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Original Contribution

HISTOLOGICAL CHANGES IN VEINED RAPA WHELK MUSCLES (*RAPANA VENOSA*) AFTER FROZEN STORAGE

M. Strateva[1], D. Stratev[2*], G. Zhelyazkov[3]

[1]Department of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria
[2]Department of Food Quality and Safety and Veterinary Legislation, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria
[3]Department of Animal Husbandry – Non-ruminants and other animals, Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria

ABSTRACT

PURPOSE: The objective of this study was to establish the effect of freezing on the histological structure of veined rapa whelk (*Rapana venosa*).

METHODS: Two hundred veined rapa whelks were collected from the Bulgarian Black Sea coast. Forty veined rapa whelks were subjected to histological analysis in the fresh state, while the remaining 160 veined rapa whelks were divided into 4 groups and frozen in a conventional freezer at -18ºC for 3, 6, 9 and 12 months, respectively.

RESULTS: The histological assessment revealed irreversible damages in the entire structure. The observed changes were the smallest after 3 months and the greatest after 12 months of frozen storage.

CONCLUSIONS: The histological finding can be used to distinguish fresh from frozen and thawed veined rapa whelks when making an official control.

Key words: *Rapana venosa*, freezing, histological changes

INTRODUCTION

Demand and supply of marine aquaculture has peaked in recent years. This wide-ranging group also includes the predatory sea snail *Rapana venosa*, which was first discovered in East Asia. It subsequently became ubiquitous due to its invasive nature. Nowadays, the meat of veined rapa whelk is considered a delicacy (1, 2), which manages to combine in its composition a set of diverse mineral substances, easily utilized by the human body (3). Sea snails of the *Rapana venosa* species belong to Phylum Mollusca, Class Gastropoda, Family Muricidae (3, 4). The nutritional value of veined rapa whelk meat is mainly determined by its physicochemical parameters. It is a preferred seafood because of its low lipid content, which is about 1%. This puts it in the category of dietetic products. As for its biological value, it is high and is determined by the content of essential amino acids (1, 3, 4). In addition to the positive economic aspects in the demand and supply of veined rapa whelk meat, the morphological features of this species are also of interest. These are easily recognizable sea creatures, possessing a large and relatively heavy shell. Its main role is to provide the necessary protection of the veined rapa whelk from external factors. The main morphological part of these sea snails is a structure denoted by the common name head-foot. Within this morphological part, other organs categorized as locomotory, sensory and nutritional are also included. The free surface of the shell in the veined rapa whelk is covered by the operculum. Its function is to close the opening of the shell after retraction of the gastropod (4–6).

Veined rapa whelk (*R. venosa*) managed to establish itself on the market as a sought-after

***Correspondence to:** *Deyan Stratev, Department of Food Quality and Safety and Veterinary Legislation, Faculty of Veterinary Medicine, Trakia University, 6000, Stara Zagora, Bulgaria, deyan.stratev@trakia-uni.bg*

food with extremely useful qualities. Unfortunately, it has a short shelf life as fresh veined rapa whelk, which requires the application of a precise method to extend the shelf life in order to improve the market economy. Freezing is a practical and relatively inexpensive method of achieving this goal. Since there is a lack of data on the comparative analysis of morphological changes occurring in the structure of veined rapa whelk (*R. venosa*) after freezing for different periods, our goal was namely related to tracking them. We therefore set out to investigate how frozen storage for 3, 6, 9 and 12 months affects the histological structure of veined rapa whelk (*R. venosa*).

MATERIAL AND METHODS

Experimental setting

Two hundred veined rapa whelks (*R. venosa*) were collected from the Bulgarian Black sea coast by the fishing trawlers of a company possessing a permit for that activity. After catching the veined rapa whelks were immediately transported to the laboratory of the Department of Veterinary Anatomy, Histology and Embryology for analysis. Forty of the fresh veined rapa whelks were subjected immediately to processing for histological analysis. From each fresh veined rapa, whelk material for histological examination was taken from the foot. The remaining 160 veined rapa whelks were divided into 4 groups and frozen in a conventional freezer at -18ºC for 3, 6, 9 and 12 months, respectively. After each of these periods, the corresponding group of veined rapa whelks was thawed at 4ºC for 24 hours. From all frozen and thawed veined rapa whelks in the experimental groups, the material was taken from the foot and subjected to histological analysis.

