



Marine and Life Sciences

Journal Homepage: <https://dergipark.org.tr/en/pub/marlife>



Structural and chemical characterization of gastroliths and calcium carbonate stability in narrow-clawed crayfish (*Pontastacus leptodactylus* (Eschscholtz, 1823)) from Atikhisar Reservoir, Türkiye

Selçuk Berber¹ • Bayram Kızılkaya² • Semih Kale^{3,4} • Sefa Acarlı² • Deniz Acarlı⁵ • Evren Tan⁶

¹Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology, Department of Marine and Inland Water Sciences, 17020, Çanakkale, TÜRKİYE

²Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology, Department of Aquaculture, Çanakkale, TÜRKİYE

³Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology, Department of Fishing and Fish Processing Technology, Çanakkale, TÜRKİYE

⁴Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology, Department of Fisheries Industry Engineering, Çanakkale, TÜRKİYE

⁵Çanakkale Onsekiz Mart University, Maritime Vocational School, Department of Motor Vehicles and Transportation Technologies, Underwater Technologies Program, Çanakkale, TÜRKİYE

⁶Independent Researcher, Çanakkale, TÜRKİYE

✉ Corresponding Author: selcukberber@comu.edu.tr

Please cite this paper as follows:

Berber, S., Kızılkaya, B., Kale, S., Acarlı, S., Acarlı, D., & Tan, E. (2026). Structural and chemical characterization of gastroliths and calcium carbonate stability in narrow-clawed crayfish (*Pontastacus leptodactylus* (Eschscholtz, 1823)) from Atikhisar Reservoir, Türkiye. *Marine and Life Sciences*, 8(1), 41-53. <https://doi.org/10.51756/marlife.1837996>

Research Article

Article History

Received: 08.12.2025

Accepted: 02.06.2026

Published Online: 17.06.2026



Keywords:

Biom mineralization

Calcium carbonate

Gastrolith

Pontastacus leptodactylus

A B S T R A C T

Narrow-clawed crayfish (*Pontastacus leptodactylus*) is an economically important freshwater species in inland fisheries. During molting, gastroliths function as temporary calcium carbonate storage structure and contribute to calcium mobilization for subsequent cuticle mineralization. In this study, the structural, chemical, and morphological properties of white and blue gastroliths extracted from mature *P. leptodactylus* individuals collected from Atikhisar Reservoir, Çanakkale, Türkiye, were investigated. Raman spectroscopy, X-ray diffraction (XRD), inductively coupled plasma-optical emission spectroscopy (ICP-OES), and scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) were used for complementary characterization. Raman spectra confirmed the presence of carbonate-related vibrational bands in both gastrolith types, while XRD patterns indicated differences in crystallinity between white and blue samples. Blue gastroliths exhibited sharper and more pronounced diffraction peaks, suggesting a higher crystalline calcium carbonate fraction, whereas white gastroliths showed broader features consistent with a greater amorphous component. ICP-OES results showed that calcium concentration was higher in blue gastroliths, while phosphorus and magnesium concentrations were relatively higher in white gastroliths. When expressed as calculated calcium carbonate equivalent, the gastroliths contained approximately 75-78% CaCO₃. SEM-EDS observations revealed layered microstructures and fibrous organic-associated features, with Ca, O, C, P, and Mg as the dominant detected elements. Overall, the results suggest that color variation in *P. leptodactylus* gastroliths is associated with differences in elemental composition, crystallinity, and the relative contribution of amorphous and crystalline calcium carbonate phases. The higher phosphorus content in white gastroliths may contribute to the stabilization of amorphous calcium carbonate, whereas the higher calcium content and sharper XRD peaks in blue gastroliths indicate a more crystalline mineral structure. These findings provide baseline data for understanding gastrolith biomineralization in Turkish populations of *P. leptodactylus* and may contribute to future studies on molting physiology, calcium metabolism, and freshwater crayfish aquaculture.

INTRODUCTION

Freshwater crayfish are ecologically and economically important decapod crustacean in inland aquatic ecosystems. Among them, the narrow-clawed crayfish, *Pontastacus leptodactylus* (Eschscholtz, 1823), is the only freshwater crayfish species of major commercial importance. It is naturally distributed in Türkiye, occurring mainly in Northern Anatolia, Thrace, Central Anatolia, and Western Anatolia (Holthuis, 1961; Geldiay and Kocataş, 1970). In addition to its economic value for inland fisheries and aquaculture, this species is biologically important because growth, survival, and reproductive performance are closely linked to successful molting and calcium metabolism.

In crayfish, growth is possible only through periodic molting because the body is covered by a rigid cuticle that does not expand continuously. The molting period involves the growth of internal organs and tissues, formation of gastroliths, and periodic replacement of the exoskeleton (Aiken and Waddy, 1992; Barım Öz, 2009, 2016). Gastroliths are paired calcified structures that develop on the lateral walls of the stomach before molting (Luquet and Marin, 2004; Erol et al., 2010). At the onset of ecdysis, these structures are released into the stomach, dissolve, and provide an internal calcium source for hardening the newly formed exoskeleton (Greenaway, 1985; Shechter et al., 2008; Erol et al., 2010). Therefore, calcium availability is a critical factor for crayfish culture, and waters used for crayfish farming should be sufficiently rich in calcium (Erol et al., 2010).

Gastroliths are biologically controlled mineral-organic composite structures formed through regulated biomineralization processes (Lowenstam and Weiner, 1989; Mann, 2001; Luquet and Marin, 2004). Their mineral fraction is mainly composed of calcium carbonate, which may occur as amorphous calcium carbonate (ACC) and/or crystalline polymorphs such as calcite and aragonite (Hikida et al., 2003; Luquet and Marin, 2004; Shechter et al., 2008; Neira-Carrillo et al., 2017). ACC is particularly important because it functions as a transient and metastable calcium reservoir that can dissolve more readily than crystalline calcium carbonate phases during molting (Addadi et al., 2003; Luquet and Marin, 2004; Neira-Carrillo et al., 2017). However, the persistence and transformation of ACC are influenced by ionic composition, organic matrix components, phosphate and magnesium incorporation, and environmental water chemistry (Addadi et al., 2003; Luquet and Marin, 2004; Neira-Carrillo et al., 2017). Therefore, evaluating the relationship between amorphous and crystalline calcium carbonate phases is essential for understanding gastrolith function and calcium mobilization in crayfish.

Previous studies on crayfish gastroliths have shown that their structure and composition may vary depending on species, molt stage, physiological condition, and environmental factors (Travis, 1963; Ueno, 1980; Shechter et al., 2008; Luquet et al., 2013). In Türkiye, previous research on *P. leptodactylus* has largely focused on population structure, growth parameters, fisheries biology, nutritional physiology, oxidative stress, chitin and chitosan extraction, color anomalies, and ecological characteristics in lakes and reservoirs (Mazlum, 2007; Berber and Mazlum, 2009; Berber et al., 2010, 2011, 2012, 2019, 2020, 2024a, 2024b, 2025; Mazlum et al., 2011, 2020, 2025; Akhan et al., 2014, 2024; Türel and Berber, 2016; Şirin and Mazlum, 2017; Berber and Kale, 2018; Küçükgülmez, 2018; Kale and Berber, 2020, 2025; Mazlum and Şirin, 2020; Boyalık and Berber, 2020; Mazlum and Uzun, 2022; Acarlı et al., 2023; Boyalık et al., 2023; Kadak et al., 2023; Gültepe et al., 2024; Gültepe and Gültepe, 2026; Uğurlu et al., 2026). However, no previous study has provided a detailed structural, chemical, mineralogical, and morphological characterization of gastroliths in Turkish crayfish populations. Atikhisar Reservoir is also a particularly relevant study site because previous studies have documented unusual color phenotypes of *P. leptodactylus* in this system. Kale et al. (2020) reported the first albino *P. leptodactylus* individual from Atikhisar Reservoir, while Kale et al. (2021) reported two blue-colored individuals from the same reservoir and suggested that the underlying causes of such color anomalies require further investigation. These earlier observations indicate that color variation in the Atikhisar population is biologically noteworthy. However, body color anomalies should not be directly equated with gastrolith mineral phase differences. Instead, gastrolith color must be evaluated through structural and chemical indicators such as Raman bands, XRD peak broadness or sharpness, elemental composition, Ca/P ratio, phosphate and magnesium content, and SEM-based morphological features.

