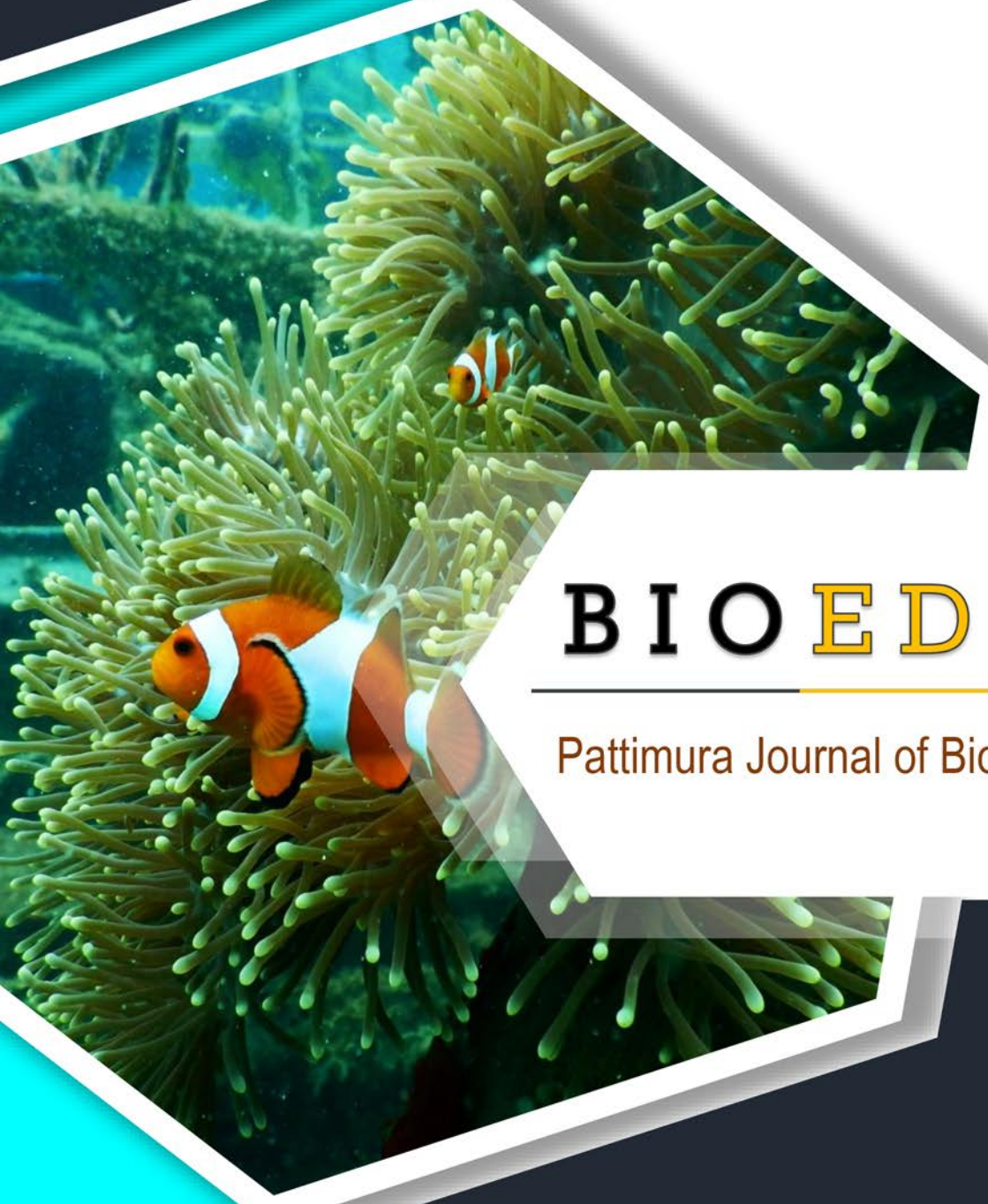
E-ISSN : 2775-4472

BIOEDUPAT
Pattimura Journal of Biology and Learning



BIOEDUPAT

Pattimura Journal of Biology and Learning

Published by:
Study Program of Biology Education
Postgraduate - Universitas Pattimura
Ambon, Indonesia
Collaborate with:
Konsorsium Pendidikan Biologi Indonesia (KPBI)

BIOEDUPAT	Volume 1	Number 1	Pages 1-44	Ambon, April 2021

BIOEDUPAT:
Pattimura Journal of Biology and Learning
https://ojs3.unpatti.ac.id/index.php/bioedupat
e-ISSN 2775-4472

Research Article

PjBL-HOTS learning model: Its application and effect on cognitive learning outcomes, critical thinking, and social attitudes

Aminah Launuru[1,*], Dominggus Rumahlatu[2], Muhammad Nur Matdoan[2]

[1] SMA Negeri 6 Leihitu, Jl. Nanihaha, Hila 97582, Indonesia
[2] Study Program of Biology Education, Faculty of Teacher Training and Education, Pattimura University, Jl. Ir. M. Putuhena, Ambon 97233, Indonesia
** corresponding author: aminalaunuru@gmail.com*

Received: 17 February 2021 Revised: 12 March 2021 Accepted: 21 April 2021

ABSTRACT

Learning in the digital era 4.0 brings changes for teachers and students. Teachers must prepare innovative digital-based learning to improve students' higher-order thinking skills. This study aims to analyze the effect of the application of the PjBL-HOTS learning model on cognitive learning outcomes, critical thinking, and social attitudes. This type of research is a quasi-experimental research design, which uses a pretest-posttest nonequivalent group design. The study used two classes, namely class X1 as the experimental class while class X2 as the control class. The research instrument is in the form of essay questions to assess students 'cognitive learning outcomes and students critical thinking. Meanwhile, a questionnaire is used to assess students' social attitudes. The data analysis used descriptive and inferential analysis techniques. The inferential analysis used ANCOVA and ANOVA analysis with a significant level of α = 0.05. The results showed that there was an effect of the PjBL-HOTS learning model in improving students' cognitive learning outcomes, critical thinking, and social attitudes. This is because the ability to think critically is always empowered by the teacher at every stage of PjBL.

Keywords: PjBL-HOTS, cognitive, critical thinking, social attitudes

To cite this article:
Launuru, A., Rumahlatu, D., & Matdoan, M. N. (2021). PjBL-HOTS learning model: Its application and effect on cognitive learning outcomes, critical thinking and social attitudes. *Bioedupat: Pattimura Journal of Biology and Learning*, Vol 1(1), 1-10. *DOI: https://doi.org/10.30598/bioedupatv1i1pp1-10*

INTRODUCTION

Higher Order Thinking Skills (HOTS) is one of the higher-order thinking skills that need to be developed in the current era of the Industrial Revolution (RI) 4.0. According to Heong et al. (2011), through higher-order thinking, students will use thinking widely. This will make students challenged to find something new. Kusuma et al. (2017) added that HOTS requires students to be able to apply the new information they get, and then manipulate the information to find new answers. The new answers are meant to be answers that are processed through creative and innovative thoughts from students.

According to Dinni (2018), HOTS consists of the ability to connect, manipulate, and change the knowledge and experience that is already owned critically and creatively in determining decisions to solve problems in new situations.

Meanwhile, according to Brookhart (2010), the HOTS category includes analytical skills, evaluation, creation, logical reasoning, critical thinking, problem-solving, creativity, and creative thinking.

Several researchers have analyzed HOTS abilities in high school students. Samosir et al. (2019) conducted a study to analyze HOTS on questions given by the teacher. Research done by Istiyono et al. (2014) is also oriented towards HOTS analysis on student academic ability tests. The research shows that students' HOTS are assessed in the form of learning evaluation. However, there is no HOTS integration in biology learning. Research conducted by Widodo & Kadarwati (2013) shows that Higher Order Thinking and Problem Based Instruction learning models can be integrated into HOT-PBI and can improve student character, cognitive, and higher-order thinking skills. Tajudin et al. (2016) also emphasized that teachers can use the HOTS learning principle by diversifying it with several learning models.

Therefore, the HOTS of these students can also be integrated into PjBL learning. The Project-Based Learning (PjBL) learning model is an innovative learning model that emphasizes the investigation process by students and produces a scientific product. Research by Rumahlatu & Sangur (2019) has shown that the application of PjBL compared to conventional learning models has been able to empower metacognitive abilities and student learning outcomes. By using the right learning model, it is hoped that Indonesia's young generation will be ready and confident to face the various challenges and changes that have occurred due to the influence of the 4.0 industrial revolution.

High school biology teachers in Ambon city have implemented many innovative cooperative learning models. These learning models are STAD, PBL, PjBL, NHT, GI, and many other models. However, the application of learning models and HOTS integration in learning to improve students' higher-order thinking skills has not been done by high school biology teachers. Research conducted by Leiwakabessy et al. (2018) show that HOTS-based evaluations conducted by biology teachers at several schools such as SMA 1 Ambon, SMA 3 Ambon, SMA 4 Ambon, SMA Siwalima Ambon, and SMA Xaverius Ambon have been able to compile HOTS questions of 93.75 %. This means that high school biology teachers have been able to evaluate HOTS, but the application of HOTS in learning has not been done. For HOTS evaluation to be effective and efficient to empower students, teachers need to design HOTS-based learning, so that the learning and evaluation process becomes one unit. Therefore, this research is important to do to examine the application of the integration of the PjBL-HOTS learning model for empowering cognitive learning outcomes, students 'critical thinking abilities, and students' social attitudes at SMA 6 Leihitu.

METHODS

Research design

This research is quasi-experimental research, which uses a pretest-posttest nonequivalent group design. The independent variables of this study are the conventional learning model (STAD) and the PjBL-HOTS learning model, while the dependent variable is the cognitive learning outcomes, critical thinking skills, and students' social attitudes. The quasi-experimental research design is shown in Table 1, as follows.

Tabel 1. Research design

Y1	X1	Y2
Y1	X2	Y2

Note:
Y1 : Pretest
Y2 : Posttest
X1 : PjBL-HOTS Learning Model
X2 : STAD Model

Sample of research

The populations used in this study were all students of class XI SMA 6 Leihitu. The research sample consisted of two classes, namely X1 as the experimental class which was taught using the PjBL-HOTS learning model, while class X2 was the control class which was taught using the conventional model (STAD).

Instruments and procedures

The instruments developed in this study were test questions and scoring rubrics. The items were prepared to refer to the competency standards in accordance with the provisions of the National Education Standards Agency (NESA), Indonesia. The items arranged are essay items that are used to measure students' critical thinking skills and cognitive

learning outcomes. The scoring rubric is a list of criteria to measure cognitive learning outcomes and critical thinking. A questionnaire is provided for students to assess their respective social attitude skills.

The implementation of learning in each class is carried out almost simultaneously and uses different learning tools, but the assessment of the dependent variable (cognitive learning outcomes, students 'critical thinking, and students' social attitudes) remains the same. The method of data collection and grouping is as follows.

1. The pretest is given to each class before the application of the learning model. The pretest results of cognitive learning outcomes and critical thinking were used as covariates in the ANCOVA statistical analysis.
2. The learning implementation in the experimental class uses the PjBL-HOTS learning stage: (a) Planning; critical in analyzing topics that become phenomena in society, think critically of relevant information to discuss the topic, doing the division of tasks in groups; (b) Creating; think critically in preparing the project stage, think critically in project implementation, create a product, think critically in preparing project reports; (c) Presenting; think critically in doing a presentation. While the control class uses the STAD learning stage which is a learning model that is always used by teachers while learning the concept of the human body defense system. The steps are as follows (a) Learning preparation: students are divided into 4-6 groups, students are grouped into heterogeneous groups based on academic ability, gender, race and ethnicity; (b) Material presentation: teacher explains the learning objectives, motivates students to work together in their group, the teacher presents the material of the human body defense system; (c) Group study activities: teacher distributes worksheets for students, the teacher guides students to discuss together; (d) Examination of group results:teacher guides students to make group presentations and asses their presentation; (e) Students work on individual test questions (Individual tests are carried out to determine the extent to which learning success has been achieved, individual tests are held on the material that has been discussed); (f) Group awards: teacher gives awards to the group that gets the highest score for the presentation phase and individual tests
3. During the learning process, students assess their social attitudes.
4. Posttest is given after the application of the learning model. The results of the posttest were used to measure students' cognitive learning outcomes and critical thinking.

Data analysis

The research data were analyzed using descriptive and inferential statistics. Descriptive statistics are used to determine the data group and the distribution of data on cognitive learning outcomes, critical thinking, and social attitudes at intervals and data classes. Inferential statistics begins with an analysis of data homogeneity and normality. The homogeneity test used the Levene test and the normality test used the Kolmogorov-Smirnov test. Furthermore, two-way covariance analysis (ANCOVA) was used to analyze: (1) the effect of the application of learning models on student cognitive learning outcomes. (2) the effect of the application of the learning model on students' critical thinking skills. While the analysis of variance (ANOVA) was used to analyze the effect of the application of the learning model on students' social attitudes. Furthermore, if there is a difference, a further test of the post hoc least significant difference (LSD) is carried out to determine the average difference which is statistically significant. Data analysis was performed using the SPSS 18.0 program.

RESULTS AND DISCUSSION

Cognitive learning outcomes

Descriptions of students' cognitive learning outcomes in the experimental and control classes are described in Table 2.

Table 2. Cognitive learning outcomes in the control and experimental class

Control Class						Experiment Class					
Pretest			Posttest			Pretest			Posttest		
Interval	F	RF (%)	Interval	F	RF (%)	Interval	F	RF (%)	Interval	F	RF (%)
27-30	8	30.77	66-70	12	46.15	27-30	9	34.62	57-63	1	3.85
31-34	9	34.62	71-74	2	7.69	31-34	8	30.77	64-70	5	19.23
35-38	5	19.23	75-78	0	0	35-38	5	19.23	71-77	2	7.69

39-42	3	11.54	79-82	10	38.46	39-42	3	11.54	78-84	6	23.08
43-46	0	0	83-86	0	0	43-46	0	0	85-91	9	34.62
47-50	1	3.85	87-90	2	7.69	47-50	1	3.85	92-98	3	11.54
Total	26	100	Total	26	100	Total	26	100	Total	26	100

Description: F: frequency; RF: relative frequency

The distribution of scores on the initial test of 100% is in the range of 27-50 scores for the control and experimental classes, this shows that students have a low level of mastery of the body's defense system material. Whereas in the final test 100% of students had improved scores in both the control and experimental classes. This means that there is an increase in the value between before and after students are taught using conventional learning models at SMA 6 Leihitu. Research by Narahaubun et al. (2020) also showed that before the application of the learning model, cognitive learning outcomes that revealed students' conceptual understanding of material were very low compared to after learning in both the experimental class and the control class. After going through the learning process, students' understanding of the concept of the human body's defense system has increased. Rumahlatu et al. (2020) also explained that cognitive learning outcomes are related to students' understanding of a concept, so it is called a conceptual understanding that can be formed through the learning process.

Critical thinking

Students' critical thinking in the experimental and control classes is described in Table 3 below.

Table 3. Results of the experimental class critical thinking

Control Class						Experiment Class					
Pretest			Posttest			Pretest			Posttest		
Interval	F	RF (%)	Interval	F	RF (%)	Interval	F	RF (%)	Interval	F	RF (%)
6-8	10	38.46	13-15	1	3.85	6-8	7	26.92	13-15	5	19.23
9-11	15	57.69	16-18	24	92.31	9-11	14	53.85	16-18	8	30.77
12-14	1	3.85	19-21	1	3.85	12-14	5	19.23	19-21	8	30.77
									22-24	5	19.23
Total	26	100	Total	26	100	Total	26	100	Total	26	100

Description: F: frequency; RF: relative frequency

The distribution of scores on the initial critical thinking test was 100% in the range of 6-14 in both the control and experimental classes (Table 3). Meanwhile, in the final test, 100% of students 'critical thinking has succeeded in improving students' critical thinking through PjBL-HOTS learning. This means that there is an increase in the value between before and after students are taught using the PjBL-HOTS and STAD learning models at SMA Negeri 6 Leihitu. However, PjBL-HOTS learning has a higher increase compared to learning using STAD, this is shown by the percentage increase in the experimental class by 50% compared to the control class using STAD learning. According to Çimer et al. (2013), the ability to think critically is a culture that is empowered continuously in learning. The same thing was conveyed by Setiawati & Corebima (2017) the ability to think critically does not appear automatically but through guided training and practice in learning until students can use it. Through the learning stages, students' critical thinking skills can be empowered in learning.

Social attitude

The social attitudes of students in the experimental and control classes are described in Table 4 below.

Table 4. Social attitudes in the experimental and control classes

Control Class			Experiment Class		
Interval	Frequency	Relative Frequency (%)	Interval	Frequency	Relative Frequency (%)
31-34	1	3.85	36-38	3	11.54
35-38	0	0	39-41	6	23.08

39-42	8	30.77	42-44	13	50
43-46	11	42.31	45-47	4	15.38
47-50	6	23.08			
Total	26	100	Total	26	100

The distribution of the value of the students' social attitudes is 100% in the range of values 31-50. This means that students of SMA Negeri 6 Leihitu can show social attitudes in the learning process in both the experimental class and the control class. Social attitude is an assessment of students' social abilities during the learning process. Descriptive analysis shows that social attitudes in students can be developed in the experimental class and the control class.

The effect of PjBL_HOTS model application on cognitive learning outcomes, critical thinking and social attitudes

Prerequisite test

Prior to data analysis and hypothesis testing, the normality and homogeneity of the data were first tested. The data normality test used the One Sample Kolmogorov-Smirnov Test statistical test, and the homogeneity test of the data used the Leven's Test of Quality of Error Variances (Table 5).

Table 5. Prerequisite test

Variable	Score	Significant	Level of Trust	Score	Significant	Level of Trust
Cognitive learning result	3.381	.061	α=0.05	.434	.434	α=0.05
Critical thinking	2.156	.100		.593	.594	
Social attitude	1.918	.172		.881	.533	

The significant value of each tested variable is greater than α = 0.05. This means that the variable data come from a homogeneous population and is normally distributed.

Hypothesis testing

The results of hypothesis testing are used to determine the effect of the independent variables on the dependent variable. The dependent variable data shows a normal and homogeneous distribution so that it is feasible to be tested using statistical tests. ANCOVA calculation results on student cognitive learning outcomes are shown in Table 6, while further tests are shown in Table 7.

