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MORPHOLOGICAL INDICATORS OF VISCERAL ORGANS OF THE SNAILS *Helix pomatia* AND *Helix aspersa* Muller UNDER THE ABIOTIC AND BIOTIC FACTORS` INFLUENCE

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Abstract

The microstructure of the stomach, intestines, hepatopancreas, kidneys, lungs, reproductive organs, and protein gland of two species of snails (Helix pomatia and Helix aspersa Muller) under the influence of abiotic (climatic) and biotic (parasite) factors was studied. In snails of the first group, the visceral organs had a typical structure. The parenchyma of the hepatopancreas, as the most vulnerable organ, is represented by glandular tubules and the system of excretory ducts that open into the intestinal cavity. Structural changes in the hepatopancreas and gonads were observed in snails of the second group, which were in the state of anabiosis under the influence of dry, hot weather. The helminthic invasion in the snails of the third experimental group caused necrosis of the hepatopancreas and replacement of the parenchyma of the gland with loose connective tissue.

Key words*: hepatopancreas, microstructure, snails.*

INTRODUCTION

Snails of the genus *Helix* belong to the family *Helicidae* of the order *Pulmonata*, class *Gastropoda* (El-Khayat et al., 2015; Bouchet et al., 2005). Snails of the species *Helix pomatia* and *Helix aspersa* are common components of the biocenosis in all climatic and geographical zones of the world (Kotsakiozi et al., 2012; Ligaszewski, 2009). The deepening of research on this class of invertebrates is due not only to their unique biological properties, but also to the growing demand for them as food. In recent years, snail farming as a business sector has been developing intensively. Technologies for snail breeding and processing are being developed and improved (Tluste & Birkhofer, 2023; Rygało-Galewska et al., 2022; Zubar & Onyshchuk, 2020; Carbone & Faggio, 2019; Cilia & Fratini, 2018; Ligaszewski et al., 2016, Zymantiene et al., 2008; S.-Rózsa, 2002).

In Ukraine, snail business entities such as Snailgroup company, the "Agroravlyk" Farm, the Ukrainian Association of Snail Producers, and others are increasing their production capacity (DSTU 7353:2013. Meat. Method of histological determination of freshness and degree of ripening. Valid from 2014-01-01. Kyiv, Ukrainian). In December 2021, the first all-Ukrainian conference was organised on the basis of the "Agroravlyk" Farm, which aims to promote snail farming, as well as to improve the level and quality of its technological processes (First All-Ukrainian conference of snail breeders, "Agroravlyk" Farm, Kyiv, Ukraine, 2021; Paska et al., 2020; Zubar & Onyshchuk, 2020; Carbone & Faggio, 2019; Zymantiene et al., 2008).

In the European Union, legal regulations are mainly focused on the control of the microbiological quality of cooked snail meat and hygienic conditions during their cultivation (Rygało-Galewska et al., 2022; Ligaszewski et al., 2016; Charrier et al., 2006). Regulation № 2073/2005 of the European Union Commission defines requirements for *Salmonella* in snail meat and for *E. coli* and coagulase-positive *Staphylococci* in the hygiene of their cultivation (Paszkiewicz et al., 2014; Regulation № 2073/2005 of the European Union Commission on microbiological criteria for foodstuffs, Brussels, Belgium, 2005).

When analysing scientific studies on the biological characteristics of snails, attention has been drawn to the very limited speed of their movement (0.047 km/h), which is why all generations of a single snail population exist in a certain territory, i.e. they are almost incapable

of migration (Becker et al., 2021; Habib et al., 2018; Lőw et al., 2016; Nowakowska et al., 2006; S.-Rózsa, 2002). The life expectancy of a snail in artificial conditions can reach 20 years, but in the "wild", due to the influence of unfavourable factors, it does not exceed 8 years (Peña et al., 2017; Kotsakiozi et al., 2012). Since snails are predominantly herbivorous organisms, plants are one of the main links in the cycle of ecosystem pollutants (Tluste & Birkhofer, 2023; Amal & Abdel-Rahman, 2020; Lobo-da-Cunha, 2019; Otitoloju et al., 2009; Hamed et al., 2007; Rabitsch, 1996).

Ecological pollutants, both organic and inorganic, have cumulative properties in relation to biotic (plants, animals) and abiotic (soil, water, air) ecosystems. In this sense, first of all, the question arises of the safety of consuming products of "spontaneous" snail farming, which is often used by collecting gastropods in the "wild" (Tluste & Birkhofer, 2023; Rygało-Galewska et al., 2022; Ali et al., 2019; Rota et al., 2016; Köse et al., 2015; Hamlet et al., 2012; Sherifa et al., 2007; Charrier et al., 2006; Gomot & Pihan, 1997).