The aim of this study was to characterize and compare white and blue gastroliths obtained from mature *P. leptodactylus* individuals collected from Atikhisar Reservoir, Çanakkale, Türkiye which will offer the first detailed investigation of gastrolith biomineralization in *P. leptodactylus* from Türkiye. For this purpose, Raman spectroscopy, X-ray diffraction (XRD), inductively coupled plasma-optical emission spectroscopy (ICP-OES), and scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) analyses were used to evaluate carbonate structure, crystallinity, elemental composition, and surface morphology. The study specifically examined whether gastrolith color differences are associated with variation in calcium and phosphorus content, Ca/P ratio, crystallinity, and the relative contribution of amorphous and

crystalline calcium carbonate phases. By addressing these relationships, this study provides the first detailed baseline data on gastrolith biomineralization in Turkish *P. leptodactylus* populations and contributes to future studies on molting physiology, calcium metabolism, environmental adaptation, and crayfish aquaculture management.

MATERIALS AND METHODS

Study Area and Sampling Material

The study material consisted of mature *P. leptodactylus* individuals collected from Atikhisar Reservoir, Çanakkale, Türkiye (Figure 1).

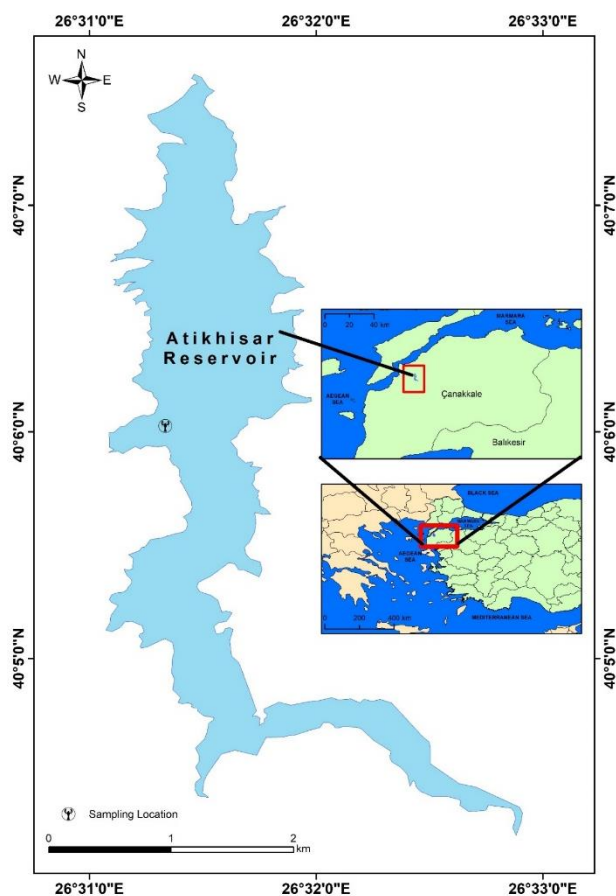


Figure 1. Map of the study area (Atikhisar Reservoir, Çanakkale, Türkiye)

Sampling was carried out between July 2020 and June 2021, and the individuals were transported alive to the laboratory. The gastric region of each specimen was carefully dissected, and the paired right and left gastroliths were removed. The extracted gastroliths were rinsed with distilled water to remove surface residues and dried at room temperature. Before analysis, the gastroliths were visually classified into two groups according to color: white gastroliths and blue gastroliths (Figure 2).

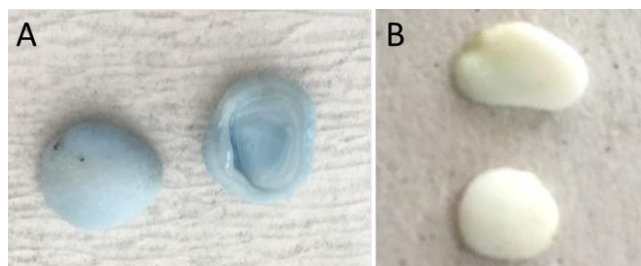


Figure 2. Representative images of blue (a) and white (b) gastroliths of *Pontastacus leptodactylus*

Raman Spectroscopy

Raman spectroscopic analyses were performed using a WITTEC Alpha 300RA Raman microspectrometer at the Central Laboratory of Çanakkale Onsekiz Mart University. Spectra were collected using a 532 nm laser source over the wavenumber range of 3785-50 cm^{-1} . Measurements were obtained from selected regions of each gastrolith sample, and replicate spectra were recorded to evaluate spectral consistency within each color group.

The Raman spectra was used to identify carbonate-related vibrational bands in the gastrolith samples. Particular attention was given to the characteristic carbonate bands observed around 1091 cm^{-1} and 716 cm^{-1} , corresponding to the symmetric stretching and in-plane bending modes of the carbonate group, respectively. The spectra obtained from white and blue gastroliths were compared with each other and evaluated together with XRD and elemental analysis results.

X-ray Diffraction (XRD) Analysis

X-ray diffraction (XRD) analyses were performed to evaluate the mineralogical structure and crystallinity-related characteristics of the gastrolith samples and to identify calcium carbonate crystalline phases. Measurements were carried out using a PANalytical Empyrean XRD system at the Central Research Laboratory of Çanakkale Onsekiz Mart University. The instrument was operated with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$), and the generator settings were 45 kV and 40 mA. Diffraction data were collected at room temperature over a $2\theta=5-70^\circ$ range with a scanning speed of 0.008° s $^{-1}$. The resulting XRD patterns were compared with the calcium carbonate standard reference card (ICDD/JCPDS No. 86-2340) to qualitatively identify crystalline calcium carbonate phases in the samples (ICDD, 2023).

Peak matching analysis was used for qualitative phase identification and for comparing crystallinity-related differences between white and blue gastroliths. No quantitative phase analysis was performed. The obtained diffraction patterns were compared with reference data for calcium carbonate polymorphs available in the literature. Peak positions, peak sharpness, peak intensity, and the

presence of broad amorphous features were evaluated qualitatively to distinguish relatively crystalline and relatively amorphous structural characteristics.

ICP-OES Elemental Analysis

Elemental analyses were performed using a PerkinElmer Optima 8000 inductively coupled plasma-optical emission spectroscopy (ICP-OES) system. Gastrolith samples were prepared using a microwave digestion system before analysis. The concentrations of Ca, P, Mg, Ba, Zn, B, and Cu were determined. Each sample was analyzed in triplicate, and the results were expressed as mean $\pm$ standard error.

The ICP-OES data were used to compare elemental composition between white and blue gastroliths. Calcium, phosphorus, and magnesium concentrations were interpreted as indicators of elemental variation between color groups, not as direct measurements of calcium carbonate polymorph abundance.

The measured Ca concentrations were also used to estimate the calculated calcium carbonate equivalent based on the stoichiometric Ca fraction of CaCO_3 . Since the molecular mass of CaCO_3 is approximately 100 g mol^{-1} and Ca accounts for 40 g mol^{-1} of this mass, calcium represents approximately 40% of CaCO_3 . Therefore, calculated CaCO_3 equivalent values were obtained as follows:

$$\text{CaCO}_3 \text{ molecular weight} = 40\text{Ca} + 12\text{C} + 3 \times 16\text{O} = 100 \text{ g mol}^{-1} \quad (1)$$

$$\text{Ca fraction in CaCO}_3 = \frac{40}{100} = 0.40 \quad (2)$$

$$\text{CaCO}_3 \text{ equivalent (\%)} = \text{Ca concentration (\%)} \times 2.5 \quad (3)$$

These values were interpreted as calculated CaCO_3 equivalents, not as direct quantitative measurements of crystalline or amorphous calcium carbonate phase abundance.

