoda, Clibanarius erythropus		Caudal	
20.	21.05.2024	23.12.2024		Clibanarius erythropus, Eriphia verrucosa		Caudal, Foot	Gobius cruentatus
21.	22.05.2024	24.12.2024	Parablennius tentacularis	Clibanarius erythropus, Palaemon adspersus	Caudal, Foot	Foot, Dorsal, Caudal	Scorpaena porcus
22.	23.05.2024	25.12.2024		Clibanarius erythropus, Palaemon adspersus, Eriphia verrucosa		Caudal, Foot	Scorpaena porcus
23.	24.05.2024	26.12.2024	Parablennius tentacularis	Clibanarius erythropus	Caudal	Dorsal, Foot, abdominal	NeoNeogobius mhandanostomusus mhandanostomus
24.	25.05.2024	27.12.2024	Parablennius tentacularis	Clibanarius erythropus, Eriphia verrucosa	Caudal	Dorsal, Foot, Hand, Caudal	
25.	26.05.2024	28.12.2024		Clibanarius erythropus, Eriphia verrucosa		Foot, Caudal, Cranial, Thoracic	
26.	27.05.2024	29.12.2024		Eriphia verrucosa, Palaemon adspersus		Foot, Caudal, Hand, Dorsal, Abdominal	NeoNeogobius mhandanostomusus mhandanostomus
27.	28.05.2024	30.12.2024		Clibanarius erythropus, Palaemon adspersus		Foot, Hand, Cranial	Scorpaena porcus
28.	29.05.2024	31.12.2024		Palaemon adspersus, Clibanarius erythropus, Eriphia verrucosa		Caudal, Foot, Cranial, Hand	Scorpaena porcus

29.	30.05.2024	1.01.2025		Clibanarius erythropus, Eriphia verrucosa		Hand, Foot, Caudal, Abdominal, Cranial	Scorpaena porcus	NeoNeogobius mhandanostomusus mhandanostomus
30.	31.05.2024	2.01.2025		Clibanarius erythropus, Eriphia verrucosa		Foot, Cranial		
31.	1.06.2024	3.01.2025		Eriphia verrucosa		Foot		
32.	2.06.2024	4.01.2025		Clibanarius erythropus		Foot, Cranial		Scorpaena porcus
33.	3.06.2024	5.01.2025	Amphipoda	Clibanarius erythropus	Diffuse contact	Foot	Scorpaena porcus	Scorpaena porcus
34.	4.06.2024	6.01.2025	Amphipoda	Clibanarius erythropus	Diffuse contact	Dorsal, Foot, Cranial	Gobius cruentatus	Scorpaena porcus
35.	5.06.2024	7.01.2025		Clibanarius erythropus, Palaemon adspersus		Foot, Abdominal		
36.	6.06.2024	8.01.2025		Clibanarius erythropus, Palaemon adspersus		Foot, Cranial, Caudal		
37.	7.06.2024	9.01.2025	Parablennius tentacularis	Clibanarius erythropus, Palaemon adspersus	Cranial	Caudal		Scorpaena porcus, NeoNeogobius mhandanostomusus mhandanostomus
38.	8.06.2024	10.01.2025	Parablennius tentacularis	Clibanarius erythropus	Caudal	Foot		Scorpaena porcus, Gobius cruentatus
39.	9.06.2024	11.01.2025		Eriphia verrucosa, Clibanarius erythropus		Cranial, Foot		Scorpaena porcus, NeoNeogobius mhandanostomusus mhandanostomus
40.	10.06.2024	12.01.2025	Parablennius tentacularis	Eriphia verrucosa	Cranial	Dorsal		Scorpaena porcus
41.	11.06.2024	13.01.2025		Clibanarius erythropus		Foot, Cranial, Dorsal, Abdominal, Caudal		
42.	12.06.2024	14.01.2025	Amphipoda	Clibanarius erythropus, Eriphia verrucosa	Diffuse contact	Foot, Cranial, Abdominal, Thoracic		
43.	13.06.2024	15.01.2025	Amphipoda	Clibanarius erythropus, Palaemon adspersus	Diffuse contact	Foot, Abdominal, Dorsal, Cranial		Scorpaena porcus, Gaidropsarus mediterraneus
44.	14.06.2024	16.01.2025	Amphipoda	Palaemon adspersus	Diffuse contact	Cranial		
45.	15.06.2024	17.01.2025		Eriphia verrucosa		Cranial		
49.	19.06.2024		Parablennius tentacularis		Cranial			
50.	20.06.2024		Eriphia verrucosa		Cranial			

