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Evaluating yield of DNA extraction methods across different tissues of moon jellyfish *Aurelia aurita* (Linnaeus, 1758)

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Abstract

Purpose: This study aimed to optimize DNA extraction from the moon jellyfish *Aurelia aurita*, a gelatinous marine invertebrate, by comparing different methodologies and evaluating the influence of tissue type and Proteinase K treatment duration on DNA yield.
Method: A total of 48 different combinations were tested using three extraction methods (salting-out, kit-based, and Trizol-based) across four tissue types (gonads, tentacles, epidermis, and gastrodermis). Each method was applied with varying Proteinase K incubation times to assess their impact on DNA yield and integrity.
Findings: Gonadal tissues consistently provided the highest DNA concentrations, likely due to their relatively high cellular density and structural robustness. Among the methods, the salting-out protocol yielded superior results, particularly with 1–3 hours of Proteinase K incubation. In contrast, highly hydrated tissues such as epidermis and gastrodermis led to underestimated yields due to distorted wet weight measurements.
Conclusion: Tissue selection, enzymatic treatment time, and extraction methodology critically affect DNA recovery from jellyfish. The salting-out method, when applied to gonadal tissue with moderate Proteinase K incubation, is recommended for efficient and reliable DNA isolation from *Aurelia aurita.*

Keywords: DNA isolation, DNA extraction, *Aurelia aurita*, salting-out method, DNA yield

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Ay denizanası *Aurelia aurita*'nın (Linnaeus, 1758) farklı dokularında DNA izolasyon yöntemlerinin verimliliklerinin değerlendirilmesi

Özet

Amaç: Bu çalışma, jelatinimsi yapısı nedeniyle DNA izolasyonu zor olan denizanası *Aurelia aurita*'dan elde edilecek DNA verimini optimize etmeyi; doku türü, enzimatik inkübasyon süresi ve farklı izolasyon yöntemlerinin etkilerini karşılaştırarak değerlendirmeyi amaçlamıştır.
Yöntem: Toplamda üç farklı DNA izolasyon yöntemi (salting-out, kit bazlı ve Trizol bazlı) dört farklı doku türü (gonad, tentakül, epidermis ve gastrodermis) üzerinde uygulanmış, her biri Proteinaz K ile 0, 1, 3 ve 24 saat inkübasyona tabi tutulmuştur. Böylece 48 farklı kombinasyon analiz edilmiştir.
Bulgular: En yüksek DNA konsantrasyonları gonad dokularında elde edilmiş olup bu durum, dokunun yüksek hücresel yoğunluğu ve yapısal bütünlüğü ile ilişkilendirilmiştir. Özellikle 1–3 saatlik Proteinaz K uygulamasıyla birlikte salting-

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out yöntemi, diğer iki yönteme kıyasla daha yüksek verim sağlamıştır. Aşırı nemli olan epidermis ve gastrodermis dokularında ise yaş ağırlık ölçümünde sapmalar oluşmuş ve bu durum biyolojik girdiye göre DNA veriminin olduğundan düşük hesaplanmasına neden olmuştur.
Sonuç: Jelatinöz denizel omurgasızlardan DNA izolasyonunda doku seçimi, enzimatik inkübasyon süresi ve kullanılan metodoloji büyük önem taşımaktadır. *Aurelia aurita* örneklerinde, özellikle gonad dokusuna 1–3 saatlik Proteinaz K uygulaması ile salting-out yöntemi kullanımı, verimli ve güvenilir bir DNA izolasyonu için önerilmektedir.

Anahtar kelimeler: DNA izolasyonu, DNA ekstraksiyonu, *Aurelia aurita*, tuzla çöktürme yöntemi, DNA verimi

1. Introduction

Cnidarians, encompassing corals, sea anemones, jellyfish, and hydra, represent a globally distributed and evolutionarily ancient lineage which evaluated as ancestral of marine ecosystems [1,2]. Among them, the class Scyphozoa is notable due to the conspicuous nature of mass bloom events, which have profound ecological and economic ramifications [3, 4, 5].