Table 6. ANCOVA cognitive learning outcomes

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	771.608[a]	2	385.804	4.919	.011
Intercept	7615.590	1	7615.590	97.101	.000
Pretest	10.051	1	10.051	.128	.722
Learning model	761.558	1	761.558	9.710	**.003**
Error	3843.065	49	78.430		
Total	311231.000	52			
Corrected total	4614.673	51			

Table 7. Further tests of students' cognitive abilities LSD

Learning Model	Average	LSD Notation
Experiment	80.6154	a
Control	72.9615	b

The independent variable of the learning model is significant at 0.003 <α = 0.05. Based on these results, there is an effect of the learning model on student cognitive learning outcomes (Table 6). The difference in notation between the experimental class and the control class (Table 7). This proves that students who are taught using the PjBL-HOTS learning model have better cognitive learning outcomes than students taught using conventional learning models. Research by Susilowati et al. (2013) shows that project-based learning affects student learning outcomes in the human digestive system material compared to conventional learning. After students complete their projects, students understand the content of the human body defense system material which is more in-depth so that students can better apply their knowledge in solving problems in real life. According to Insyasiska et al. (2015) in project-based learning, students experience the project as a whole themselves, starting from selecting topics, deciding on approaches, conducting experiments, drawing conclusions, and communicating the results of the project. Several studies on the PjBL learning model show that PjBL is constructivist based to shape student concepts. In this study, PjBL is combined with HOTS to become PjBL-HOTS which still reflects PjBL's constructivist to form student concepts independently.

The PjBL-HOTS stage contains a combination of project stages with critical thinking skills. At the critical stage in analyzing the topic, students are provided with the experience of finding information and reading this information to compile a project implementation plan, to project reports. At this stage students can develop cognitively by reading, taking notes, and detailing the stages of the project and report implementation. Klein et al. (2009) explain that project-based learning can empower students to pursue their knowledge. Anazifa & Hadi (2016) project-based learning also improves students' learning abilities. The two opinions above indicate that PjBL learning can empower students to understand the material they are learning independently. Meanwhile, this learning is based on the PjBL-HOTS integration which is also able to improve students' cognitive learning outcomes because there are already critical thinking stages in the project learning stage.

The next stage is critical thinking on relevant information to be used to solve problems/phenomena, division of tasks into groups, compiling work stages, implementation to product manufacturing. In each stage of project learning, critical thinking skills are continuously trained so that students become accustomed to them. According to Husnah (2017), how to improve critical thinking skills is to read critically, develop observation skills (observe), increase curiosity, and discussion. Students can practice critical thinking during reading to find information for the group division process, the preparation of project stages, and project implementation. Reading in this process is not just reading material about the body's immune system in general but reading critically, meaning that after reading students get results from reading, namely tasks to be carried out by groups, project stages for project implementation in the immune system. The same thing was also conveyed by Christina & Kristin (2017) that critical thinking is the ability to obtain information for solving a problem by finding this information from various sources. Walfajri & Harjono (2014) added that critical thinking is a person's ability to make decisions, analyze problems and overcome the problems they face. Therefore, critical thinking skills are very important to use in project learning (PjBL) to improve students' cognitive abilities. Huda & Rahman (2020) explain that students have a good cognitive way of thinking that is influenced by critical thinking habits so that it can also affect student learning achievement. Ramdani and Badriah's (2018) research shows that there is a positive correlation between critical thinking and student learning outcomes because there is the suitability of stages in the learning model that focuses on authentic problems so that it can accommodate critical thinking and cognitive learning outcomes.

Based on this opinion, HOTS's ability to think critically in combination with the PjBL learning stages can affect cognitive learning outcomes compared to the STAD learning model commonly used by teachers. Various studies have shown that STAD can improve student cognitive learning outcomes. Hasan et al. (2016) show that STAD can improve cognitive learning outcomes in its two cycles of application. The descriptive analysis also shows that STAD can improve student learning outcomes before and after the application of the learning model. However, further tests showed a significant difference between the STAD model and the PjBL-HOTS combination in improving cognitive learning outcomes for students at SMA N 6 Leihitu.

In addition to the contribution of critical thinking in the PjBL learning stages in influencing student learning outcomes, the next analysis is an analysis of the PjBL-HOTS combination in influencing critical thinking. The results of the ANCOVA calculation on students' critical thinking skills are shown in Table 8, while the LSD advanced test is shown in Table 9.

Table 8. ANCOVA students' critical thinking skills

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	46.549[a]	2	23.274	4.667	.014
Intercept	278.075	1	278.075	55.757	.000
Critical early	15.779	1	15.779	3.164	.081
Learning model	21.781	1	21.781	4.367	**.042**
Error	244.374	49	4.987		
Total	16146.000	52			
Corrected total	290.923	51			

Table 9. LSD further test of students' critical thinking skills

Learning Model	Average	LSD Notation
Experiment	18.2308	a
Control	16.6923	b

Inferential analysis using the ANCOVA test shows that there is an effect of the learning model on students' critical thinking 0.042 <α 0.05 (Table 8). The LSD advanced test showed that the difference in notation between the experimental class and the control class so that the PjBL-HOTS learning model applied to the experimental class was able to improve students' higher-order thinking compared to the conventional model (STAD) in the control class (Table 9). The results showed that the PjBL-HOTS learning model is a learning model that can improve students' critical thinking skills. The use of PjBL is one effective way to train students' HOTS skills (Sambite et al., 2019). HOTS is a thought process that not only memorizes but also involves deep understanding and critical analysis thinking processes (Lukitasari et al., 2018). This constructivist-based PjBL learning model is very suitable to be used to improve HOTS, especially students' critical thinking. According to Stephani (2016), critical thinking will occur when students are allowed to analyze various information then make a decision and put the decision into practice. Through critical thinking, students can identify problems, seek information, and find solutions to these problems are stages to develop critical thinking (Putri et al., 2018).

In PjBL-HOTS learning, the series of PjBL stages are always integrated with critical thinking components. Therefore, each stage that is integrated with HOTS can improve students' critical thinking skills, compared to the STAD learning model that has been used by teachers in teaching the concept of the human body defense system. STAD is a cooperative learning model that focuses on group discussion activities, after which a test is conducted to check understanding at the end of the lesson. However, PjBL learning integrates HOTS in every lesson to improve critical thinking skills. Several studies have shown that PjBL can increase students' HOTS. According to Fitriani et al. (2015), there are differences in the ability to think critically in the control class and the experimental class because the stages of the learning model support the development of this critical thinking ability. Therefore, HOTS integration at the PjBL stage can also improve students' critical abilities. The same results were also conveyed by Wahid & Karimah's (2018) study of the Creative Problem Solving (CPS) learning model which is integrated with HOTS which can empower students to increase the ability of students to think highly. The results of ANOVA calculations on students' social attitudes are shown in Table 10 below.

Table 10. ANOVA of the ability of social attitudes

	Sum of Squares	df	Mean Square	F	Sig.
Between groups	27.769	1	27.769	2.193	.145
Within groups	633.000	50	12.660		
Total	660.769	51			

Inferential analysis using the ANOVA test shows that there is no influence of the learning model on students' social attitudes (Table 10). Social attitude is one of the important aspects that need to be presented in the learning process, especially Biology-Science. PjBL-HOTS learning and STAD (conventional) learning are also able to

empower students' social attitudes because students can interact with other students, as well as interact with teachers. According to Gusviani (2016), social attitudes can be raised in core activities through exploration by the teacher, it can also be raised in the final activity, namely by providing advice or motivation so that students can form individuals who have good social attitudes as provisions for life in society.

The PjBL-HOTS learning stage emphasizes interaction with groups for project planning, implementation, and presentation. According to Astalini et al. (2018), social attitudes in science learning imply that students can control themselves to achieve learning goals through understanding, experimenting, being active, and liking science learning.

The same thing was conveyed by Sanjiwana et al. (2015) so that the students 'social attitudes that are formed do not deviate from the expected aspects of social attitudes, it is the teacher's task to monitor students' social attitudes according to the learning stages. At this stage, students plan the division of tasks in groups, implement projects, make products and present them in groups to increase student cooperation. Student cooperation attitudes must be honed from childhood so that students can socialize with their friends, respect friends' opinions, and work will feel lighter when done together (Listiani & Purwanto, 2018).

According to Primandari et al. (2019) social attitudes in the form of responsibility, discipline, honesty, courtesy, group work can be trained in every lesson because students are more relaxed because it is done with peers. The PjBL-HOTS learning stages also empower responsibility, honesty and discipline, and are polite to group mates and teachers. Sukerta et al. (2014) reported that student activeness in discussion was supported by student social interaction during the learning process. Therefore, social attitudes are one of the psychological aspects of students that are very important to be nurtured and developed.

CONCLUSION

The PjBL-HOTS learning model affects students 'cognitive learning outcomes, critical thinking, and students' social attitudes. This is because the critical thinking stage is always empowered in the PjBL learning stage starting from project planning, implementation, and product presentation. Empowerment of critical thinking can affect the mastery of student cognitive learning outcomes in studying the immune system.

REFERENCES

Anazifa, R., & Hadi, R. (2016). Environmental education through project-based learning (Project-Based Learning) in biology learning. *Proceedings of the Symbion of the Biology Education Study Program, FKIP, Ahmad Dahlan University*, 453–462.

Astalini, Kurniawan, D., & Putri, A. (2018). Identification of the attitude of the social implications of science, interest in increasing science learning time, and interest in a career in science for junior high school students in Muaro Jambi Regency. *Tarbiyah Journal: Scientific Journal of Education*, 7(2), 93–108.

Brookhart, SM (2010). *How to assess higher-order thinking skills in your classroom*. Alexandria, Virginia USA.

Christina L. V ., & Kristin, F. (2017). The effectiveness of the group investigation (G I) type of learning model and cooperative integrated reading and composition (CIRC) in increasing the creativity of critical thinking and social studies learning outcomes for grade 4 students. *Scholaria: Journal of Education and Culture*, 6 (3), 217–230.

Çimer, A., Timuçin, M., & Kokoç, M. (2013). Critical thinking level of biology classroom survey: Ctlobics. *The Online Journal of New Horizons in Education*, 3(1), 15–24.

Dinni, H. N. (2018). HOTS (High Order Thinking Skills) and its relation to mathematical literacy skills. *Prism 1: Proceedings of the National Mathematics Seminar,* 170–176.

Fitriani, A., Indrowati, M., & Karyanto, P. (2015). Students' critical thinking skills in biology learning through the application of accelerated learning for class X SMA Negeri Karangpan and Karanganyar. *Journal of Biological Education,* 7(2), 56–67.

Gusviani, E. (2016). Analysis of the emergence of spiritual attitudes and social attitudes in science learning activities for grade IV SD using KTSP and the 2013 curriculum. *Eduhumaniora,* 7(2), 1–10.

Heong, Y. M, Othman, W., Yunos, J. B. M., Kiong, T., Hassan, R., & Mohamad, MM (2011). The level of Marzano higher-order thinking skills among technical education students. *International Journal of Social Science and Humanity,* 1(2), 121–125.

Huda, M., & Rahman, L. (2020). The relationship between critical thinking skills and learning outcomes of elementary school students. *Journal of Character Education: Journal of Child and Character Education,* 2(2), 42–47.

Husnah, M. (2017). The relationship between the level of critical thinking on student physics learning outcomes by applying the problem-based learning model. *Journal of Physics and Science Learning*, 1(2), 1–12.

Insyasiska, D., Zubaidah, S., & Susilo, H. (2015). The effect of project-based learning on learning motivation, creativity, critical thinking skills, and cognitive abilities of students in biology learning. *Journal of Biological Education,* 7(1), *9–21.*

Istiyono, E., Mardapi, D., & Suparno, S. (2014). Development of high-level physics thinking skills test (PysTHOTS) of high school students. *Journal of Educational Research and Evaluation*, 18(1), 2–5.

Klein, J., Taveras, S., King, SH, Commitante, A., Bey, LC., & Stripling, B. (2009). *A Guide To project-based learning in middle schools: Inspiring students to engage in deep and active learning.* NYC Department of Education.

Kusuma, M., Rosidin, U., Abdurrahman., & Suyatna, A. (2017). The Development of higher-order thinking skill (HOTS) assessment instrument in physics study. *Journal of Research & Method in Education*, 7(1), 26–32.

Leiwakabessy, F., Hetharia, M., & Manuputty, R. (2018). Development of biological teacher capabilities in the development of test instruments based on high order thinking skills (HOTS) In high school in Ambon city that have applied 2013 Curriculum. *Proceeding Book of The 3rd International Seminar on Education November 07th*, 2, 76–86.

Listiani, S., & Purwanto, A. (2018). The application of a project-based learning model using used goods to improve students' scientific attitudes. *Proceedings of the Seminar and National Discussion on Basic Education*, 24–29.

Lukitasari, M., Handhika, J., & Murtafiah, W. (2018). Higher-order thinking skills: Using e-portfolio in project-based learning. *In Journal of Physics: Conference,* 983(12047), 1–7.

Narahaubun, S. S, Rehena, J. F., & Rumahlatu, D. (2020). Empowering students' critical thinking skills, information literacy, and cognitive learning outcomes through the RBL-TPS model. *Indonesian Journal of Biological Education*, 6 (2), 243–256. https://doi.org/10.22219/jpbi.v6i2.11456

Primandari, P., Sulasmono, B. S., & Setyaningtyas, E. (2019). The difference in the effect of the cooperative model type TGT and STAD with cheerful interactive multimedia on social attitudes and cognitive learning outcomes in thematic learning in grade 5 SD. *Basicedu's Journal*, 3(3), 83–91.

Putri, R. L, Dwiastuti, S., & Karyanto, P. (2018). The effect of higher-order thinking skills questions in problem-based learning model toward student's critical thinking. *Proceeding Biology Education Conference*, 15(1), 324–328.

Rumahlatu, D., & Sangur, K. (2019). The influence of project-based learning strategies on the metacognitive skills, concept understanding, and retention of senior high school students. *Journal of Education and Learning*, 13(1), 104–110.

Rumahlatu, D., Sangur, K., & Liline, S. (2020). The effect of complex instruction team product (CITP) learning model on increase student's skills. *International Journal of Instruction*, 13(1), 587–606. doi: https://doi.org/10.29333/iji.2020.13138a

Said, H., Kasuba, F., & Sirajudin, N. (2016). Application of learning model student teams education division (STAD) to improve learning outcomes and activities of class VII students on the ecosystem concept at SMP Negeri 6 Bibinoi. *Journal of Bioeducation*, 4(2), 551–561.

Sambite, F. C, Mujasam, M., Widyaningsih, S., & Yusuf, I. (2019). Application of project-based learning based on simple teaching aids to increase the HOTS of students. *Periodic Scientific Physical Education*, 7(2), 141–147.

Samosir, A., Hasruddin., & Dongoran, H. (2019). Analysis of the quantity and quality of biology teacher and student questions on the excretion system material. *Pelita Pendidikan Journal*, 7(1), 9–15.

Sanjiwana, P. P. C. M., Pudjawan, K., & Margunayasa, IG (2015). Analysis of the Social Attitudes of Class V Students on Learning with the 2013 Curriculum. *PGSD Journal, Ganesha Education University, PGSD Department*, 3(1), 11–18.

Setiawati, H., & Corebima, A. (2017). Empowering critical thinking skills of the students having different academic abilities in biology learning of Senior High School through PQ4R - TPS Strategy: *The International Journal of Social Sciences and Humanities Invention,* 4(5), 3521–3526.

Stephani, M. R (2016). The Role of Teachers in Efforts to Increase Higher Order Thinking Through Guided-Discovery Teaching Styles in Physical Education Learning. *Journal of Physical Education d 's Sport*, 1(2), 34-42.

Sukerta, K., Lasmawan, I. W, & Natajaya, I. N. (2014). The influence of the cooperative learning model of the stad type on social studies learning achievement with the covariable social attitudes of fifth-grade elementary school students of Cluster III South Kuta District, Badung Regency, Bali. *PENDASI: Indonesian Journal of Basic Education,* 4(1), 1–11.

Susilowati, I., Iswari, R., & Sukaesih, S. (2013). The effect of project-based learning on student learning outcomes regarding the human digestive system. *Journal of Biology Education*, 2(1), 82–90.

Tajudin, Nor'ain, M., & Chinnappan, M. (2016). The link between higher-order thinking skills, representation, and concepts in enhancing TIMSS tasks. *International Journal of Instruction*, 9(2), 1–9.

Wahid, A. H., & Karimah, R. A. (2018). Integration of higher-order thinking skills (HOTS) with creative problem-solving models. *Modeling: Journal of the PGMI Study Program,* 5(1), 82–98.
Walfajri, R., & Harjono, N. (2014). Improved critical thinking skills and thematic learning outcomes of science content through problem-based learning models for grade 5 SD. *Basicedu's Journal*, 3(1), 16–20.
Widodo, T., & Kadarwati, S. (2013). Higher-order thinking based on problem-solving to improve learning outcomes-oriented to character building students. *Education Horizons,* 2013(1), 161–171.

BIOEDUPAT:
Pattimura Journal of Biology and Learning
https://ojs3.unpatti.ac.id/index.php/bioedupat
e-ISSN 2775-4472

Research Article

The density of Siasia (*Sipunculus nodus*) population based on the differences in the substrate of the sea grasses beds on the waters of Saparua Island

Yoin Meissy Matulessy[1,*], Ali Awan[2], Sintje Liline[2]

[1] Postgraduate Student of Biology Education, Pattimura University,
Jl. Dr. Tamaela, Ambon, Maluku 97114, Indonesia
[2] Study Program of Biology Education, Faculty of Teacher Training and Education, Pattimura University,
Jl. Ir. M. Putuhena, Ambon, Maluku 97233, Indonesia
** corresponding author: matulessy03@gmail.com*

Received: 17 February 2021 Revised: 15 March 2021 Accepted: 21 April 2021

ABSTRACT

Sipuncula, a marine biota which is commonly called a peanut worm, is a marine biota that is a little "controversial". Some literature also refers to these animals as "unsegmented marine worms" or unsegmented marine worms. Siasia (*Sipunculus nodus*) usually lives in coastal areas, especially around seagrass beds, mangrove forests, and coral reefs. These biotas tend to inhabit the bottom of the waters, especially in the substrate, so they are categorized as infauna benthic organisms. Siasia (*S. nodus*) can inhabit soft and hard substrate areas. Based on its ecological function, seagrass forms associations with various kinds of marine life as food providers, shelters, and places to live, causing high diversity of marine life. The basic substrate in the form of flat stones and gravel is a good living environment for macrozoobenthos because it is rich in organic compounds so it has a big population density and diversity.The bottom of the waters in the form of sand and fine sediment is not a good living environment for benthic animals. Factors affecting density and diversity are environmental conditions, habitat, and diet. The same factors also affect the nutritional content of Siasia (*S. nodus*). So, it is feared that when the population density of Siasia (*S. nodus*) becomes high in the water there will be competition for food which will have a direct impact on the nutritional content of Siasia (*S. nodus*). The population density of each water is different, so it is necessary to research the population density of Siasia (*S. nodus*) based on differences in the substrate of the seagrass area in the waters of Saparua Island, Maluku Province, Central Maluku Regency. The highest population density value of Siasia (*Sipunculus nodus*) in Saparua Island waters is found in Siri-sori State and the lowest is in Paperu Country.