Surface Morphology and Element Mapping (SEM-EDS) Analysis

The surface morphology and local elemental composition of gastroliths were examined using a Gemini FE-SEM system at Pamukkale University İLTAM Laboratory. Prior to imaging, the samples were sputter-coated with a thin conductive gold-palladium layer (Au-Pd, 80/20 alloy) to reduce surface charging and improve image quality. The coating process was carried out under vacuum conditions. SEM observations were performed under high-vacuum conditions at accelerating voltages of 10-15 kV.

Energy-dispersive X-ray spectroscopy (EDS) was used to determine the local distribution of Ca, O, C, P, and Mg on selected regions of the gastrolith surface. SEM images were obtained from both intact and powdered gastrolith samples. SEM-EDS data were used to support morphological

observations and local elemental characterization; they were not used as a substitute for bulk elemental quantification.

Data Analysis

Quantitative ICP-OES data were expressed as mean $\pm$ standard error. Ca/P ratios were calculated from the mean Ca and P concentrations to support comparison of elemental composition between the two gastrolith color groups. Raman, XRD, and SEM-EDS results were evaluated descriptively and used as complementary evidence for interpreting structural, mineralogical, and morphological differences between white and blue gastroliths. XRD data were interpreted qualitatively based on phase matching and crystallinity-related peak characteristics; no quantitative phase refinement was performed.

RESULTS

Raman Spectroscopy Results

The Raman spectra of white and blue gastrolith samples are presented in Figure 3. Both gastrolith types exhibited carbonate-related vibrational bands consistent with calcium carbonate. The characteristic symmetric stretching vibration (ν_1) of the carbonate group was observed as a strong band at approximately 1091 cm^{-1} , while the in-plane bending vibration (ν_4) was observed at approximately 716 cm^{-1} . Additional bands were detected at approximately 155 and 210 cm^{-1} , which may be associated with lattice or metal-carbonate interactions. However, the specific cations responsible for these low-wavenumber bands could not be assigned based on Raman data alone.

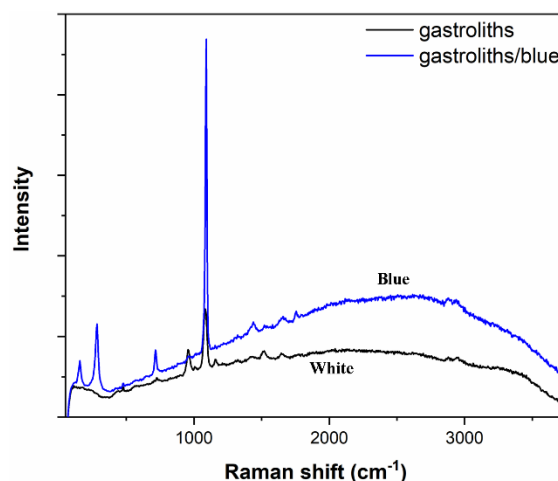


Figure 3. Raman spectra of white and blue gastrolith samples. Characteristic carbonate-related bands are observed at approximately 1091 cm^{-1} and 716 cm^{-1} .

The Raman spectra of the blue gastroliths showed more clearly defined carbonate-related bands than those of the white gastroliths. In both color groups, the presence of the 1091 cm^{-1} and 716 cm^{-1} bands confirmed that calcium

carbonate was the main carbonate-bearing component of the gastrolith structure. No further phase quantification was made based solely on Raman spectra.

ICP-OES Elemental Composition

The ICP-OES results for white and blue gastrolith samples are presented in Table 1. Calcium (Ca) was the dominant measured element in both gastrolith types, followed by phosphorus (P) and magnesium (Mg). Barium (Ba) was detected at low concentrations, whereas zinc (Zn), boron (B), and copper (Cu) were not detected in either gastrolith type.

Table 1. ICP-OES elemental composition of white and blue gastroliths

Element	Unit	White Gastrolith	Blue Gastrolith
Zn	ng/g	ND	ND
P	mg/g	30.138±0.098	22.365±0.703
Ba	ng/g	140.917±0.587	122.203±3.391
B	ng/g	ND	ND
Mg	mg/g	4.463±0.011	4.127±0.0092
Ca	mg/g	297.715±0.636	315.748±10.808
Cu	ng/g	ND	ND

Note: ND: Not detected. Values are presented as mean ± standard error

The Ca concentration was higher in blue gastroliths than in white gastroliths. The mean Ca concentration was 315.748±10.808 mg/g in blue gastroliths and 297.715±0.636 mg/g in white gastroliths. In contrast, P concentration was higher in white gastroliths, with values of 30.138±0.098 mg/g in white gastroliths and 22.365±0.703 mg/g in blue gastroliths. Mg concentration also showed a slightly higher value in white gastroliths, with 4.463 ± 0.011 mg/g in white gastroliths and 4.127±0.009 mg/g in blue gastroliths. Ba concentration was 140.917 ± 0.587 ng/g in white gastroliths and 122.203 ± 3.391 ng/g in blue gastroliths.

Based on mean Ca and P concentrations, the calculated Ca/P ratio was approximately 9.9 in white gastroliths and 14.1 in blue gastroliths. The calculated CaCO₃ equivalent, estimated from measured Ca concentrations using the stoichiometric Ca fraction of CaCO₃, was approximately 74.4% in white gastroliths and 78.9% in blue gastroliths. These values represent calculated CaCO₃ equivalents and should not be interpreted as direct quantitative measurements of crystalline or amorphous calcium carbonate phase abundance.

XRD Results

The XRD patterns of white and blue gastrolith samples are shown in Figure 4. The diffraction patterns were evaluated qualitatively by comparison with calcium carbonate reference data. The observed diffraction peaks were consistent with crystalline calcium carbonate,

particularly calcite-related reflections.

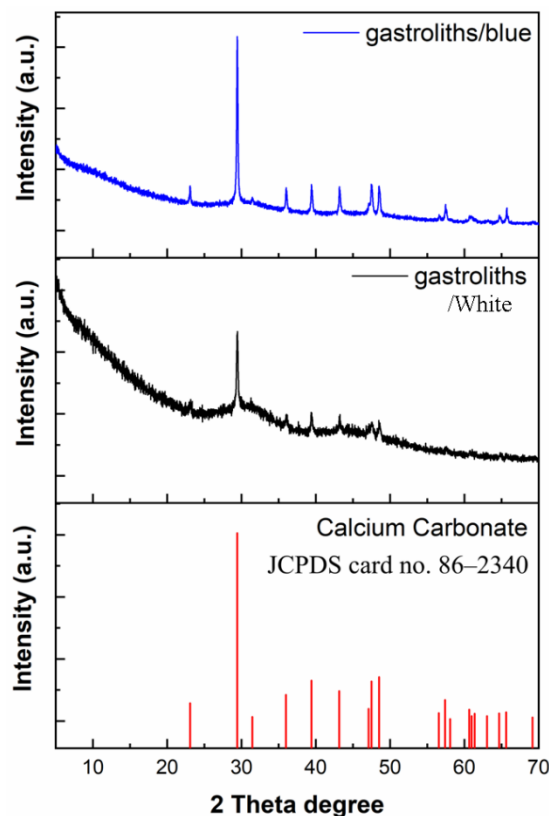


Figure 4. XRD patterns of white and blue gastrolith samples. The blue gastrolith shows sharper and more intense diffraction peaks, whereas the white gastrolith shows broader features qualitatively consistent with a lower degree of crystallinity and a stronger amorphous contribution.

The white gastrolith sample exhibited broader and lower-intensity diffraction features, including a broad region over approximately 2θ=20–40°. This pattern indicates a lower degree of crystallinity and the presence of a more pronounced amorphous contribution in the white gastrolith sample. In contrast, the blue gastrolith sample showed sharper and more intense diffraction peaks, indicating a higher degree of crystallinity relative to the white gastrolith.

The XRD results therefore indicate a clear crystallinity-related difference between the two gastrolith color groups. However, no quantitative phase analysis or Rietveld refinement was performed. Therefore, the XRD data were used only for qualitative phase identification and comparison of crystallinity-related features between white and blue gastroliths.

