Proceeding of International Conference on Arts and Humanities: International Conference on Education Innovation, and International Conference on Research and Academic Community Services

The Effectiveness of Various Concentrations of Alcohol as Preservative of Bamboo Shells (Ensis leei) and Blood Clams (Anadara granosa)

Sugimin Sugimin[1,*] , Rizki Yulia Oxi [1], Rizki Yulia[1], Trisiswanti[1], Rizqita[1], Eza Alfian[1], Arista[1], Anggi Maulia[1]

[1] *Departemen of Biology, Universitas Negeri Surabaya*
[*]*Corresponding author. Email: sugimin@unesa.ac.id*

ABSTRACT
Laboratory activities and/or field practicum activities are one of the important activities. Preservation of specimens is an activity that is often carried out. Preservation of specimens is intended to maintain the condition of living things as they are in nature and their morphological structure (only slight changes) and free from bacteria and fungi that can cause decay. Bivalves are one of the best-preserved laboratory specimens. Bivalves that are often encountered in the field include blood clams (Anadara granosa) and bamboo shells (Ensis leei). Preservation of bivalves was carried out by dry preservation method and wet preservation using 70% alcohol. The purpose of this study was to determine the effectiveness of various concentrations of alcohol preservative solution for bivalves specimens. The research stages included dilution of alcohol solutions with various concentrations (90%, 85%, 80%, 75%, and 70%), bivalves sampling, preservation of bivalves samples in the laboratory, care and observation of bivalves specimens. The effectiveness of the alcohol solution was seen from the turbidity of the alcohol and the morphology of the bivalves. The data were analysed by quantitative descriptive method. The results of this study alcohol with a concentration of 70% effectively used as a preservative for blood clams and bamboo shells.

Keywords: *Alcohol concentration, Preservatives, Bamboo clams, Blood shells*

1. INTRODUCTION

Laboratory activities and/or field practicum activities are one of the important activities to be carried out. One of the practicum activities that are often carried out is the preservation of specimens. Preservation of specimens is intended to maintain the state of living things as in their habitate and the morphological structure (n this case only slight changes) and free from bacteria and fungi that can cause decay [1]. The SITH ITB Zoological Museum uses a collection storage method with a preservation method to reduce evaporation [2] In addition, specimen preservation is also used as a learning medium for students, for example in the effectiveness and efficiency of practicum implementation. for example, the effectiveness and efficiency of the implementation of learning and practicum activities, as carried out at the Taxonomy Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya.

In the learning process and the function of specimens as learning media, preserved specimens in the laboratory must be in good condition, therefore, periodic maintenance of preserved specimens is necessary. Treatment of preserved specimens can be done in 2 ways, namely by wet preservation and dry preservation. The wet preservation method is carried out by preserving the specimen in a preservative liquid. Wet preservation is usually done for animals/plants whose size is large enough, or animals that have a soft body structure by being immersed in a preservative solution. While the dry preservation method is carried out by drying the preserved specimens until the water content becomes very low so that the destroying organisms do not work. Dry preservation of sufficiently large

preserved specimens in intact form is usually carried out by drying in in the sun or in the oven. Small preserved specimens such as spores, powders extracts, fungi, plankton and slices of tissue or organs are preserved in the form of preparations microscopic (slides).

Bivalves are one of the best-preserved laboratory specimens. Bivalves that are often encountered in the field include blood clams (Anadara granosa) and bamboo shells (Ensis leei). In the laboratory, these types of bivalves are preserved by dry preservation (bivalve shell preservation). Preservation of wet specimens is rarely performed in bivalves. Research conducted by [3], preservation of shellfish specimens wrapped in cotton or cloth that has been soaked with formalin (2%) or alcohol (70%). After that the specimen is placed in a thick plastic bag and then stored in a plastic container or box to be brought to the laboratory. In the laboratory, it is transferred to a bottle that already contains a preservative solution (70% alcohol).

Wet preserved animals or wet collections use a preservative liquid, namely 70% alcohol. This liquid has antibiotic and antiseptic properties suitable for long-term preservation. This method is carried out so that wet-preserved specimens are not contaminated with bacterial spoilage organisms that can enter and live in the specimens. This method changes the preventive policy of wet collection preservation which used to use 4% formalin in the 1990s [2]. [4] showed that the shrimp preserved with 70% alcohol were overgrown with fungus. Therefore, this research is needed on the effectiveness of various concentrations of alcohol solution in this case with a higher concentration of alcohol solution as a preservative for bivalves specimens. Parameters of the effectiveness of the use of alcohol solution can be seen from the change in color, turbidity of alcohol, and morphological changes of preserved specimens.