Keywords: Density, proximate, *Sipunculus nodus*

To cite this article:
Matulessy, Y. M., Awan, A., Liline, S. (2021).The density of Siasia (*Sipunculus nodus*) population based on the differences in the substrate of the sea grasses beds on the waters of Saparua Island. *Bioedupat: Pattimura Journal of Biology and Learning,* Vol 1(1), 11-16.*DOI: https://doi.org/10.30598/bioedupatv1i1pp11-16*

INTRODUCTION

Sipuncula, a marine biota commonly called peanut worm, is a marine biota that is a little "controversial" (Silaban & Silaban, 2019). Some literature also refers to these animals as "unsegmented marine worms" or unsegmented marine worms (Barnes, 1987; Hutching & Johnson, 2003). Taxonomically, Sipuncula is not included in the Phylum Annelida Class Polychaeta, which is a taxon where the majority of marine worms can be grouped. Sipuncula, also known as Sipunculida (Tree of a life web project, 2008), is a biota that belongs to a

separate phylum under Kingdom Animalia, namely Phylum Sipuncula. Thus, although it is often called a "worm" the use of this term is still a matter of debate among scientists. The presence of Sipuncula in marine and estuary ecosystems is relatively less well known when compared to the marine worm Polychaeta.

Sipuncula is described at glance as a marine animal that looks like a worm but without segments. Its body is divided into the main body (trunk) and an introvert that can be pulled inward or behind. The ratio of the length of the two parts varies for each type. Sipuncula is a minor phylum in the large bilaterian animal group, namely a group of animals that are triploblastic, bilaterally symmetrical, and formed from three kinds of seed layers (endodermis, mesoderm, and ectoderm (Suwignyo & Sugiarti, 2005).

Kendari people generally process Siasia (*Sipunculus nodus*) as a food substitute for fish which is believed to have health benefits and is also used as herbal/natural medicine. Silaban & Nanlohy (2011) state that marine worms originating from Nalahia waters have the highest protein content of 17.13%. Zhang et al. (2011) state that in China sea worms have long been used as traditional medicine in treating tuberculosis, regulating gastric and spleen functions, as well as restoring health caused by pathogens. Purwaningsih (2014) states that the ethanol extract of sea worms has potential as antidiabetes through in vitro tests which can inhibit the activity of the a-glucosidase enzyme by 16-24 ppm.

Sipuncula of the species (*S. nudus*) is known mainly in the Maluku region as a food ingredient and as bait to catch fish such as tatu fish and garopa. *S. nudus* is known as "Siasia" by the people of Ambon Island. On the island of Rhun (Banda Islands) it is called "Kariong" and in the Bangka-Belitung Islands it is called "kekuak". Siasia (*S. nodus*) on the island of Saparua is used as a coastal water resource for the community. The capture of this biota by the local community has been carried out regularly. Siasia (*S. nodus*) is one of the biotas that is usually sought after when the seawater recedes by the local community. Usually, Siasia is consumed fresh (kohokoho, the general term for the Maluku people), sautéed, and sauteed with soy sauce. But a lot of people, who never know at all, or know but have never tasted it because the community's knowledge is still very low about the benefits of Siasia (*S. nodus*).

Silaban (2012) states that Siasia (*S. nodus*) usually lives in coastal areas, especially around seagrass areas, mangrove forests, and coral reefs. These biotas tend to inhabit the bottom of the waters, especially in the substrate. They have been categorized as infauna benthic organisms. Siasia (*S. nodus*) can inhabit soft and hard substrate areas. Based on its ecological function, seagrass forms associations with various kinds of marine life as food providers, shelters, and places to live, causing high diversity of marine life (Supono & Arbi, 2010). The basic substrate in the form of flat stones and gravel is a good living environment for macrozoobenthos because it is rich in organic compounds so that it has great density and diversity (Odum, 1994). Furthermore, Koesbiono (1979) said that the bottom of the waters in the form of sand and fine sediment is an unfavorable living environment for benthic animals.

Factors affecting population density and diversity are environmental conditions, habitat, and diet. The same factors also affect the nutritional content of Siasia (*S. nodus*). It is feared that when the population density of Siasia (*S. nodus*) becomes high in the water there will be competition for food which will have a direct impact on the nutritional content of Siasia (*S. nodus*). The population density of each water is different. So it is necessary to research the population density of Siasia (*S. nodus*) based on differences in the substrate on the seagrass area in the waters of Saparua Island, Maluku Province, Central Maluku Regency.

METHODS

Sampling

This research was conducted for 8 months starting from December 2019 to August 2020. It was carried out in the waters of Saparua Island (Figure 1). Sampling was carried out in several villages, including Paperu Village (rocky), Siri-soriAmalatu Village (rocky sand substrate), Kulur Village (sandy substrate), Ihamahu Village (sandy substrate), and Itawaka Village (rocky sandy substrate).

Sampling is done by using the roaming method. And then observations were made on the surface of the sediment. If there is some kind of dune and the roots of the seagrass are slightly raised, then the Sipuncula usually hides under the area. The bigger the dune that is formed and the roots of the seagrass are lifted, the bigger the body size and vice versa (Silaban & Silaban, 2019).

When sampling is carried out, positions are plotted using a machete. Then after using a machete, a sample of Siasia (*Sipunculus nodus*) was taken and was put in a labeled plastic sample. Data collection on the population density of Siasia (*S. nodus*) was carried out by counting the number of individual animals contained in squares at the research location and also documentation in the form of photographs.

Figure 1. Research Location Map

Population Density Calculation

The population density of Siasia (*Sipunculus nodus*) was analyzed using the population density formula (Brower, 1997) as follows:

$$D = \frac{Ni}{A}$$

Where :

D = Population Density of Siasia (*S. nodus*) (ind / m^2)

Ni = Number of Individuals

A = Area of the sampling plot (m^2)

Environmental parameter measurement

Environmental parameters calculation includes salinity, temperature, pH, and substrate (observed). The tools that were used are thermometer and pH meter by placing these tools at two different points, namely at the point near the coast of Station 1 (shallow) and the other at a point near the reef of Station 2 (deep). This tool is placed to determine the fluctuation of temperature and light intensity at the research location at a predetermined time range. The salinity meter uses a hand refractometer, where these measurements are carried out three times at each station. Substrate observation was carried out physically by observing the surface of the sediments found in each research location.

RESULTS AND DISCUSSION

Environmental parameter

Environmental parameters measured in this study are salinity, temperature, and water pH. The following are the results of environmental parameter measurements in 5 villages (Paperu, Siri-sori Amalatu, Kulur, Ihamahu, and Itawaka) with different substrates in the waters of the island of Saparua, Central Maluku district. The results of environmental parameters can be seen in Table 1.

Table 1. Results of measurements and observations of environmental parameters

Location	Temperature (°C)	Salinity (‰)	pH	Substrate
Ihamahu Village	26.4-28.5	29.8-34.3	7.4-7.7	Rocky, sandy
Paperu Village	29.9-36.5	36.5-41.8	7.7-7.9	Rocky
Itawaka Village	27.1-29.6	31.1-33.1	7.2-7.6	Rocky, sandy
Kulur Village	25.9-27.7	28.3-32.5	7.1-7.5	Sandy
Siri-sori Amalatu Village	29.2-35.9	34.9-38.5	7.6-7.8	Rocky, sandy

Temperature

Based on measurements made using a thermometer, the results show that the highest temperature is in Paperu, which ranges from 29.9-36.5°C and is followed by Siri-sori Amalatu Village with values ranging from 29.2-35.9°C The lowest temperature measurement values were in Kulur Village, namely 25.9-27.7°C and followed by Ihamahu and Itawaka villages which had temperature measurement values ranging between 26.4-

28.5 and 27.1-29.6°C. This is because in the waters of Paperu and Siri-sori Amalatu only a few rivers flow fresh water into the sea. So the temperature value of the water in the 2 villages namely Paperu and Siri-sori Amalatu is higher than the waters in 3 other villages (Ihamahu, Itawaka, and Kulur). The factors that influence the temperature value are rainfall, evaporation, river run-off, and season (Barus, 2004). In general, the temperature range in all research locations (Paperu, Siri-sori Amalatu, IhamahunItawaka, and Kulur) obtained during the study was tolerable by Siasia (*S. nudus*). This is because the temperature obtained is below the tolerance limit for the balance of the population structure of benthic animals in general.

According to Effendi (2003), the activity of microorganisms requires different optimum temperatures. Every 10°C increase in temperature will increase the decomposition process and oxygen consumption by 2-3 times. However, this increase in temperature is accompanied by a decrease in dissolved oxygen levels so that the presence of oxygen is often unable to meet the oxygen needs of aquatic organisms for metabolism and respiration. According to Ghufran & Baso (2007) in other words, the higher the water temperature, the lower the solubility of oxygen in the water, and vice versa.The higher the solubility of oxygen, the lower the water temperature. The indirect effect of temperature on the environment is to affect metabolism, the solubility of gases, including oxygen, and various chemical reactions in water. Welch (1980) states that the temperature that is dangerous for macrozoobenthos ranges from 35°C- 40°C.

Salinity

Based on the results of salinity measurements carried out using a refractometer. The results show that the highest salinity is in Paperu. Which is between 36.5-41.8 ‰ and followed by Siri-sori Amalatu Village with values ranging from 34.9-38.5 ‰. The lowest salinity measurement value is in Kulur Village, which is 28.3-32.5 ‰ and is followed by Ihamahu and Itawaka Villages which have salinity measurement values ranging between 29.8-34.3 and 31.1-33.1 ‰. This is because the results of water temperature measurements in 3 Villages: Ihamahu, Itawaka, and Kulur are lower than the water temperatures in 2 other countries, namely Paperu and Siri-soriAmalatu so that the evaporation rate is also low. The relationship between evaporation and salinity is directly proportional. The higher the level of evaporation in water, the higher the salinity and vice versa (Nybakken, 1992).

Furthermore, Nybakken (1992) also explained that the fluctuation of salinity in the intertidal area can be caused by two things. Firstly, due to heavy rain so that the salinity will decrease and secondly due to very high evaporation during the day. So, the salinity will be very high. In general, the salinity range in all study locations (Paperu, Siri-sori Amalatu, Ihamahu, Itawaka, and Kulur) obtained during the study was tolerable by Siasia (*S. nodus*). This is because the salinity obtained is below the tolerance limit for the balance of benthic animal population structures in general.

Mudjiman (1981) states that the salinity range considered suitable for macrozoobenthic life ranges from 15-45 ‰. The report from Irwan (1997) added that in low and high salinity waters, macrozoobenthos such as snails, worms (Annelida) and shellfish can be found. Payung (2017) states that salinity greatly affects the balance in the body of organisms through changes in water density and changes in osmotic pressure. The higher the salinity, the greater the osmotic pressure so that the organism must have the ability. Adapt to changes in salinity to some extent through osmoregulation mechanisms.

pH

Based on the results of pH measurements made using a ph meter, the results have almost the same values from one location to another. The highest pH measurement value ranged from 7.7-7.9 in Paperu Village while the lowest was between 7.1-7.5 in Kulur Village. Variations in pH values are influenced by many factors, including temperature, dissolved oxygen, alkalinity, the presence of various anions and cations as well as the type and stage of organisms (Pescod, 1973).

In general, the pH range in all research locations (Paperu, Siri-sori Amalatu, Ihamahu, Itawaka, and Kulur) obtained during the study was tolerable by Siasia (*S. nudus*). The life of aquatic organisms is greatly influenced by pH value fluctuations. In general, aquatic organisms are tolerant of a neutral pH value range. The ideal pH for aquatic organisms is generally between 7-8.5. Water conditions that are very acidic or very alkaline will threaten the survival of the organism because it will cause metabolic and respiration disorders (Odum, 1994).

Substrate

Based on the results of substrate observations carried out physically by observing the sediment surface in 5 villages, the results are varied. The substrate that was mostly found is the rocky sandy substrate. It is found in Ihamahu, Itawaka, and Siri-sori Amalatu villages. The least found substrate is the sandy and rocky substrate in Kulur and Paperu villages. Besides the existence of basic substrate organic compounds in the form of flat stones and gravel, it is a good living environment for macrozoobenthos so that it has great population density and

diversity (Odum, 1994). Furthermore, Koesbiono (1979) said that the bottom of the waters in the form of sand and fine sediment is not a very good living environment for benthic animals.

According to Seki (1982), the main organic components found in water are amino acids, proteins, carbohydrates, vitamins, and hormones which are also found in water. Only 10% of the organic material settles as a substrate to the bottom of the water. The basic substrate is the main factor affecting the life, development and diversity of macrozoobenthos (Hynes, 1976).

Population density

The results of the calculation on the population density of Siasia (*Sipunculus nodus*) in the waters of the island of Saparua show quite varied values. The results of observing the presence of individuals in each village can be seen in Table 2. The results of calculating the density of Siasia (*S. nodus*) can be seen in Table 3.

Table 2. Observations of the presence of individuals in each villages

Location	Number of Individuals in Squares to												Total of Individuals
	1	2	3	4	5	6	7	8	9	10	11	12	
Ihamahu	1	-	2	1	-	1	2	2	1	-	-	1	11
Paperu	-	2	1	-	2	-	-	1	-	1	1	-	8
Itawaka	2	-	1	2	2	1	2	2	2	2	3	2	21
Kulur	1	1	1	2	1	1	3	1	1	2	1	-	15
Siri-sori Amalatu	3	2	2	3	1	2	2	1	3	2	2	1	24

Table 3. Population density calculation result

Location	Subtrate Type	Value
Ihamahu	Rocky, sandy	1.21
Paperu	Rocky	0.89
Itawaka	Rocky, sandy	2.32
Kulur	Sandy	1.67
Siri-sori Amalatu	Rocky, sandy	2.67
Total		8.76

Based on the population density calculation results that have been obtained, each location shows different values. The total value of the 5 research locations is 8.76 ind / m^2. The highest value was in Siri-sori Amalatu Village which had a value of 2.67 ind / m^2 and was followed by Itawaka Village with a value of 2.32 ind / m^2. The lowest density calculation value was found in Paperu Village with a value of 0.89 ind / m^2 and followed by Ihamahu Village with a value of 1.21 ind / m^2. For 1 other village, namely Kulur, it has a value close to Ihamahu Village, which is 1.67 ind / m^2.

This difference occurs due to environmental conditions that do not support the life of the *S. nodus*, diet, type of substrate, and capture. In addition, other activities that can damage the seagrass ecosystem which can have an impact on the population density of *S. nodus* is the local community making the research location a place for boats to dock, the activities of catching gastropods and other marine animals which are carried out at low tide which are mentioned by the local community as "bameti" and tourism / recreational activities such as swimming. One of the activities of the ship that is suspected of destroying the seagrass is the entry and exit of ships into the coastal area. Tourism activities such as swimming on the beach are also thought to damage the seagrass. In contrast to the existing research station in Siri-Sori Amalatu Village which is quite far from residential areas so that no community activity is found, this makes seagrass cover still well preserved and the opportunities for fishing are less, this is shown in the condition of the sediment surface which is still awake.

CONCLUSION

Based on the results and discussion stated, it can be concluded that: The population density value of Siasia (*Sipunculus nodus*) in the waters of the island of Saparua shows quite a large difference. The highest population density value is in Siri-soriAmalatu Village and is followed by Itawaka Village. Meanwhile, the lowest population density calculation value was found in Paperu Village and followed by Ihamahu Village. For 1 other country, Kulur has a value that is close to the Ihamahu Village.

REFERENCES

Barnes, R. D. (1987). *Invertebrete zoologi*. Edisi ke-5. Sounders College Publising, Orlando, New York. pp. 893.

Barus, T. A. (2004). Faktor-faktor lingkungan abiotik dan keanekaragaman plankton sebagai indikator kualitas perairan danau toba (Environmental abiotic factors and the diversity of plankton as water quality indicators in lake toba, North Sumatera, Indonesia). *Jurnal Manusia dan Lingkungan*, 11(2), 64-72. (In Indonesian).

Brower, J., & Zar, J. (1997). *Field and laboratory methods for general ecology.* Spiral- bound, USA.

Effendi, H. (2003). *Telaah kualitas air bagi pengelolaan sumber daya dan lingkungan perairan* [*study of water quality for management of aquatic resources and environment*]. Kanisius, Yogyakarta. (In Indonesian).

Gufran, M. H., & Baso, B. T. (2007). *Pengelolaan kualitas air dalam budi daya perairan* [*Management of water quality in aquaculture*]. Rineka Cipta, Jakarta. (In Indonesian).

Hutching, P. A., & Johnson, R. T. (2003). *Australian aphroditidae (Polychaeta) delta database*. In R.S. Wilson, P.A. Hutchings dan C.J. Glasby (eds). Polychaetes: An Interactive Identification. Csiro, Melbourne.

Hynes, H. B. N. (1976). The *ecology with of running water*. Lipverpool University Press, England.

Irwan, D. (1997). *Prinsip-prinsip ekologi dan organisasi ekosistem & komunitas lingkungan* [*Ecological principles and organization of environmental ecosystems & communities*]. Bumi Aksara, Jakarta. (In Indonesian).

Koesbiono. (1979). *Dasar-dasar ekologi umum* [*General ecology basics*]. Sekolah Pasca Sarjana Program Studi Lingkungan, IPB. Bogor. (In Indonesian).

Mudjiman, A. (1981). *Budidaya udang windu* [*Tiger prawn cultivation*]. PT. Penebar Swadaya, Jakarta. (In Indonesian).

Nybakken, J. W. (1992). *Biologi laut suatu pendekatan ekologis* [*Marine biology an ecological approach*]. PT. Gramedia Pustaka Utama, Jakarta. (In Indonesian).

Odum, E. P. (1994). *Dasar-dasar ekologi* [*Ecological basics*]. Edisi Ketiga. Universitas Gadjah Mada Press, Yogyakarta. (In Indonesian).

Payung, W. R. (2017). *Keanekaragaman makrozoobentos (epifauna) pada ekosistem mangrove di sempadan sungai tallo kota makassar* [*Diversity of macrozoobenthos (epifauna) in the mangrove ecosystem on the tallo riverbank of the city of Makassar*]. Skripsi. Departemen Ilmu Kelautan Fakultas Ilmu Kelautan dan Perikanan Universitas Hasanuddin Makassar. (In Indonesian).

Pescod, M. B. (1973). *Investigation of rational effluent and stream standart for tropical countries, AIT*, Bangkok.

Purwaningsih, S. (2014). *Pengembangan pangan fungsional sebagai antidiabetes dari beberapa moluska yang mempunyai aktivitas antioksidan tinggi* [*Development of functional food as antidiabetic of several mollusks which have high antioxidant activity*]. Institut Pertanian Bogor, Bogor. (In Indonesian).

Seki, H. (1982). *Organic Material in Aquatik Ecosystem.* CRC Press. Inc, Folrida.