SEM-EDS Results

SEM images and EDS spectra of white and blue gastrolith samples are presented in Figure 5. SEM observations showed that both gastrolith types had a layered microstructure. The surface of the blue gastrolith appeared relatively denser and more compact, whereas the white gastrolith showed a more irregular and porous surface morphology.

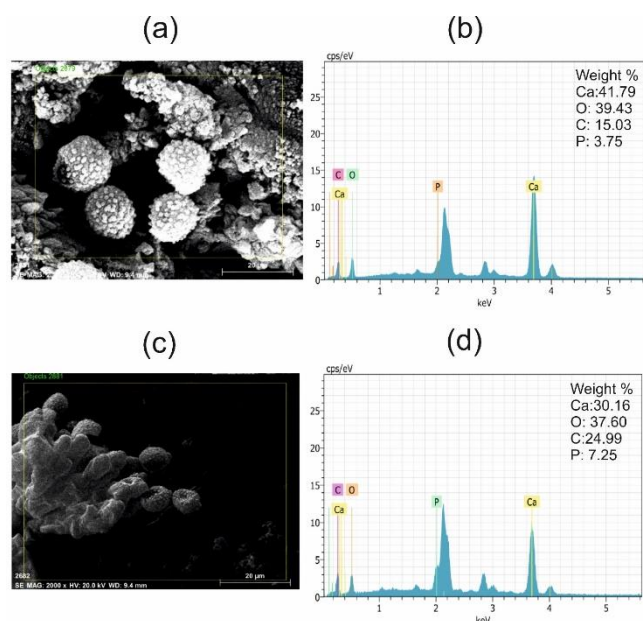


Figure 5. SEM images and EDS spectra of white and blue gastroliths. (a) Surface morphology of white gastrolith; (b) EDS spectrum obtained from the selected region of white gastrolith; (c) surface morphology of blue gastrolith; (d) EDS spectrum obtained from the selected region of blue gastrolith. Scale bars and magnifications are shown on each SEM micrograph.

Fibrous structures were observed on the surfaces of both gastrolith types. These structures were described as organic-associated fibrous features or mineral-organic surface formations. Their biological origin could not be confirmed within the scope of this study. Therefore, they were not interpreted as fungal structures or microbial colonies.

EDS analyses performed on selected regions of the gastrolith surfaces detected Ca, O, C, P, and Mg as the main elements. These local elemental data were consistent with the calcium carbonate-rich nature of the gastroliths and with the presence of additional phosphate- and magnesium-associated components. Since EDS provides local surface or near-surface elemental information, the EDS results were used descriptively and were not treated as bulk elemental quantification.

DISCUSSION

Structural and Mineralogical Interpretation of Gastroliths

The present study provides the first detailed structural, chemical, mineralogical, and morphological characterization of gastroliths in Turkish populations of *P. leptodactylus*. Previous studies from Türkiye have examined molting, growth, nutrition, oxidative stress, population biology, and color anomalies in crayfish populations, and have emphasized the biological importance of gastrolith formation during the molting cycle (Aiken and Waddy, 1992; Barım Öz,

2009, 2016; Erol et al., 2010; Kale et al., 2020, 2021). However, the mineralogical and elemental characteristics of gastroliths have not previously been characterized in detail in Turkish crayfish populations. In this respect, the present study fills a clear gap by evaluating white and blue gastroliths through Raman spectroscopy, XRD, ICP-OES, and SEM-EDS analyses.

Gastrolith formation is a key component of the molting process in freshwater crayfish. During this period, gastroliths act as temporary calcium storage structures and later dissolve to provide calcium for post-molt exoskeleton hardening. This functional role is consistent with classical studies on calcium balance and molting in crustaceans (Greenaway, 1985; Ziegler et al., 2005), as well as with studies describing gastrolith formation and structure in crayfish and other decapod crustaceans (Travis, 1963; Ueno, 1980; Hikida et al., 2003; Shechter et al., 2008). The present findings support this framework by showing that *P. leptodactylus* gastroliths are calcium carbonate-rich structures with distinct mineralogical and morphological organization.

Raman spectroscopy confirmed the presence of carbonate-related vibrational bands in both white and blue gastroliths. The strong band at approximately 1091 cm^{-1} and the band at approximately 716 cm^{-1} are consistent with carbonate group vibrations and support the calcium carbonate-rich nature of the gastrolith structure. This interpretation is consistent with previous studies showing that gastroliths and calcium carbonate biominerals can be characterized through complementary structural and spectroscopic approaches (Hikida et al., 2003; Luquet and Marin, 2004; Neira-Carrillo et al., 2017). However, Raman data alone were not used to quantify phase abundance, because phase-level interpretation requires integrated evaluation with XRD and elemental data.

XRD analysis indicated that white and blue gastroliths differed in crystallinity-related features. The blue gastroliths showed sharper and more intense diffraction peaks, whereas the white gastroliths exhibited broader and lower-intensity features, including a broad region suggestive of a stronger amorphous contribution. These observations are consistent with studies showing that gastroliths may contain both amorphous calcium carbonate (ACC) and crystalline calcium carbonate phases (Hikida et al., 2003; Luquet and Marin, 2004; Shechter et al., 2008; Neira-Carrillo et al., 2017). However, because no quantitative phase analysis or Rietveld refinement was performed, these differences should be interpreted qualitatively rather than as exact phase percentages.

Biom mineralization Framework and ACC Transformation

Gastroliths are not simple mineral deposits; they are biologically controlled mineral-organic composite structures. The general concept that organisms regulate inorganic mineral formation through organic matrices and controlled microenvironments is well established in biomineralization research (Lowenstam and Weiner, 1989; Mann and Ozin, 1996; Ozin, 1997; Mann, 2001). Within this framework, crustacean gastroliths represent a specialized calcium storage strategy in which calcium carbonate is deposited before molting and later mobilized for cuticle mineralization (Luquet and Marin, 2004; Luquet, 2012).

The occurrence of amorphous and crystalline calcium carbonate phases in gastroliths is particularly important. ACC is metastable and can serve as a transient calcium reservoir that is more readily mobilized than stable crystalline phases. Addadi et al. (2003) emphasized that organisms can take advantage of the disordered nature of ACC in biomineralization processes. Similarly, Luquet and Marin (2004) described crustacean calcium carbonate deposits as storage systems in which amorphous and crystalline forms may play different functional roles. More recent biomineralization models also describe biomineral formation as a dynamic process in which amorphous precursor phases can transform into more ordered crystalline phases over time (Gilbert et al., 2022). The present findings are compatible with this model: broader XRD features and higher P/Mg values in white gastroliths suggest a relatively stronger amorphous contribution, while sharper XRD peaks and higher Ca/P ratio in blue gastroliths suggest a relatively more crystalline structure.

The interpretation of ACC stabilization must nevertheless remain cautious. The higher P content in white gastroliths may be associated with stabilization of amorphous calcium carbonate, because phosphate can influence calcium carbonate precipitation and delay crystallization in biomineral systems (Luquet and Marin, 2004; Neira-Carrillo et al., 2017). Mg may also influence crystallization pathways by modifying calcite formation and calcium carbonate transformation behavior (Addadi et al., 2003). However, the present study did not directly measure transformation kinetics, organic matrix composition, or ACC stability under controlled experimental conditions. Therefore, the observed elemental differences should be interpreted as indirect evidence supporting possible ACC stabilization, not as direct proof of a causal mechanism.

Elemental Composition, Ca/P Ratio, and Mineral Phase Interpretation

ICP-OES results showed that Ca was the dominant measured element in both gastrolith types, followed by P and Mg. This agrees with the expected calcium carbonate-rich composition of crustacean gastroliths and with previous descriptions of gastroliths as calcium storage structures (Greenaway, 1985; Luquet and Marin, 2004; Shechter et al., 2008). The higher Ca concentration and higher Ca/P ratio in blue gastroliths are consistent with the sharper XRD peaks observed in these samples, suggesting a more crystalline calcium carbonate-rich structure. In contrast, white gastroliths showed higher P and slightly higher Mg concentrations, broader XRD features, and more irregular surface morphology, suggesting a relatively stronger amorphous contribution.