in both seasons were determined to be minaret snails (*Turritella communis*). It was determined that certain species (e.g. *Rapana thomasiana Crose*) were active in the spring, but these species were not observed in the winter months. Species such as *Parablennius tentacularis, Amphipoda, Eriphia verrucosa and Palaemon adspersus* showed differences in seasonal distribution and regional preference. The frequency of participation of these species in the deformation process, their selective behaviors towards the body region and their timing reveal the role of biological interaction on the rate of decay.

In addition, various predatory fish species were observed in the study area in both periods. It is evaluated that these species affect the decay process by creating an indirect pressure on necrophagous organisms. The increase in predatory species diversity, especially in the winter period, indicates that ecological competition may be an important factor in necrophagous-fauna interactions.

Seasonal distribution of scavengers participating in the

deformation process and their effects on decay showed remarkable differences. In the spring period, T*urritella communis, Rapana thomasiana Crose, Parablennius tentacularis, Amphipoda and Eriphia verrucosa* species were detected; while in the winter period, new species such as *Clibanarius erythropus, Neogobius melanostomus and Palaemon adspersus* were added to this list. The increasing species diversity, especially in the winter period, reveals that the level of biological interaction is high despite the low temperature conditions.

Figure 3. Feeding behavior of Rapana venosa in the underwater environment, with activity concentrated around the mouth, nose, and eye regions of the rat carcass.

A seasonal difference was also observed in terms of the body parts targeted by scavengers. In the spring, deformation was generally concentrated in distal regions such as the head, ears, hands and tail, while in the winter, larger and softer tissue regions such as the nose, feet, chest and abdomen were also affected. This situation shows that the selective behavior of organisms on the body may change as the decay stages progress, as seen in Figure 3, and sheds light on the evaluation of lesions in forensic analyses.

Microbial Taxonomy

The biofilm formation seen in Figure 4 and Figure 5 corresponds to the advanced stages of decay. The samples taken were sent to BM Software Consulting and Lab. System Ltd. (Trade Registry Number: 195281) for microorganism research. As a result of the taxonomic evaluation of the samples taken during this period, it was determined that microorganisms belonging to the Archaea domain could only be identified up to the class level (9, 10). This limitation is associated with the inadequacy of the existing phylogenetic databases and the fact that the genetic diversity of this group has not been defined to a large extent. In contrast, species belonging to the Bacteria domain could be identified in detail up to the genus level thanks to more comprehensive reference data.

Figure 4. Image of the biofilm layer formed on rat carcasses retrieved from the underwater environment during the winter-period experiment.

This indicates that bacterial diversity may provide more functional results in forensic applications in terms of detailed evaluation of the microbial composition in the decay environment.

Figure 5. Image of the biofilm layer formed on rat carcasses retrieved from the underwater environment during the spring-period experiment.

Read quality for the V1–V9 region

Prior to bioinformatic analysis, quality control of sequencing data was performed using FastQC software. When Figure 6 is examined, the overall quality level of raw data obtained from the Illumina platform was found to be high; Phred scores of 35 and above starting from the 9th base position showed that most of the sequencing was performed with high accuracy. Partial quality decreases observed at the starting positions were evaluated as technical artifacts, and the remaining data segments were d s.

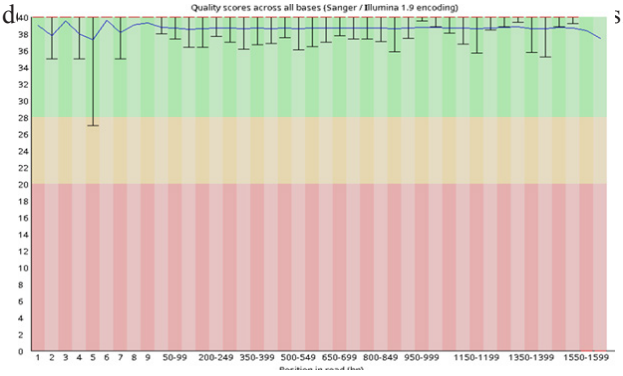

Figure 6. Positional distribution graph of read quality scores across all bases according to Sanger/Illumina 1.9 encoding.