It is known that the animal body reacts with appropriate structural and functional changes to the accumulation of exotoxins in it. Therefore, attention has been drawn to the possibility of using snails as environmental bioindicators and biological test systems for organic and inorganic pollutants not only in their habitat but also in feed and livestock products obtained from these areas (Yasmeen S. M. Abd El Mageed et al., 2023; Carbone & Faggio, 2019; Parvate & Thayi, 2017; Rota et al., 2016; Sharaf et al., 2015; El-Khayat et al., 2015; Mohammadein et al., 2013; Almedros & Porcel, 1992; Janssen & Dallinger, 1991).

MATERIALS AND METHODS

The goal of the study is to determine the structural parameters in the organs of the visceral part of the body of the snails *Helix* under the influence of habitat conditions and pathogenic factors (helminthiasis).

To achieve this goal, the following objectives have been set:

1. To collect the snails *Helix pomatia* living in different natural environmental conditions in the temperate continental climate zone of Europe.
2. To study the anatomical features of the body structure of the snails *Helix pomatia* and to select material for histological examination.
3. To development the technique of making histological preparations from the visceral part of the snail body and their microscopy with the identification of organs.
4. To compare the histological structure of the organs of the visceral part of the body of the snails *Helix pomatia* collected under different natural environmental conditions.
5. Coproscopic examination of the snails *Helix aspersa* to detect helminthic invasion and determine the state of the microstructure of the visceral organs of the infested snails.

To conduct the planned research, 3 groups of snails of the same age, 10 individuals each, have been used.

The *first group* (*control group*) includes the snails *Helix pomatia*, which have been collected during their life activity in May in cool and moderately humid weather on the territory of a household plot where chemical plant protection products were not used during the spring and summer season.

The *second group* includes the snails *Helix pomatia* collected from the same household plot, but in hot weather – in August. The snails were found in a state of anabiosis, fixed mainly to the bark of tree trunks.

The *third group* includes the snails *Helix aspersa* Muller from a specialised farm collected in May under weather conditions similar to those of the first group. Snails with low motor activity and reduced feed consumption have been used.

Snails were dissected according to the method described in *Atlas of Animal Anatomy and Histology* (Lőw et al., 2016) (Figure 1).

Figure 1. Preparation for dissection (Own source)

The visceral part of the snail's body was separated from the foot with scissors along the lower surface of the mantle edge, starting from the pneumostome.

Samples for histological examination were fixed during the week in a neutral aqueous formalin solution, with the first day in 5% followed by re-fixation in 10% solution. According to the classical method of making paraffin blocks, the sampled material was passed through a battery of alcohols of increasing concentration and embedded in paraffin. Histological sections of 7-10 μm thick were stained with haematoxylin and eosin (Horalskyi et al., 2019). Histological preparations from the visceral part of the snail's body were examined using *Jenamed-2* light microscope.

Coproscopic examination for parasitosis has been performed using the native smear and Fülleborn methods. A native smear was prepared on a slide, a drop of distilled water was added, in which a lump of snail secretions was thoroughly stirred and covered with a cover slip. According to the Fülleborn method, the snail secretions were stirred in a beaker with a cooled saturated solution of sodium chloride, filtered through a metal strainer and extracted for 40 minutes. Three drops were taken from the surface of the sample with a metal loop onto a degreased slide and covered with a cover slip. Microscopy in both cases was performed at low microscope magnification (MBS, objective – 10^x, eyepiece – $12,5^x$) (Halat et al., 2004).

The experiment was conducted in accordance with generally accepted principles of humane treatment of animals (Law of Ukraine "On the Protection of Animals from Cruelty Treatment", No. 3447-IV as of 21.02.2006, Kyiv; European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, Strasbourg, 1986). The work was done at the Department of Normal and Pathological Morphology of the State Biotechnological University (Kharkiv, Ukraine).

RESULTS AND DISCUSSIONS

When studying the anatomical structure of the snails, it has been revealed that the body of the snail is firmly connected to the calcareous shell and consists of a head, body and foot. The visceral part of the body is located in the distal whorls (Figures 2, 3).

