animals

MDPI

Article

Energy Processes During *Rigor Mortis* in the Adductor Muscle of the Lion's Paw Scallop (*Nodipecten subnodosus*): Effects of Seasonality and Storage Temperature

Edgar Iván Jiménez-Ruiz [1], Víctor Manuel Ocaño-Higuera [2,*], María Teresa Sumaya-Martínez [1], Enrique Márquez-Ríos [3], Saúl Ruíz-Cruz [3], Dalila Fernanda Canizales-Rodríguez [2], Orlando Tortoledo-Ortiz [4], Alba Mery Garzón-García [5], José Rogelio Ramos-Enríquez [2], Santiago Valdez-Hurtado [6], María Irene Silvas-García [3] and Nathaly Montoya-Camacho [2]

1 Unidad de Tecnología de Alimentos, Secretaría de Investigación y Posgrado, Universidad Autónoma de Nayarit, Ciudad de la Cultura s/n, Tepic 63000, Mexico; edgar.jimenez@uan.edu.mx (E.I.J.-R.); teresa.sumaya@uan.edu.mx (M.T.S.-M.)
2 Departamento de Ciencias Químico Biológicas, Universidad de Sonora, Blvd. Luis Encinas y Rosales s/n, Hermosillo 83000, Mexico; dalila.canizales@unison.mx (D.F.C.-R.); rogelio.ramos@unison.mx (J.R.R.-E.); nathaly.montoya@unison.mx (N.M.-C.)
3 Departamento de Investigación y Posgrado en Alimentos, Universidad de Sonora, Blvd, Luis Encinas y Rosales s/n, Hermosillo 83000, Mexico; enrique.marquez@unison.mx (E.M.-R.); saul.ruizcruz@unison.mx (S.R.-C.); maria.silvas@unison.mx (M.I.S.-G.)
4 Coordinación de Nutrición, Centro de Investigación en Alimentación y Desarrollo, A.C. Carretera Gustavo Astiazarán Rosas, No. 46, Hermosillo 83304, Mexico; otortoledo@ciad.mx
5 Ingeniería Industrial, Universidad del Valle, Sede Regional Caicedonia, Carrera 14 No 4-48, Caicedonia 762540, Valle del Cauca, Colombia; garzon.alba@correounivalle.edu.co
6 Unidad Académica Navojoa, Universidad Estatal de Sonora, Blvd, Manlio Fabio Beltrones 810, Col. Bugambilias, Navojoa 85875, Mexico; santiago.valdez@ues.mx
* Correspondence: victor.ocano@unison.mx

check for updates

Academic Editors: Marcelo Hermes-Lima, Daniel Carneiro Moreira and Víctor M. Cubillos

Received: 10 September 2025
Revised: 5 October 2025
Accepted: 10 October 2025
Published: 12 October 2025

Citation: Jiménez-Ruiz, E.I.; Ocaño-Higuera, V.M.; Sumaya-Martínez, M.T.; Márquez-Ríos, E.; Ruíz-Cruz, S.; Canizales-Rodríguez, D.F.; Tortoledo-Ortiz, O.; Garzón-García, A.M.; Ramos-Enríquez, J.R.; Valdez-Hurtado, S.; et al. Energy Processes During *Rigor Mortis* in the Adductor Muscle of the Lion's Paw Scallop (*Nodipecten subnodosus*): Effects of Seasonality and Storage Temperature. *Animals* **2025**, *15*, 2953. https://doi.org/10.3390/ani15202953

Simple Summary

The lion's paw scallop (*Nodipecten subnodosus*) is a high-value mollusk species cultivated in northwestern Mexico, but its postmortem biochemical changes during storage are still poorly understood. This study investigated the effect of seasonality, storage temperature, and transportation on energy-related metabolites associated with rigor mortis in the adductor muscle. Scallops were stored at 0, 5, and 10 °C for 48 h across all four seasons, and the concentrations of ATP, ADP, AMP, glycogen, arginine phosphate, and arginine were quantified. The adenylate energy charge (AEC) was also determined in freshly harvested and transported scallops. The results showed that ATP depletion and AEC decline were strongly affected by storage temperature and seasonality, with significant implications for the postharvest quality and shelf life of this species.