Findings from Archaea-Based Metagenomic Analysis

In this study, metagenomic sequencing targeting the V1–V9 regions of the 16S rRNA gene was applied to analyze microbial DNA obtained from environmental samples. A total of 15,138 high-quality reads were obtained and all sequences were above the quality thresholds. Sequencing was performed using the Illumina NovaSeq platform.

Data Processing and Analysis Workflow

Raw data obtained after sequencing were subjected to quality control processes and bioinformatic analyses were performed using FastQC and QIIME2 software. Reads with a Phred score below 20 and primer and barcode sequences were excluded from the analysis; chimeric sequences were extracted using the DADA2 algorithm. In the following stage, operational taxonomic units (OTUs) were created and taxonomic assignments were made (11).

Taxonomic Composition

The taxonomic analysis results revealed that only one archaeal phylum was dominant in the studied sample. As can be seen from Figure 8, all sequences obtained at the Phylum level were classified as *Aenigmarchaeota*. As can be seen from Figure 7, all archaeal sequences at the Class level were determined to be compatible with the Deep Sea Euryarchaeotic Group (DSEG). At the lower taxonomic levels of order, family and genus, the data were evaluated in the "Incertae Sedis" (uncertain position) category and detailed classification could not be made. These results indicate that the existing reference databases belonging to the *Aenigmarchaeota* phylum are limited in scope and that previously undescribed or low-known archaeal species may exist in the sampled environment.

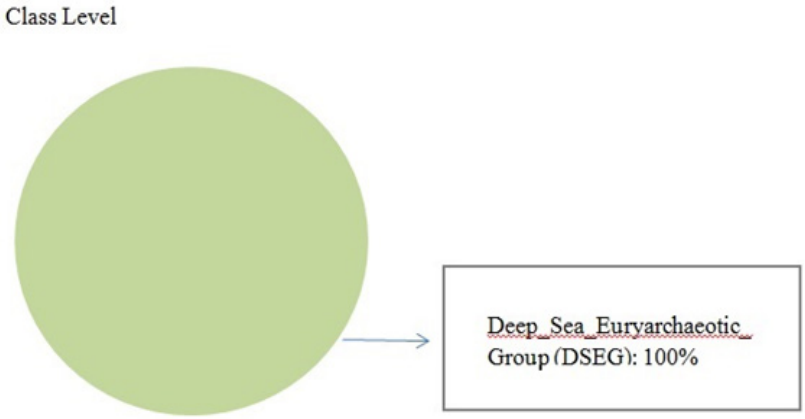

Figure 7 At the class level, the Deep Sea Euryarchaeotic Group (DSEG) was identified and found to be dominant at 100%.

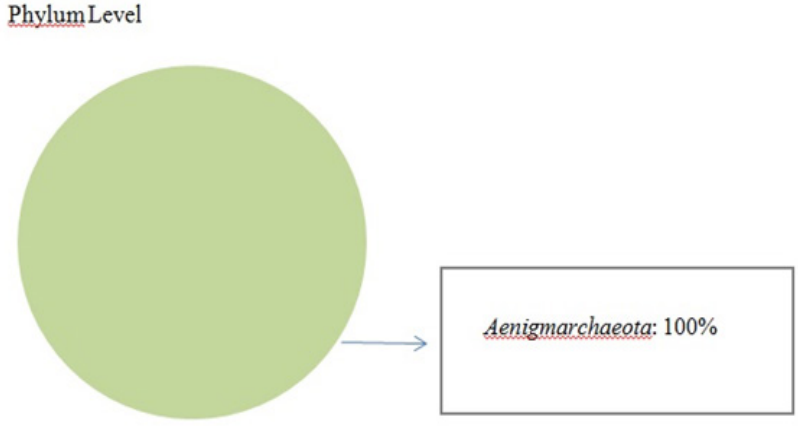

Figure 8. At the phylum level, only organisms belonging to the phylum Aenigmarchaeota were present, exhibiting 100% dominance.

The phylum *Aenigmarchaeota* belongs to the domain Archaea and includes microorganisms that generally exist in extreme environmental conditions, especially deep-sea hydrothermal vents and sediments (12). This group is characterized by small cell and genome structures and develops symbiotic or parasitic life strategies due to the lack of central biosynthetic pathways. These features allow *Aenigmarchaeota* to survive in oxygen-free and nutrient-limited environments by interacting with different microbial communities. Hydrogenase enzymes detected in their genomes support this adaptability by contributing to energy production processes through hydrogen metabolism.

