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Chapter- 13

Diaphonization: Enhancing Efforts Toward Achieving SDGs 10, 14, and 15

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Abstract:

Diaphonization, also known as clearing and staining, is an invaluable technique for visualizing and studying the skeletal structures of animals. This study explores the application of diaphonization to examine bone development and soft tissues and determine causes of death in various animal species. By rendering tissues transparent and selectively staining bones and cartilage, diaphonization allows for detailed observation of skeletal morphology without dissection, thus preserving the integrity of specimens. This method enhances the understanding of morphological and anatomical structures and aids in determining the phylogenetic relationships between species. Diaphonization directly contributes to achieving Sustainable Development Goal (SDG) 4 by improving educational tools, indirectly supports SDG 10 by reducing educational inequalities, and SDG 14 and 15 by providing knowledge about the habitat of different species. Additionally, it plays a significant role in research, conservation, forensic science, and embryological studies.

Introduction:

Diaphonization, also known as clearing and staining, is a remarkable scientific technique used to study the internal anatomy of organisms without the need for dissection. It is also employed to investigate causes of death in various species and was first developed in 1977 by

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scientists G. Dingerkus and L.D. Uhler (Chitra et al., 2020), this process involves rendering biological specimens transparent while simultaneously staining bones and cartilage with vibrant dyes, enabling detailed examination of internal structures. Though the technique was first introduced in the early 20th century, it has become an invaluable tool in zoology, developmental biology, and paleontology.

Diaphonization combines elements of chemistry and biology to preserve specimens and highlight their intricate features. Specific chemical solutions are used to make soft tissues transparent, providing an unobstructed view of skeletal and cartilaginous components (Rehman et al., 2015). This method offers insights into the anatomy and development of organisms and also serves as an effective tool for education, museum displays, and comparative analysis with model specimens.

Diaphonization typically involves several key steps: fixation, staining, and clearing. The specimen is treated with a preservative agent during fixation to prevent decomposition and maintain tissue integrity. In the staining step, dyes such as Alcian Blue for cartilage and Alizarin Red for bones are applied, creating a striking contrast against the now-transparent soft tissues (Dawson, 1926). Finally, the clearing step uses solutions like glycerin to render the tissues transparent, revealing the internal architecture in vivid detail.

Diaphonization provides a unique glimpse into the complexity of anatomy, allowing scientists and enthusiasts alike to deepen their understanding of the structural intricacies of life. Through this captivating process, we can explore the hidden beauty of nature and gain valuable insights into phylogenetic relationships and developmental patterns that shape the diversity of life on Earth.

Materials & Method

Diaphonization is a technique that makes biological specimens transparent while simultaneously staining specific tissues, usually for research and educational purposes. This process allows scientists and researchers to study the internal structures of organisms without dissection (Chitra et al., 2020). This process has four major steps, viz., fixation, staining, clearing, and mounting or preservation, The following steps are discussed below-

1. Fixation: The fixation process starts with a 10% formalin deep bath of dead specimens for a minimum of 2 weeks, which preserves their tissues and prevents decay. After that, it can be transferred to the next step, the staining method.

2. Staining: Specific dyes are used to stain different tissues. For example, Alcian Blue and Alizarin Red S are two dyes that are mostly used to stain bones and cartilage. Alizarin Red S binds to calcium, providing a clear contrast against cleared tissues. Alcian Blue is used to stain cartilage, highlighting cartilage structures distinctively against the transparent background (Lipman, 1935).

3. Clearing: First, trypsin digestion. In this process, trypsin digests the cell protein, and the tissue appears transparent. After that, the specimen is treated with a series of solutions, such as glycerin or other clearing agents, that render the transparent tissues much softer and more

transparent. This step allows the stained structures to be visible through the now-translucent body of the specimen (Cumley et al., 1939).

4. Preservation / Mounting: The cleared and stained specimen can be mounted in glycerin and thymol crystal for viewing. Cleared and stained specimens are stored in glycerin, which maintains transparency and provides a medium for long-term storage.