Aurelia aurita (Linnaeus, 1758), the moon jellyfish, has emerged as one of the most studied species within Cnidaria: Scyphozoa due to its wide distribution, ecological plasticity, and physiological adaptability. Its thermal tolerance and well-documented morphological features have positioned it as a model organism in numerous ecological studies [6, 7, 8]. The ecological relevance of *A. aurita* extends even further when considering its response to anthropogenic pressures. Jellyfish blooms are now increasingly frequent, severe, and geographically widespread, with substantial impacts on biodiversity, fisheries, and infrastructure [9, 10, 11]. These outbreaks are strongly correlated with human-induced changes, including overfishing, nutrient enrichment, habitat modification, and global warming [12,13]

Beyond its ecological footprint, *A. aurita* presents a compelling model for understanding fundamental principles of developmental biology. While bilaterians typically maintain a single morphology encoded by one genome, cnidarians like *A. aurita* employ a shared genomic architecture to orchestrate multiple, functionally distinct body forms. Insects and amphibians undergo metamorphosis via well-characterized hormonal cascades involving nuclear receptors (e.g., EcR, TR, USP, RxR) [8, 14-16]. However, in early-branching metazoans, the regulatory landscape of metamorphosis remains underexplored. A deeper understanding of these ancestral pathways in cnidarians may offer pivotal insights into the evolutionary origins of complex life cycles and developmental systems drift.

At the molecular level, *A. aurita* holds a unique position in metazoan evolution. Comparative genomic analyses have shed light on conserved genes involved in apoptosis, neurogenesis, and stress response, with ancient nuclear receptor subfamilies (e.g., ligand-activated and orphan NRs) identified in cnidarians [17]. Moreover, *A. aurita* exhibits remarkable regenerative capabilities, mediated by processes like stem cell proliferation and radial pattern re-establishment [18]. Despite these insights, jellyfish genomics remains an emerging field. The rapid advancement of high-throughput sequencing technologies continues to unravel the genomic underpinnings of jellyfish development, resilience, and environmental adaptation.

Central to all such studies is the ability to isolate high-quality DNA. However, extracting genomic DNA from jellyfish poses considerable challenges owing to their high-water content, abundance of mucopolysaccharides, and fragile cellular composition. This study aimed to evaluate three distinct DNA extraction protocols for their efficiency in isolating genomic material from *A. aurita* specimens collected along the Tarabya Coast, Istanbul (Türkiye). The outcomes of this methodological comparison support downstream applications such as sequencing and eDNA analysis and contribute to broader efforts to integrate cnidarians into molecular research frameworks.

2. Materials and methods

2.1. Jellyfish collection

In this study, *Aurelia aurita* individuals were collected from the Tarabya Coast, situated in Sarıyer, Istanbul, Türkiye. To ensure consistency, individuals of moderate size (average bell diameter ~12 cm) and weight (~20 g) with clearly visible gonads were selected. To avoid tissue damage, specimens were gently retrieved from the water surface using hand scoops. Immediately after collection, they were immersed in 100% ethanol to prevent degradation and transported to the laboratory under cooled conditions. Upon arrival, samples were preserved at –20°C until further processing.

2.2. Experimental design and tissue sampling

A matrix-based experimental design systematically explored how various biological parameters affect DNA isolation efficiency. This design incorporated three key variables: tissue type, sample mass, and enzyme treatment process. Four anatomically distinct tissues (epidermis, gastrodermis, gonads, and tentacles) were dissected from ethanol-fixed individuals using sterile scalpels, following the anatomical landmarks shown in Figure 1. The identification and classification of tissue regions in this study were based on previously published anatomical descriptions [19].

Tissue type and quantities were selected based on structural abundance. Accordingly, more voluminous tissues, such as the epidermis and gastrodermis, were sampled in quantities ranging from 25 mg to 150 mg, while gonads and tentacles were analyzed in smaller amounts. Proteinase K treatment was tested at three different incubation times: 1 hour, 3 hours, and 24 hours to assess the impact of enzymatic breakdown. This multifactorial approach enabled a comprehensive evaluation of how these biological variables influence DNA yield and purity, particularly in tissues with differing biochemical profiles.