Uncovering Bacterial Community Structure through Metagenomic Profiling

In this study, high-throughput sequencing (NGS) method targeting V1–V9 regions of 16S rRNA gene was applied to determine microbial diversity in environmental samples. As a result of sequencing performed with Illumina NovaSeq platform, a total of 15,138 reads were obtained and no low-quality sequences were found in the analysis. The obtained data were analyzed using QIIME2 software (13).

Raw Data Processing and Quality Control

Quality assessment of raw sequencing data was performed using FastQC software. Prior to analysis, low quality and off-target sequences were filtered, and chimeric sequences were eliminated using the DADA2 algorithm. As a result of these quality control processes, a high-confidence dataset was obtained for analysis.

Taxonomic Assignment and Community Structure

Sequencing data were clustered to allow the creation of operational taxonomic units (OTUs) and taxonomic assignments were made for each OTU. When Figure 9 is examined, according to the taxonomic analysis results, the most dominant microbial phyla in the sampled environment are *Planctomycetota, Campylobacterota* and *Pseudomonadota*. It was determined that Planctomycetes, *Campylobacteria, Campylobacterales* and *Pirellulales* groups were prominent at the class and order levels, respectively. At the genus level, *Sulfurovum, Halodesulfovibrio* and *Synechococcus_CC9902* species are particularly notable. These findings reveal that bacterial populations with high sulfur reduction capacity and adapted to anaerobic conditions are dominant in the environment where the decay process continues.

Analyses at genus level revealed that various bacterial species were represented at different rates in the sampled marine environment. In particular, it was determined that the genus *Sulfurovum* was clearly dominant and constituted a significant part of the microbial community. However, it was observed that various species such as *Halodesulfovibrio, Synechococcus_CC9902, Clostridium, Aliiroseovarius* and *Anaerovorax* were also found at significant rates. When the general distribution was evaluated, it was understood that microbial diversity was high and species density was concentrated around certain genera.

The genus *Sulfurimonas*, which was predominantly detected in this study, consists of bacteria belonging to the class Epsilonproteobacteria, which have chemosynthetic capacity and obtain energy by oxidizing sulfur compounds. These microorganisms are widespread in marine sediments, especially those rich in sulfur and where oxygen is limited. The metabolic activity of *Sulfurimonas* species using thiosulfate, sulfur and sulfite as electron donors reveals their ability to adapt to anaerobic conditions observed in underwater decay environments (10).

Sulfurimonas species are widely distributed in marine ecosystems due to their role in the sulfur cycle and their

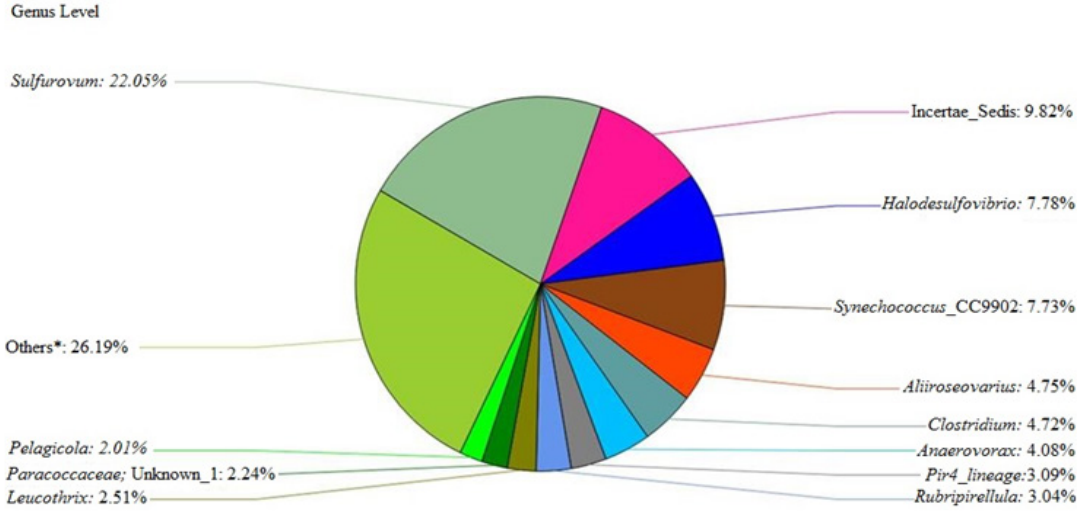

Figure 9. Distribution of microbial composition at the genus level.

ability to adapt to anaerobic conditions (14). The capacity of these microorganisms to adapt to different redox potentials makes them functional in variable environmental conditions such as underwater decay environments. In this respect, the *Sulfurimonas* genus has a dominant role in the microbial dynamics of the decay process observed in our study.