2. RESEARCH METHODS

The type of research used is an observational experiment, namely by conducting experiments on preserved bivalves specimens with various concentrations of alcohol and observing for 3 months. Bivalvia sampling was carried out on the southern coast of Madura Island. Preservation and treatment of bivalves specimens was carried out at the Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Surabaya. This research was conducted from May to July 2022. The variables of this research included diluted alcohol content, namely alcohol with levels of 90%, 85%, 80%, 75%, and 70%. As well as the types of preserved bivalves, namely blood clams and bamboo shells, Parameters of the effectiveness of alcohol content as an appropriate preservative were measured from the turbidity of the alcohol (the longer the alcohol concentration became turbid, the better the alcohol was used as a specimen preservative) and the physical changes of bivalves specimens. The research was carried out with the following stages.

2.1. Alcohol Dilution

Alcohol dilution with various concentrations ranging from the highest concentration to the lowest concentration, starting from a concentration of 90%, 85%, 80%, 75%, and 70%. The alcohol dilution formula follows the formula: and 70%. The alcohol dilution formula follows the formula: and 70%. The alcohol dilution formula follows the formula:

$$M1 \times V1 = M2 \times V2$$

2.2. Bivalve Specimen Collection

At this stage, the bivalve specimen used is a new specimen. Bivalve specimens used were feather clams, blood clams, bamboo shells. Bivalve specimens that have been obtained are put in a cool box, previously filled with blue ice to prevent spoilage when traveling to the laboratory. (3) Preservation of Bivalves Specimens, Bivalves that have been obtained are brought to the Taxonomy Laboratory to be preserved with alcohol in a preservation jar. Bivalve specimens were preserved for 1 x 24 hours. After being preserved with alcohol for 1 x 24 hours, the alcohol is replaced with a new one. (4) Treatment and Observation of Bivalves Specimens, Bivalvia specimens that have been replaced with alcohol are then arranged and observed for 3 months. Observations were made by paying attention to the alcohol turbidity and bivalves morphology, The data was collected by observing once a week on preserved bivalves specimens. Observations are made by filling in the instrument/observation table. The data obtained were analyzed by quantitative descriptive methods by describing the results of the collected observation tables.

3. RESULTS AND DISCUSSION

A1B1 Treatment (blood clams with 70% alcohol concentration), alcohol was turbid after the first turn until the second day. On the third to tenth day, the alcohol remains clear. On the first day of observation, the shell of the blood clams was slightly opened and a precipitate of crushed mussel meat was formed. In the A1B2 treatment (bamboo shells with 70% alcohol concentration), it was not much different from the A1B1 treatment. During the first 24 hours, a precipitate is formed from the crushed flesh of bamboo shells. The shell of the bamboo clam is exposed and the flesh of the bamboo clam is rough.

Table 1. Table of Observations of Blood Clams and Bamboo Shells with Alcohol Concentrations of 70%, 75%, 80%, 85%, and 90%.Short cut keys for the template