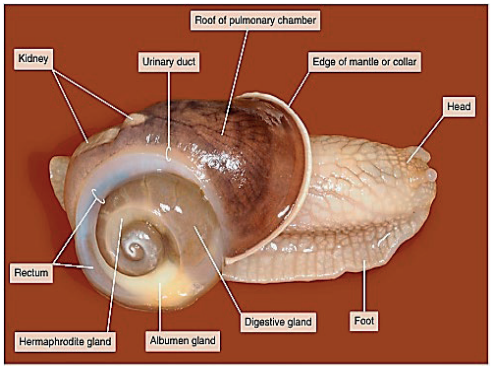

Figure 2. Structure of the snail's body after shell removal (Lőw et al., 2016)

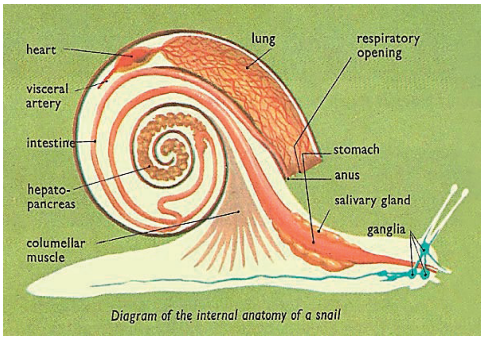

Figure 3. Diagram of the body structure of a snail. Retrieved from https://www.daviddarling.info/encyclopedia/S/snail.html

It has been determined that the snail *Helix pomatia* (Grape snail) belongs to the pulmonary snails and is a hermaphrodite. Under unfavorable conditions (too high or low air temperature, drought), snails retract their bodies into the shell and close its outer opening with an epiphragm formed by congealed mucus with minerals, which makes it strong. During this period, snails are in a state of anabiosis, which can last for 3-5 months (Noothuan et al., 2021; Kotsakiozi et al., 2012; Pirger et al., 2004).

Histological studies have shown that the section covers the visceral part of the snail's body and allows to identify almost the entire complex of internal organs: stomach, intestines, hepatopancreas, and gonad. A special feature of the digestive system of a snail is the presence of an organ called the digestive gland, or hepatopancreas, which combines the functions

of the liver and pancreas and performs an excretory function (Figures 2, 3).

In snails, there is no separation of visceral organs by serous membranes due to the very limited size of the secondary body cavity – coelom (Figure 4). Only the pericardial cavity is formed.

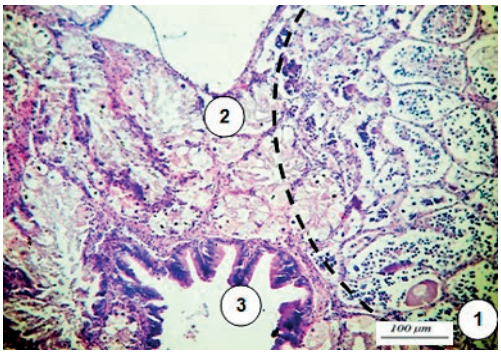

Figure 4. Fragment of the visceral part of the body of the snail *Helix pomatia* of the first group: 1 - hermaphroditic gonad; 2 - hepatopancreas; 3 - intestine. H & E (Own source)

On the side of the mantle cavity, there is a separating barrier formed by a single-layer single-row low prismatic epithelium (Figure 5).

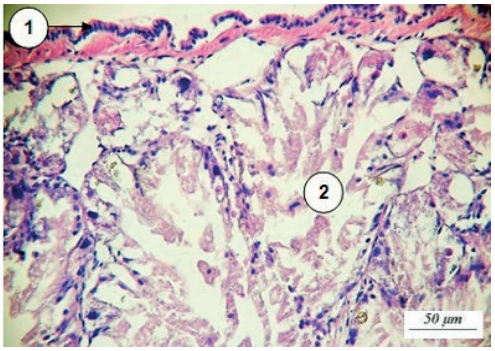

Figure 5. Fragment of the visceral part of the body of the snail *Helix pomatia* of the first group: 1 - single-layer epithelium from the side of the mantle cavity; 2 - hepatopancreas. H & E (Own source)

According to the results of our study, the hepatopancreas of the snails of each group showed peculiarities of a microscopic structure. The hepatopancreas of the snails of the first group has a typical structure. Its parenchyma is represented by glandular tubes and a system of excretory ducts that open into the intestinal cavity (Figure 6).