DISCUSSION

This study aimed to evaluate the underwater decay process under the unique hydrological and biochemical characteristics of the Black Sea with a multi-faceted approach and systematically revealed the effects of environmental, biological and microbial factors on postmortem change. The findings are remarkable in terms of both forensic medicine applications and the field of forensic microbiology. It is known that the dynamics of decay of corpses in underwater environments are more complex than in terrestrial conditions (1). As observed in this study, one of the most obvious factors affecting the decay process is the species diversity and the order of action of necrophagous organisms. The increased diversity of scavengers, especially detected in the winter season, shows how biological activity is shaped by temperature. This finding is consistent with the environmental temperature-decay relationship emphasized in previous studies (7).

The increase in organ volume observed in subjects in the ventral position during the study was explained by osmotic water uptake, not by gas accumulation. In underwater environments, buoyancy is primarily determined by the accumulation of gases generated by decomposition and the osmotic uptake of water into the body. These two processes can vary significantly depending on the position of the body and environmental conditions (1, 17). In cases of ventral recumbency, water seepage into internal organs through natural openings or osmotic uptake through soft tissues can significantly increase organ volume. However, this volume increase is not always due to gas accumulation and can be misleading in terms of buoyancy. Indeed, gas production from decay is slowed in underwater environments by factors such as low temperature, decreased oxygen levels, and hydrostatic pressure, which can delay or completely prevent the surfacing process (18). The literature has shown that gases accumulate more easily in the thoracic and abdominal cavities in corpses in the supine position, facilitating buoyancy. Conversely, gas accumulation is less likely to occur in ventrally positioned corpses due to compression, and the corpse remains submerged for longer periods (19). Therefore, factors such as corpse position, osmotic water uptake, and microbial gas production play an interactive role in determining buoyancy dynamics during underwater decomposition. The combined evaluation of these parameters is critical for accurate estimation of the underwater postmortem interval. This situation shows that assumptions based solely on gas accumulation may be insufficient in detecting corpses that do not float to the surface. In this context, it is understood that variables such as depth, temperature and pressure should be integrated in estimating the timing of surfacing of underwater corpses.

Microbial analysis results have shown that underwater decay environments host unique microbial communities. The dominance of bacteria such as *Sulfurimonas* and *Halodesulfovibrio*, which have sulfur metabolism, reveals that anaerobic processes that begin with the decrease in oxygen levels during decay can be monitored at the microbial level. The potential of these species to be evaluated as biomarkers in decay stages can contribute to forensic environmental microbiology (12, 14).

In addition, sulfate reduction processes carried out by these bacteria may support symbiotic interactions by forming substrates used by methanogenic archaea species in energy production. This is especially important for the survival of archaea with limited metabolic capacity, such

as *Aenigmarchaeota*. The fact that species belonging to the Archaea domain could only be identified at the class level points to the limitations of existing phylogenetic databases. However, it is known that the archaea species belonging to the *Aenigmarchaeota* phylum identified can coexist with symbiotic life forms in extreme environments such as the deep sea (15, 6). This provides an important clue that the archaeal presence observed together with bacterial species within the scope of the study may indicate a symbiotic or metabolic cooperation (10, 16).

PH measurements were recorded not only as an environmental parameter but also to assess the potential impact of microbial activity on decay. Throughout the measurements, pH values remained constant in both seasons and remained in the alkaline range (8.1–8.8) (See Table 1). This suggests that optimal pH conditions, allowing marine microbial communities to remain metabolically active, persisted in both periods. Especially in the early stages of decomposition, pH levels near the skin surface may be a determining factor in the rate of microorganism colonization. However, since no significant pH difference was observed between periods and the measured variation remained within a narrow range of ±0.3 pH units, a separate statistical significance analysis was not performed for this parameter. This variation was not expected to create a statistically significant difference considering the current sample size. Therefore, factors affecting the initiation of microbial colonization were evaluated holistically, including temperature.