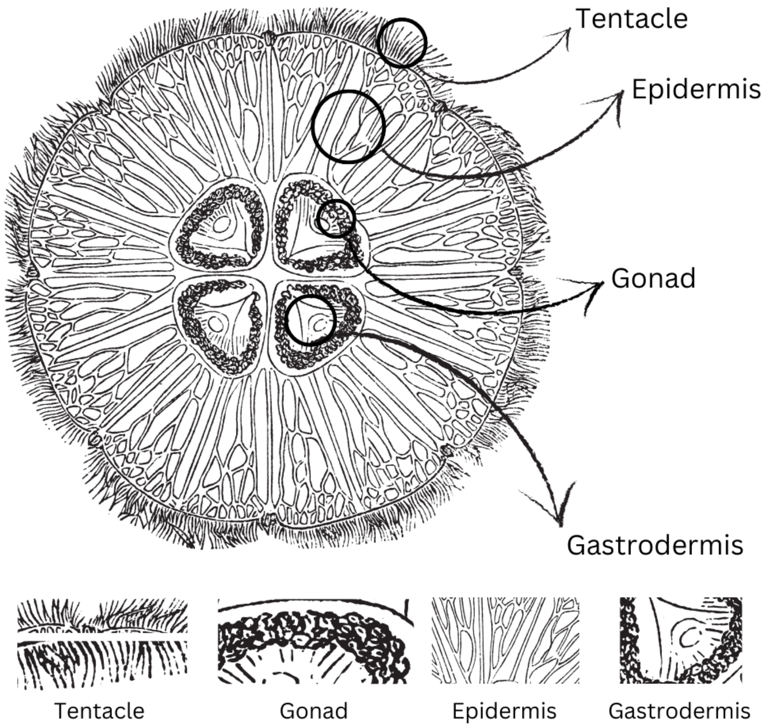

Figure 1. Illustration of *Aurelia aurita* showing the four dissected tissue types, tentacle, gonad, epidermis, and gastrodermis, used for comparative DNA extraction analysis.

2.3. DNA extraction protocols

Before DNA isolation, extracted tissue parts were rinsed with sterile distilled water, weighed precisely, and pre-frozen at –20°C for 3 hours to improve homogenization efficiency. This pre-treatment was selected based on preliminary optimization trials, which demonstrated that physical disruption was most effective after controlled pre-freezing under these specific conditions.

2.3.1. Kit-based DNA isolation

Genomic DNA was extracted using the Bio Basic Canada One-4-All Genomic DNA Miniprep Kit, with minor protocol optimizations adapted to jellyfish tissue characteristics. Each frozen sample was homogenized in 180 µL ACL buffer using sterile pestles. Following homogenization, 20 µL of Proteinase K was added and incubated at 56°C for up to 24 hours. After digestion, 200 µL CL buffer and 200 µL cold ethanol (99%) were added sequentially. Lysates were passed through spin columns and centrifuged. Wash steps using CW1 and CW2 buffers were followed by a dry spin, and DNA was eluted in 100 µL CE buffer and stored at –20°C for further analysis. The DNA extraction kit used in this study was selected because it had previously been applied successfully in studies involving marine organisms [20].

2.3.2. Trizol-based DNA isolation

Secondly, DNA was extracted using a phenol-based reagent (One Step-RNA Reagent, Bio Basic), analogous to Trizol. As a well-established and routinely used procedure, the TRIzol-based method was also included in this study for

DNA extraction due to its general effectiveness and reliability in molecular biology workflows [21]. Samples were homogenized in 1 mL of reagent and incubated at room temperature for 5 minutes. After adding 0.2 mL chloroform, the mixture was vortexed vigorously and centrifuged at 12,000 × g for 15 minutes at 2–8°C. The upper aqueous phase (containing RNA) was discarded, and DNA was precipitated from the interphase by adding 0.3 mL ethanol. Following a brief incubation, samples were centrifuged at 2,000 × g to pellet DNA. The pellet was washed twice with 0.1 M sodium citrate in 10% ethanol and then once with 75% ethanol. After air drying, DNA was resuspended in 0.5 mL of 8 mM NaOH and stored at –20°C.

2.3.3. Salting-out method

The third method involved a salt-precipitation protocol using freshly prepared TNES buffer (50 mM Tris-HCl, 400 mM NaCl, 20 mM EDTA, 0.5% SDS), modified from Sugrue et al. (2025) [22].
Tissues were homogenized post-freezing and incubated in 300 µL TNES with 100 µg/mL Proteinase K at 37°C for varying durations (1 h, 3 h, overnight). After digestion, 85 µL of 5 M NaCl was added, and samples were centrifuged at 14,000 rpm. Supernatants were collected, and DNA was precipitated by ethanol addition. Pellets were washed in 70% ethanol, dried, and resuspended in 50 µL of sterile water before storage at –20°C. The salting-out method, a classical technique that has been widely applied to marine organisms and whose efficiency has been validated in numerous studies, was also utilized in this study as a reliable approach for DNA extraction [23].