Although the overall P concentration was higher in white gastroliths, SEM-EDS analysis revealed a higher local P percentage in the selected area of blue gastroliths, 7.25%, compared with 3.75% in white gastroliths (Figure 5). This difference likely reflects the localized and semi-quantitative nature of SEM-EDS analysis, which measures elemental composition only at the selected surface area, rather than the bulk composition of the entire sample. Therefore, the SEM-EDS result indicates a locally enriched P region in the blue gastrolith rather than contradicting the higher bulk P concentration observed in white gastroliths.

This interpretation is consistent with studies showing reciprocal changes between gastrolith calcification and cuticle mineralization during the molt cycle in crayfish (Shechter et al., 2008). It is also compatible with Luquet et al. (2013), who reported comparative ultrastructural and compositional differences among freshwater crayfish gastroliths from different families. The present study extends these observations to Turkish *P. leptodactylus* populations by showing that visually distinct gastrolith color groups differ in elemental composition and crystallinity-related characteristics.

The calculated CaCO_3 equivalent values were approximately 74.4% in white gastroliths and 78.9% in blue gastroliths. These values were calculated from ICP-OES-derived Ca concentrations using the stoichiometric Ca fraction of CaCO_3 . Therefore, they are useful for comparing the calcium carbonate equivalent potential of the two gastrolith groups. However, they should not be interpreted as direct measurements of crystalline or amorphous calcium carbonate phase abundance. ICP-OES provides elemental concentrations, whereas phase interpretation depends on

structural data from Raman and XRD analyses.

Gastrolith Color Variation

The comparison of white and blue gastroliths indicates that color variation is associated with differences in elemental composition, crystallinity, and surface morphology. Blue gastroliths were characterized by higher Ca concentration, higher Ca/P ratio, and sharper XRD peaks, whereas white gastroliths had higher P and Mg concentrations, broader XRD features, and a more porous surface appearance. These findings suggest that gastrolith color may reflect differences in the relative contribution of amorphous and crystalline calcium carbonate phases.

However, gastrolith color should not be interpreted as a direct standalone indicator of mineral phase composition. Color may be influenced by mineral organization, organic matrix composition, hydration state, pigment-associated compounds, and light scattering properties. Therefore, the color difference observed in this study should be considered an associated visual feature rather than definitive evidence of phase identity. The interpretation is reliable only when color is evaluated together with Raman, XRD, ICP-OES, and SEM-EDS data.

This point is particularly relevant for Atikhisar Reservoir, where unusual color phenotypes of *P. leptodactylus* have previously been reported. Kale et al. (2020) documented albinism in Turkish crayfish from Atikhisar Reservoir, while Kale et al. (2021) reported a blue color anomaly in *P. leptodactylus* from the same reservoir. These studies show that color-related variation in this population is biologically noteworthy. However, external body color anomalies and gastrolith color differences should not be treated as the same phenomenon. The present study does not test the genetic or physiological mechanisms underlying body color anomalies; instead, it shows that gastrolith color variation is accompanied by measurable structural and elemental differences.

Surface Morphology and Mineral-Organic Organization

SEM observations revealed that both white and blue gastroliths had a layered microstructure, supporting the view that gastroliths are mineral-organic composite structures formed under biological control. Such organization is consistent with broader biomineralization concepts in which organisms regulate mineral deposition through organic matrices, spatial confinement, and controlled chemical environments (Lowenstam and Weiner, 1989; Mann and Ozin, 1996; Mann, 2001). The presence of fibrous organic-associated structures in gastroliths is also consistent with

previous studies emphasizing the role of chitin and matrix proteins in gastrolith mineralization (Glazer et al., 2013; Luquet et al., 2013).

The blue gastroliths appeared relatively denser and more compact, whereas the white gastroliths showed a more irregular and porous morphology. This difference is consistent with the Raman, XRD, and ICP-OES results, which collectively suggest a higher crystalline contribution in blue gastroliths and a stronger amorphous component in white gastroliths. The fibrous structures observed on gastrolith surfaces may represent organic matrix-associated formations or mineral-organic aggregates. However, their biological origin cannot be confirmed without further analyses such as microbial identification, organic matrix characterization, or molecular testing. Therefore, these structures should not be identified as fungal or microbial colonies based only on SEM images. If microbial colonization is considered in future work, it should be tested directly rather than inferred morphologically; this caution is necessary because similar surface-associated biological interpretations require specific microbiological confirmation.

Ecophysiological and Aquaculture Implications

Molting is a critical process in crayfish growth because the rigid cuticle must be periodically replaced to allow body enlargement. Barım Öz (2009, 2016) emphasized that the molting period includes internal tissue growth, gastrolith formation, and periodic replacement of the exoskeleton. Erol et al. (2010) further stated that gastroliths form on the lateral walls of the stomach and dissolve at the onset of molting, thereby providing calcium for hardening the new shell. This physiological role explains why calcium availability is important for crayfish culture and why calcium-rich water conditions may support successful post-molt mineralization.

The findings of the present study suggest that gastrolith composition may provide useful information about calcium storage and mobilization processes in *P. leptodactylus*. The higher Ca concentration and crystallinity-related features of blue gastroliths may reflect a more advanced or more crystalline mineral state, whereas the higher P and Mg levels in white gastroliths may indicate greater preservation of amorphous or less crystalline calcium carbonate. Such differences may be related to molt stage, individual physiology, environmental water chemistry, or a combination of these factors. However, because molt stage, hemolymph chemistry, and water chemistry were not experimentally monitored in this study, these explanations should be tested in future controlled studies.

Environmental factors may also influence gastrolith mineralization. Luquet and Marin (2004) noted that crustacean mineral storage strategies are shaped by physiological and environmental context, while Takesue et al. (2008) showed in biocarbonate systems that organic matter and calcification rate can affect trace element incorporation. Although bivalve shells and crayfish gastroliths are not directly equivalent systems, this comparison supports the broader point that carbonate biominerals can reflect both biological regulation and environmental chemistry. Therefore, future studies on *P. leptodactylus* gastroliths should include direct water chemistry measurements, particularly calcium hardness, pH, alkalinity, temperature, and magnesium availability.

Biomimetic and Applied Relevance

The present study also has potential relevance for biomimetic calcium carbonate research. Gastroliths are natural examples of biologically controlled calcium carbonate storage, in which amorphous and crystalline mineral phases coexist within a mineral-organic matrix. This makes them useful conceptual models for understanding controlled mineral stabilization and transformation.

Kapusuz and Ercan (2019) reported that different calcium carbonate polymorphs incorporated into silk-fibroin scaffolds influenced calcium phosphate mineralization, with polymorph-dependent effects on apatite formation. This finding is relevant to the present study because white and blue gastroliths differed in crystallinity-related characteristics and in P/Ca balance. However, the comparison should be interpreted as conceptual rather than direct experimental equivalence. The present study did not test biocompatibility, apatite formation, or scaffold performance. Therefore, gastroliths should be described as a potential natural model for future biomimetic studies, not as an immediately validated biomaterial source.

Within this cautious framework, the higher P content and broader XRD features observed in white gastroliths may be of interest for future work on phosphate-associated ACC stabilization, while the more crystalline structure of blue gastroliths may provide comparative insight into mineral maturation. Future studies could isolate gastrolith mineral phases and test their dissolution behavior, apatite-forming capacity, and interaction with organic matrices under controlled conditions. Such work would be required before any applied conclusion can be drawn for bone grafts, tissue scaffolds, or related biomaterials.

Limitations and Future Research

Several limitations should be considered when interpreting the findings. First, XRD analysis was used qualitatively, and no quantitative phase analysis was performed. Therefore, the relative abundance of amorphous and crystalline calcium carbonate phases could not be calculated precisely. Second, ICP-OES provided bulk elemental concentrations but did not directly identify calcium carbonate polymorphs. Third, SEM-EDS provided local elemental information from selected regions and should not be interpreted as whole-sample elemental quantification. Fourth, the study did not include direct measurements of molt stage, hemolymph chemistry, water chemistry at the time of sampling, organic matrix composition, or microbial identity.