The earlier onset of microbial colonization in winter is considered a result of multidimensional environmental and biological interactions that, contrary to classical expectations, cannot be explained solely by temperature changes. Comparative analysis results showed that there was no statistically significant relationship between seasonal temperature differences and the onset time of microbial colonization ($p > 0.05$); the observed difference was limited to only one day. This suggests that the microbial activity observed early in the winter period may be related to the metabolic adaptability of anaerobic microorganisms to low temperature conditions, their symbiotic relationships, and the adaptation mechanisms of species living in deep water environments to high hydrostatic pressure. In this context, the process may be driven by the interaction of environmental parameters such as oxygen levels, nutrient accumulation, microbial diversity, and hydrostatic pressure, rather than temperature. The influence of extremophile microorganisms adapted to anaerobic conditions and deep-sea pressures is particularly striking in this process. It is thought that sulfate-reducing bacteria, such as Sulfurovum and Halodesulfovibrio, identified in the study, can remain active in anaerobic environments and contribute to the acceleration of microbial activity by forming symbiotic relationships with archaeal species with limited metabolic capacity (12).

In addition, some species of the kingdom Archaea are known to maintain energy production even under extreme conditions such as low temperature, high pressure, and limited nutrients (14). This suggests that microbial communities observed in marine sediments have different metabolic adaptations than terrestrial systems. Furthermore, the stable pH levels observed in the study (8.10–8.35) create an optimal activity range for certain groups of marine microorganisms, enabling metabolic processes to be maintained even in cold environmental conditions (3).

From a methodological perspective, the Shore A type durometer used in the study was applied for the first time in the literature for the measurement of biological tissue hardness under underwater conditions and allowed the objective monitoring of the decay process. This approach has the potential for standardization for similar forensic investigations to be conducted at different depths and

environmental conditions in the future.

This study shows that the evaluation of the postmortem process in the underwater environment together with its biological, environmental and microbial aspects is of vital importance in forensic medicine applications in terms of accurate time of death estimation, distinction of traumatic lesions and preservation of evidence integrity in the field. Especially in crime scene investigations to be carried out in marine environments, knowing the effect of biotic factors on deformation will increase the reliability of forensic interpretations.

Studies on microbial communities in marine environments specific to the Black Sea are limited, and regional data, particularly those focusing on decay processes, are insufficient. The Sulfurimonas and Halodesulfovibrio species identified in this study are sulfur-metabolizing and typically occur in low-oxygen sediments (12, 13). However, there is no direct record of these species previously reported in decay environments in the Black Sea.

Similarly, there is no evidence that archaeal sequences belonging to the phylum Aenigmarchaeota have been reported in samples from the Black Sea. In this context, our results are considered to provide a pioneering and original contribution to the microbial flora of the Black Sea.

Limitations

The evaluation of only the spring (April–May) and winter (December–January) periods in this study was a deliberate methodological choice to compare the effects of seasonal water temperature extremes on postmortem decay processes. Spring represents the period when biological activity in the benthic zone increases due to the breeding season, while winter represents the period when biotic diversity is at its lowest. This contrast allowed for a more explicit observation of the effects of environmental stressors and biotic interactions on decomposition. Furthermore, considering the hydrological structure of the Black Sea, these two periods, when water temperature differences are most pronounced, provide a meaningful framework for seasonal comparisons of decomposition dynamics (2).

However, excluding summer and autumn periods within this methodological approach may limit the generalizability of the findings to the entire year. Therefore, including all seasons in future studies will allow for more comprehensive and comparative assessments.

CONCLUSION

This study evaluated underwater postmortem decay processes in hydrological and biochemical conditions specific to the Black Sea with a multi-faceted approach and made original contributions to the forensic science literature in terms of both content and method. The findings obtained within the scope of the study revealed that underwater decay dynamics are shaped not only by physical and biological factors but also by microbial interactions.

Seasonal comparisons showed that variables such as environmental temperature and living interactions significantly affected the rate of decay and the pattern of deformation. The increased necrophage diversity detected especially in the winter season supported the role of biological pressure in the decay process; however, no buoyant samples were observed in the spring months. This situation shows that assumptions based solely on gas formation may be insufficient in evaluating the probability of corpses floating on the water surface.