2.4. DNA quantification

DNA concentration and purity were assessed using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, USA) [24]. A 2 µL aliquot of each sample was analyzed, and absorbance values at 260 nm and 280 nm were recorded. The A260/A280 ratio was used to evaluate sample purity. Measurements were conducted in duplicate, and buffer-only blanks were used to calibrate the instrument.

2.5. Statistical analysis

To assess whether DNA yields varied significantly among tissue types, one-way ANOVA was applied using tissue type as the independent variable and DNA concentration as the response variable. In cases where statistical significance was observed ($p < 0.05$), Tukey's HSD post hoc test was performed to identify specific pairwise differences [25]. Results were visualized using boxplots, with statistically significant comparisons annotated using letters or asterisks, depending on the plotting approach.

3. Results

Various sample masses were tested for each tissue type to explore the impact of tissue quantity on DNA yield. In gonadal tissues, a strong positive correlation between tissue mass and DNA concentration was observed. Yields increased from 0.22 µg/µL at 2 mg to a peak of 1.11 µg/µL at 10 mg, indicating that extraction efficiency was significantly enhanced by even minimal increases in sample size (Figure 2).

In tentacle tissues, a moderate increase in DNA yield was recorded, reaching a maximum of 0.24 µg/µL at 20 mg. However, higher sample amounts did not result in further improvements, suggesting a plateau effect. Epidermal tissues did not show a consistent pattern. While the highest yield (0.059 µg/µL) was obtained at 100 mg, smaller amounts produced fluctuating results, likely due to interference from mucopolysaccharide-rich matrices or low cellular content. Gastrodermal tissues yielded the lowest overall DNA concentrations. A gradual improvement was noted with increasing mass, rising from 0.015 µg/µL at 15 mg to 0.035 µg/µL at 75 mg.

When the overall DNA yield was compared across tissue types, gonads yielded the highest average concentration (0.60 µg/µL) and the maximum individual value (2.89 µg/µL), followed by tentacles (0.17 µg/µL), epidermis (0.04 µg/µL), and gastrodermis (0.02 µg/µL). These findings indicate that both tissue type and quantity play critical roles in DNA extraction efficiency, with gonads emerging as the most productive and consistent tissue source for *A. aurita*.

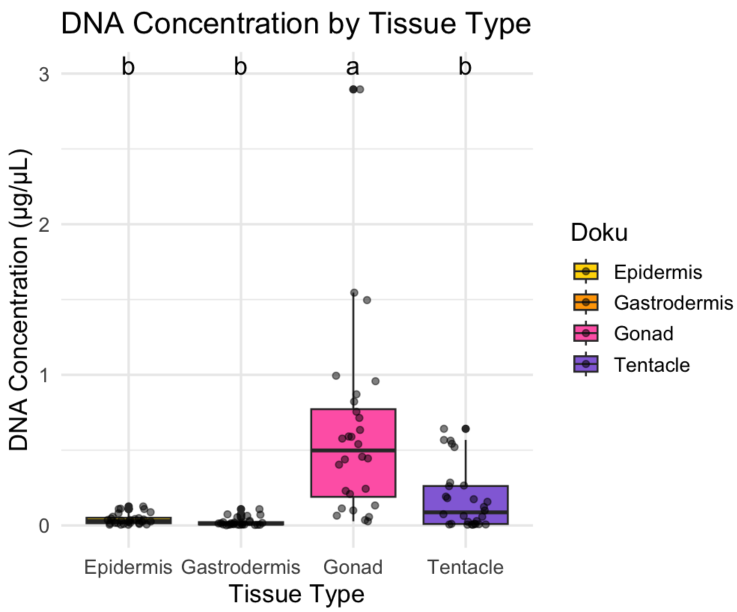

Figure 2. Boxplot showing DNA yield (µg/µL) across different tissue types. Gonadal tissues produced significantly higher DNA concentrations compared to epidermis, gastrodermis, and tentacle samples. Different letters above the boxes indicate statistically significant differences based on Tukey's HSD post-hoc test ($p < 0.05$).