Histological analysis

The material from the foot for the histological examination was taken after careful removal of the shells. The obtained samples were fixed immediately in 10% buffered formalin for 48 hours. The samples were then rinsed in water and processed according to a standard paraffin embedding procedure. Sections of 6 μm thickness were obtained by rotary microtome (YD-335A, China) and stained by the hematoxylin/eosin method under standard protocol. By means of an N-200 M microscope (Hangzhou Sumer Instrument Co.,Ltd, China) the histological assessment was made. Digital camera OptikamB5 (OPTIKA MICROSCOPES, Italy) and software PROVIEW (Optika Srl, Ponteranica, Italy) were used to make the photodocumenting of the observed sites.

RESULTS

Histological assessment of fresh veined rapa whelk (*R. venosa*) foot

The histological analysis of the muscle foot in fresh veined rapa whelk showed the presence of epithelial tissue, connective tissue, muscle tissue, adipose tissue **(Figure 1. A; B; C; D).** Epithelial tissue was located at the terminal border of the foot. It was represented by a single-layer prismatic epithelium with the presence of cilia. Numerous mucocidal cells (secretory goblet cells) were also observed among the main epithelial cells. The subepithelial zone was characterized by the presence of connective tissue in which various projections of muscle fibres were intertwined. Adipose tissue covered the middle part of the foot. It was essentially mono-vacuolar white adipose tissue. In addition, in the middle part, larger sizes of muscle fibres were observed, which were again located in different projections (oblique, transverse, longitudinal). Placed amidst the connective tissue, again in the middle part of the foot, the specific granulated basophilic cells known as *Leydig cells* were distinguished.

Histological assessment of frozen veined rapa whelk (*R. venosa*) foot

Freezing for 3 months revealed damage to the muscle structure, damage of the typical orientation of muscle fibres and combining them in a common eosinophilic mass. Epithelial tissue was affected and had broken integrity, which resulted in the loss of connection between it and the underlying connective tissue **(Figure 2. A; B).** More pronounced damage of muscles and presence of more optical void spaces between the muscles were found after 6 months of freezing **(Figure 2. C; D).** The histological assessment revealed a significant level of structural damage after freezing for 9 months **(Figure 2. E; F).** Freezing for 12 months revealed the most pronounced changes in the foot structure. Tissue looked deformed and irreversibly damaged to the greatest extent compared to the above-mentioned previous periods **(Figure 2. G; H).**