Abstract

The lion's paw scallop (*Nodipecten subnodosus*) is a commercially valuable pectinid whose postharvest quality strongly depends on storage and handling conditions. This study investigated the combined effects of seasonality, postmortem time, and storage temperature on energy metabolism in the adductor muscle, focusing on metabolites associated with *rigor mortis* and freshness. Adult scallops (~10 cm shell height) were harvested in four seasons (spring, summer, autumn, winter), transported under commercial conditions for approximately 2 h, and stored at 0, 5, and 10 °C for 48 h. Muscle samples were collected every 8 h and analyzed for ATP, ADP, AMP, glycogen, arginine phosphate (Arg-P), and free arginine using HPLC and enzymatic assays. In addition, the adenylate energy charge

(AEC) was determined in freshly harvested and post-transport specimens. Initial ATP concentrations ranged from 4.2 to 6.5 μmol/g, with higher levels in winter, while Arg-P varied from 3.1 to 4.8 μmol/g. Seasonality significantly influenced all metabolites except arginine, and transport markedly reduced ATP and AEC, particularly in spring and autumn. Storage at 0 °C resulted in rapid ATP depletion (<1.0 μmol/g within 12 h) and AMP accumulation (>3.0 μmol/g), indicating accelerated energy collapse. In contrast, scallops stored at 5 and 10 °C maintained ATP levels above 2.5 μmol/g for up to 24 h, delaying *rigor mortis*, reducing postmortem contraction, and preserving muscle texture and appearance. Overall, these findings demonstrate that moderate refrigeration represents a physiologically suitable and technologically advantageous strategy to optimize scallop postharvest handling, extend shelf life, and enhance product quality for the fresh seafood market.

Keywords: *Nodipecten subnodosus*; energy metabolism; seasonality; storage temperature; transportation; *rigor mortis*

1. Introduction

The lion's paw scallop, *Nodipecten subnodosus* (Sowerby I, 1835), is one of the largest pectinid species, reaching up to 218 mm in shell height (SH) and inhabiting tropical and subtropical waters along the Pacific and Gulf of California coasts of Baja California, Mexico, down to Peru [1–3]. In aquaculture conditions, it attains a mean SH of 140 mm in approximately three years. This high-value species represents a key target for aquaculture development in the Baja California Peninsula [1,4]. Its commercial appeal stems from its large adductor muscle, rapid growth (reaching commercial size of 7 cm within 8 months), and premium market price (approximately USD 25/kg), as well as its favorable sensory attributes [5–7]. These characteristics cause stimulated increasing efforts to optimize its cultivation, processing, and postharvest management, particularly as production shifts from fishery-based exploitation to aquaculture-based supply chains.

Postmortem muscle quality in aquatic species is strongly influenced by physiological status at harvest, environmental stressors, and storage conditions [8]. The death of the organism triggers a cascade of postmortem biochemical changes, which include the cessation of circulation and oxygen supply, a shift to anaerobic metabolism, rapid ATP depletion, accumulation of lactic acid, pH decline, and the initiation of *rigor mortis*. These changes ultimately affect the physicochemical integrity of the muscle, determining its texture, water-holding capacity, and shelf-life [9–11]. Among these processes, *rigor mortis* plays a central role. It is defined as the progressive stiffening of the muscle caused by the irreversible formation of actomyosin complexes following ATP depletion, resulting in the loss of elasticity and contractile function [12–14]. The onset, intensity, and duration of *rigor mortis* are governed by species-specific metabolism, physiological condition at slaughter, and postharvest handling, particularly temperature [15,16].

In pectinids, energy metabolism after death relies primarily on two biochemical reserves: arginine phosphate, a high-energy phosphagen unique to invertebrates, and glycogen. Arginine phosphate supports rapid ATP regeneration via arginine kinase during the early postmortem phase, while glycogen fuels longer-term ATP synthesis through anaerobic glycolysis [16–20]. The mobilization of these substrates governs the postmortem energy balance and, consequently, the progression of *rigor mortis*. Seasonal fluctuations in environmental temperature and reproductive stage have been shown to influence preharvest energetic reserves and stress tolerance in mollusks [21,22], potentially affecting postmortem biochemical responses.

Previous research on postmortem energy metabolism has focused on other molluscan species such as *Zygochlamys patagonica* [23], *Mizuhopecten yessoensis* [24], *Haliotis midae* [25], *Haliotis discus* [26], *Bathymodiolus platifrons* [27], *Patinopecten yessoensis*, and *Pecten albicans* [20], demonstrating species-specific patterns in ATP degradation, arginine phosphate utilization, and rigor onset under different storage conditions. However, little is known about how these processes are modulated in *Nodipecten subnodosus*, particularly under the combined effects of seasonal variation and refrigerated storage.

Understanding the postmortem energetic profile of *Nodipecten subnodosus* is essential for optimizing storage protocols, minimizing quality loss, and improving its marketability in the fresh seafood sector. Therefore, the aim of this study was to evaluate the effects of seasonality and storage temperature on the concentrations of key energy-related metabolites and the development of *rigor mortis* in the adductor muscle of *N. subnodosus*.