To assess the influence of Proteinase K incubation time, samples were treated for 0, 1, 3, and 24-hours. On average, the highest DNA concentration (0.29 µg/µL) was obtained after 1-hour incubation, followed by 24-hours (0.24 µg/µL) and 3-hours (0.19 µg/µL). Samples without enzymatic digestion yielded negligible DNA (~0.016 µg/µL), confirming the necessity of Proteinase K treatment for effective lysis in jellyfish tissues (Figure 3).

Further inspection revealed several notable outliers. A 10 mg gonad sample incubated for 1-hour yielded the highest concentration (2.89 µg/µL) recorded in the study. Other high-yielding samples were obtained at 3-hours (1.49 µg/µL) and 24-hours (1.54 µg/µL) as well. These outliers suggest that while 1-hour incubation generally yields the most consistent results, extended digestion periods can enable higher recovery in specific tissues, particularly those with greater cellular density such as gonads. Interestingly, these high-yielding samples were primarily processed using the salting-out method, suggesting a synergistic interaction between enzyme incubation and extraction strategy. While 1-hour digestion produced the most consistent mean results, the highest individual yields were achieved at 3- and 24-hours, particularly when combined with salting-out. This underscores the importance of optimizing tissue type, extraction protocol, and enzymatic treatment.

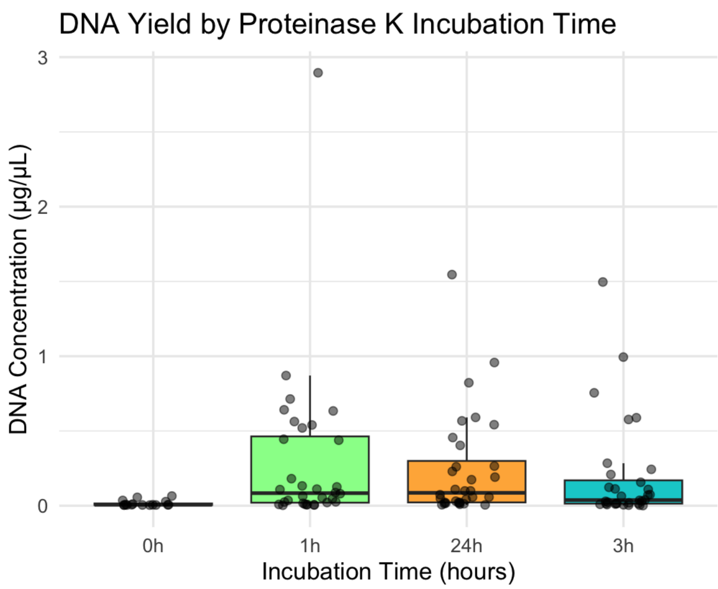

Figure 3. Boxplot illustrating DNA concentration (µg/µL) based on varying Proteinase K incubation times (0h, 1h, 3h, and 24h). A general increase in DNA yield was observed with increased incubation time, with the highest concentration detected at 1h, though the difference between 1h and 24h was minimal in some tissues.

Among the three DNA isolation methods tested, the salting-out protocol yielded the highest average DNA concentration (0.36 µg/µL) and the maximum individual recovery (2.89 µg/µL). Although this method exhibited the highest variability (SD = 0.53, n = 48), it proved to be highly effective and versatile. The silica column-based kit provided a lower average yield (0.12 µg/µL, max 0.76 µg/µL) but offered several practical benefits, including ease of use, shorter processing time, and improved consistency (SD = 0.20). These features make it a suitable choice for routine applications where ultra-high yields are not essential. By contrast, the Trizol-based extraction method resulted in the lowest yields across all tissue types, with an average DNA concentration of 0.016 µg/µL and a maximum of only 0.065 µg/µL (SD = 0.019, n = 16). This poor performance is likely attributable to the incompatibility of phenol-chloroform chemistry with jellyfish tissue composition, particularly their high-water content and mucopolysaccharide-rich nature, which may disrupt phase separation and DNA precipitation (Figure 4).

Notably, the salting-out method demonstrated greater performance in gonadal tissues, suggesting that its salt-based chemistry may be more compatible with the structural and biochemical properties of jellyfish cells. However, its high variability indicates that extraction outcomes may depend heavily on protocol precision and operator consistency.