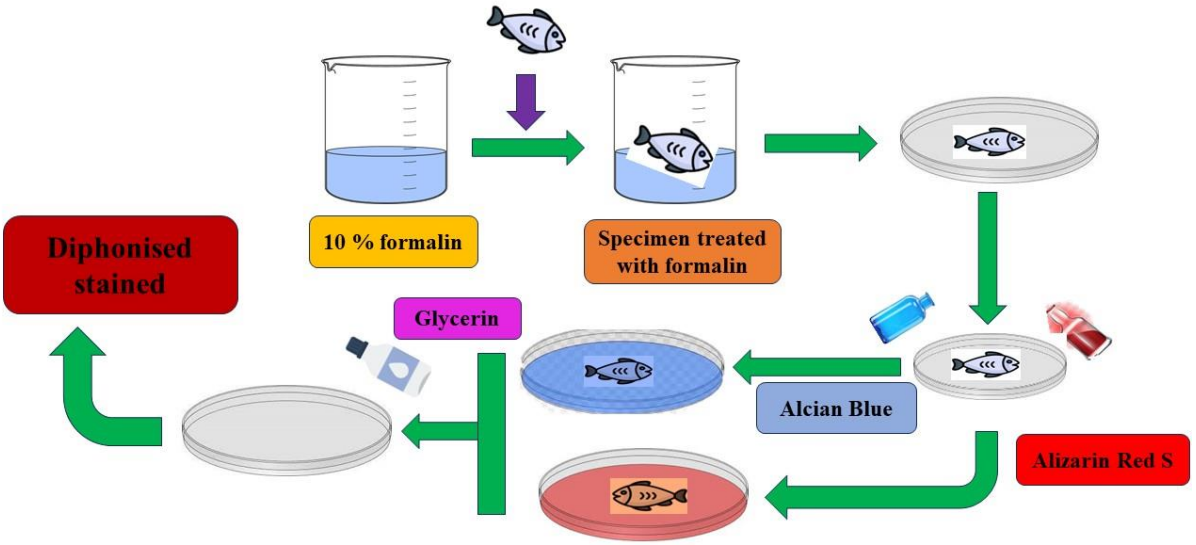

Figure 1. Method of diaphonization on a fish sample.

The Diaphonization Process can involve the following branches of biology:

Physiological System:

I. Vascular Staining: A dye such as Alcian Blue is often used to visualize the circulatory system. This dye stains glycosaminoglycans, which are present in the blood vessels, highlighting them in blue. Alternatively, specific vascular stains that bind to blood vessels can be used (Susaki *et al.*, 2020).

II. Optional Skeletal Staining: If both the circulatory and skeletal systems are to be visualized, Alizarin Red may be used to stain bones, allowing for a dual visualization.

The stained specimen is then treated with clearing agents, such as glycerol or benzyl alcohol/benzyl benzoate (BABB). These agents make the soft tissues transparent without disrupting the stained structures. Depending on the size and density of the specimen, the clearing process can take several days to weeks (Lee,1963).

Developmental Biology:

I. **Embryonic Development:** Studying the circulatory system development in embryos helps researchers understand congenital disabilities and the fundamental processes of vascular formation (Rueda-Esteban et al., 2017).

II. **Growth Patterns:** Observing changes in the circulatory system over developmental stages provides insights into growth-related changes and how they correlate with overall organismal development (Rueda-Esteban et al., 2017).

Evolutionary study:

- **Phylogenetic Relationships:** Diaphonization supports the reconstruction of evolutionary relationships by enabling the comparison of anatomical features across species, making it a valuable tool for phylogenetic studies.
- **Adaptations and Evolution:** By examining anatomical, morphological, and physiological traits, diaphonization aids in understanding the adaptive features of different species. It is also used to determine the ancestors of various species through parsimony analysis, which ultimately helps construct phylogenetic relationships.