Silaban, B. (2012). *Profil Nutrisi Sipuncula (Cacing Kacang); Biota laut yang kontrovertif di Pulau Nusalaut, Maluku Tengah* [*Nutritional Profile of Sipuncula (Peanut Worm); Controversial marine life on Nusalaut Island, Central Maluku*]. Laporan hasil penelitian dosen pemula yang dibiayai dengan PNBP Lembaga Penelitian Universitas Pattimura. (In Indonesian).

Silaban, B, br., Nanlohy, E. E. E. M. (2012). Profil nutrisi sipuncula (cacing kacang) biota laut yang kontrovertif di pulau Nusalaut, Maluku Tengah. *Jurnal Triton*, 7(2):32-41. (In Indonesian).

Silaban, B., & Silaban, R. (2019). *Cacing kacang (sipuncula): Kajian biologi, ekologi, penangkapan dan pemanfaatan* [*Peanut worms (sipuncula): Study of biology, ecology, capture and use*]. CV. Budi Utama, Yogyakarta. (In Indonesian).

Supono., U. Y., & Arbi. (2010). Struktur komunitas ekhinodermata di padang lamun perairan kema, Sulawesi Utara [Structure of Echinoderm Community in Kema Seagrass Field, North Sulawesi]. *Oseanologi dan Limnologi di Indonesia*, 6(3), 329-342. (In Indonesian).

Suwignyo., & Sugiarti. (2005). *Avertebrata Air* [*Aquatic Invertebrates*]. *Jilid 1,* Swadaya, Jakarta. (In Indonesian).

Tree of Life Web Project. (2008). *Sipuncula. Peanut Worms. Version 09 January 2008 (temporary)*. http://tolweb.org/Sipuncula/2487/2008. 01.09 in The Tree of Life Web Project, http://tolweb.org/ [13 Maret 2021].

Welch, E. B. (1980). *Ecological Effect of Wastewater*. Cambridge University Press, Cambridge London, New York New Rochelle.

Zhang, C. X., Zi, R. D., Qiu, X. C. (2011). Anti inflammatory and anti-nociceptive activities of *Sipunculus nudus* L. Extract. *Journal of Ethnopharmacology,* 137(3), 1177-1182.

BIOEDUPAT:
Pattimura Journal of Biology and Learning
https://ojs3.unpatti.ac.id/index.php/bioedupat
e-ISSN 2775-4472

Research Article

Decreasing SGPT level and macrophage activity through CD68 expression in the Balb/c mice (*Mus musculus*) liver infected with *Salmonella typhi* after treating with atung seeds (*Parinarium glaberimmun* Hassk)

Eifan B. Pattiasina[1,*], Pieter Kakisina[2], Ferymon Mahulette[3]

[1] Postgraduate Student of Biology Education, Pattimura University,
Jl. Dr. Tamaela, Ambon, Maluku 97114, Indonesia
[2] Department of Biology, Faculty of Mathematics and Natural Science, Pattimura University,
Jl. Ir. M. Putuhena, Ambon, Maluku 97233, Indonesia
[3] Study Program of Biology Education, Faculty of Teacher Training and Education, Pattimura University,
Jl. Ir. M. Putuhena, Ambon, Maluku 97233, Indonesia
** corresponding author: pattiasina2905@gmail.com*

Received: 16 February 2021 Revised: 10 March 2021 Accepted: 21 April 2021

ABSTRACT

Normally macrophages are always in the body and spread in various body tissues such as liver tissue (Kupffer cells). Macrophages in tissue can be identified by the expression of several markers, in humans the marker is CD68. The increase and decrease in macrophage activity in the liver can also be indicated by an increase in SGPT levels so that atung seeds have the ability to inhibit the growth of *S. tyhpi* bacteria which contain tannin compounds which can damage microbial cell walls and form bonds with microbial functional proteins so that bacterial growth is inhibited. The purpose of this study was to determine the SGPT levels and macrophage activity. The method used is laboratory experiment. The results showed an increase in SGPT levels in the positive control (87.00 ± 2,915) and a concentration of 25% (84.20 ± 3,962) and a decrease in SGPT levels in the negative control (50.80 ± 2.168 *), 50% concentration (78.20 ± 3.114 *) and concentrations. 75% (58.20 ± 3,834), decreased macrophage activity in the liver also occurred at a concentration of 50% and at a concentration of 75%, the liver was normal, which was indicated by the resulting brown expression.

Keywords: SGPT, Macrophage Activitty, CD68, Salmonella typhi, atung seeds

To cite this article:
Pattiasina, E. B., Kakisina, P., & Mahulette, F. (2021). Decreasing SGPT level and macrophage activity through CD68expression in the Balb/c mice (*Mus musculus*) liver infected with *Salmonella typhi* after treating with atung seeds (*Parinarium glaberimmun* Hassk). *Bioedupat: Pattimura Journal of Biology and Learning*, Vol 1(1), 17-23. *DOI: https://doi.org/10.30598/bioedupatv1i2pp17-23*

INTRODUCTION

Infection-causing bacteria can usually be killed using drugs containing synthetic antibiotics. Therapy of infection with synthetic antibiotics can bring its own problems, namely the presence of bacterial resistance to these antibiotics and symptoms that indicate side effects with antibiotics. Efforts to find other SGPT alternatives in

the treatment of infection are the use of traditional medicines derived from plants. The use of plant materials as medicine is because plants contain natural compounds that have the potential to be antibacterial, such as flavonoids, tannins, steroids, polyphenols, terpenoids, alkaloids, and saponins. Atung is a type of wild plant that is included in the Rosaceae tribe, is a megatherm plant and is very limited in the tropics. Atung is consumed as an additional ingredient in the preparation of raw fish-based dishes and also in rujak (salad) mixtures in Maluku. The atung plant is thought to be able to prevent diarrhea or stomach pain if you consume foods such as raw fish or salad (Sopaheluwakan, 2009). Atung seeds contain very high fatty oil, namely 42.68%, and other contents, respectively, protein (5.38%), crude fiber (4.26%), ash (2.14%), tannins (1.65%), water (8 - 13.15%), ethanol soluble components 95% (7.30%) and components that are not detected around 23.20% (Adawiyah, 1998).

Tannins have antibacterial activity related to their ability to inactivate microbial cell adhesin as well as activate enzymes, and interfere with protein transport in the inner layer of cells (Cowan, 1999). Tannins also targeted polypeptides cell wall so that the formation of the cell walls becomes less perfect. This causes bacterial cells to become lysed due to osmotic and physical pressure so that the bacterial cells will die (Sari, 2011). The content of compounds in plants can trigger the system and can activate cellular components of the immune system, for example the function of phagocytosis without affecting humoral or cellular immunity (Tursinawati & Dharmana 2015). People have started to choose traditional medicines to increase the immune system so that they are not susceptible to various diseases (Meyer et al., 2008).

Macrophages play an important role in an immune response, not only for innate immune response, but also for inducing adaptive immune response. Macrophages are phagocytes, which are the first known antigen presenting cells (APC). Macrophages and neutrophils are the first defense of the innate immune system against microbes and play an important role in bacterial phagocytosis (Samaranayake, 2012). Macrophages in tissue can be identified by the expression of several marker proteins, in humans the marker is CD68 (Nucera et al., 2010). CD68 is type I glycoprotein transmembrane mainly localized in the endosome compartment, due to its intracellular localization (Saito et al., 2003) and its characteristic structure (Holness et al., 1993). Macrophages can be found in circulation and in networks (Baratawidjaja & Rengganis, 2010). Among the cytokines produced are interleukin-1 (IL-1) which is needed to stimulate the proliferation of T and B cells, fibroblasts and vascular endothelial growth factors which are needed to repair damaged tissue (Nah et al., 2008; Franca et al., 2010).

The destruction of cells causes changes in transport function and membrane permeability resulting in the release of the SGPT enzyme in the cytoplasm into the blood circulation. Alanine Aminotransferase (SGPT), is an enzyme found in liver cells and is effective in diagnosing hepatocellular destruction. SGPT enzymes are mostly found in the liver, so it is considered to be more specific to detect SGPT disease compared to AST (Sadikin, 2002). Tannin compounds in atung seeds are thought to reduce SGPT levels and macrophage activity through CD68 expression in the liver of *S. typhi*-infected mice. This requires a comprehensive research to prove it.

METHODS

Research design

This type of research is a laboratory experiment to determine the decrease in macrophage activity through the expression of CD68 in the liver of mice treated with steeping atung seeds. The design in this study was a completely randomized design (CRD). In this study, there were 3 treatments of concentrating the steeping of atung seeds, namely 25%, 50% and 75%, as well as control, each treatment there were 5 replications of SGPT levels. The research design is shown in Table 1.

Table 1. Research design

Treatment	Repetition				
	1	2	3	4	5
Kontrol Positif (K^+)	K1	K2	K3	K4	K5
Kontrol Negatif (K^-)	K1	K2	K3	K4	K5
P1	P 1.1	P 1.2	P 1.3	P 1.4	P 1.5
P2	P 2.1	P 2.2	P 2.3	P 2.4	P 2.5
P3	P 3.1	P 3.2	P 3.3	P 3.4	P 3.5

The population in this study were Balb / c mice (*Mus musculus*), atung fruit seeds, *Salmonella typhi*. The samples in this study were male Balb / c mice, old atung fruit seeds, Salmoella typhi with a cell number of 3×10^8.

Preparation of Salmonella typhi bacterial suspension

S. typhi bacteria that have been identified by gram staining, are rejuvenated first so that a culture age of 18-24 hours is obtained. Then the bacteria were suspended in physiological NaCl solution. The suspension density was adjusted according to the standard 1 Mc solution. Farland corresponds to 3 x 108 cells / mL (Forbes et al., 2007).

Observation of CD68 expression using immunohistochemistry

- Observation of CD68 expression was carried out using immunohistochemical staining methods
- The liver histological preparations were washed with PBS (Phosphate Buffer Saline) pH7.4 for 3 minutes.
- Endogenous perioxide removal using 3% H2O2 for 20 minutes. Then the preparations were washed with PBS pH 7.4 for 3 minutes, 3 times.
- Blocking, the preparation was dripped with primary CD68 antibody, then incubated at 4□C for one night. After that the preparations were washed with PBS pH 7.4 for 3 minutes, 3 times.
- The preparations were dropped with a secondary antibody labeled AP (Alkaline Phosphatase) 1: 2500 (anti IgG AP Labeled), incubated at room temperature for 60 minutes. After that the preparations were washed with PBS pH 7.4 for 3 minutes, 3 times.
- The preparations were dripped with SAN-HRP (Strep Avidin Horseradish Peroxidase) and incubated at room temperature for 60 minutes. After that the preparations were washed with PBS pH 7.4 for 3 minutes, 3 times.
- The application of chromogen by dripping 3,3-diaminobenzidine (DAB) solution, and incubating it at room temperature for 20 minutes, then washing it with distilled water for 5 minutes, 3 times.
- Counterstain uses hematoxylin. Incubated at room temperature for 20 minutes. Then drop the tap water, wash it with distilled water for 5 minutes 3 times.
- Dehydration was done with a solution of alcohol with a grade of 70%, 80%, 90% and 100% and absolute alcohol I and II for 1 minute each.
- Clearing was carried out with xylol I and xylol II each for 3 minutes, then the preparations were dried and aired
- Mounting using an entellant, then covered with a glass slide, labeled with the name of the preparation and then observed under a microscope
- If CD68 is detected, it will turn brown.

SGPT level calculation

SGPT levels were measured using a spectrophotometer (Shimadzu UV-1201 V). SGPT is determined based on enzymatic reactions. The serum is mixed with the reagent kit at a temperature of 25/30. 200 microliter of serum was taken and 1000 microliter of reagent kit. After homogeneity, read the absorbance at 1, 2, and 3 minutes using a spectrophotometer with a wavelength of 334 nm. The results of the SGPT activity are expressed in units / liter (U / L) which is the number of enzymes in one liter of serum that can produce NAD + at the same time unit. SGPT levels using the formula: SGPT (U / L) = ΔAbs. / Min x 1768. SGPT level data were analyzed using ANOVA, while macrophage activity through CD68 expression in the liver of *S. typhi* infected mice after steeping atung seeds was analyzed descriptively.

RESULTS AND DISCUSSION

The results of SGPT levels in Balb / C mice (*Mus musculus*) infected with *S.typhi* after steeping atung seeds (*Parinarium glaberimmum* Hassk) showed that there was a decrease in SGPT levels. The test results can be seen in Table 2.

Tabel 2. Average SGPT Levels after Steeping Atung Seed (*Parinarium glaberimmmum* Hassk) in Balb / C Mice (*Mus musculus*)

Treatment	Average SGPT Level (U/L)
Kontrol (-)	50.80±2.168[a]
Kontrol (+)	87.00±2.915[d]
Levels 25%	84.20±3.962[d]
Levels 50%	78.20±3.114[b]
Levels 75%	58.20±3.834[c]

Note: Superscript the same letter shows no significant difference in the level of confidence 95% (p<0,05).

The Duncan test results on the average SGPT level in mice showed that there were differences in each treatment group, this was indicated by the giving of an asterisk to the average value. In the negative group it was

different from the positive control, treatment levels were 25%, 50% and 75%. The positive control has a difference with the treatment concentration of 50% and 75%, while for the 25% level treatment has no difference with the positive control. This is because the results of the average SGPT level and standard deviation in the Duncan test, positive control with 25% level treatment, showed no difference.

Table 1 shows the average SGPT level in the negative group with an average SGPT level of 50.80 U / L. This value is used as a reference to see the difference in each treatment because the negative control is not treated. In the positive control, the average SGPT level was 87.00 U / L. This value is greater than negative control. The positive group of mice was induced by Salmoella typhi which could damage the liver cells of the mice, causing a high average SGPT level.

At a concentration of 25%, the average SGPT level is 84.20 U / L. When compared with positive controls, the average SGPT value was still high. At a concentration of 50%, it showed an average SGPT level of 78.20 U / L and experienced a significant decrease when compared to the positive control and the level of 25%. The result of SGPT levels at 75% is a concentration that has an average SGPT level of 58.20 U / L which indicates that at 75% levels of liver cells are in a normal state with a significant decrease in SGPT levels when compared to negative controls which are the inner group normal condition (no treatment).

From the measurement results of SGPT levels, histopathological observations were made of macrophage activity through the expression of CD68. The results of macrophage activity through CD68 expression can be seen in Figure 1.

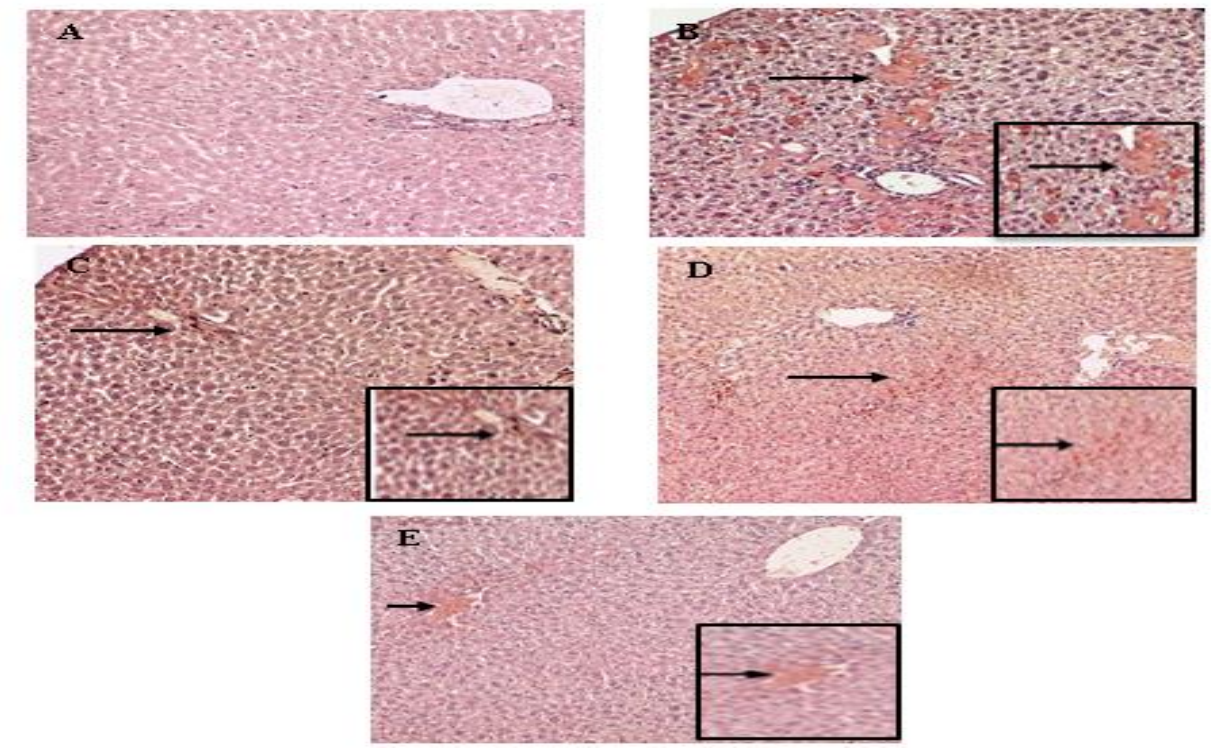


Figure 1. Macrophage activity through CD68 expression in the liver of Balb / C mice infected with Salmonella tphi after steeping atung seeds. Negative control (A), positive control (B), concentration 25% (C), concentration 50% (D), concentration 75% (D). The staining was carried out using the indirect immunohistochemical method showing that cells expressing CD68 were shown as brown cells observed under a light microscope with a magnification of 1000x. The preparations were ligated with CD68 monoclonal antibody, a secondary antibody labeled peroxidase, then characterized by double staining using DAB and hematoxylin eosin. The arrows indicate macrophage activity.

Figure 1 shows the activity of macrophages through CD68 expression in the liver of mice infected with *Salmonella typhi* after steeping atung seeds which shows that the activity of macrophages expressed through CD68 is marked in brown in histopathology, namely the activity of macrophages increases in the phagocytosis process when a lot of brown color is produced. and there is a decrease in macrophage activity if the brown color is low. Serum Glutamic Pyruvic Transaminase (SGPT) is the main enzyme found in liver cells and is effective in diagnosing hepatocellular destruction (Kee & Lefever, 2014). SGPT normal levels are expressed in the U / L

range and will increase if there is liver damage (Kosasih, 2008). SGPT levels will increase due to *Salmonella typhi* and produce endotoxins and will multiply and attack liver tissue resulting in an increase in SGPT levels (Widyastuti, 2016).