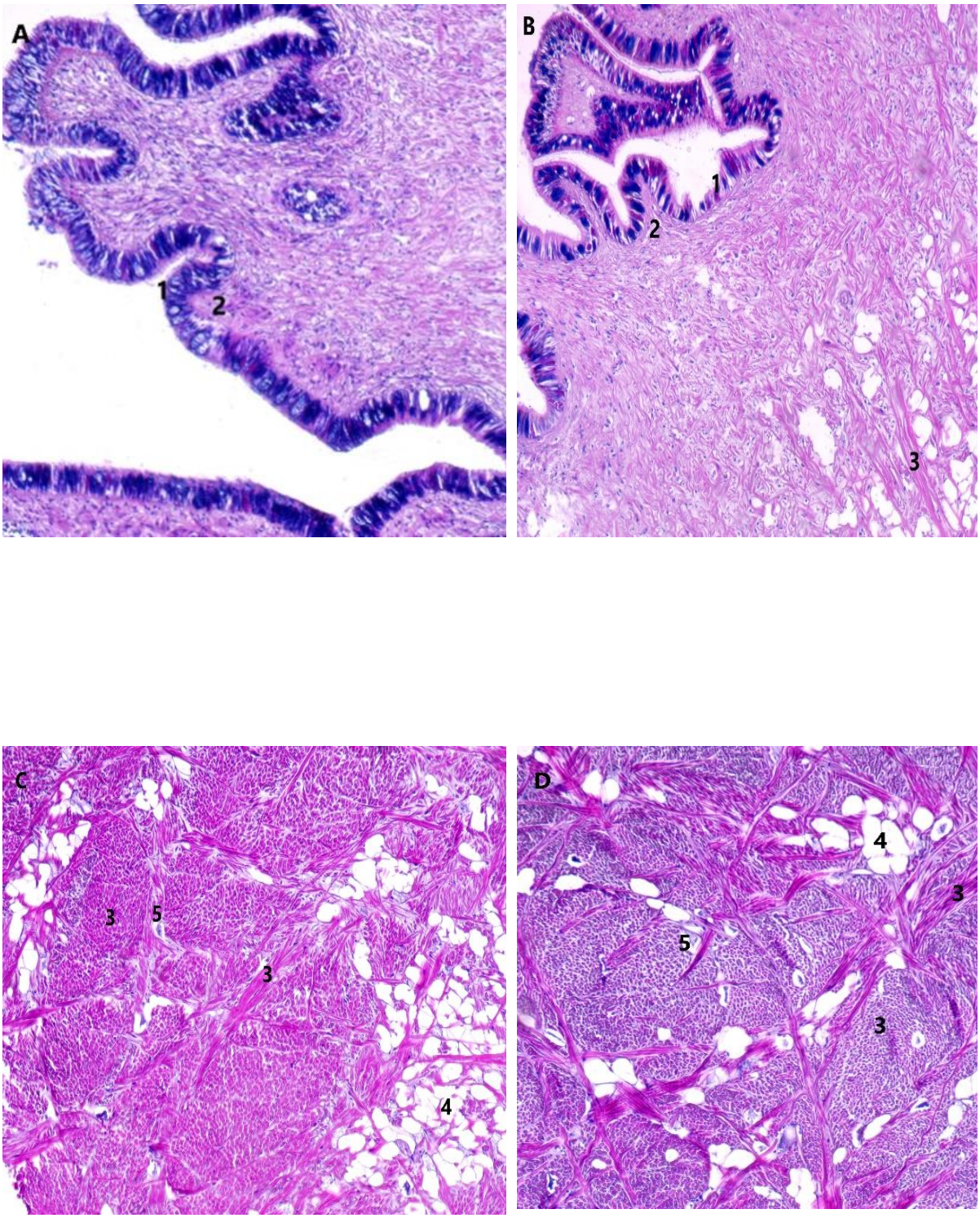

Figure 1: Muscles of foot in fresh veined rapa whelk (*R. venosa*) (H&E) (10x; scale bar = 100 μm)
A; B; C; D: cross section of foot of fresh veined rapa whelk (*R. venosa*)
Key: 1: epithelial tissue; 2: connective tissue; 3: muscle fibres – normal histological structure; 4: adipose tissue; 5: Leydig cell