2. Materials and Methods

2.1. Experimental Organisms

Adult specimens of *Nodipecten subnodosus* (approximately 10 cm in shell height) were obtained from a suspended lantern net culture system located in Bahía Tortugas, Baja California Sur (BCS), Mexico (27°41′30″ N, 114°53′45″ W). Specimen collection was carried out with the assistance of trained aquaculture personnel, following the harvesting technique normally used at the site.

Sampling was conducted in four separate seasons: spring, summer, autumn, and winter. For each sampling, 120 individuals were collected. Six specimens were immediately frozen in liquid nitrogen to represent the initial condition (harvested organisms), while the remaining scallops were transported to a processing facility equipped for adductor muscle extraction (Marimex del Pacífico, S.A. de C.V., La Paz, BCS). The transport was performed by specialized personnel and lasted approximately 2 h. During this time, the scallops were kept in plastic containers covered with moistened cotton cloth to minimize desiccation. Ambient temperature during transport varied by season, with average values of 15 °C in spring, 19 °C in summer, 22 °C in autumn, and 18 °C in winter. This additional information provides a clearer context for interpreting the post-transport (0 h) energetic condition of the organisms.

2.2. Storage Experiment

Following shucking, the adductor muscles were immediately packed in polyethylene bags, with six muscles per bag. For each season, samples were randomly assigned to three storage temperatures: 0, 5, and 10 °C. The storage experiment was conducted over a 48 h period, during which samples were collected every 8 h for biochemical analysis, since preliminary data and previous studies [28–30] have shown that the most relevant postmortem biochemical changes in scallops, including those related to *rigor mortis* energetics, occur within this time frame. An exception was made for the determination of adenylate energy charge (AEC) which were evaluated only in initial (harvested) and post-transport (0 h) in each season.

At each sampling point, adductor muscle samples were flash-frozen in liquid nitrogen and stored at −80 °C until analysis of ATP, ADP, AMP, AEC, glycogen, arginine phosphate, and arginine concentrations. The 0 h time point corresponded to samples taken immediately after transport and muscle extraction. For storage at 0 °C, samples were placed directly on crushed ice. For 5 and 10 °C conditions, calibrated General Electric refrigerators (Model GMR02BANMWH, Singapore) were used to maintain the desired temperature.

2.3. ATP, ADP, AMP

Extraction, identification, and quantification of adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP), and adenosine 5′-monophosphate (AMP) were performed using the reverse-phase high-performance liquid chromatography (HPLC) method described by Ryder [31], with modifications. Muscle samples were homogenized in 0.6 M perchloric acid, and the resulting extracts were filtered and diluted prior to injection.

A volume of 20 µL of the diluted extract was injected into a Varian ProStar 240 chromatograph (Varian Inc., Lake Forest, CA, USA) equipped with a C18 reversed-phase column (4.6 × 150 mm; Varian Inc., CA, USA). The mobile phase consisted of a phosphate buffer (0.04 M KH_2PO_4 and 0.06 M K_2HPO_4), with a flow rate of 1.0 mL/min. Detection was carried out at 254 nm using a UV-Vis detector (Varian ProStar 325, Varian Inc., CA, USA). Retention times were compared against commercially available standards to identify each nucleotide.

2.4. AEC

The adenylate energy charge (AEC) was calculated to evaluate the energetic status of the adductor muscle during postmortem storage. AEC was determined using the concentrations of ATP, ADP, and AMP obtained by HPLC, following the formula proposed by Guida et al. [32]:

$$AEC = [(ATP) + ½ (ADP)]/[(ATP) + (ADP) + (AMP)]$$

where ATP = adenosine 5′-triphosphate, ADP = adenosine 5′-diphosphate, and AMP = adenosine 5′-monophosphate. AEC values were calculated for both freshly harvested (initial) and post-transport samples at defined storage times (0, 8, 24, and 48 h) across all seasonal replicates.

2.5. Glycogen

Glycogen content in the adductor muscle was quantified following the method of Racotta et al. [33], with minor modifications. Briefly, 0.3 g of muscle tissue was homogenized in 1 mL of cold 10% trichloroacetic acid (TCA) using an Ultra-Turrax T18 Basic homogenizer (IKA Works Inc., Wilmington, NC, USA) for 1 min. The homogenate was centrifuged at 3000× *g* for 15 min at −5 °C (IEC-MULTI RF, Thermo Fisher Scientific, Asheville, NC, USA). A 0.1 mL aliquot of the supernatant was mixed with 1 mL of 95% ethanol and centrifuged again under the same conditions. The resulting pellet was dried in a vacuum concentrator (Vacufuge Plus, Eppendorf, Hamburg, Germany) at 45 °C for 15 min, and then resuspended in 0.1 mL of distilled water.