The results showed that the average SGPT levels in mice infected with *S. typhi* after steeping atung seeds with a concentration of 75% contained secondary metabolites, namely tannins with high concentrations so that the SGPT level decreased, namely the average SGPT level was 58.20 U / L with standard deviation 3,834, when compared with the *S. typhi* infected group of mice after being treated with steeping atung seeds with a concentration of 25% had an average SGPT level of 84.20 U / L with a standard deviation of 3,962 and a concentration of 50% with an average value of SGPT levels 78.20 U / L with a standard deviation of 3.114. The decrease in SGPT levels in the liver of *S. typhi*-infected mice (*Mus musculus*) was treated with the infusion of atung seeds (*Parinarium glaberimmum* Hassk) with a concentration of 75% lower than the concentrations of 25% and 50%. It is assumed that the tannin content in the infusion of atung seeds with a high concentration of 75% so that the ability to inhibit the growth of *S. typhi* is better than the concentrations of 25% and 50%. The results of this study indicated that there was a decrease in the levels of SGPT in mice (*Mus musculus*) infected with *S. typhi* treated with 25% to 75% concentration of atung seeds, so it can be said that the decrease in SGPT levels was in line with the increase in the concentration of atung seed steeping (*Parinarium glaberimmum* Hassk). This opinion is in accordance with Lee et al. (2013) which states that tannins in low concentrations are not able to inhibit bacterial growth, whereas at high concentrations tannins work by forming stable bonds with bacterial proteins so that the bacterial protoplasm is coagulated. Furthermore, Sari (2011) stated that besides that tannins also have a target on cell wall polypeptides so that the formation of the cell walls becomes less than perfect. Because lysis occurs in the bacterial cell wall, it causes osmotic and physical pressure so that the bacterial cell will die.

The *S. typhi* infection given will stimulate macrophages to activate and move to the source of infection. The lipopolysaccharide content in the cell walls of *S. typhi* is a signal for macrophages to activate. Macrophage activation has a high ability to carry out phagocytosis. These cells will destroy all foreign bodies such as germs, damaged cells, tumor cells, colloid objects, and large molecules (Chanana et al., 2007). Normally macrophages are always in the body and scattered in various body tissues such as lungs (alveolar macrophages), liver tissue (Kupffer cells), joint spaces (synovial cells type A), central nervous system (Schwann cells or microglia), space serous (pleural and peritoneal macrophages), binding tissue (histiocytes). Macrophages in tissue can be identified by the expression of several markers, one of which is CD68 (Nucera et al., 2010).

CD68 in the liver of mice (Mus musculus) infected with *S. typhi* after steeping atung seeds (*Parinarium glaberimmum* Hassk) can be seen in Figure 1. Histopathological preparations of the mice's liver can be seen from the activity of macrophages using the immunohistochemical method. Immunohistochemistry is a method of staining the substance or active ingredient in tissues using the basic principles of immunology, namely the binding of the active ingredient (antigen) to a specific active site by an anti-active ingredient (antibody). The results of the antigen and antibody reactions can be identified in the specimen when the antibody is bound by a marker.

The results showed that the histopathological preparations of the mice's liver could be seen the presence of macrophage activity using the immunohistochemical method through the expression of CD68. The presence of macrophage activity in the histopathological liver tissue of mice can be visualized with a brownish color. The brown color is the result of the interaction between the antigen that binds to the primary antibody (110kDA and CD68) and the secondary antibody in the form of Strep Avidin horseradish peroxidase (SA-HRP) and the diamino benzidine (DAB) substrate. Chromogen DAB (3,3- diaminobenzidine tetrahydrocloride) contains H_2O_2 peroxide as a marker substance that will form a complex with the peroxidase enzyme in the SA-HRP complex (Strepvidin horseradish peroxidase). The complex formed from DAB chromogen will form a dark brown color. This chromogen has a very strong bond with peroxide so that the dehydration and clearing processes will not change color.

In the negative control group, CD68 in the liver of mice was not expressed, this was because the negative control group was the untreated group so that there was no interaction between the antigen that binds to the primary antibody (110kDA and CD68) and the secondary antibody in the form of Strep Avidin horseradish peroxidase (SA- HRP) as well as the diamino benzidine (DAB) substrate. In the positive control group of mice infected with S. typhi after steeping atung seeds showed that CD68 in the liver of the expressed mice were marked with a brown color, this was due to the interaction between antigens that bind to primary antibodies (110kDA and CD68) and secondary antibodies. in the form of Strep Avidin horseradish peroxidase (SA-HRP) and diamino benzidine (DAB) substrate. The identification of macrophage activity in the liver histology of mice showed a bond between antigen and primary antibody visualized with brownish color on the hepatocytes and synocytes.

The positive control mice infected with *S. typhi* and not treated with steeping atung seeds showed CD68 in the liver of the expressed mice which was marked by a brown color which was produced a lot when compared to the *S.typhi* infected group after steeping atung seeds with a concentration of 25%, 50% and 75%. This is because CD68 can be significantly regulated in macrophages that respond to inflammatory stimuli (O'Reilly et al., 2003; O'Reilly et al., 2007) and CD68 as a myeloid-specific marker, which is expressed in abundance by macrophages. (Betjes et al., 1991). In addition, CD68 is also used as a histochemical / cytochemical marker of inflammation associated with monocyte / macrophage involvement (Ferenbach, 2008). CD68 is also used to identify cells from macrophage lineages such as histiocyte tissue, Kupffer cells, and osteoclasts (Brooks et al., 2009). Several studies have demonstrated a significant upregulation of CD68 expression in macrophages in response to inflammatory stimuli such as exposure to oxLDL (Ramprasad et al., 1996; Yoshida et al., 1998; Tsukamoto et al., 2002) and chronic stimulation with bacterial lipopolysaccharide (LPS) or inflammatory cytokine interferon γ (IFN-γ).

The activity of macrophages in the infusion of atung seeds with a concentration of 25% increased, it is thought that the tannin content was in low concentrations so that it was not able to inhibit *S. typhi*, when compared with concentrations of 50% and 75%. Whereas for the 50% concentration there was a decrease in macrophage activity when compared to the 25% concentration. At a concentration of 75% there was a decrease in the activity of macrophages which was marked by CD68 in the liver of the mice which was expressed to produce a small amount of brown color, this indicated that the tannin content was at a concentration of 75% in large quantities so that the tannin content in atung seeds had the ability to inhibit the growth of *S. typhi* and can damage microbial cell walls and form bonds with microbial functional proteins and cause inhibited bacterial growth (Sudira et al., 2011).

CONCLUSION

From the results of the research conducted, it can be concluded that the infusion of atung seeds (*Parinarium glaberimmum* Hask) with a concentration of 75% was able to reduce SGPT levels in mice (*Mus musculus*) infected with *S. typhi* after steeping atung seeds (*Parinarium glaberimmum* Hassk) when compared with a concentration of 25% and 50%. Steeping atung seeds (*Parinarium glaberimmum* Hask) with a concentration of 75% was able to reduce the activity of macrophages through the expression of CD68 in the liver of mice (*Mus musculus*) infected with *S. typhi* after being treated with the infusion of atung seeds (*Parinarium glaberimmum* Hask) which is characterized by a brown color produced less when compared with concentrations of 25% and 50%.

REFERENCES

Adawiyah, D. R. (1998). Studi pengembangan metode ekstraksi komponen antimikroba biji buah atung (*Parinarium glaberrimum* Hassk) [Study on the development of antimicrobial component extraction methods of atung fruit seeds (*Parinarium glaberrimum Hassk*)]. S2 thesis. PPs. IPB: Bogor. (In Indonesian)

Baratawidjaja, K. G., & Rengganis, I. (2010). *Imunologi Dasar* [*Basic Immunology*]. 9th ed. BP FKUI, Jakarta:. (In Indonesian)

Betjes, M. G., Haks, M. C., & Tuk, C. W. (1991). Monoclonal antibody EBM11 (anti-CD68) discriminates between dendritic cells and macrophages after short-term culture. *Immunobiology*, 183, 79–87. doi: https://doi.org/10.1016/S0171-2985(11)80187-7

Brooks, E., Simmons-Arnold, L., & Naud, S. (2009). Multinucleated giant cells' incidence, immune markers, and significance: a study of 172 cases of papillary thyroid carcinoma. *Head and Neck Pathology*, 3, 95–99. doi: https://doi.org/10.1007/s12105-009-0110-9

Chanana, V., Ray, P., Rishi, D. B., & Rishi, P. (2007). Reactive nitrogen intermediates and monokines induce caspase-3 mediated macrophage apoptosis by anaerobically stressed *Salmonella typhi*. *Clinical & Experimental Immunology*. 150(2): 368-374. doi: https://doi.org/10.1111/j.1365-2249.2007.03503.x

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12, 564-582.

Ferenbach, D., & Hughes, J. (2008). Macrophages and dendritic cells: what is the difference?. *Kidney International*, 74, 5–7. doi: https://doi.org/10.1038/ki.2008.189

Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2007). *Bailey and Scott's Diagnostic Microbiology*. 12th ed. Missouri: Mosby.

Franca, A. C. H., Eduardo, L. F., Joanna, C. M., Valeria, C. C., Uender, C. R. P., & Claudemir, B. (2010) Immunomodulatory effects of herbal plants plus melatonin on human blood phagocytes. *International Journal of Phytomedicine*, 2, 354–62. doi: https://doi.org/10.5138/ijpm.2010.0975.0185.02050

Holness, C. L., da Silva, R. P., Fawcett, J., Gordon, S., & Simmons, D. L. (1993). Mac-rosialin, a mouse macrophage-restricted glycoprotein, is a member of the lamp / lgp family. *Journal of Biological Chemistry*, 268, 961–966.

Kee., & Lefever, J. (2014). *Pedoman pemeriksaan laboratorium dan diagnostik* [*Laboratory and Diagnostic Examination Guidelines*]. Edition 6. EGC, Jakarta.

Kosasih, E. N., & Kosasih, A. S. (2008). *Tafsiran hasil pemeriksaan laboratorium klinik* [*Interpretation of Clinical Laboratory Examination Results*]. Karisma Publishing Group, Tangerang.

Lee, J. H., Park, J. H., Cho, H. S., Joo, S. W., Cho, M. H., & Lee, J. (2013). Anti-biofilm activities of quercetin and tannic acid against Staphylococcus aureus. *Biofouling*, 29(5), 491-499.

Meyer, S. L. F., Lakshman, D. K., Zasada, I. A., Vinyard, B. T., & Chitwod, D. J. (2008). Dose response effects of clove oil from *Syzygium aromaticum* on the root-knot nematode Meloidogye incognita. *Pest Management Science,* 64, 223-229. doi: https://doi.org/10.1002/ps.1502

Nah, S. S., Choi, I. Y., Lee, C. K., Oh, J. S., Kim, Y. G., Moon, H. B., & Yoo. (2008). Effects of advanced glycation and products on the expression of COX2, PGE2 and no in human osteoarthritic chondrocytes. *The Journal of Rheumatology*, 47, 425-431. doi: https://doi.org/10.1093/rheumatology/kem376

Nucera, S., Biziato, D., & Palma, M. D. (2010). The interplay between macrophages and angiogenesis in development tissue injury and regeneration. *The International Journal of Developmental Biology*, 55, 495-503. doi: https://doi.org/10.1387/ijdb.103227sn

O'Reilly, D., & Greaves, D. R. 2007. Cell-type-specific expression of the human CD68 gene is associated with changes in Pol II phosphorylation and short-range intrachromosomal gene looping. *Genomics*, 90: 407–415. doi: https://doi.org/10.1016/j.ygeno.2007.04.010

O'Reilly, D., Quinn, C. M., & El-Shanawany, T. (2003). Multiple ets factors and interferon regulatory factor-4 modulate CD68 expression in a cell type-specific manner. *Journal of Biological Chemistry*, 278, 21909–21919. doi: https://doi.org/10.1074/jbc.M212150200.

Ramprasad, M. P., Terpstra, V., & Kondratenko, N. (1996). Cell surface expression of mouse macrosialin and human CD68 and their role as macrophage receptors for oxidized low density lipoprotein. *Proceedings of the National Academy of Sciences,* 93, 14833–14838. doi: https://doi.org/10.1073/pnas.93.25.14833

Sadikin, M. (2002). *Biokimia enzim* [*Enzyme biochemistry*]. Widya medika, Jakarta. (In Indonesian).

Saito, G., Swanson, J. A., & Lee, K. D. (2003). Drug delivery strategy utilizing conjugation via reversible disulfide linkages: role and site of cellular reducing activities. *Advanced drug delivery reviews*, 55(2), 199-215.

Samaranayake, L. 2012. *Essential Microbiology for Dentistry*. 4rd ed. Elsevier, Hongkong. pp. 100-115.

Sari, F. P., Sari, S. M. (2011). *Ekstraksi zat aktif antimikroba dari tanaman yodium* (*Jatropha multifi*da Linn) *sebagai bahan baku alternatif antibiotik alami* [Extraction of antimicrobial active substances from iodine plants (*Jatropha multifida* Linn) as alternative raw materials for natural antibiotics]. Fakultas Teknik Universitas Diponegoro: Semarang. (In Indonesian).

Sudira, I. W., Merdana, I., & Wibawa, I. (2011). Uji daya hambat ekstrak daun kedondong (*Lannea grandis* Engl) terhadap pertumbuhan bakteri *erwinia carotovora* [Inhibition test of kedondong leaf extract (*Lannea grandis* Engl) on the growth of the bacteria *Erwinia carotovora*]. *Buletin Veteriner Udayana,* 3(1), 45-50. (In Indonesian).

Tursinawati, Y., & Dharmana, E. (2015). Efektivitas pemberian kombinasi produk herbal antibiotik terhadap infeksi Salmonella typhimurium pada mencit Balb/c. *University Research Coloquium*, (2), 231-237.

Tsukamoto, K., Kinoshita, M., & Kojima, K. (2002). Synergically increased expression of CD36, CLA-1 and CD68, but not of SR-A and LOX-1, with the progression to foam cells from macrophages. *Journal of Atherosclerosis and Thrombosis*, 9, 57–64. doi: https://doi.org/10.5551/jat.9.57

Widyastuti, R. (2016). Hubungan kadar SGPT (*Serum Glutamic Pyruvic Transminase*) dengan titer widal antigen O *Salmonella tyhpi* pada penderita demam typhoid [Relationship between SGPT (Serum Glutamic Pyruvic Transminase) levels with *Salmonella tyhpi* widal antigen O titer in typhoid fever patients]. *The Journal of Muhammadiyah Laboratory Technologist,* 2(1), 43- 53. (In Indonesian).

Yoshida, H., Quehenberger, O., & Kondratenko, N. (1998). Minimally oxidized low-density lipoprotein increases expression of scavenger receptor A, CD36, and macrosialin in resident mouse peritoneal macrophages. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 18, 794–802. doi: https://doi.org/10.1161/01.atv.18.5.794

BIOEDUPAT:
Pattimura Journal of Biology and Learning
https://ojs3.unpatti.ac.id/index.php/bioedupat
e-ISSN 2775-4472

Research Article

Problem-based learning model: Its application and effect on learning outcomes

La Amaludin [1,*], Johanis F. Rehena [2], Hermalina Sinay [2]

[1] Madrasah Ibtidaiyah Swasta, Jl. Trans Seram, Kairatu 97566, Indonesia
[2] Study Program of Biology Education, Faculty of Teacher Training and Education, Pattimura University, Jl. Ir. M. Putuhena, Ambon 97233, Indonesia
** corresponding author: la.amaludin05@gmail.com*

Received: 17 February 2021 Revised: 20 March 2021 Accepted: 21 April 2021

ABSTRACT

The problem-based learning model is a model that plans a problem that is given by educators to be solved by students. This PBL learning model has learning conditions oriented to concrete problems. This research aimed to determine the effect of the problem-based learning (PBL) model on the learning outcomes of class VIII students on Plant Motion Material at MTs Al-Muhajirin Waihatu. The type of research used in this research is descriptive quantitative analyzed using inferential statistics. This research was conducted for three months starting from August 10 to October 10, 2020. The location of this research was carried out in class VIII on Plant Motion Material at MTs Al-Muhajirin Waihatu with a sample of 90 students. To analyze the data obtained through questionnaires and test results, students were processed using descriptive analysis with the SPSS 20 application. The results showed that the application of the problem-based learning (PBL) learning model to the learning outcomes of students in plant motion reached the final qualifications of students (Test Formative) describes 90 students (100%) who succeeded in achieving the MCC value (minimum completeness criteria). The problem-based learning model has a significant effect on the learning outcomes of class VIII students of MTs Al-Muhajirin Waihatu, this can be seen from the significant level of 0.626 with the strong category.

Keywords: Problem based learning, learning outcome

To cite this article:
Amaludin, L., Rehena, J. F., & Sinay, H. (2021). Problem-based learning model: Its application and effect on learning outcomes. *Bioedupat: Pattimura Journal of Biology and Learning,* Vol 1(1), 24-31. *DOI: https://doi.org/10.30598/bioedupatv1i1pp24-31*

INTRODUCTION

Education has an important role in human life. The level of human quality can be seen from the quality of education. Education is an activity that is carried out on purpose and in a planned manner carried out by adults who have the knowledge and skills to provide competence to students so that they have basic knowledge, skills, and values that reflect thinking and acting for the creation of the desired human resources, as al-insan-al-Kamil (Idi, 2017). Biology lessons are interesting and fun lessons that are related to everyday life so that biology learning can be carried out properly and achieving maximum learning objectives, students must be able to understand the concepts or material provided by the teacher during the learning process (Kurniawan, 2013). In fact, in learning, of course, some steps need to be considered. These steps are in line with the learning model that is implemented so that the learning model is easily accepted by students. In line with this, educators need to

change their mindset to help students become more active by applying a scientific approach (Saputri & Febriani, 2017).

The problem-based learning (PBL) learning model is a model that plans a problem that is given by educators to be solved by students. This PBL learning model has learning conditions oriented to concrete problems. In line with that, learning a basic concept of a subject matter requires a learning model that orientates students to problems in the real world to train students' thinking skills (Trianto, 2015). Students' thinking skills can be guided by solving problems in small groups using a problem-based learning model. A problem given to students will be solved with the ability they have to build concepts in the material they are learning. Thus, with the problem-based learning model, it can train students' ability to solve problems. Learning outcomes are the result of an interaction between teaching and learning activities and are usually indicated by the test scores given by the teacher (Bakkenes et al., 2010). Skaggs & Bodenhorn (2006) states that learning outcomes indicate learning achievement, while learning achievement is an indicator of the degree of change in student behavior.

The results of the researchers' initial observations at MTs Muhajirin Waihatu are the researchers saw that the learning outcomes were still low. The low learning outcomes of students are due to educators who dominate the learning process. In addition to these factors, educators still use conventional learning models where learning is still centered on educators so that the achievement of student learning outcomes is low. To solve this problem, it is necessary to apply the problem-based learning (PBL) learning model in a condition oriented to concrete problems so that student learning outcomes increase. Furthermore, it is measured how much influence the problem-based learning (PBL) learning model has on student learning outcomes.