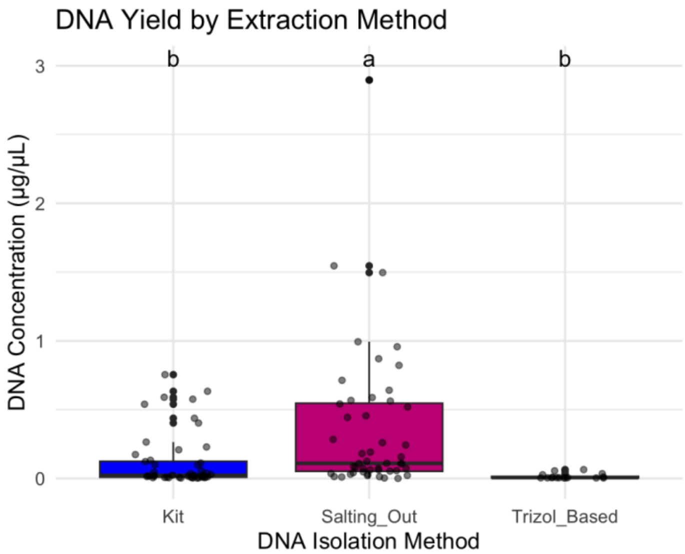

Figure 4. Comparison of DNA concentrations obtained using three different extraction methods: Salting-out, Kit-based, and Trizol-based protocols. Salting-out method yielded significantly higher DNA concentrations than the other two methods. Letters above the boxplots indicate statistically significant groupings based on Tukey's HSD post-hoc test ($p < 0.05$).

Taken together, the results indicate that DNA yield is significantly affected by tissue type, extraction method, and enzymatic incubation duration. Gonadal tissues consistently yielded the highest concentrations, with statistically significant differences compared to epidermis, gastrodermis, and tentacles (Figure 2). Enzyme incubation also played a pivotal role: a marked increase in DNA recovery was noted after 1-hour of digestion, with some improvements observed at 24-hours (Figure 3). Among all methods, the salting-out technique produced significantly higher yields than both the kit-based and Trizol protocols (Figure 4). These findings were further reinforced through a combined analysis of tissue type and enzyme exposure, where salting-out extraction applied to gonadal tissue under extended incubation conditions resulted in the most efficient DNA recovery overall (Figure 5).

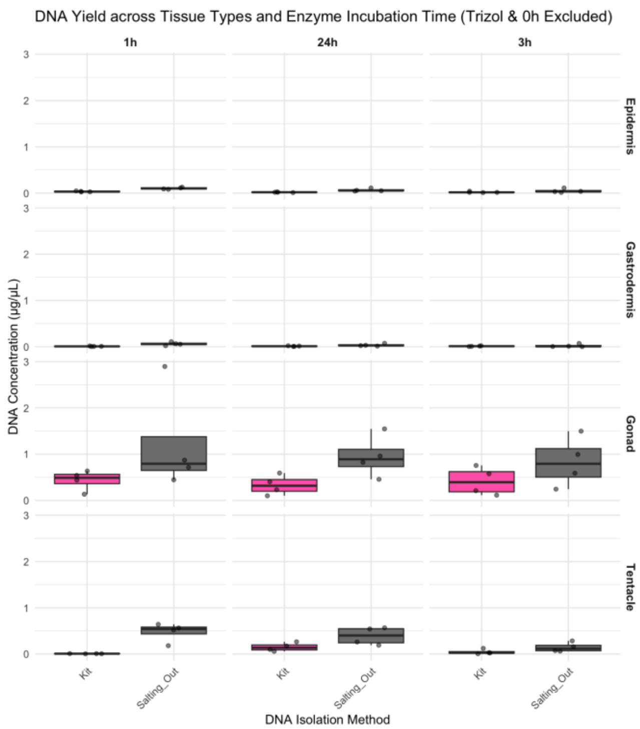

Figure 5. Comparison of DNA concentrations (µg/µL) across tissue types and enzyme incubation times using Salting-out and Kit-based extraction methods. Data were grouped by tissue type (rows) and Proteinase K incubation duration (columns: 1h, 3h, 24h). Boxplots display the distribution and variation of DNA yield, indicating notably higher efficiency in gonadal tissue, especially with Salting-out extraction under prolonged incubation.