Future studies should combine gastrolith characterization with molt-stage determination, controlled water chemistry measurements, organic matrix analysis, and quantitative mineralogical techniques. Such studies would clarify how calcium, phosphate, magnesium, and organic matrix components regulate ACC stabilization and transformation into crystalline calcium carbonate phases. Longitudinal sampling across the molt cycle would also be valuable for determining whether white and blue gastroliths represent different physiological states, mineralization stages, or individual-level variation.

Overall, the present study demonstrates that white and blue gastroliths of *P. leptodactylus* from Atikhisar Reservoir differ in elemental composition, crystallinity-related features, and surface morphology. These differences suggest that gastrolith color variation is associated with the balance between amorphous and crystalline calcium carbonate components. By providing the first detailed characterization of gastrolith biomineralization in Turkish *P. leptodactylus* populations, this study contributes to the understanding of calcium metabolism, molting physiology, biomineralization processes, and potential biomimetic research directions in freshwater crayfish.

CONCLUSION

This study presents the first detailed structural, chemical, mineralogical, and morphological characterization of gastroliths in Turkish populations of *P. leptodactylus*. By using Raman spectroscopy, XRD, ICP-OES, and SEM-EDS analyses together, the study demonstrated that white and blue gastroliths from Atikhisar Reservoir differ in elemental composition, crystallinity-related features, and surface morphology.

Raman spectroscopy confirmed the presence of carbonate-related vibrational bands in both gastrolith types, supporting the calcium carbonate-rich nature of the gastrolith structure. XRD patterns indicated that blue gastroliths had sharper and more intense diffraction peaks, suggesting a relatively higher degree of crystallinity, whereas white gastroliths showed broader and lower-intensity features consistent with a stronger amorphous contribution. However, because XRD data were evaluated qualitatively and no quantitative phase analysis was performed, these findings should not be interpreted as exact amorphous/crystalline phase percentages.

ICP-OES results showed that blue gastroliths had higher Ca concentration and a higher Ca/P ratio, while white gastroliths had relatively higher P and Mg concentrations. The calculated CaCO_3 equivalent values, estimated from Ca concentrations, were approximately 78.9% in blue gastroliths and 74.4% in white gastroliths. These values represent calculated calcium carbonate equivalents and should not be considered direct measurements of calcium carbonate polymorph abundance. Together with the XRD and Raman findings, the elemental data suggest that blue gastroliths are associated with a more crystalline calcium carbonate-rich structure, whereas white gastroliths may retain a relatively greater amorphous calcium carbonate component.

SEM-EDS observations revealed layered gastrolith microstructures and local elemental signals dominated by Ca, O, C, P, and Mg. Blue gastroliths showed a relatively compact and dense surface morphology, whereas white gastroliths exhibited a more irregular and porous surface structure. Fibrous surface features were observed, but their biological origin could not be confirmed within the scope of this study. Therefore, these structures should be interpreted cautiously as organic-associated or mineral-organic surface features rather than as confirmed microbial or fungal formations.

Overall, the findings indicate that gastrolith color variation in *P. leptodactylus* is associated with differences in crystallinity, elemental composition, Ca/P ratio, and surface morphology. Nevertheless, color should not be used as a standalone indicator of mineral phase composition. Instead, it should be interpreted together with complementary structural, chemical, and morphological data. The results provide baseline information for future studies on gastrolith biomineralization, calcium metabolism, molting physiology, and mineral storage strategies in Turkish *P. leptodactylus* populations.

Future research should include molt-stage determination, direct water chemistry measurements, organic matrix characterization, and quantitative mineralogical analyses to

clarify the mechanisms controlling ACC stabilization and transformation into crystalline calcium carbonate phases. Such studies would also help determine whether white and blue gastroliths represent different mineralization stages, physiological conditions, or individual-level variation. From an applied perspective, the present findings may contribute to future work on crayfish aquaculture, mineral supplementation strategies, and biomimetic calcium carbonate systems; however, these applications require further experimental validation.

ACKNOWLEDGEMENTS

This study was funded by the Scientific Research Coordination Unit of Çanakkale Onsekiz Mart University, Project No. FHD-2021-3516. The authors would also like to thank Mr. Mert Akgün from Çanakkale Onsekiz Mart University for his kind support during the interpretation of the findings.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' Contributions

SB: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Data curation, Writing - original draft, Writing - review & editing, Project administration, Supervision.

BK: Conceptualization, Data curation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing.

SK: Conceptualization, Investigation, Methodology, Resources, Visualization, Writing - review & editing, Supervision.

SA: Writing - original draft, Data curation.

DA: Investigation, Methodology, Resources.

ET: Investigation, Methodology.

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Funding

This study was funded by the Scientific Research Coordination Unit of Çanakkale Onsekiz Mart University, Project No. FHD-2021-3516.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI Disclosure

Generative AI tools (e.g., ChatGPT 5.0, DeepSeek) were used only for language editing and grammatical review of selected sections. The authors reviewed and validated all AI-assisted outputs and take full responsibility for the final content.