According to Riyanto (2009), the application of the problem-based learning model is 1) The teacher gives problems to students who are in small groups. 2) Then, each group discusses the problem their basic knowledge and skills. 3) Students also formulate problems and hypotheses. 4) Students actively seek information and data related to problems that have been formulated, students are diligent in discussing with their groups to solve the problems given by reporting the data that has been obtained. 5) The closing discussion activity is carried out when the process has obtained the right solution. The ability to solve problems can be identified into five indicators, namely: 1) Providing simple explanations, 2) Building basic skills, 3) Concluding, 4) Providing further explanations, 5) Organizing strategies and techniques (Suprijono, 2016). Effect of Problem based learning models. Regarding the learning outcomes above, it is supported by the opinion of Hasibuan (2018) that the factors that influence learning outcomes are classified into two, namely internal factors, which can be in the form of interests, talents, intelligence, perceptions and so on relating to students as individuals.

METHODS

Types of research

This research is designed using descriptive quantitative research that is analyzed using inferential statistics. Quantitative research is to see the effect of the problem-based learning (PBL) model on the learning outcomes of class VIII students on Plant Motion Material at MTs Al-Muhajirin Waihatu. This research was conducted from 10 August-10 October 2020. The population in this study included all students of class VIII at MTs Al-Muhajirin Waihatu, totaling 90 people consisting of 3 classes. The sample in this study was a population sample, namely all students of class VIII at MTs Al-Muhajirin Waihatu, totaling 90 students. The independent variable (free) is the PBL learning model (X) and the dependent variable is learning outcomes (Y).

Validity and reliability test

Validity test

All factor analysis processes use a computer program Statistics Package Social Science (SPSS) 20.0 for windows. The calculation of the validity test uses SPSS 20 with the criteria for deciding whether an instrument is valid or not, namely by comparing r count with r table, with a confidence level of 95% and a significance level of 5%. If r count> r is critical, then the statement item is valid, whereas if r count <r is critical, then the statement item is invalid.

Reliability test

Similar to the validity test, to measure the reliability test, the formula for testing the reliability of the instrument is Alpha Cronbach, and by using the Statistical Package Social Science (SPSS) 20 program for windows.

Classic Assumption Test

Normality test

The way to detect whether the residuals are normally distributed or not is by statistical tests. The statistical test is the Kolmogorov-Smirnov test if the sig (2-tailed) value is greater than 5%, then the data is normally distributed. The basis for decision making can be made based on probability (Asymtotic Significance), namely:

1) If the probability> 0.05 then the distribution of the population is normal.
2) If the probability <0.05 then the population is not normally distributed.

Heteroscedasticity

The basis of this heteroscedasticity test analysis:

1) If there is a certain pattern such as the dots forming a regular pattern (wavy, widened then narrowed), it indicates that heteroscedasticity has occurred.
2) If there is no clear pattern, and the dots spread above and below the 0 on the Y axis, there is no heteroscedasticity.

Hypothesis test

Simple linear regression

The general form of the simple linear regression equation is: Y = a + b + bx**,** where:

Y = Problem based learning model in the predicted dependent variable
a = constant number
b = variable regression coefficient X (PBL model)

T-test

Decision making is based on the probability value obtained from the results of data processing through the SPSS Parametric Statistics program as follows:

$T_{count} > T_{table}$, then = the independent variable affects the dependent variable.
$T_{count} < T_{table}$, then = the independent variable does not affect the dependent variable.

Coefficient of Determination (r^2)

The criteria used to express the relationship between the data distribution of the independent variable (X) and the dependent variable (Y) are stated to be linear if the coefficient of linearity F count is greater than or equal to the value of the F table at the 5 percent (5%) significance level. Decision-making on probability figures, if the probability of the analysis results is ≤ 0.05 then H0 is rejected and H1 is accepted, indicating that there is an influence of the learning environment and self-concept on the learning outcomes of students' biology. Data processing using a computer program Statistic package social science (SPSS) 20.0 for Microsoft Office Word For Windows.

RESULTS AND DISCUSSION

Learning outcomes

The results of the students' initial tests were obtained from the motion material in plants for the 2020/2021 academic year which was carried out by researchers before the activity and learning process using the problem-based learning (PBL) learning model in class VIII MTs Al-Muhajirin Waihatu showed that the highest score obtained by students was 69, 09 and the lowest score obtained by students was 40.73. To more clearly the frequency distribution and percentage of learning outcomes of class VIII students of MTs Al-Muhajirin Waihatu can be seen in the following Table 1.

Tabel 1. Qualification of the presentation of the pre-test interval value

Value Interval / Score Value	Frequency	Relative Frequency (%)	Qualification
81-100	0	0	Very good
71-80	0	0	Good
61-70	42	46.67	Adequate
<60	48	53.33	Less
Jumlah	**90**	**100**	

The data above can be concluded that the ability level of students 'initial test results before participating in the learning process using the problem-based learning (PBL) learning model shows that the students' initial test is low where 42 students with a percentage of 46.67% indicate qualifications (sufficient), 48 participants students with a percentage of 53.33% showed qualifications (lacking), no students were able to achieve qualifications (good and very good).

The data above also reveals that the initial test results achieved by the majority of students do not meet the minimum completeness criteria (KKM), which is 67 for individuals and 85% for classical completeness. It is recognized that there are 17 students or 18.89% who meet the minimum completeness criteria. This is because the students' knowledge of the material has not been taught so that the level of mastery is at sufficient and insufficient qualifications.

The final test results show the learning outcomes (cognitive, affective, and psychomotor) of students obtained from the learning process using the problem-based learning (PBL) learning model in class VIII MTs Al-Muhajirin Waihatu shows that the highest score obtained by students is 85.00 with very good qualifications and the lowest score obtained by students is 69.00 with sufficient qualifications. Learning outcome data which is a combination of the value of the cognitive, affective, and psychomotor aspects can be seen in the following Table 2.

Tabel 2. Classification of the post-test score presentation

Value Interval / Score Value	Frequency	Relative Frequency (%)	Qualification
81-100	49	54.44	Very good
71-80	34	37.78	Good
61-70	7	7.78	Adequate
<60	0	0	Less
Jumlah	**90**	**100**	

From the table above, it can be concluded that as many as 49 students with a percentage of 54.44% showed qualifications (very good) to master the indicators with a value (81-100), 34 students with a percentage of 37.78% showed qualifications (good) to master the indicators with a value (71-80), 7 students with a percentage of 7.78% showed qualifications (sufficient) to master the indicators with a value (61-70) when compared with (MCC) which had been determined by the average score of achievement of students on the formative test. It can be said that the attainment of learners' knowledge is in the complete category of the MCC which is determined both individually and classical. This proves that after the learning process using the problem-based learning (PBL) learning model on the learning outcomes of students in plant motion to achieve the final ability qualifications of students (Formative Test), it describes 90 students (100%) successfully achieving the MCC value (completeness criteria minimal).

Research Janah et al. (2018) also show that before the application of the learning model, learning outcomes that reveal students' conceptual understanding of the material is very low compared to after learning. After going through the learning process, students' understanding of plant motion material has increased. Rumahlatu & Sangur (2019) also explain that learning outcomes are related to students' understanding of a concept formed through the learning process (contextual). It can be seen in the research conducted by Anisa et al. (2018) regarding readiness for independent study. Highly independent students will make them ready to respond/answer in lessons or ready to accept lessons well.

Assumption Requirements Test

Normality test

The normality test is intended to determine whether each variable is normally distributed or not. Data normality testing was carried out using the Statistical Package Science (SPSS) 20.0 computer program by observing the significance of Kolmogorov Smirnov. If the Kolmogorov Smirnov significance value is greater than 0.05, the data is normally distributed and vice versa, if the Kolmogorov Smirnov significance value is less than 0.05, the data is not normally distributed. For more details about the normality test of each variable studied, it can be seen in the following Table 3.

Table 3. Summary of the results of the variable normality test

Variable	Significance	α	Information
Problem based learning model (X)	0,075	0,05	Normal
Learning Outcome (Y)	0,200	0,05	Normal

The table above shows that all variables show a Kolmogorov-Smirnov significance value greater than 0.05. The problem-based learning model variable has a Kolmogorov Smirnov significance value of 0.075 while learning outcomes have a significance value of 0.200. It can be concluded that the data from the problem-based learning model variables and learning outcomes are normally distributed.

Linearity test

The linearity test is intended to test whether the variables affect linearly or not, whether it violates the linearity assumption or not. The variables X and Y affect linearly if they form a linear line. The results of the linearity test between variables using SPSS 20.0 can be seen in the following table:

Table 4. Summary of linearity test results

Variable	Sig	α	Info
Problem based learning model (X) and learning outcome (Y)	0,00	0,05	Linear

The variables X and Y have a linear effect if the significance value of linearity is less than 0.05. Based on the table above, both problem-based learning model variables have a linear effect on learning outcomes. This is indicated by the significance value of the effect of the problem-based learning model with learning outcomes is 0.00 and the significance value is less than 0.05. Thus, there is a linear relationship between problem-based learning models and student learning outcomes.

Pituitary Test

The results of hypothesis testing are used to determine the effect of the independent variables on the dependent variable. The dependent variable data shows a normal and homogeneous distribution so that it is feasible to be tested using statistical tests. The results of the ANOVA calculation on learning outcomes are shown in Table 5, while the coefficient values are shown in Table 6 and Table 7. Residuals Statistics.

Table 5. Anova

	Model	Sum of Squares	df	Mean Square	F	Sig.
1	Regression	4325.362	1	4325.362	18.873	.000
	Residual	343.260	88	3.901		
	Total	4668.622	89			

Table 6. Coefficients

	Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	44.057	2.121		1.913	.049
	Model PBL	.605	.018	.625	33.300	.000

Table 7. Residuals Statistics

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	57.30	86.95	74.36	6.971	90
Residual	-2.401	13.339	.000	1.964	90
Std. Predicted Value	-2.446	1.806	.000	1.000	90
Std. Residual	-1.216	6.754	.000	.994	90

The results of the measurement of the problem-based learning model associated with the learning outcomes of students processed using SPSS 20.0 for windows obtained the results of simple regression analysis with a coefficient "b" of 44.057 and a constant "a" of 0.605 so that the regression line equation $\hat{Y}$ = 44.057 + 0.605 X. The test on the table shows that the value of F count = 18.873 is greater than the F table at α 0.05, namely 3.04 with a significant 0.00 much smaller than α 0.05. This result means that the regression with the equation $\hat{Y}$ = 44.057 + 0.605 X is significant ($p < 0.05$). The regression equation above illustrates that each increase of one score in the problem-based learning (X) learning model causes an increase of 0.605 scores of student learning outcomes at a constant of 44.057. A simple analysis of the score of the problem-based learning model (X) and the score of student learning outcomes (Y) shows the rxy coefficient of 0.626.

The test results show that t = 1.913 is greater than t table 1.66 at α = 0.05 and α = 0.01. This means that the power of the effect of the problem-based learning model (X) on student learning outcomes (Y) is significant. The results of the simple linear regression analysis show that the problem-based learning model has a positive effect on the learning outcomes of students at MTs Al-Muhajirin Waihatu. Based on this test, it was obtained a value of 0.626 so that it was concluded that the influence of the problem-based learning model with learning outcomes was high, while the simultaneous contribution or contribution of the problem-based learning model variable and learning outcomes was 39.18% while 60.82% was influenced by other factors.

The results of simple linear regression analysis show that there is an influence between the problem-based learning model with the learning outcomes of students on plant motion material in class VIII MTs Al-Muhajirin Waihatu. Thus, it is concluded that if the learning process is carried out using the problem-based learning model for students, there will be an influence on the learning outcomes of class VIII students on the material of plant motion in MTs Al-Muhajirin Waihatu. So, that it is known that the hypothesis H0 is rejected and hypothesis H1 is accepted. Although in the descriptive data, it was found that students obtained a score of problem-based learning model which was quite high, but when it was related to learning outcomes it contributed to the influence category with a strong category (0.626).

Learning in the 2013 curriculum requires a change in the mindset of educators to further activate students by applying a scientific approach which aims to improve critical thinking skills. Through these scientific steps, it is hoped that the critical thinking skills of students will develop. Learning curriculum 2013 requires scientific learning, one of which is the PBL model (Azmi et al., 2017). The problem-based learning (PBL) model is a model that plans a problem that is given by educators to be solved by students. This PBL model has a learning condition that is oriented to concrete problems. In line with that, learning a concept and the basis of a subject matter requires a learning model that orientates students to problems in the real world to train students' critical thinking skills (Trianto, 2015). So PBL is a model for solving a problem and stimulating critical thinking of students on learning materials to achieve learning objectives.

The problem-based learning model can affect the learning outcomes of students in biology subjects at MTs Al-Muhajirin Waihatu, this is as seen from the frequency distribution table, it is known that the problem-based learning model is the X variable obtained through giving questionnaires to class students. VIII MTs. Al-Muhajirin Waihatu as a respondent located in Kairatu District, West Seram Regency which includes 3 (three) classes with 90 students having a questionnaire score range 88-137. The highest score for the questionnaire obtained by students was 137 while the lowest score was obtained value 88. Based on the table above shows that most of the students' answers to the questionnaire analysis results of the problem-based learning model were in the high enough categories, namely there were 29 people with a percentage of 32.22% with a score range of 110 - 119.

The effect of the problem-based learning model on student learning outcomes is a positive linear role, meaning that the problem-based learning model is quite high followed by an increase in the learning outcomes of these students. The results of the regression analysis of the problem-based learning model with learning outcomes showed that there was a significant influence with the moderate category, namely 0.626 so that the environment in the study contributed 62% while 48% was influenced by other factors. Other factors that are intended do not come from the variables studied.

The results of the inferential analysis that has been carried out to test the first hypothesis regarding the effect of the problem-based learning model with learning outcomes indicate that there is a positive influence between the problem-based learning model and learning outcomes at α=0.05%. This shows that if the problem-based learning model is good, the learning outcomes will be good. The regression results show that the value between Problem based learning model and student learning outcomes is 0.626. Referring to the interpretation of the r-value proposed by Arikunto (2010), this percentage is in the medium category. The problem-based learning model contributed 62% to learning outcomes.

The problem-based learning model is a condition that affects the learning process. Because a supportive problem-based learning model it will allow students to learn well, but the problem-based learning model itself will run even better if it is supported by students' conditions because critical thinking skills will provide abilities that can be recognized by students based on characteristics. Physical and psychological disregard that is general or specific, but both problem-based learning models are needed in supporting students in learning activities so that it will help the success of students in learning activities. Because learning outcomes are a process of changing behavior that has been done and done which can be measured based on numbers and values.

The results above illustrate that the problem-based learning model is an indicator in the learning process that affects the achievement of student learning outcomes. It can be said that internal and external factors are two factors in the life of students who can deliberately achieve learning outcomes. From the discussion of the effect of the problem-based learning model on learning outcomes, it shows that the strength of the problem-based learning model with learning outcomes is very high, while the simultaneous contribution of problem-based

learning model variables and critical thinking skills with learning outcomes is 39% while 61% is influenced by other factors.

The internal and external factors that were studied did not contribute 100% to learning outcomes, so other internal and external factors were not studied that also affected the achievement of learning outcomes. It is seen in research conducted by Raharjo (2010) regarding independent learning readiness. Highly independent students are more ready to respond/answer in lessons or to accept lessons well. Other external factors that support learning outcomes are studied by (Daud, 2012). The results showed that in general learning motivation and learning outcomes were classified as good. This means that if students are motivated to learn, their learning outcomes will be good (high). Conversely, if students have bad habits in learning, their learning outcomes will be bad (low).

From the research that supports that in addition to internal and external factors, the problem-based learning model examined in this study also proves that there are other internal and external factors that support so that learning outcomes can be achieved by students in class VIII MTs Al-Muhajirin Waihatu. Recapitulation of the relationship between the effect of the Problem-based learning model on student learning outcomes. Overall, the variable between the problem-based learning model (X) on learning outcomes (Y) with a regression coefficient value of 0.626 is a strong category.

The influence of the Problem-based learning model on learning outcomes above is supported by the opinion of Taiyeb & Mukhlisa (2015) that the factors that influence learning outcomes are classified into two, namely internal factors of students, which can be in the form of interests, talents, intelligence, perceptions and so on. relating to students as individuals. And external factors in the form of the influence of the surrounding environment, namely including the natural environment and socio-cultural environment, as well as the non-social or instrumental environment, which includes curriculum, programs, learning facilities, teachers.

CONCLUSION

The application of the problem-based learning (PBL) learning model to the learning outcomes of students in plant movements to achieve the final ability qualifications of students (Formative Tests) describes 90 students (100%) successfully achieving the KKM value (minimum completeness criteria). The problem-based learning model has a significant effect on the learning outcomes of class VIII students of MTs Al-Muhajirin Waihatu, this can be seen from the significant level of 0.626 with the strong category.

REFERENCES

Arikunto, S. (2010). Metode peneltian. Rineka Cipta, Jakarta.

Azmi, M. K., Rahayu, S., & Hikmawati, H. (2017). Pengaruh model problem based learning dengan metode eksperimen dan diskusi terhadap hasil belajar fisika ditinjau dari sikap ilmiah siswa kelas X MIPA SMA N 1 Mataram. *Jurnal Pendidikan Fisika dan Teknologi*, 2(2), 86-94.

Bakkenes, I., Vermunt, J. D., & Wubbels, T. (2010). Teacher learning in the context of educational innovation: Learning activities and learning outcomes of experienced teachers. *Learning and instruction*, 20(6), 533-548.

Daud, F. (2012). Pengaruh kecerdasan emosional (EQ) dan motivasi belajar terhadap hasil belajar Biologi siswa SMA 3 Negeri Kota Palopo. *Jurnal Pendidikan dan Pembelajaran*, 19(2), 243-255.

Idi, A. (2017). *Sosiologi Pendidikan* [*Sociology of Education*]. Rajagrafindo Persada, Jakarta.

Hasibuan, E. K. (2018). Analisis kesulitan belajar matematika siswa pada pokok bahasan bangun ruang sisi datar di smp negeri 12 bandung. *Axiom: Jurnal Pendidikan dan Matematika*, 7(1), 1-11.

Kurniawan, A. D. (2013). Metode Inkuiri Terbimbing Dalam Pembuatan Media Pembelajaran Biologi Untuk Meningkatkan Pemahaman Konsep Dan Kreatifitas Siswa SMP. *Jurnal Pendidikan IPA Indonesia*, 2(1), 8-11.

Raharjo, S. B. (2010). Pendidikan karakter sebagai upaya menciptakan akhlak mulia. *Jurnal Pendidikan dan Kebudayaan*, 16(3), 229-238.

Riyanto, Y. (2009). *Paradigma Baru Pembelajaran Sebagai Referensi bagi Pendidikan dalam Implementasi Pembelajaran yang Efektif* [*New Paradigm of Learning as a Reference for Education in the Implementation of Effective Learning*]. Kencana, Jakarta.