4. Conclusions and discussion

In this study, we conducted a systematic comparison with 48 combinations of three DNA extraction methods, salting-out, kit-based, and Trizol-based, across four anatomically distinct tissues of *Aurelia aurita*, while also evaluating the effect of varying Proteinase K incubation times on DNA yield. Among the tissues tested, gonadal samples consistently outperformed others, providing the highest DNA concentrations and emerging as the most reliable source for molecular analyses in jellyfish. This can likely be attributed to their higher cellular density and more robust structure, especially when compared to the more hydrated, gelatinous, and fragile nature of epidermal, gastrodermal, and tentacle tissues.

Over the years, a variety of DNA isolation methods have been employed in jellyfish-related molecular studies. There are studies in the literature that comparatively evaluate different DNA isolation methods across plant taxa [26].For instance, in phylogenetic and mitochondrial sequencing analyses, CTAB-based extraction protocols were utilized due to their effectiveness in isolating high- molecular-weight DNA [27]. In other cases, alternative protocols such as the PPLPP method (preserve, precipitate, lyse, precipitate, and purify) have been applied to improve DNA recovery and purity from preserved tissue samples [28]. More recent approaches, particularly those involving species identification via microarrays and high-throughput sequencing, have increasingly favored the use of commercial kits [29-33]. This preference is largely due to the speed, reproducibility, and convenience of kit- based protocols, especially in environmental DNA (eDNA) applications where DNA may be present in low quantities or highly degraded. However, these methods often rely on small, homogenized samples and generally overlook tissue-specific differences in DNA yield and quality, an aspect that is critical in gelatinous organisms such as jellyfish.

A key technical insight from our study is the significant impact of tissue hydration on perceived sample mass. Despite efforts to standardize wet weights, tissues like epidermis and gastrodermis, though appearing equal in weight, often contained far less actual cellular material. This discrepancy likely led to overestimation of input and underestimation of DNA yield per unit of biological content. For example, a 150 mg epidermal sample might represent only ~50 mg of actual cellular mass. This issue was further complicated by the physical fragility of such tissues, which often disintegrated during dissection or enzymatic processing.

Among extraction methods, salting-out consistently outperformed both the kit-based and Trizol-based approaches in terms of yield and reproducibility. Its compatibility with jellyfish matrices may stem from its simpler, non-phase-dependent chemistry, which seems less hindered by the high viscosity and mucopolysaccharide-rich composition of jellyfish tissues. Trizol, despite its success in other systems, proved ineffective here, likely due to interference during phase separation and the inefficient precipitation of DNA in hydrated matrices.

Enzymatic digestion time was also found to be a critical variable. While 1–3 hours of Proteinase K incubation generally improved yields, longer exposures (e.g., 24 hours) offered limited additional benefit and sometimes even compromised sample quality. This was particularly true for fragile tissues, where over-digestion likely led to DNA degradation. On the other hand, gonadal tissue benefited from longer digestion periods, likely due to its denser cellular composition and stronger structural integrity, which allowed for more complete lysis and DNA release.

Based on these findings, we recommend using gonadal tissue in combination with the salting-out method and a 1–3 hour Proteinase K incubation period for optimal DNA extraction from *Aurelia aurita*. Additionally, future protocols should consider incorporating pre-lysis dehydration steps, such as freeze-drying or ethanol concentration, to correct for tissue hydration differences and improve inter-sample comparability.

Overall, our results emphasize the importance of biological and methodological considerations when developing DNA isolation protocols for gelatinous marine organisms. This study not only highlights an efficient workflow for jellyfish, but also lays a foundation for broader applications in population genetics, barcoding, and genomic research across cnidarian species.

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Conflicts of interest: No Conflict of Interest.

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Ethical statement: This study does not require ethical approval.

Author contributions: Merve Nur AYDEMİR was responsible for the supervision of the study and conceptualized and designed the study, conducted the literature review, and carried out the critical revision of the manuscript. Sümeyra Zeynep ÇALICI actively participated in the experimental procedures, contributed to data collection, manuscript writing, and literature review. Both Merve Nur AYDEMİR and Sümeyra Zeynep ÇALICI also provided biological materials and laboratory equipment. Zeynep Aslıhan AVŞAROĞLU, Buse BAYRAK, Hatice Buse YAVUZ, and Nida KELEL actively participated in the experimental procedures and contributed to data collection and hands-on work throughout the study. All authors reviewed and approved the final version of the manuscript.

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