Specimens	The alcohol turbidity			Bivalves morphology		
Day	1	5	10	1	5	10
A1B1	Cloudy, yellowish (+)	Limpid	Limpid	Slightly open shell (1 out of 10 pieces, a crumbled shellfish precipitate forms)	Slightly open clam shells (2 of 10)	Slightly open clam shells (3 of 10)
A2B1	Cloudy, yellowish (++)	yellowish (+)	Limpid	Slightly open shell (2 out of 10 pieces) a crumbled clam meat precipitate formed	Slightly open clam shells (3 out of 10)	Open clam shells (4 of 10 pieces)
A3B1	Cloudy, yellowish (+++)	yellowish (++)	Limpid	Slightly open shell (1 out of 10 pieces) a crumbled clam meat precipitate formed	Slightly open shell (3 out of 10 pieces) a crumbled clam meat precipitate formed	Open clam shells (4 of 10 pieces)
A4B1	Cloudy, yellowish (+++)	yellowish (++)	Limpid	Slightly open shell (1 out of 10 pieces) a crumbled clam meat precipitate formed	Slightly open shell (3 out of 10 pieces) a crumbled clam meat precipitate formed	Slightly open shell (4 out of 10 pieces)
A5B1	Cloudy, yellowish (++++)	yellowish (++)	Limpid	Slightly open shell (1 out of 10 pieces) a crumbled clam meat precipitate formed	Slightly open shell (4 out of 10 pieces) a crumbled clam meat precipitate formed	Slightly open shell (5 out of 10 pieces)
A1B2	Cloudy, yellowish (+)	Limpid	Limpid	At the bottom of the specimen jar formed deposits of crushed bamboo shell meat. The shell has opened.	No precipitate is formed, rough textured meat	No precipitate is formed, rough textured meat
A2B2	Cloudy, yellowish (++)	yellowish (+)	Limpid	At the bottom of the specimen jar formed deposits of crushed bamboo shell meat. The shell has opened.	No precipitate is formed, rough textured meat	No precipitate is formed, rough textured meat
A3B2	Cloudy, yellowish (++)	yellowish (++)	Limpid	At the bottom of the specimen jar formed deposits of crushed bamboo shell meat. The shell has opened.	No precipitate is formed, rough textured meat	No precipitate is formed, rough textured meat
A4B2	Cloudy, yellowish (+++)	yellowish (+++)	Limpid	At the bottom of the specimen jar formed deposits of crushed bamboo shell meat. The shell has opened.	No precipitate is formed, rough textured meat	No precipitate is formed, rough textured meat
A5B2	Cloudy, yellowish (++++)	Yellowish (+++)	Limpid	At the bottom of the specimen jar formed deposits of crushed bamboo shell meat. The shell has opened.	No precipitate is formed, rough textured meat	No precipitate is formed, rough textured meat

Information:

A1B1 = alcohol 70%, blood clams

A2B1 = alcohol 75%, blood clams

A3B1 = alcohol 80%, blood clams

A4B1 = alcohol 85%, blood clams

A5B1 = alcohol 90%, blood clams

A1B2 = alcohol 70%, bamboo shells

A2B2 = alcohol 75%, bamboo shells

A3B2 = alcohol 80%, bamboo shells

A4B3 = alcohol 85%, bamboo shells

A5B4 = alcohol 90%, bamboo shells

A2B1 treatment (blood clams with 75% alcohol concentration) in the first 24 hours, the color of the alcohol was cloudy yellowish. The alcohol was then changed daily until the fifth day. On the sixth day, the alcohol is clear. The A2B2 treatment (bamboo shells with 75% alcohol concentration) was also not much different from the A2B1 treatment. The first 24 hours during the alcohol preservation process became cloudy and more meat deposits were formed than in the A1B2 treatment. On the fifth day, the alcohol was still yellowish in color. At the turn of the sixth day until

now, the alcohol alcohol remains clear.

A3B1 treatment (blood clams with 80% alcohol concentration) in the first 24 hours experienced more turbidity than A3B1 treatment. At the fifth turn the color of the alcohol remained yellowish but not as dense as the second, third and fourth days. The shell of the blood clam in the first 24 hours is slightly open. The specimen bottle also formed more precipitate than the A3B1 treatment. Treatment A3B2 (bamboo shells with an alcohol concentration of 85%) until the change of alcohol on the sixth day, the alcohol was yellow although not as thick as the change of the first day to the fifth day. The shells of the bamboo shells are all exposed. The flesh of the bamboo shells has a rough texture.

A4B1 treatment (blood clams with an alcohol concentration of 85%) in the first 24 hours was cloudy and dark yellow in color, there were deposits from shellfish meat. Alcohol continues to be replaced every day, at the turn of the fifth day, alcohol is clear yellow. Alcohol was changed daily until the eighth day. A4B2 treatment (bamboo shells with an alcohol concentration of 85%), in the first 24 hours, the alcohol was thick yellow and cloudy. The flesh of the bamboo shells was crushed so that a precipitate formed at the bottom of the specimen jar. On the next day there was no precipitate but the alcohol was yellow until the seventh day. The flesh of the bamboo shells has a rough texture.