Rumahlatu, D., & Sangur, K. (2019). The influence of project-based learning strategies on the metacognitive skills, concept understanding, and retention of senior high school students. *Journal of Education and Learning*, 13 (1), 104–110.

Saputri, D. A., Febriani, S. (2017). Pengaruh model problem based learning (PBL) terhadap kemampuan pemecahan masalah peserta didik pada mata pelajaran biologi materi pencemaran lingkungan kelas X MIA SMA N 6 Bandar Lampung [The influence of problem based learning (PBL) model on students' problem-solving ability in biology subjects environmental pollution material class X MIA SMA N 6 Bandar Lampung]. *Journal of Biological Education Tadris*, 8(1), 40-52.

Suprijono, A. (2016). *Model Pembelajaran Emansipatoris* [*Emancipatory Learning Model*]. Pustaka Pelajar, Surabaya.

Skaggs, G., & Bodenhorn, N. (2006). Relationships between implementing character education, student behavior, and student achievement. *Journal of Advanced Academics*, 18(1), 82-114.

Taiyeb, A. M., & Mukhlisa, N. (2015). Hubungan Gaya Belajar dan Motivasi Belajar dengan Hasil Belajar Biologi Siswa Kelas XI IPA SMA Negeri 1 Tanete Rilau [The Relationship between Learning Style and Learning Motivation with Biology Learning Outcomes of Class XI IPA Students of SMA Negeri 1 Tanete Rilau]. *Bionature*, 16(1), 8-16. doi: https://doi.org/10.35580/bionature.v16i1.1563

Trianto. (2015). *Model Pembelajaran Terpadu* [*Integrated Learning Model*]. Bumi Aksara, Jakarta.

BIOEDUPAT:
Pattimura Journal of Biology and Learning
https://ojs3.unpatti.ac.id/index.php/bioedupat
e-ISSN 2775-4472

Research Article

Development of *sasi* culture based mangrove forest conservation instruction package to improve ecological knowledge of Amahai State Society, Central Maluku District

Eva Moy Wonley[1,*], Fredy Leiwakabessy[2], Stevin Melay[2]

[1] Postgraduate Student of Biology Education, Pattimura University,
Jl. Dr. Tamaela, Ambon, Maluku 97114, Indonesia
[2] Study Program of Biology Education, Faculty of Teacher Training and Education, Pattimura University,
Jl. Ir. M. Putuhena, Ambon, Maluku 97233, Indonesia
** corresponding author: wonley133@gmail.com*

Received: 17 February 2021 Revised: 24 March 2021 Accepted: 21 April 2021

ABSTRACT

Instructional Package is one of the learning media that can be used in the learning process and can increase public knowledge. This research aims to determine, (1) Development of an Instructional Package for Mangrove Forest Conservation Based on Sasi Culture in Amahai Region, (2) Knowing the Effectiveness of this Instructional Package in Improving Community Knowledge About Mangrove Forest Conservation Based on Sasi Culture in Amahai Region. And analyzed qualitatively and quantitatively. This research is development research using the dick and Carey model to produce an instructional package which will be tested in Amahai and Sehati villages with a sample for each village of 30 people using a test instrument to measure knowledge both pre-test and post-test, the behaviour group used the instructional package while the control group did not use the instructional package. The mean values obtained in the behavioral group were: for the pre-test 25% and the post-test 29%, while the mean scores for the control group were 25% for the pre-test and 26% for the post-test. To find out the effectiveness of the instructional package, normality is tested using Kolmogorov Smirnov with pre-test and post-test data, and the gain scores are declared normal, and the variance homogeneity test using levena is the value of the two groups is homogeneous and the test is different (t-test). with the significance value used is α 0.05. Thus, it can be concluded that the instructional package is effective and feasible to be used as a learning resource and can be used in the learning process.

Keywords: Instructional package, ecological knowledge, sasi culture, mangrove forest conservation

To cite this article:
Wonley, E. M., Leiwakabessy, F., & Melay, S. (2021). Development of *sasi* culture based mangrove forest conservation instruction package to improve ecological knowledge of Amahai State Society, Central Maluku District. *Bioedupat: Pattimura Journal of Biology and Learning,* Vol 1(1), 32-38. *DOI: https://doi.org/10.30598/bioedupatv1i1pp32-38*

INTRODUCTION

Local wisdom is a legacy passed on by ancestors from a long time ago, with the values of a life that contain forms of religion, culture, and customs (Pattinama & Pattipeilohy, 2003). One form of inheritance that is still known and carried on by the people of Maluku is sasi. Sasi is a form of local wisdom that plays a role in managing natural resources and the environment (Zulfikar, 2008). Sasi was enforced to prevent environmental damage (Fatma, 2015). Some coastal natural resources in Maluku where the management system applies sasi, for example, sasi for coastal resources such as Lola, action rock, sea cucumber, algae, fish, coral reefs and

mangrove forests, etc. However, in this paper, the author focuses more research on forest natural resources, Mangroves.

Mangrove forest is a woody plant that grows around the coastline and has high adaptation to brackish salinity, and continues to live with the environmental conditions it occupies (Dahuri, 2003). Efforts to maintain mangrove forest conservation need to be done with the aim that millions of mangroves will not be exhausted and even damaged due to continuous exploitation without thinking about future generations (Putrawan, 2014). So that there need to be activities that must be carried out to maintain the conservation of mangrove forests, one of the activities is to provide understanding to the community about knowledge of conservation concepts through the development of instructional packages.

The instructional package contains learning materials for mangrove forest conservation based on sasi culture in the form of useful textbooks for the community. With the consideration that so far there have not been any textbooks (pocketbooks) or instructional packages as a source of knowledge about mangrove forest conservation based on the sasi culture so that it makes people see that the conservation actions that have been implemented so far are only a habit (Melay, 2018). Therefore, it is necessary to make a pocketbook so that basic ecological knowledge is owned and improved. Knowledge of local wisdom is very important because knowledge of local wisdom contains knowledge, practices, and beliefs about the relationship between living things and their environment that develops and is culturally inherited. Thus the people of Maluku, especially the Amahai region, have traditional various ways to manage the environment to maintain the quality of natural resources. So that it will further support efforts to improve the welfare and quality of human life, and can preserve the ability and utilization of living natural resources and their ecosystems in a harmonious and balanced manner. One of the conservation strategies is to use culture or local wisdom as learning materials (Knapp et al., 2013). The objectives of this study were: 1. To develop an instructional package for mangrove forest conservation based on the Sasi culture in Amahai region. 2. Knowing the effectiveness of this instructional package in increasing community knowledge about the conservation of mangrove forests based on the Sasi culture in Amahai region..

METHODS

The stages that will be carried out are based on the references used in the outline are: (1) Information collection, namely by gathering information and needs analysis to determine the goals to be achieved from the resulting product. (2) Product Formulation and Development, namely by identifying and formulating objectives, determining the potential to be achieved by learners, designing model designs. Instructional package and develop instructional package material made. (3) Field testing, namely by testing the effectiveness of the instructional package produced through the quasi-experimental method. The results of field trials are used as material for dissemination and recommendations.

Instructional package development steps In general, the steps for developing an instructional package in this study are as follows:

1. Planning Stage

   The planning stage is the initial stage for the Instructional Package product development activities. The steps for the activities carried out are:

   a) Observation, which aims to gather information about conservation knowledge and the learning tools developed. The data obtained is used as material to formulate goals to be achieved from the instructional package developed.
   b) Analysis and assessment of the need to develop a basic framework for the material based on the Cultural Conservation Instructional Package.

2. Development Stages

   This stage is the preparation of the design and development of the Instructional Package and compiling a draft of conservation ecology material. The Instructional Package development procedure refers to the Dick and Carey model, with the following steps:

   a) Identify objectives and formulate competencies and learning outcomes, and develop the points of the Instructional Package.
   b) Designing Instructional Package model designs and Instructional Package material framework.
   c) Develop an instrument (benchmark reference test) to measure people's knowledge based on the objectives to be achieved, then validate the benchmark reference test.
   d) Develop Instructional Package material on the concept of conservation ecology based on the Instructional Package design.

3. Effectiveness Test Phase

Testing of the effectiveness of the Instructional Package was carried out through an experimental method with the design of the pre-test and post-test control and behavioral groups. The experimental design is as follows:

Tabel 1. Research design

O	X	O
O	C	O

Note:
X = Behavior, using the developed Instructional Package
C = Control
O = Observation / Pre-Test and Post Test

Data analysis in this study was carried out quantitatively and qualitatively. Qualitative data analysis was carried out to process data from observations. After the data is obtained, then the data is summarized, selected, and focused on according to the Instructional Package variables, which are then used as the basis for developing an effective instructional package for the community. Data can be related to needs analysis, trends in the development of Instructional Package materials, the results of content feasibility evaluation, and presentation of the instructional package model developed. Testing the instrument validity, reliability, and effectiveness of the Instructional Package used quantitative data analysis. The effectiveness test data is the result of an assessment of public knowledge about conservation based on Sasi culture in the experimental group and the control group. The learning outcomes were tested for differences using the t-test at the significance level (α) 0.05 (Shirley, 2004). Before being analyzed, the normality test was carried out using the Kolmogorov Smirnov (KS) test, and the homogenetic test using the Levena test at the significance level (α) 0.05. Quantitative data analysis was carried out with the SPSS version 16 program.

RESULTS AND DISCUSSION

Based on the results of the pre-test that has been obtained, it describes that the two groups of people already have an initial knowledge about mangrove forest conservation based on sasi culture even though the value of the control group is higher than the behavior group. However, the post-test or the final test experienced very different changes. It can be seen based on the gain score that the behavior group given the dominant instructional package was higher than the control group. To see the difference in the effectiveness test of the ecological instructional package for mangrove forest conservation based on sasi culture in the behavior group and the control group, an analysis was carried out. The following is the presentation of data for the knowledge of the behavior and control groups that have been calculated statistically.

Tabel 2. Description of pretest and post test score data from the treatment group and the control group related to public knowledge about the ecology of conservation based on sasi culture

		Group	
		Experiment	**Control**
Skor pre-test	Total	30	30
	Mean	25	25
	Modus	27	26
	Median	26	26
	Score Range	10	13
	Deviation Standard	2.816	2.949
	Variance	7.066	8.133
Skor post-test	Total	30	30
	Mean	29	26
	Modus	28.29 and 30	27
	Median	29	27
	Score Range	4	10
	Deviation Standard	1.1 65	2.515
	Variance	1.5	6.3
Gain score	Total	30	30
	Mean	4	1.26
	Modus	2	1
	Median	3	1

Score Range	7	3
Deviation Standard	2.079	0.639
Variance	4.36	7.86

Data analysis of effectiveness test results

To test the effectiveness of the t-test the data must be tested for normality and homogeneity first. In this study, the t-test was carried out in the control group and the treatment group to know the difference in knowledge between the two groups.

Normality test

In the normality test of the pre-test, post-test and gain score data for each treatment group and control group, the Kolmogorov Smirnov test was used based on the following hypothesis testing:

counted ≤ dtab: Data are normally distributed

counted ≥ dtab: Data are not normally distributed

If the results of the normality test obtained are normally distributed data, the data for the pre-test, post-test, and gain scores for the control and treatment groups can be tested further, namely the normal test and the difference test (t-test).

Tabel 3. The results of the analysis of the Normality Test using Kolmogorov Smirnov in the treatment group and the control group on the score data on the public's answer to the knowledge of mangrove forest conservation based on sasi culture

Test Group	N	Statistic Test	
		d-hit	d-tab(0,05)
Pre-test Experiment Group	30	0.161	0.24
Post-test Experiment Group	30	0.178	
Gain Skor Experiment Group	30	0.207	
Pre-test Control Group	30	0.135	
Post-test Control Group	30	0.232	
Gain Skor Control Group	30	0.238	

The table above shows that the data for the pre-test (initial test), post-test (final test) and gain scores for the control and treatment groups were declared normal because based on the Kolmogorov Smirnov test the results obtained were calculated ≤ in the table.

Homogeneity test

The variance homogeneity test used levena to the control and treatment groups consisting of pre-test and post-test with the following test conditions:

H0: σ'12 = σ'22 = ... = σ'k2 (homogeneous data)

H1: there is at least one σ'i2 which is not the same

Table 4. The results of the analysis of the homogeneity test using the Levena test in the treatment and control groups on the score data on the community's answer about conservation knowledge based on sasi culture

Test group	N	Statistic test	
		f-hit	f-tab (0,05)
Pre-test Experiment Group	30	0.254	2.76
Post-test Experiment Group	30	0.273	
Gain Skor Experiment Group	30	0.827	
Pre-test Control Group	30	0.617	
Post-test Control Group	30	0.485	
Gain Skor Control Group	30	0.693	

The table above shows that the data for the pre-test score (initial test), post-test (final test), and score gain for the control and treatment groups are stated to be homogeneous because based on the Levene test the value of Fcount ≤ Ftable is obtained so that H0 is accepted.

Different test (t-test)

The different test (t-test) is applied if the data analysis aims to determine the differences in certain variables in two groups. The results of this test analysis were carried out to test the hypothesis of the difference in the parameter between the treatment group and the control group as a comparison test between two independent samples. The hypothesis used for the different test (t-test) in this study is:

H0: $\mu 1 = \mu 2$

H0: $\mu 1 \neq \mu 2$ or one of them is not the same

The test criteria are determined based on the results of the calculation of the test statistic value with the following conditions:

H0: Accepted if $|t_{count}| < |t_{table}|$

H0: Rejected if $|t_{count}| > |t_{table}|$

With a significant level $\alpha = 0.005$

Table 5. Analysis results in different tests in the behavior group and the control group on conservation knowledge based on sasi culture

Test Group	Db	Statistic Test	
		t-hit	f-tab
Pre test-post test Experiment group	29	6,410	
Pre test-post test Control group	29	1,790	
Post test Experiment and Control group	29	4,117	1,699
Gain score test Experiment and Control group	29	5,791	

**p<0,05

In the table above, the different test results (t-test) have been presented. These results show that the test scores for the pre-test and post-test between the experimental group and the control group are very significantly different. High post-test scores were obtained by the experimental group because the experimental group was given treatment while the pre-test scores for both groups were almost the same. The following shows a graph of increasing knowledge in the form of differences in the pre-test and post-test scores in the treatment group and the control group, as well as a bar histogram for the gain scores of the treatment group and the control group.

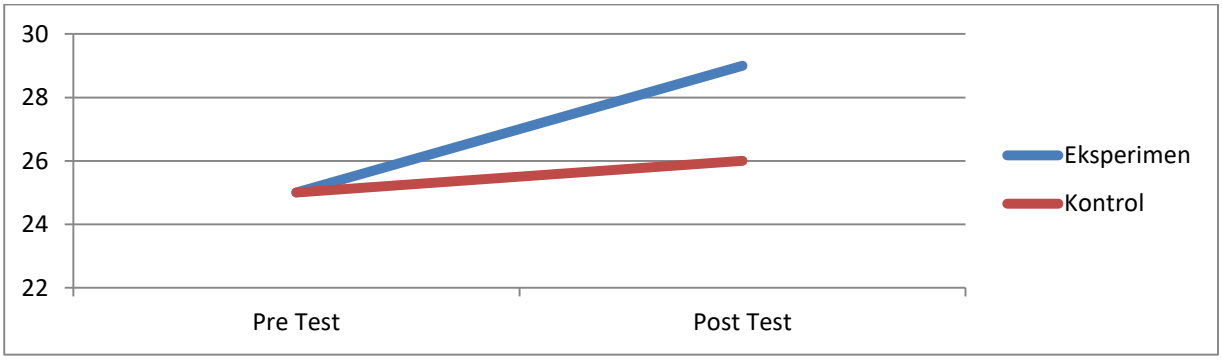


Figure 1. Graph of increasing knowledge in the form of differences in the pre-test and post-test scores in the treatment group and the control group

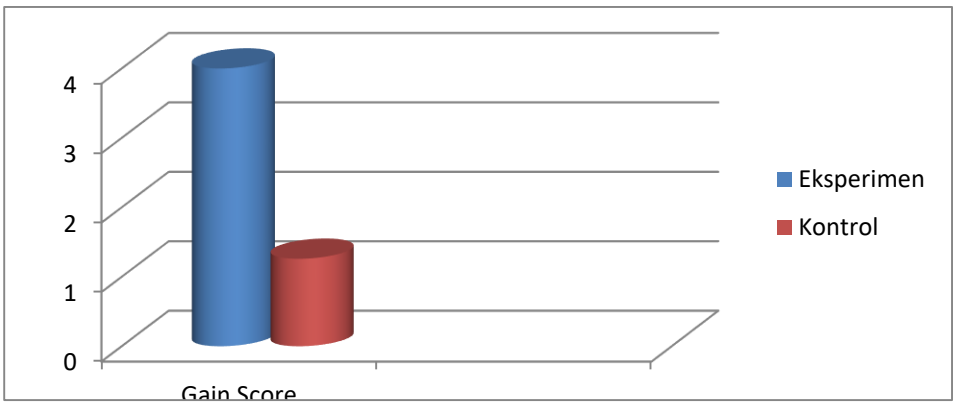


Figure 2. Bar histogram for the gain scores of the treatment group and the control group

The development of an instructional package for mangrove forests based on the Sasi culture in increasing the ecological knowledge of the Amahai people of Central Maluku district is the title of this research. The instructional package contains materials to conserve natural resources in Maluku, especially in an Amahai region so that it is maintained and not threatened with extinction. KOMA BUSA is the result of the development of an Instructional Package which is designed in the form of a pocketbook, the KOMA BUSA being developed is expected to make readers understand the contents of the reading comprehensively and conceptually. Also, at the end of the instructional package, there is information about the existence of mangrove forests in Amahai region and the contents of the instructional package contain efforts to actualize knowledge related to the ecological concept of mangrove forest conservation, the actualization effort is to present moral messages in the instructional package.

Several prominent Indonesian studies have examined traditional knowledge which greatly influences the use of natural resources. These studies include 1. Utilization of Indonesian Traditional Knowledge Based on Regional Potential as Development Capital (Heryanto), 2. Legal Protection of Intellectual Property Rights to Traditional Knowledge Against Legal Economic Benefits Gaining (Karlina Softarto), 3. Protection of Community Traditional Knowledge On Utilization of Genetic Resources (SDG) (Nurhayati Qodriyatun). In this case, the community is the main actor in managing the environment and utilizing natural resources, so good and broad knowledge is needed from the community. If the broader the knowledge, the better and correct the management of natural resources and vice versa if there is less knowledge from the community, the less way the community manages natural resources. Thus, information sources and socialization are needed to increase public knowledge. This research proves that information sources can increase knowledge, as seen in the t-test conducted between the treatment group and the control group, which illustrates the difference in the level of knowledge the community has regarding the concept of mangrove forest conservation based on sasi culture, where the treatment group uses the instructional package as a source. Learning has a much higher value than the control group which was not given an instructional package as a learning resource.