REFERENCES

- Acarlı, S., Kızılkaya, B., Vural, P., Berber, S., Kale, S., & Acarlı, D. (2023). Antioxidant radical scavenging capacity and total carotenoid content of narrow-clawed crayfish (*Pontastacus leptodactylus*, Eschscholtz, 1823) in Atıkhisar Reservoir (Çanakkale, Türkiye). *Oceanological and Hydrobiological Studies*, 52(4), 471-483. <https://doi.org/10.26881/oahs-2023.4.08>
- Addadi, L., Raz, S., & Weiner, S. (2003). Taking advantage of disorder: Amorphous calcium carbonate and its roles in biomineralization. *Advanced Materials*, 15(12), 959-970. <https://doi.org/10.1002/adma.200300381>
- Aiken, D. E., & Waddy, S. L. (1992). The growth process in crayfish. *Reviews in Aquatic Sciences*, 6(3-4), 335-381.
- Akhan, S., Bektaş, Y., Berber, S., & Kalaycı, G. (2014). Population structure and genetic analysis of narrow-clawed crayfish (*Astacus leptodactylus*) populations in Turkey. *Genetica*, 142, 381-395. <https://doi.org/10.1007/s10709-014-9782-5>
- Akhan, S., Çağatay, İ. T., Berber, S., Taştan, B., Taştan, Y., & Dalar, T. (2024). Prevalence, molecular identification and genotyping of the crayfish plague pathogen, *Aphanomyces astaci*, in major narrow-clawed crayfish (*Pontastacus leptodactylus* Eschscholtz, 1823) populations from Türkiye. *Journal of Veterinary Research*, 68(4), 515-524. <https://doi.org/10.2478/jvetres-2024-0061>
- Barım Öz, Ö. (2009). Kabuk değiştirme dönemindeki tatlı su istakozu (*Astacus leptodactylus* Esch. 1823)'na ait bazı dokularda oluşan oksidatif stresi üzerine vitamin E, C, astaksantin ve β -karotenin etkisinin belirlenmesi. *Veterinary Sciences*, 4(3), 39-46. <https://izlik.org/IA94ZW22PU>
- Barım Öz, Ö. (2016). Kabuk değiştirme döneminde kerevit (*Astacus leptodactylus*, Esch. 1823) yemine ilave edilen vitamin E ve C'nin büyüme, oksidatif stres, vitamin A, E, C ve beta karoten üzerine etkileri. *Atatürk Üniversitesi Veteriner Bilimleri Dergisi*, 11(3), 296-304. <https://doi.org/10.17094/ataunivbd.282986>
- Berber, S., Acarlı, S., Bayraklı, B., Kale, S., Kızılkaya, B., Vural, P., & Acarlı, D. (2024b). Monthly variation of fatty acids, lipid quality index and metal content of *Pontastacus leptodactylus* (Eschscholtz, 1823) in Atıkhisar Dam Lake (Çanakkale, Türkiye). *Environmental Science and Pollution Research*, 31(18), 27014-27036. <https://doi.org/10.1007/s11356-024-32858-1>
- Berber, S., Akhan, S., Bektaş, Y., & Kalaycı, G. (2020). Meat yield and length-weight relationship of freshwater crayfish (*Pontastacus leptodactylus* (Eschscholtz, 1823)) population in nine different inland water resources in Turkey. *Acta Natura et Scientia*, 1(1), 82-95. <https://doi.org/10.29329/actanatsci.2020.313.10>
- Berber, S., & Kale, S. (2018). Comparison of juvenile *Astacus leptodactylus* growth raised in cages in rice fields to other crayfish juvenile growth studies. *Turkish Journal of Fisheries and Aquatic Sciences*, 18(2), 331-341. https://doi.org/10.4194/1303-2712-v18_2_12
- Berber, S., Kale, S., & Acarlı, D. (2024a). Cheliped loss and abnormalities of the narrow-clawed crayfish, *Pontastacus leptodactylus* (Eschscholtz, 1823) (Crustacea: Decapoda: Astacidae). *Nauplius*, 32, e20240502. <https://doi.org/10.1590/2358-2936e20240502>
- Berber, S., Kale, S., Acarlı, D., & Demirci, A. (2025). Reproduction of the narrow-clawed crayfish, *Pontastacus leptodactylus* (Decapoda: Astacidae: Astacidae), in western Turkey. *Journal of Crustacean Biology*, 45(4), ruaf064. <https://doi.org/10.1093/jcbiol/ruaf064>
- Berber, S., Kale, S., Bulut, M., & İzci, B. (2019). Tatlısu istakozunun (*Pontastacus leptodactylus*) çeltik (*Oryza sativa* L.) ile birlikte polikültür yetiştiriciliğinde ideal stok oranlarının belirlenmesi üzerine bir araştırma. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 22(6), 953-964. <https://doi.org/10.18016/ksutarimdogavi.544561>
- Berber, S., & Mazlum, Y. (2009). Reproductive efficiency of the narrow-clawed crayfish, *Astacus leptodactylus*, in several populations in Turkey. *Crustaceana*, 82(5), 531-542.
- Berber, S., Mazlum, Y., Demirci, A., & Türel, S. (2012). Structure, growth, mortality and size at sexual maturity of various populations *Astacus leptodactylus* Eschscholtz, 1823 (Crustacea: Decapoda) in Turkey. *Marine Science and Technology Bulletin*, 1(1), 21-27. <https://izlik.org/IA95FH49KH>
- Berber, S., Yıldız, H., Ateş, A. S., Bulut, M., & Mendeş, M. (2010). A study on the relationships between some morphological and reproductive traits of the Turkish crayfish, *Astacus leptodactylus* Eschscholtz, 1823 (Crustacea: Decapoda). *Reviews in Fisheries Science*, 18(1), 131-137. <https://doi.org/10.1080/10641260903491003>
- Berber, S., Yıldız, H., Özen, Ö., Mendeş, M., & Palaz, M. (2011). Temporary timing of reproductive traits with respect to environmental variables in Turkish crayfish in Yenice Reservoir. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 17(3), 477-486.
- Boyalık, F., & Berber, S. (2020). An investigation of the reproductive properties of crayfish (*Pontastacus leptodactylus* (Eschscholtz, 1823)) in Kocahıdır Reservoir. *Fresenius Environmental Bulletin*, 29, 7765-7772.

- Boyalık, F., Berber, S., & Kale, S. (2023). Meat yield and the length-weight relationships of the narrow-clawed crayfish, *Pontastacus leptodactylus* (Eschscholtz, 1823). *Momona Ethiopian Journal of Science*, 15(2), 189-215. <https://doi.org/10.4314/mejs.v15i2.4>
- Erol, K. G., Özkök, R., Küçükbara, R., & Çınar, Ş. (2010). Potential mortality causes on juvenile stage in Freshwater crayfish *Astacus leptodactylus* (Eschscholtz, 1823) farming. *Süleyman Demirel Üniversitesi Eğirdir Su Ürünleri Fakültesi Dergisi*, 6(2), 23-30. <https://izlik.org/JA26WT47CF>
- Geldiay, R., & Kocataş, A. (1970). *Türkiye Astacus (Decapoda) popülasyonlarının dağılışı ve taksonomik tespiti* (Yayın No. 94). Ege Üniversitesi Fen Fakültesi İlmi Raporlar Serisi.
- Gilbert, P. U. P. A., Bergmann, K. D., Boekelheide, N., Tambutté, S., Mass, T., Marin, F., Adkins, J. F., Erez, J., Gilbert, B., Knutson, V., Cantine, M., Ortega-Hernández, J., & Knoll, A. H. (2022). Biomineralization: Integrating mechanism and evolutionary history. *Science Advances*, 8(10), eabl9653. <https://doi.org/10.1126/sciadv.abl9653>
- Glazer, L., Tom, M., Weil, S., Roth, Z., Khalaila, I., Mittelman, B., & Sagi, A. (2013). Hemocyanin with phenoloxidase activity in the chitin matrix of the crayfish gastrolith. *Journal of Experimental Biology*, 216(Pt 10), 1898-1904. <https://doi.org/10.1242/jeb.080945>
- Greenaway, P. (1985). Calcium balance and moulting in the Crustacea. *Biological Reviews*, 60(3), 425-454. <https://doi.org/10.1111/j.1469-185X.1985.tb00424.x>
- Gültepe, Y., Berber, S., & Gültepe, N. (2024). Modeling and predicting meat yield and growth performance using morphological features of narrow-clawed crayfish with machine learning techniques. *Scientific Reports*, 14, 18499. <https://doi.org/10.1038/s41598-024-69539-5>
- Gültepe, Y., & Gültepe, N. (2026). A sample model for applying feature selection and machine learning techniques to estimate and manage crayfish populations. *Scientific Reports*, 16, 14540. <https://doi.org/10.1038/s41598-026-45535-9>
- Harris, L. V., Hutchinson, I. B., Ingley, R., Marshall, C. P., Olcott Marshall, A., & Edwards, H. G. M. (2015). Selection of portable spectrometers for planetary exploration: A comparison of 532 nm and 785 nm Raman spectroscopy of reduced carbon in Archean cherts. *Astrobiology*, 15(6), 420-429. <https://doi.org/10.1089/ast.2014.1220>
- Hikida, T., Nagasawa, H., & Kogure, T. (2003). Characterization of amorphous calcium carbonate in the gastrolith of crayfish, *Procambarus clarkii*. In I. Kobayashi & H. Ozawa (Eds.), *Biomineralization: Formation, diversity, evolution and application* (pp. 81-84). Tokai University Press.
- Holthuis, L. B. (1961). Report on a collection of Crustacea Decapoda and Stomatopoda from Turkey and the Balkans. *Zoologische Verhandelingen*, 47(1), 1-67.
- ICDD. (2023). Powder Diffraction File (Card No. 86-2340). International Centre for Diffraction Data. Calculated from Inorganic Crystal Structure Database Collection Code 79657; primary crystal data from Maslen, E. N., Streltsov, V. A., Streltsova, N. R., & Ishizawa, N. (1995). Electron density and optical anisotropy in rhombohedral carbonates. III. Synchrotron X-ray studies of CaCO₃, MgCO₃ and MnCO₃. *Acta Crystallographica Section B: Structural Science*, 51(6), 929-939. <https://doi.org/10.1107/S0108768195006434>
- Kadak, A. E., Küçükgülmez, A., & Çelik, M. (2023). Preparation and characterization of crayfish (*Astacus leptodactylus*) chitosan with different deacetylation degrees. *Iranian Journal of Biotechnology*, 21(2), e3253. <https://doi.org/10.30498/ijb.2023.323958.3253>
- Kale, S., & Berber, S. (2020). Trend analysis and comparison of forecast models for production of Turkish crayfish (*Pontastacus leptodactylus* Eschscholtz, 1823) in Turkey. *Yüzüncü Yıl University Journal of Agricultural Sciences*, 30(Additional Issue), 973-988. <https://doi.org/10.29133/yyutbd.761275>
- Kale, S., & Berber, S. (2025). Developing an artificial neural network (ANN) to estimate growth model of narrow-clawed crayfish (*Pontastacus leptodactylus*) in Yenice Reservoir (Çanakkale, Türkiye). *Momona Ethiopian Journal of Science*, 17(1), 82-98. <https://doi.org/10.4314/mejs.v17i1.6>
- Kale, S., Berber, S., Acarlı, D., Demirkıran, T., Vural, P., Acarlı, S., Kızılkaya, B., & Tan, E. (2020). First report of albinism in Turkish crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823) (Crustacea, Decapoda, Astacidae). *Acta Natura et Scientia*, 1(1), 36-42. <https://doi.org/10.29329/actanatsci.2020.313.5>
- Kale, S., Berber, S., Acarlı, D., Demirkıran, T., Vural, P., Acarlı, S., & Kızılkaya, B. (2021). Blue color anomaly in Turkish crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823) (Crustacea, Decapoda, Astacidae) from Atikhisar Reservoir in Çanakkale, Turkey. *Acta Natura et Scientia*, 2(1), 1-5. <https://doi.org/10.29329/actanatsci.2021.314.1>
- Kapusuz, D., & Ercan, B. (2019). Incorporation of calcium carbonate polymorphs into silk-fibroin scaffolds enhances calcium phosphate mineralization. *Celal Bayar University Journal of Science*, 15(4), 387-394. <https://doi.org/10.18466/cbayarfbe.573625>
- Küçükgülmez, A. (2018). Extraction of chitin from crayfish (*Astacus leptodactylus*) shell waste. *Alinteri Journal of Agriculture Science*, 33(1), 99-104. <https://doi.org/10.28955/alinterizbd.432139>
- Lowenstam, H. A., & Weiner, S. (1989). *On biomineralization*. Oxford University Press.
- Luquet, G. (2012). Biomineralizations: Insights and prospects from crustaceans. *ZooKeys*, 176, 103-121. <https://doi.org/10.3897/zookeys.176.2318>