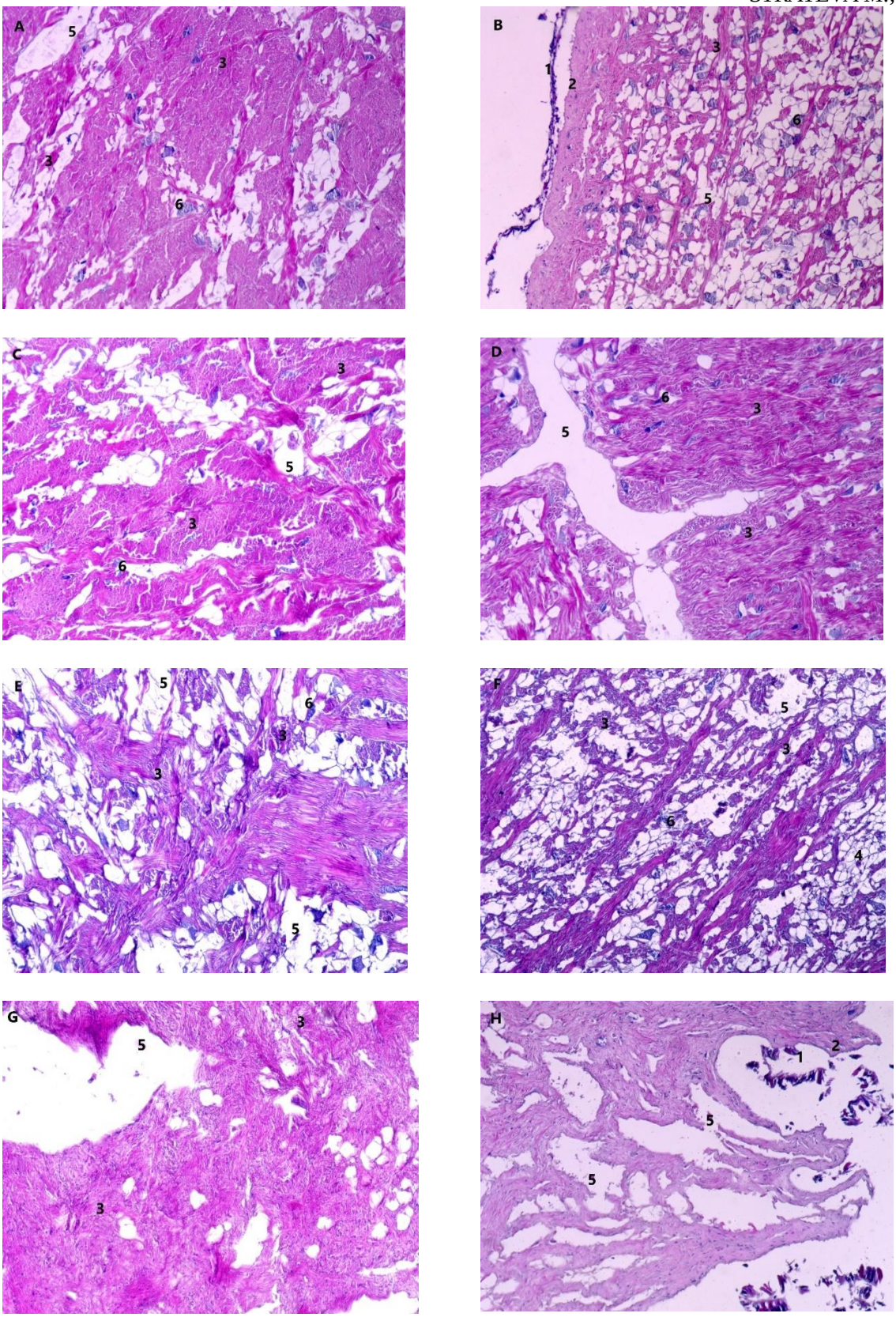

Figure 2: Frozen veined rapa whelk (*R. venosa*) foot (H&E) (10x; scale bar = 100 µm)
A; B: cross section of veined rapa whelk (*R. venosa*) foot frozen for 3 months at -18ºC
C; D: cross section of veined rapa whelk (*R. venosa*) foot frozen for 6 months at -18ºC
E; F: cross section of veined rapa whelk (*R. venosa*) foot frozen for 9 months at -18ºC
G; H: cross section of veined rapa whelk (*R. venosa*) foot frozen for 12 months at -18ºC
Key: 1: epithelial tissue; 2: connective tissue; 3: muscle fibres – damaged histological structure; 4: adipose tissue; 5: optical void spaces among structural components; 6: Leydig cell

DISCUSSION

Meat products are extremely vulnerable to spoilage. For the food industry, it is of great importance that these products retain both their high quality and remain safe for consumption after long-term storage (7). These aspects are important because the occurrence of death in animals results in the formation of a number of undesirable changes, ultimately leading to meat spoilage (8). Negative manifestations can be limited and even stopped if low temperatures are used. The safest and most popular method by which these low temperatures are achieved is freezing (9). One of the greatest benefits of its application is its ability to maintain the high quality of the original raw material while extending its shelf life (10). The extended shelf life of meat products is achieved thanks to the inhibition of a number of enzymatic and microbial processes. Regardless, freezing causes ice crystals to form. These, in turn, have a detrimental effect on muscle fibres and lead to their severe and irreversible damage (11). Damage to the fibres also occurs due to a change in the distribution of water in the muscle structure. Loss of water is found after thawing, with which valuable substances dissolved in it are also lost. In addition, water losses result in weight loss. These changes are definitely undesirable but they are also inevitable. All this leads to a decrease in the biological and economic value of the product. Moreover, during freezing, myofibrillar proteins are also affected, which is the main reason for the manifestation of protein denaturation and lipid oxidation, through which a rancid taste occurs and again a decrease in the quality of meat products (7, 12). The changes that occur during freezing largely depend on the rate at which this process takes place. This means that the changes and their manifestation are directly related to the freezing method. Tissue damage occurs due to the formation of ice crystals. They are the reason for the shrinkage of structural components and the occurrence of osmosis resulting in dehydration of the tissue (9). Despite the negative aspects stated so far, the best way to achieve an increased shelf life of meat products according to Zhang et al. (7) is freezing. Traditional methods of freezing have the strongest harmful effect on tissue structures, as it is clear that they result in loss of water, and hence of the substances dissolved in it. New freezing methods provide new opportunities to reduce losses but at this stage, more research is needed in this direction, as many of them are only applicable in laboratory environments (13). Freezing at a slow rate results in the formation of large ice crystals. They are unevenly distributed and are the cause of mechanical and gross tissue damage due to the compressive deformation they exert on them. The tissue is extensively damaged and the lesions are severe. The resulting voids are the result of disruption of the integrity of the muscle fibres and their surrounding connective tissue (14). On the other hand, rapid freezing results in the formation of intracellular ice crystals of small size (15).