In microbial analyses, the dominance of sulfate-reducing bacteria such as Sulfurimonas and Halodesulfovibrio revealed microbial profiles that could be evaluated as environmental biomarkers under anaerobic digestion conditions. In addition, it was suggested that the metabolic products of these bacteria could support the symbiotic life of methanogenic archaea such as Aenigmarchaeota,

which have limited biosynthetic capacity. This finding indicates that microbial symbiosis in underwater digestion environments should be considered for potential forensic applications.

From a methodological point of view, the first use of the Shore A type durometer in measuring tissue hardness under underwater conditions puts the study in a technically pioneering position in the literature. The objective hardness data obtained by this device allowed for the standardized monitoring of the decay stages and enabled the temporal characterization of deformation.

In general, this study shows how environmental and microbial factors can be decisive in basic forensic processes such as determining the time of death, distinguishing traumatic lesions and preserving the integrity of evidence in the clarification of marine forensic cases. The results obtained are a valuable reference source for forensic medicine experts, underwater crime scene investigation teams and environmental microbiology researchers. In this context, extending similar studies with different depths, seasons and species will contribute to better modeling of marine decay processes.

Declarations

Conflict of Interest

The authors declare that they have no conflict of interest related to this article.

Funding

The authors declare that no financial support was received for this study.

KAYNAKLAR

1. Caruso, J. L. (2016). Decomposition changes in bodies recovered from water. Academic Forensic Pathology, 6(1), 19–27 [internet]. Academic Forensic Pathology; 2016 [Accessed on: July 31, 2025]. Doi: https://doi.org/10.23907/2016.003

2. Murray JW, Yakushev EV. The suboxic transition zone in the Black Sea. In: Neretin LN, editor. Past and present water column anoxia. Vol. 64. Dordrecht: Springer; 2006. p. 105–138 [internet]. Springer Nature; 1999 [Accessed on: July 31, 2025]. Doi: https://doi.org/10.1007/978-94-011-4568-8_6

3. Jessen GL, Lichtschlag A, Ramette A, Pantoja S, Rossel PE, Schubert CJ, Struck U, Boetiuset A. Hypoxia causes preservation of labile organic matter and changes seafloor microbial community composition (Black Sea). Sci Adv. 2017;3(2):e1601897 [internet]. Science Advances; 2017 [Accessed on: July 31, 2025]. Doi: https://doi.org/10.1126/sciadv.1601897

4. De Donno, A., Campobasso, C. P., Santoro, V., Leonardi, S., Tafuri, S., & Introna, F. (2015). Bodies in sequestered and non-sequestered aquatic environments: A comparative taphonomic study using decompositional scoring system. Science & Justice, 55(1), 64-70. [internet]. Science & Justice; 2014 [Accessed on: August 1, 2025]. Doi: https://doi.org/10.1016/j.scijus.2014.10.003

5. Dalal, J., Sharma, S., Bhardwaj, T., & Dhattarwal, S.k. (2023). Assessment of post-mortem submersion interval using total aquatic decomposition scores of drowed human cadavers. Journal of Forensic Sciences, 68(2), 549-557. [internet]. Journal of Forensic Sciences; 2023 [Accessed on: August 1, 2025]. Doi: https://doi.org/10.1111/1556-4029.15220

6. Soysal Z, Çakalır C. Forensic Medicine. Vol. 1. [in Turkish]. Istanbul: Istanbul University, Cerrahpaşa Faculty of Medicine Publications; 1999. p.472-3

7. Simmons T, Cross PA, Adlam RE, Moffatt C. The influence of insects on decomposition rate in different environments. J Forensic Sci. 2010;55(3):882–888 [internet]. Journal of Forensic Sciences; 2010 [Accessed on: July 31, 2025]. Doi: https://doi.org/10.1111/j.1556-4029.2010.01402.x

8. De Pelsmaeker N, Ferry N, Stiegler J, Selva N, von Hoermann C, Müller J, Heurich M. Seasonal variability of scavenger visitations is independent of carrion predictability. Basic Appl Ecol. 2024;79:57–64 [internet]. Basic and Applied Ecology; 2024 [Accessed on: July 31, 2025]. Doi: https://doi.org/10.1016/j.baae.2024.05.005

9. De Teske A., Sørensen KB. Uncultured archaea in deep marine subsurface sediments: Have we caught them all? ISME J. 2008;2(1):3–18 [internet]. Isme Journal; 2008 [Accessed on: July 31, 2025]. Doi: https://doi.org/10.1038/ismej.2007.90