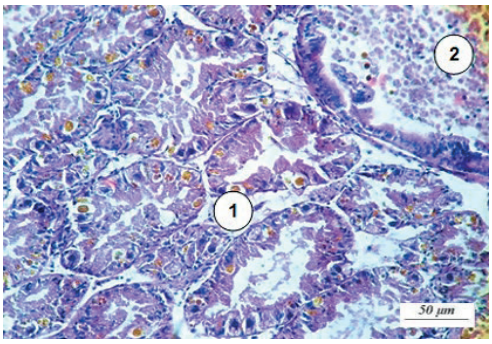

Figure 6. Fragment of the hepatopancreas of the snail *Helix pomatia* of the first group: 1 - hepatopancreas; 2 - stomach cavity. H & E (Own source)

Glandular tubes contain 4 types of cells: digestive cells, excretory cells, calcium cells and thin cells. The cytoplasm of digestive cells contains numerous secretory granules and sometimes green granules of various sizes. Excretory cells are distinguished by the presence of a vacuole with a large yellow granule in the cytoplasm. Calcium cells are predominantly triangular in shape compared to other cell types, smaller in height, with large nuclei and optically dense cytoplasm. Sometimes spherical calcium granules – spherules – are found in their cytoplasm. The granules are also present in the lumen of the excretory ducts and the gastric cavity (Figure 7). Thin cells are undifferentiated cambial cells (Lobo-da-Cunha, 2019; Lőw et al., 2016).

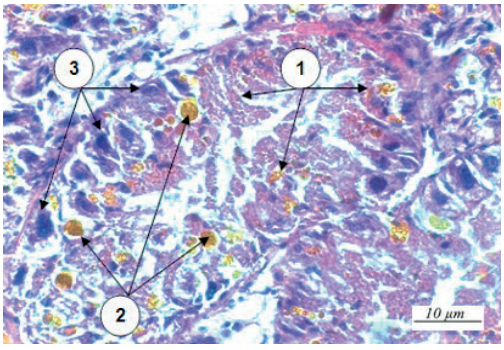

Figure 7. Cellular composition of the hepatopancreas of the snail *Helix pomatia* of the first group: 1 - digestive cells; 2 - excretory cells; 3 - calcium cells. H & E (Own source)

According to the literature, the hepatopancreas is the organ that to the greatest extent deposits

toxic substances and ensures their detoxification, which is why it is highly responsive to environmental factors (Yasmeen S. M. Abd El Mageed et al., 2023; Carbone & Faggio, 2019; Parvate & Thayi, 2017; Rota et al., 2016; Sharaf et al., 2015; El-Khayat et al., 2015; Mohammadein et al., 2013; Almedros & Porcel, 1992; Janssen & Dallinger, 1991).

In snails of the second group, which were in a state of anabiosis, significant structural changes occurred in the digestive, excretory and genital organs. Due to the closure of the shell opening by the epiphragm and the absence of communication between the organism and the external environment, it is impossible for the excretory organs to remove metabolic products. This situation leads to a change in the function of the hepatopancreas. The hepatopancreatic glandular cells transform and differentiate into excretory cells. The glandular tubes are dominated by cells with vacuoles with yellow granules in the cytoplasm. The excretion products accumulate in the lumen of the glandular tubes and the hepatopancreatic ducts, as well as in the stomach and intestinal cavity. The colour of the excretory products suggested that they are in a colloidal state, possibly as a result of water reabsorption (Figures 8, 9).

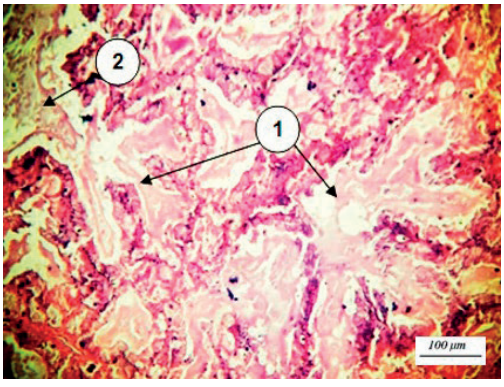

Figure 8. Hepatopancreas of the snail *Helix pomatia* of the second group: 1 - glandular tubes filled with compacted excretory products; 2 - excretory duct with excretory products. H & E (Own source)

Snails, like many invertebrate hermaphroditic organisms, have a sexual organ, the spermatheca, for the accumulation and storage of male reproductive products (Rogers & Reeder, 1987).

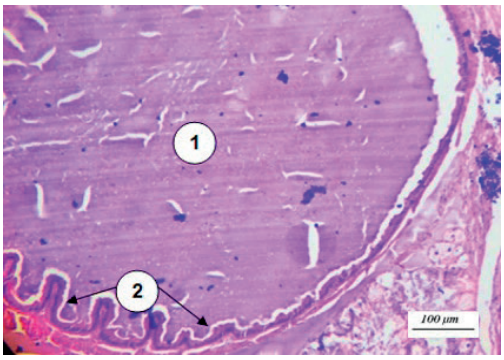

Figure 9. The stomach of a snail Helix *pomatia* of the second group: 1 - excretory products in a compacted state in the stomach cavity; 2 - stomach wall. H & E (Own source)

In snails of the second group, lysis of sperms in the spermatheca is observed, which is evidence of the limited viability of sperm and its autonomous disposal during a period of prolonged absence of sexual activity (Figure 10).