Paleontological studies:

Diaphonization is a powerful tool that bridges the gap between modern organisms and their ancient counterparts. By studying the internal structures of modern analogs, paleontological studies can infer the structural anatomy, function, and evolutionary pathways of fossilized species.

Osteological and chronological study:

Diaphonization offers several advantages for studying bone and cartilage:

- **Enhanced Visibility:** The technique allows for the clear visualization of bone and cartilage structures, which can be challenging to distinguish with traditional imaging methods.
- **Non-destructive Analysis:** Unlike dissection, diaphonization preserves the specimen, enabling detailed study without damaging or altering the original anatomy.

Figure 2. Morphology of the bones and cartilage of a tetra fish using the diaphonization technique.

Applications of Diaphonization:

1. Developmental Biology:

- **Skeletal Development:** Diaphonization allows researchers to study the development of the skeletal system from embryonic stages to adulthood, revealing patterns of bone ossification and cartilage formation.
- **Congenital Defects:** The technique identifies and understands congenital skeletal abnormalities and malformations in embryos, providing insights into developmental disorders.

2. Comparative Anatomy:

- **Species Comparison:** Diaphonization helps researchers understand evolutionary adaptations and morphological variations by comparing bone and cartilage structures across different species.
- **Functional Morphology:** The technique provides insights into how skeletal structures are adapted for specific functions, such as locomotion, feeding, or flight.

3. Contributing to Species Recovery Programs:

- **Studying Endangered Species:** Diaphonization is particularly useful for studying endangered and threatened species. It provides critical information about their biology and ecology, which informs conservation strategies.
- **Guiding Breeding Programs:** By understanding anatomical and developmental aspects, conservationists can develop better breeding programs for endangered species, ensuring genetic diversity and health.

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5. Cause of death determination

This technique allows for a detailed examination of skeletal structures without dissection. While diaphonization is not typically used directly for determining the cause of death, it can be a complementary tool in forensic investigations and biological studies to provide insights into certain aspects of an organism's death or condition at the time of death.

Diaphonization can assist in determining the cause of death in two ways are

a. **Fractures or Trauma:** The process can reveal fractures, dislocations, or other skeletal injuries that might indicate trauma, accidents, or violence leading to death.
b. **Bone Density Analysis:** Conditions like osteoporosis or nutritional deficiencies can be observed, providing clues about the individual's health before death.

6. Role of Diaphonization in Achieving Sustainable Development Goals (SDGs)

Diaphonization plays a significant role in advancing several Sustainable Development Goals (SDGs) through its applications in research, education, and conservation:

SDG 4 (Quality of Education):

Diaphonization is a valuable technique used in research and education to render biological tissues transparent while selectively staining specific structures, such as bones and cartilage. This process enables detailed visualization of anatomy without dissection, making it especially useful in developmental biology and comparative anatomy. By providing clear views of skeletal structures and circulatory systems, diaphonization helps researchers and students observe bone and cartilage development and anatomical abnormalities. Diaphonized specimens in educational settings allow students to better understand spatial relationships within intact organisms, offering a practical, visual complement to textbook learning.

SDG 10 (Reduced Inequalities):

Diaphonization lowers costs and contributes to reducing inequalities in education. It provides durable, reusable anatomical specimens, reducing the need for costly live animals and dissection materials. These high-quality models improve learning efficiency, decreasing reliance on supplementary resources. Furthermore, digitized diaphonized specimens can be shared widely in virtual classrooms, making quality education accessible to students in resource-limited areas.

SDG 14 (Life Below Water) & SDG 15 (Life on Land):

Diaphonization aids in understanding the adaptive features of various species, providing valuable insights into their habitats and ecological diversity. By visualizing the internal structures of species from different environments, this technique enhances our knowledge of biodiversity and helps inform conservation strategies. Consequently, diaphonization supports the preservation of species and ecosystems, aligning with the goals of conserving marine (SDG 14) and terrestrial life (SDG 15) (Mukherjee et al., 2022).

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