Muscle fibres constitute predominantly the foot of the sea snail *Pomacea canaliculata* (16). Peña et al. (17) confirmed the statement that the foot of *P. canaliculata* is covered with epithelial tissue represented as prismatic ciliated epithelium with the presence of secretory cells. The surface of the muscular foot is wavy. In the subepithelial space of the foot, there are muscles in the form of muscle fibres, adipose tissue and fibrous connective tissue. Adipose tissue is located amidst the muscle fibres. Our results are in agreement with Arrighetti et al. (16) and Peña et al. (17), confirming the presence of the found structural components. In another histological study of a gastropod foot by Chaparro et al. (18), the presence of muscle fibre bundles was revealed. According to this study, the type of these muscle fibres is smooth muscle with eccentrically positioned nuclei. Their orientation is different, since transverse, oblique and longitudinal muscle fibres appear simultaneously. This histological finding was confirmed in the present study of *R. venosa*, too. Thus, our results coincided with those stated by Chaparro et al. (18) regarding the position and shape of muscle fibres. According to Haszprunar (19) and Tiley et al. (20), the body of the sea snail *Lobatus gigas* is also made of multidirectional smooth muscle fibres forming a dense connective tissue network in which specific metabolizing cells called *Leydig cells* are found. According to Tiley et al. (20), the main muscles are made up by the so-called columnar muscle, which is part of the foot. The outer surface of the foot has well-defined indentations, which were also recognized in the present results. Concerning the epithelial covering of the foot, Tiley et al. (20) think that it is able to secrete mucus through the mucous cells in its composition, thereby helping the movement of the gastropods. The statement by Chaparro et al. (18) regarding the epithelial

cover in the lower (ventral) part of the foot also complies with the results obtained by us. In our opinion, secretory prismatic ciliated epithelium forms the border zone of the foot and with this we are in agreement with the facts described by Chaparro et al. (18). According to Avila-Poveda et al. (21), the main function of the foot is to do the movement. It is formed by several basic interconnected components such as collagen and muscle fibres surrounded by connective tissue. The epithelium of the foot is ciliated and there is a difference in the height of the cells of the epithelial layer. According to them, the taller cells are located on the lateral surfaces of the foot, while the smaller ones are positioned on the ventral surface. The cilia on the epithelial surface are precisely the structures that help the distribution and movement of the secretion and thus take an active part in the processes of nutrition, movement and protection in the presence of adverse factors from the surrounding environment. Loeher and Moore (22) also claim that the foot secretes mucus composed of goblet cells. In addition, there are epithelium cells with and without cilia, as well as cells secreting mucopolysaccharides. Almeida et al. (23) and McDermott et al. (24) state that due to the mucus secreted by the foot movement or attachment of the gastropod to a substrate are made possible. Moreover, Chaparro et al. (18) reported that epithelium tissue is separated by a basal membrane from the underlying net straight muscle fibres and connective tissue. D'Ambrosio et al. (25) agree with the above authors and in their study they confirm the discussed morphological characteristics in the structure of the foot. In their results they revealed muscle fibres located in various directions and epithelium tissue on the surface secreting mucus. The main components of the gastropod foot according to them are also muscle and connective tissue. They also confirmed that the foot had uneven surface. Gato (26) agree with the points made so far stating that the foot is entirely made up of muscle fibres positioned in all directions. These are eosinophilic coloured structures by the H&E method. According to Leonard et al. (27), the consequences from freezing reveal the occurrence of changes in the muscle fibres. The effects of freezing are obvious when interpreting the microscope image of the muscles. It shows change in the position of the muscle fibres being unevenly distributed. The occurrence of optical void spaces among muscle fibres is due to the broken integrity of the connective tissue between them. Muscles look like disintegrating and lose their volume. After freezing void spaces can be seen both in the structure of fibres themselves and in the extracellular zones. After freezing we observed damage of muscle fibres and presence of increasing void spaces among them. The disintegration of muscle fibres is significant. Adipose tissue is also affected and has broken integrity, with muscle tissue being disorganized and deformed.

CONCLUSION

The histological assessment of veined rapa whelk (*R. venosa*) foot after freezing showed irreversible damage in the entire structure. The observed changes tended to increase in presence, being the smallest after 3 months and the greatest after 12 months of frozen storage. The results from the present study could help in the need to distinguish fresh from frozen and thawed veined rapa whelks in the official control by the relevant institutions, since the results are definitive and cannot be manipulated.

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Abbreviations:

H&E - Hematoxylin and eosin

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