For colorimetric quantification, 1 mL of anthrone reagent (0.1% in 76% sulfuric acid) was added, and the mixture was incubated at 90 °C for 5 min. The reaction was stopped by cooling the tubes in ice. Absorbance was measured at 620 nm using a UV-Visible spectrophotometer (Cary 100 Bio, Varian Inc., Mulgrave, VIC, Australia). Glycogen concentration was expressed as glycosyl units, using a glucose standard curve.

2.6. Arginine Phosphate

Arginine phosphate content was determined using the same perchloric acid extracts prepared for nucleotide analysis. Quantification was performed by high-performance liquid chromatography (HPLC) according to the method of Viant et al. [34]. Analyses were carried out using a Varian ProStar 240 chromatograph (Varian Inc., Lake Forest, CA, USA) equipped with a SUPELCOSIL® LC-NH_2 column (4.6 × 250 mm; Supelco, Bellefonte, PA, USA). The mobile phase consisted of a 0.02 M KH_2PO_4 buffer (pH 2.6) and acetonitrile (72:28, *v*/*v*), delivered at a flow rate of 1.2 mL/min. Detection was performed

at 205 nm using a UV-Vis detector (Varian ProStar 325, Varian Inc., Lake Forest, CA, USA). Identification and quantification of arginine phosphate were based on comparison with analytical-grade standards.

2.7. Arginine

Arginine concentration was quantified using the same perchloric acid extracts employed for nucleotide analysis, following the method of Sato et al. [35]. A 20 µL aliquot of each extract was injected into a Varian HPLC system (Varian Inc., Lake Forest, CA, USA) equipped with a reversed-phase C18-A column (4.6 × 100 mm; Varian Inc.) maintained at 40 °C using a Metatherm® column heater. A gradient elution was performed using two mobile phases: solution A (20% acetonitrile in 0.25 M Tris-HCl buffer, pH 9.5) and solution B (80:20 acetonitrile:water, *v*/*v*). The linear gradient progressed from 0% to 50% solution B over 20 min, followed by a final washing step with 100% solution B. Detection was carried out by fluorescence, using a Varian ProStar 363 detector with excitation and emission wavelengths of 325 and 425 nm, respectively. Identification and quantification were based on the retention time and peak area of analytical-grade arginine standards.

2.8. Statistical Analysis

The experiment was structured under a completely randomized design with a factorial arrangement to evaluate the effects of season (spring, summer, autumn, and winter), storage temperature (0, 5, and 10 °C), and postmortem storage time (0, 8, 16, 24, 32, 40, and 48 h) on the concentrations of energy-related metabolites and the progression of *rigor mortis* in *Nodipecten subnodosus*. For each treatment combination, six biological replicates were analyzed (n = 6). The data were subjected to analysis of variance (ANOVA) to assess the main effects and interactions among factors. Differences were considered statistically significant at $p < 0.05$. When significant differences were detected, Tukey's multiple range test was applied to perform pairwise comparisons among means. All statistical analyses were carried out using the NCSS 2000 software package [36].

3. Results and Discussions

3.1. ATP, ADP and AMP

Adenosine triphosphate (ATP) is the primary energy carrier in muscle tissue and a key determinant of postmortem biochemical stability. After death, the progressive hydrolysis of ATP initiates *rigor mortis* and affects various quality attributes, including texture, water-holding capacity, and shelf-life [10]. In mollusks, ATP degradation also serves as a biochemical marker of physiological status and stress response [37,38]. Recent studies on scallops such as *Pecten maximus* and *Argopecten irradians* have demonstrated that postmortem ATP stability is influenced by storage temperature, reproductive stage, and seasonal variation [39,40].

In this study, ATP concentrations in the adductor muscle varied significantly ($p < 0.05$) with both storage temperature and season (Figure 1). Initial ATP levels (from lagoon-harvested organisms) were highest in summer and lowest in spring. A significant decline in ATP was observed after transport to the shucking site (day 0) in spring, summer, and autumn ($p < 0.05$), while winter samples showed no significant changes ($p > 0.05$). During storage, ATP levels decreased in all treatments but remained significantly higher at 5 and 10 °C compared to 0 °C ($p < 0.05$). A transient increase in ATP was detected at 8 h in spring and autumn samples stored at 5 and 10 °C, whereas at 0 °C the decline was continuous and more pronounced.