10. Rinke C, Schwientek P, Sczyrba A, Ivanova NN, Anderson IJ, Cheng JF, Darling A, Malfatti S, Swan BK, Gies EA, Dodsworth JA, Hedlund BP, Tsiamis G, Sievert SM, Liu WT, Eisen JA, Hallam SJ, Kyrpides NC, Stepanauskas R, Rubin EM, Hugenholtz P, Woyke T. Insights into the phylogeny and coding potential of microbial dark matter. Nature. 2013;499(7459):431–437 [internet]. Nature; 2013 [Accessed on: July 31, 2025]. Doi: https://doi.org/10.1038/nature12352

11. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LBR, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JGC. (2019).

Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology, 37, 852-857. [internet]. Nature Biotechnology; 2019 [Accessed on: July 31, 2025]. Doi: http://doi.org/10.1038/s41587-019-0209-9

12. Han Y, Perner M. The globally widespread genus Sulfurimonas: Versatile energy metabolisms and adaptations to redox clines. Front Microbiol. 2015;6:989 [internet]. Frontiers in Microbiology; 2015 [Accessed on: July 31, 2025]. Doi: https://doi.org/10.3389/fmicb.2015.00989

13. Inagaki F, Takai K, Kobayashi H, Nealson KH, Horikoshi K. Sulfurimonas autotrophica gen. nov., sp. nov., a novel sulfur-oxidizing ε-proteobacterium isolated from hydrothermal sediments in the Mid-Okinawa Trough. Int J Syst Evol Microbiol. 2003;53(6):1801–1805 [internet]. International Journal of Systematic and Evolutionary Microbiology; 2003 [Accessed on: July 31, 2025]. Doi: https://doi.org/10.1099/ijs.0.02682-0

14. Castelle CJ, Wrighton KC, Thomas BC, Hug LA, Brown CT, Wilkins MJ, Frischkornet KR, Tringe SG, Singh A, Markillie LM, Taylor RC, Williams KH, Banfield KF. Genomic expansion of domain Archaea highlights roles for organisms from new phyla in anaerobic carbon cycling. Curr Biol. 2015;25(6):690–701 [internet]. Current Biology; 2015 [Accessed on: July 31, 2025]. Doi: https://doi.org/10.1016/j.cub.2015.01.014

15. Garritano AN, Majzoub ME, Ribeiro B, Damasceno T, Modolon F, Messias C, Vilela C, Duarte G, Hill L, Peixoto R, Thomas T. Species-specific relationships between deep sea sponges and their symbiotic Nitrosopumilaceae. ISME J. 2023;17(6):1517– 1519 [internet]. Isme Journal; 2023 [Accessed on: July 31, 2025]. Doi: https://doi.org/10.1038/s41396-023-01439-4

16. Pernthaler A, Dekas AE, Brown CT, Orphan VJ. Diverse syntrophic partnerships from deep-sea methane vents revealed by direct cell capture and metagenomics. Proc Natl Acad Sci USA. 2008;105(19):7052–7057 [internet]. Proceedings of the National Academy of Sciences; 2008 [Accessed on: July 31, 2025]. Doi: https://doi.org/10.1073/pnas.0711303105

17. Madea, B., & Doberentz, E. (2010). Commentary on: Heaton, V., Lagden, A., Moffatt, C., & Simmons, T. Predicting the postmortem submersion interval for human remains recovered from U.K. waterways. Journal of Forensic Sciences, 55(2), 302–307 [internet]. Journal of Forensic Sciences; 2010 [Accessed on: October 31, 2025]. Doi: https://doi.org/10.1111/j.1556-4029.2010.01517.x

18. Pechal, J. L., Crippen, T. L., Tarone, A. M., Lewis, A. J., Tomberlin, J. K., & Benbow, M. E. (2013). Microbial community functional change during vertebrate carrion decomposition. PLOS ONE, 8(11), e79035 [internet]. PLOS ONE; 2013 [Accessed on: October 31, 2025]. Doi: https://doi.org/10.1371/journal.pone.0079035

19. Rodriguez, W. C. III, & Bass, W. M. (1985). Decomposition of buried bodies and methods that may aid in their location. Journal of Forensic Sciences, 30(3), 836–852 [internet]. Journal of Forensic Sciences; 1985 [Accessed on: November 1, 2025]. Doi: https://doi.org/10.1520/JFS11017J