- Luquet, G., Fernández, M. S., Badou, A., Guichard, N., Le Roy, N., Corneillat, M., Alcaraz, G., & Arias, J. L. (2013). Comparative ultrastructure and carbohydrate composition of gastroliths from Astacidae, Cambaridae and Parastacidae freshwater crayfish (Crustacea, Decapoda). *Biomolecules*, 3(1), 18-38. <https://doi.org/10.3390/biom3010018>
- Luquet, G., & Marin, F. (2004). Biomineralisations in crustaceans: Storage strategies. *Comptes Rendus Palevol*, 3(6-7), 515-534. <https://doi.org/10.1016/j.crpv.2004.07.015>
- Mann, S. (2001). *Biomineralization: Principles and concepts in bioinorganic materials chemistry*. Oxford University Press.
- Mann, S., & Ozin, G. A. (1996). Synthesis of inorganic materials with complex form. *Nature*, 382, 313-318. <https://doi.org/10.1038/382313a0>
- Mazlum, Y. (2007). Stocking density affects the growth, survival, and cheliped injuries of third instars of narrow-clawed crayfish, *Astacus leptodactylus* Eschscholtz, 1823 juveniles. *Crustaceana*, 80(7), 803-815.
- Mazlum, Y., Demirci, A., Şimşek, E., & Demirci, S. (2025). An assessment on harvest, exports, and marketing income relationships of freshwater crayfish in Turkey. *Momona Ethiopian Journal of Science*, 17(1), 99-120. <https://doi.org/10.4314/mejs.v17i1.7>
- Mazlum, Y., Güner, Ö., & Şirin, S. (2011). Effects of feeding interval on growth, survival and body composition of narrow-clawed crayfish, *Astacus leptodactylus* Eschscholtz, 1823 juveniles. *Turkish Journal of Fisheries and Aquatic Sciences*, 11(2), 283-289. <https://doi.org/10.4194/trjfas.2011.0213>
- Mazlum, Y., & Şirin, S. (2020). The effects of using different levels of calcium carbonate (CaCO₃) on growth, survival, molting frequency and body composition of freshwater crayfish juvenile, *Pontastacus leptodactylus* (Eschscholtz, 1823). *Kahramanmaraş Sütçü İmam University Journal of Agriculture and Nature*, 23(2), 506-514. <https://doi.org/10.18016/ksutarimdog.vi.614826>
- Mazlum, Y., & Uzun, C. (2022). Impact of stocking density on the survival, growth and injury of narrow-clawed crayfish (*Pontastacus leptodactylus*) reared in a flowing brackish water system. *Acta Natura et Scientia*, 3(2), 163-183. <https://doi.org/10.29329/actanatsci.2022.352.08>
- Mazlum, Y., Yazıcı, M., & Gürlek, O. G. (2020). Evaluation of stocking densities and feed types on growth and survival of narrow-clawed crayfish *Pontastacus leptodactylus* (Escholtz, 1823) reared under laboratory conditions. *Fresenius Environmental Bulletin*, 29(9A), 8283-8293.
- Neira-Carrillo, A., Fernández, M. S., Hevia, G. P., Arias, J. L., Gebauer, D., & Cölfen, H. (2017). Retrosynthesis of CaCO₃ via amorphous precursor particles using gastroliths of the red claw lobster (*Cherax quadricarinatus*). *Journal of Structural Biology*, 199(1), 46-56. <https://doi.org/10.1016/j.jsb.2017.05.004>
- Ozin, G. A. (1997). Morphogenesis of biomineral and morphosynthesis of biomimetic forms. *Accounts of Chemical Research*, 30, 17-27.
- Shechter, A., Berman, A., Singer, A., Freiman, A., Grinstein, M., Erez, J., Aflalo, E. D., & Sagi, A. (2008). Reciprocal changes in calcification of the gastrolith and cuticle during the molt cycle of the red claw crayfish *Cherax quadricarinatus*. *Biological Bulletin*, 214(2), 122-134. <https://doi.org/10.2307/25066669>
- Şirin, S., & Mazlum, Y. (2017). Effect of dietary supplementation of calcium chloride on growth, survival, moulting frequency and body composition of narrow-clawed crayfish, *Astacus leptodactylus* (Eschscholtz, 1823). *Aquaculture Nutrition*, 23(4), 805-813. <https://doi.org/10.1111/anu.12447>
- Takesue, R. K., Bacon, C. R., & Thompson, J. K. (2008). Influences of organic matter and calcification rate on trace elements in aragonitic estuarine bivalve shells. *Geochimica et Cosmochimica Acta*, 72(22), 5431-5445. <https://doi.org/10.1016/j.gca.2008.09.003>
- Travis, D. F. (1963). Structural features of mineralization from tissue to macromolecular levels of organization in the decapod crustacean. *Annals of the New York Academy of Sciences*, 109, 177-245. <https://doi.org/10.1111/j.1749-6632.1963.tb13467.x>
- Türel, S., & Berber, S. (2016). The effects of calcium supplemented diets on growth performance of crayfish (*Astacus leptodactylus* Eschscholtz, 1823). *Adıyaman University Journal of Science*, 6(1), 96-109. <https://izlik.org/IA65RB95XX>
- Ueno, M. (1980). Calcium-transport in crayfish gastrolith disk: Morphology of gastrolith disk and ultrahistochemical demonstration of calcium. *Journal of Experimental Zoology*, 213(2), 161-171. <https://doi.org/10.1002/jez.1402130202>
- Uğurlu, E., Mazlum, Y., Duysak, Ö., & Yazıcı, M. (2026). Chitin and chitosan production from crayfish exoskeleton waste: A sustainable approach to value-added biomaterials. *Waste and Biomass Valorization*, <https://doi.org/10.1007/s12649-026-03567-2>
- Ziegler, A., Fabritius, H., & Hagedorn, M. (2005). Microscopical and functional aspects of calcium-transport and deposition in terrestrial isopods. *Micron*, 36(2), 137-153. <https://doi.org/10.1016/j.micron.2004.09.006>