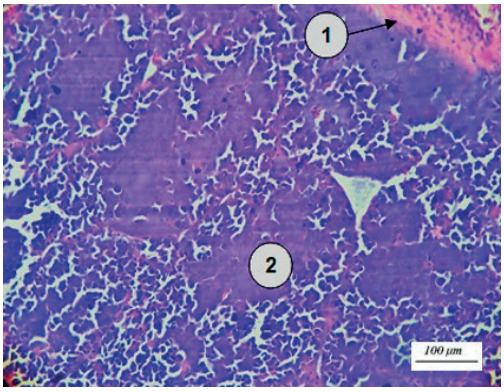

Figure 10. Spermatheca of the snail *Helix pomatia* of the second group: 1 - wall of the spermatheca; 2 - lysis of sperms in the spermatheca. H & E (Own source)

At the same time, the hermaphroditic gonad shows no destruction of ova and spermatogonia, which indicates their resistance to hypoxia and other unfavourable conditions of the internal environment cellular metabolism during hibernation.

In the snails *Helix aspersa* Muller of the third group, according to the results of coproscopic studies, in native smears and by the Fülleborn method, larval and imaginal stages of nematodes of the order *Strongylata,* family *Strongylidae*, have been detected (Figure 11).

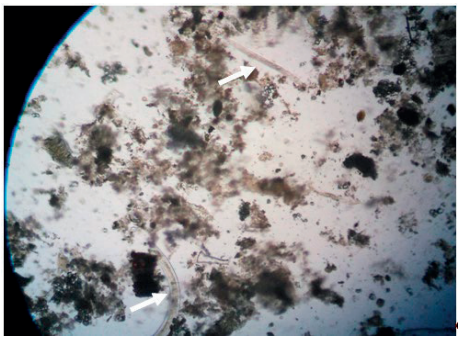

Figure 11. Helminths of the *Strongylidae* family in the native faecal smear of the snails *Helix aspersa* of the third group. Native preparation, magnification x125 (Own source)

Morphological analysis of the histopreparations from the visceral organs of the snails *Helix aspersa* enables to determine the effect of one of the biotic factors on their organism. The digestive gland of the snails retained its typical structure, but in some places, glandular tubes of the hepatopancreas are represented by a structureless mass in which individual swollen epithelial cells with nuclei in the stage of pyknosis, rexis and lysis are visible, indicating a process of necrosis.

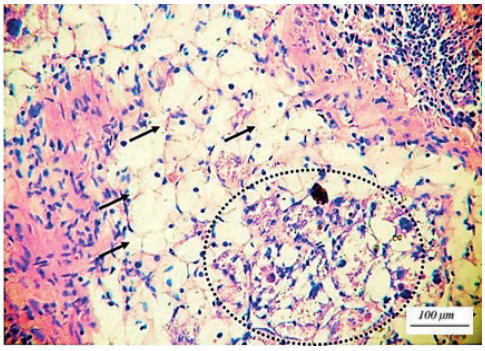

Figure 12. A fragment of the hepatopancreas of the snail *Helix aspersa* of the third group: the area of necrosis is marked with a circle; arrows indicate the replacement of parenchyma by the connective tissue. H & E (Own source)

These morphological manifestations should be considered as changes in hepatopancreatic tissues to the destructive effects of metabolic products of the detected nematodes. Destroyed areas of the hepatopancreatic parenchyma are replaced by the connective tissue (Figure 12).

Connective tissue layers are also increased between microscopically intact glandular tubes. Such structural changes are caused by prolonged exposure to the metabolic products of the detected nematodes and lead to the development of hepatopancreatic cirrhosis.

CONCLUSIONS

Based on the results of our research and analysis of the literature, we have come to the following conclusions:

1. The organism of the snails *Helix* reacts with clear structural changes in visceral organs to the action of adverse environmental factors.
2. In the snails *Helix pomatia*, which were in a state of anabiosis, transformation and differentiation of glandular cells of the parenchyma into excretory cells takes place in the hepatopancreas. Excretory substances in a compacted state accumulate in the digestive organs. Lysis of sperm is observed in the spermatheca.
3. In the snails *Helix aspersa*, which have been exposed to prolonged helminthic invasion, foci of necrosis are found in the hepatopancreas, which are replaced by the connective tissue, causing cirrhosis of the organ.

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