The treatment of A5B1 (blood clams with 90% alcohol concentration) in the first 24 hours of alcohol was thick yellow and a white precipitate formed at the bottom of the specimen jar, and the shells were slightly open. Alcohol continued to be replaced until the ninth day. On the tenth day of observation, more and more blood clam shells were opened, but the alcohol was already clear in color so it didn't need to be replaced anymore. In the A5B2 treatment (bamboo shells with 90% alcohol concentration), in the first 24 hours the alcohol was dark yellow, a white precipitate was formed at the bottom of the specimen bottle from crushed bamboo shell meat. The shell of the bamboo shell is also exposed. The flesh of the bamboo scallops is coarsely textured. Alcohol continued to be replaced until the ninth day. On the tenth day of alcohol change, the alcohol is clear in color.

In this study, the higher alcohol concentration, the more turbid the alcohol solution was, so the alcohol in the specimen bottle needed to be changed every day. This is because the alcohol which was originally clear, becomes yellow after mixing with the preserved specimens. In addition, the higher alcohol concentration causes the preserved specimens to wrinkle. Alcohol is a good preservative but alcohol has the disadvantage that it dehydrates the specimen and dissolves certain pigments such as proteins and lipids from the specimen [5]. Alcohol also causes shrinkage of the specimen so that the texture of the specimen becomes rough. Alcohol at higher concentrations acts as a dehydrator which means it removes and replaces water in cells, tissues, or whole-body specimens with alcohol. Alcohol is hypertonic so it causes the water or solution in the specimen to shrink, this is known as crenation. Crenation is the event that the concentration of the solution outside the cell is higher than the concentration of the solution inside the cell.[6]. The reduced water causes changes in the protein in the specimen and causes texture specimens become rough [7]. In line with research [8] Ethanol concentrations at or above 90% make insects more fragile and result in more shrivelling, but insects with stronger or sclerotized exoskeletons actually retain more of their appendages at higher concentrations. However, insect morphology is severely damaged if allowed to dry.

4. CONCLUSION

Alcohol with a concentration of 70% is effective as a preservative solution in bamboo shells and blood clams. A higher alcohol concentration makes the color of the alcohol as a preservative change so that the alcohol is replaced more often. The meat of the bamboo mussels becomes rough after being preserved and the fragile condition of the mussel meat is separated from the shell so that a precipitate is formed. The shell of the blood clam was slightly exposed. Researchers hopes there are further studies related to alcohol concentrations lower than 70% alcohol or other preservatives as preservatives for specimens in the laboratory due to the inefficient and economical use of alcohol.

ACKNOWLEDGMENTS

The researcher would like to thank the research and community services (LPPM Unesa) and the Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya.

REFERENCES

[1] Rahayoe, A.S. Kombinasi Alkohol dan Asap Cair sebagai Alternatif Pengawet Spesimen Cacing Tanah (Pheretima sp.). Indonesian Journal of Laboratory. Vol 1 (3), 1-12. 2019.

[2] Kaenuwihanulaha, JI., Damayanib, NA., Anwar, RK.. Identifikasi dan penyimpanan koleksi Museum Zoologi Sekolah Ilmu dan Teknologi Hayati (SITH) Institut Teknologi Bandung Jatinangor. Informatio: Journal of Library and Information Science Vol. 1(1), 99-120. 2021.

[3] Pratiwi, R. Biota Laut : Bagaimana Mengkoleksi dan Merawat Biota Laut. Oseana, Volume XXXI, Nomor 2 : 1 – 9. 2006.

[4] Suharna, N. dan Rahayu, RD. Efektivitas Alkohol dan Campurannya dengan Formalin sebagai Bahan Pengawet Spesimen Binatang untuk Udang. Berita Biologi Volume 5, Nomor 1, April 2000

[5] National Park Service. Storage Concerns For Fluid-Preserved Collections. Conserve O Gram Vol 11 No 3, May 1999.

[6] Yustina dan Darmadi. 2019. Buku Ajar Fisiologi Hewan. Riau : Program Studi Pendidikan Biologi FKIP Universitas Riau.

[7] Coffey, Donavyn. Why is alcohol used to preserve things?. September 05, 2021. https://www.livescience.com/why-alcohol-preserves.html

[8] Marquina, Daniel., Ronquist , Fredrik & Łukasik, Piotr. The effect of ethanol concentration on the preservation of insects for biodiversity studies. The Prepint Server For Biology. March 6, 2020. https://doi.org/10.1101/2020.03.05.978288