For the instructional package that has been developed, the authors feel that this product is easy to learn because it uses language that is clear and simple so that it is sufficiently influential and effective in increasing public knowledge regarding the ecological knowledge of mangrove forest conservation based on the sasi culture in Amahai region that previously the community did not know. The importance of mangrove forests for the next generation and the community only carries out sasi as a customary tradition or habit which is carried out from generation to generation to protect natural resource products. However, with the presence of sasi, the government and residents have also made mangrove forests in the Amahai region a tourist place for nature-loving visitors and facilities and infrastructure for learning, as well as improving the economic standard of living for the coastal communities of Amahai State..

CONCLUSION

The development of an Instructional Package for Mangrove Forest Conservation Based on the Sasi Culture in the Amahai State Community of Central Maluku Regency was successfully developed with the development stages of Borg and Gall which were carried out in ten stages. The framework of the instructional package for mangrove forest conservation based on sasi culture begins with a strengthening of the concept, then contains materials, namely: ecology and scope, conservation, sasi culture, mangrove forests, the instructional package that is made ends with information about the existence of mangrove forests in the amahai country. Then the Instructional Package Effectiveness Test in Increasing Public Knowledge about Mangrove Forest Conservation Based on the Sasi Culture which was tested for normality, homogeneity, and t-test differences with the results achieved between the treatment and control groups, namely the treatment group was very satisfying because it was given an instructional package. So it is effective if KOMA BUSA is used in the learning process.

REFERENCES

Borg, W. R., & Gall, M.D. (1989). *Educational research: An intoduction.* Fifth Edition. Longman, New York. pp.773-775.

Dahuri, R. (2003). *Keanekaragaman hayati laut (aset pembangunan berkelanjutan indonesia)* [Marine biodiversity (Indonesia's sustainable development assets)]. Penerbit PT. Gramedia Pustaka Utama, Jakarta.

Fatma, P. (2015)*. Pengaruh Kemampuan Kerja, Lingkungan Kerja, dan Masa Kerja Terhadap Produktivitas Karyawan PT. Djarum SKM II Kudus.* Skripsi. Universitas Muria Kudus.

Knapp, CN., Cochran, J., Chapin III, FS., Kofinas, G., & Sayre, N. (2013). Putting local knowledge and context to work for Gunnison sage-grouse conservation. *Human-Wildlife Interactions*, 7(2), 195-213.

Melay, S. (2018). *Pengembangan paket instruksional ekologi berbasis budaya sasi dalam meningkatkan pengetahuan masyarakat tentang konservasi sumber daya alam* [*Development of an ecological instructional*

package based on the sasi culture in increasing public knowledge about natural resources conservation]. Disertasi. Universitas Negeri Jakarta.

Putrawan. M. (2014). *Konsep-konsep dasar ekologi dalam berbagai aktivitas lingkungan* [*Basic ecology concepts in various environmental activities*]. Alfabeta, Bandung.

Pattinama, W., & Pattipeilohy, M. (2003). *Upacara sasi ikan lompa di negeri Haruru* [*Lompa fish sasi ceremony in Haruru Country*]. Ambon: Kementrian Kebudayaan dan Pariwisata. Balai Kajian dan Nilai Tradisional

Shirley, D. (2004). Statistik for research. Jhon Wiley dan Sons, West Virginia. pp. 184-186.

Zulfikar Judge & Marissa Nurizka. (2008). Peran hukum adat sasi laut dalam melindungi kelestarian lingkungan di desa Eti Kecamatan Seram Bagian Barat [The role of sasi laut customary law in protecting environmental sustainability in Eti Village, West Seram District]. *Lex Jurnalica*, 6(1), 30-61.

BIOEDUPAT:
Pattimura Journal of Biology and Learning
https://ojs3.unpatti.ac.id/index.php/bioedupat
e-ISSN 2775-4472

Research Article

People behavior and *anopheles* mosquitous bionomic and its correlation with malaria parasite prevalence and case fatality rate (CFR) in West Seram Regency

Johanis F. Rehena[1,*], Sriyanti I. A. Salmanu[1], Zasendy Rehena[2]

[1] Study Program of Biology Education, Faculty of Teacher Training and Education, Pattimura University, Jl. Ir. M. Putuhena, Ambon, Maluku 97233, Indonesia
[2] Faculty of Public Health, Universitas Kristen Indonesia Maluku, Jl. O. T. Pattimaipauw, Ambon, Maluku 97115, Indonesia

** corresponding author: johanisrehena@yahoo.com*

Received: 18 February 2021 Revised: 30 March 2021 Accepted: 21 April 2021

ABSTRACT

WHO mentions that 300-500 million of people in the world are infected with malaria every year, 110 million of people perform the symptoms, and 2.7 million are even died. In 2005, malaria patients in West Seram Regency were 7.760 people. The research design was Survey and Case control in order to investigate the behavior of local society, *Anopheles* mosquito bionomic, malaria prevalence and case fatality rate (CFR). Purposive sampling was taken from the people of 2 villages in 3 districts with the highest numbers of patients. 30 people were taken from each village so total sample was 180 respondents. *Anopheles* bionomics involved habitat and breeding. Malaria cases in 2012-2016 obtained from public health center, health department office and public hospitals. Data analysis was done by using Pearson correlation test. The results showed that people behavior strongly agreed in overcoming the spread of *Anopheles* mosquitoes and malaria. Natural breeding habitat in swamps area where mangrove trees grow and sago trees at trenches, rice fields, ponds and water on the boat. *Anopheles* larvae species encountered was *Anopheles sundaicus* that actively bite all night inside and outside the house with peak activity at *02.00-03.00 a.m*. Average larval density was 5.00, 9.00 and Average density of *Anopheles* mosquitoes was 5.09 - 9.85. The Prevalence Rate of Malaria is still high and Case Fatality Rate (CFR) is low. There is a significant correlation between people behavior and prevalence rate and no correlation with Case Fatality Rate (CFR).

Keywords: People behavior, bionomic anopheles, prevalence rate, CFR

To cite this article:
Rehena, J. F., Salmanu, S. I. A., & Rehena, Z. (2021). People behavior and *anopheles* mosquitous bionomic and its correlation with malaria parasite prevalence and case fatality rate (CFR) in West Seram Regency. *Bioedupat: Pattimura Journal of Biology and Learning,* Vol 1(1), 39-44. *DOI: https://doi.org/10.30598/bioedupatv1i1pp39-44*

INTRODUCTION

Malaria is still a public health problem, especially in the tropical areas because its prevalence is sufficiently high. Malaria generally attacks developing countries including Indonesia, especially in socio-economic communities in rural area (Hafriani, 2012). The limitations of multifactorial information on epidemiological and bionomic determinants of malaria vectors have resulted in no effective and efficient specific ways of preventing malaria transmission applied with satisfactory outcomes in rural areas. Until today, many mosquito vector control efforts are done by spraying with several types of insecticides. This effort has been done for decades with high

cost, but the results are not maximized, it is proven that mosquitoes are still one of the main problems of human health (Sumantri & Rosidati, 2015).

Malaria is currently a public health problem in 90 countries, populated by 2.4 billion of people or 40% of the world's population. WHO mentions that 300-500 million of people in the world are infected with malaria every year, 110 million of people perfom the symptoms, and 2.7 million are died. In Southeast Asia, 10-11 countries are infected with malaria and *the population at risk* in this region are 1.35 billion. This shows that most of the world's malaria sufferers are in Southeast Asia (Padley et al., 2008; Bjorge, 2004 in Hidajati, 2006). Indonesia is a malaria endemic area, about 60% of Indonesia's population live in malaria endemic areas and according to Household Health Survey (SKRT) data in 2001 there were 15 million malaria patients every year and caused 1.2% or 23.483 mortality (Fahmi, 2004).

Based on the data, in Maluku Province. Malaria morbidity rate in Maluku every year is caused by geographical condition of Maluku which is coastal area and swamp area. According to the information from the Sub-directorate of Communicable Disease Eradication and Environmental Sanitation of Maluku Province, most of the areas in Maluku are included as high malaria endemic areas, with case finding rates between 57 cases per 1000 inhabitants annually. In 2004, the numbers of malaria patients in Ambon city were 7.285, Central Maluku Regency were 16.611, Southwest Maluku Regency were 4,402, Southeast Maluku Regency were 8.782, and Buru Island Regency were 4.663, so malaria morbidity rate in Maluku province was 41.743 people (Health Dept. osf Maluku Province, 2005). In 2005, malaria patients inWest Seram regency were 7.760 people (Health Dept. of West Seram regency, 2006). Efforts to overcome malaria have been widely practiced, but the morbidity and mortality rates of malaria in some countries remain high.

Soedarto (1995) states that gametocytes, microgamete, and macrogamete found in the human body take place in the life cycle stage of plasmodium, which is through the stage of schizogoni living in red blood cells and will form stages into tropozoid, schizon, and merozoit. This stage takes place with different time according to plasmodium species. The plasmodium cycle takes place inside the human body and the body of the mosquito, in a mosquito's body lasting a sexual life cycle (sporogami), so *Anopheles* mosquito acts as a definitive host. Rampengan (1997) says that a malaria patient can be infested more than one type of plasmodium, such infection is a mixed infection between *Plasmodium falciparum* and *Plasmodium vivax* or with *Plasmodium malariae*. This mixed infection is usually encountered in areas with high rates of transmission. *Anopheles* mosquitoes are a major vector of malaria and its transmission through female *Anopheles* bites containing infective sporozoites. *Anopheles* mosquitoes are dependent on their species, such as *Anopheles sundaicus* which are in brackish water, shallow creeks, fish ponds, and trenches.

The asexual cycle is when a sporozoite infection of the female anopheles liver gland is inserted into the human blood through the mosquito's puncture, and within just thirty minutes it enters the liver parenchymal cells and begins the eryoeryrocytic stage of the life cycle. The sexual cycle occurs in the body of a mosquito, where the gametocytes with the blood are not digested by other cells. In macrogamete (male) chromatin divides into 6-8 nuclei that move parasitic to the edge (Zein, 2005).

Based on the results of the bionomic survey*, Anopheles sp* mosquitoes in Kairatu District and Taniwel District of West Seram Regency, the species found were *Anopheles aconitus*, *Anopheles balanbacensis* and *Anopheles subpictus* as malaria vectors (Rehena, 2005). The density of *Anopheles sp* mosquitoes from the results of a survey in 2006 at Kairatu District, mosquito density with man biting rate (MBR) was 0.9 in coastal areas and mountain areas was 0.8 (Rehena, 2006).The results of survey in June-August 2007 found that *anopheles* larvae density was 7.3, while the adult mosquito with man biting rate (MBR) was 5.

Mosquito behavior always requires 3 places for its survival: (a).Blood Seeking Behavior: mosquitoes in different blood-seeking behaviors are *Culexy* mosquitos which are active in the morning, at noon, and in the evening or at night. *Aedes* mosquitoes look for active blood during the day, and *Anopheles* is actively looking for blood in the evening and at night. (b). Resting Behavior: a process of waiting for egg maturation and when mosquitoes are still actively looking for blood, in the process mosquitoes usually break on the walls of the house. (c) Breeding Behavior: Mosquitoes have the ability to choose spurs or places to breed with their needs, some prefer brackish water, in clear water and some others prefer dirty water. Non-sanitary waste water can be a breeding medium for pathogenic microorganisms (Pulungan et al., 2012).

Health behaviors or behaviors to nurture and promote this conducive health contain multiple dimensions. Changes of people behavior that are not in accordance with the values of health or negative behavior then need to be altered. While the change of healthy society to be maintained. The development of healthy behavior is primarily intended to familiarize healthy life for children should start as early as possible. The concept of health education is an effort to influence and invite other people, groups and individuals and communities to implement healthy life behavior (Notoatmodjo, 2003). The objective of the study is to investigate the condition of people behavior, anopheles mosquito bionomic, prevalence and CFR of malaria in West Seram Regency.

METHODS

This research employed Survey and Case Control design, it is intended to investigate the behavior of society, *Anopheles* mosquito bionomic and view disease progression (prevalence) and case fatality rate (CFR). The population in this research was the people of West Seram Regency. *Anopheles* mosquitoes Bionomic as well as cases of malaria. The sample in this research is the people of Taniwel, Piru and Kairatu Disctrict. Purposive sampling procedures and technique with consideration of the object under research are widely expected that this technique can be used to investigate the behavior of people at homes, *Anopheles* mosquitoes bionomics at each location in in swamp areas, ponds, rice fields, mangroves area in Taniwel, Piru and Kairatu District. Data collection of malaria was in Puskesmas (Public Health Center) in Taniwel, Piru, and Kairatu, also at Health Department Office of West Seram Regency and Piru Public Hospital.

Determining the number of the sample was based on the consideration; the need of analysis so that the sample taken is the people of 2 villages in 3 districts that have sufficiently high number of patients, then 30 people of each village so total sample are 180 respondents. *Anopheles* mosquitoes Bionomic in swamp areas, ponds, rice fields, mangrove areas; Anopheles larvae was found. Malaria cases from 2012 - 2016 are collected from Public Health Center, Health Department and Public Hospital. The instrument used in this research is questionnaire of behavior of society and observation sheet. The equipment used includes: Small bottle / film roll for mosquito larva, pipette, aspirator / mosquito catcher, petri, microscope, loop, preparation / cover. Composition of Ingredients: 70% alcohol, 5% formalin, egg white.

The independent variable consists of People behavior, Anopheles mosquito Bionomic, the density of larvae and *Anopheles* mosquitoes. The dependent variable consists of number of people with malaria (Prevalence), Case fatality rate (CFR) of malaria.

Research data on people behavior was tested with Likhert scale. *Anopheles* mosquitoes Bionomic were analyzed descriptively. The prevalence of malaria disease was analyzed using the formula of Idram et al. (2002) and Chandra (1995) as follows:

$$\text{Prevalence Rate (PR)} \frac{\text{number ofold/newpatients in certain period}}{\text{population at risk of malaria}} x\ 100\%$$

$$\textbf{(CFR)} \frac{\text{number of deaths of a disease}}{\text{total number of malaria case}} \text{x } 100\%$$

Anopheles Mosquito Bionomic is determined by the formula Chandra (1995), as follows:

$$\text{Mosquito Density Formula: MBR} \frac{\text{number of mosquito biting man}}{\text{number of catching time } (\frac{\text{hour}}{\text{person}})}$$

$$\text{Larvae Density Formula}: \frac{\text{number of larvae obtained}}{\text{number of cuts performed}}$$

The correlation between people behavior with Prevalence Rate and Case Fatality Rate (CFR) of malaria was done by Pearson correlation test. The analysis was performed with SPSS version 20.0.

RESULTS AND DISCUSSION

Description of research setting

West Seram Regency as research setting is geographically located at $1^0$19, - $1^0$16 Lat. S and 129^0 1, -127 20, Lon. E with total area of 85,953.40 km^2 which consists of 6,948,40 km^2 (8.08) wide plains and an area of 79005 km^2 km^2 (91.92%) with the boundaries of West Seram District are as follows:

a. North Seram is bordered by Seram sea
b. The south by the Banda Sea
c. The east is bordered by Central Maluku Regency
d. The west is bordered by the Buru Sea

West Seram Regency consisting of 11 districts, 92 villages and 112 orchards with population in 2013 recorded as 178,781 people with annual population growth rate of 1% and population density (soul / km^2) is around 42.

People behavior

Behavior is influenced by attitudes but also by subjective norms that is our beliefs about what others remind us to do, attitude toward behavior along with subjective norms form an intention to behave. Behavior broadly, certainly can not only be reviewed in relation to human attitudes. Human behavior is not simple to understand and to predict because so many internal and external factors of the past, present and future dimensions affect humans (Azwar, 1995). The behavior of the people towards the use of malaria drugs (Klorokuin, promakuin and traditional medicine), the use of mosquito repellent (*Bakar and baigon*), the habit of hanging clothes and traveling outside the house with a test using a Likhert scale to180 respondents in 6 villages of West Seram Regency who strongly agreed. There is a significant correlation between the behavior of society with Prevalence Rate of malaria disease after correlation analysis was carried out.

Anopheles mosquito bionomic

Tabel 1. Correlation is behavior prevalence malaria

Correlations

		Behavior people	Prevalence malaria
Behavior people	Pearson Correlation	1	-0.948**
	Sig. (2-tailed)		0.004
	N	6	6
Prevalence malaria	Pearson Correlation	-0..948**	
	Sig. (2-tailed)	0.004	1
	N	6	6

Table 2. Mosquito bionomic in West Seram Regency

Bionomics	Taniwel	Piru	Kairatu
Eating Behavior	-Biting man, evening and night 16.00-24.00 Peak: 02.00-03.00.	-Biting man, evening and night 16.00-06.00 Peak: 02.00-03.00.	-Biting man, night 16.00-05.00 Peak: 02.00-03.00.
Breeding Behavior	-Lay eggs in the pool, boats, tub of water, coconut	-Lay eggs in the pool, well, water barrel, boats, swamp	-Lay eggs in the rice field, pool, bathtubs
Resting/ Sleeping Behavior	-Outdoor -Indoor -Hanger	-Indoor -Outdoor -Hanger	-Indoor -Outdoor -Hanger
Species	-Sundaicus	-Sundaicus	-Sundaicus

Qomariah (2004) states that spawning places, mosquito habitats can be divided into *container habitats* and *ground water habitats*. Container habitats consist of natural containers and artificial containers. Groundwater puddle is a puddle of water with the ground at the bottom. Rehena (2005) also asserts that the species that have groundwater inundation habitat is *Anopheles sp*, Anopheles mosquito breeding behavior carried out after absorbing the blood of prey or host until the egg maturity in the stomach and ready to reproduce i.e., lay eggs in the water. Health Department (1987) states that breeding behavior is also very definite according to species and where the breed is where anopheles mosquito species placed the eggs well in the place of direct sunlight as well as the shade.

Table 3. Larva bionomic in West Seram Regency

Bionomic	Taniwel	Piru	Kairatu
Larva location / container	- Pool - Boat - Bathtub - Coconut	-Pool -Well -Barrel -Boat -Swamp	-Rice field -Pool -Barrel -Coconut
Amount of larva	- 200	-150	-120

Larva species	-Sundaicus - Aconitus	- Sundaicus - Letifer	-Sundaicus - Aconitus - Subpictus

Gandahusada (2000) states that anopheles mosquitoes prefer resting places inside the house or endophilic and outdoors or exophthalic. Rehena (2005) states that the determination of malaria vector in Uraur in Kairatu Village of Kairatu District, 3 species of Anopheles mosquito were found suc as; *Anopheles aconitus, Anopheles subpictus and Anopheles balanbacensis*. Natural containers are commonly found in forest areas or plantation areas, but natural containers are also found elsewhere, such as logged-over areas, bamboo segments, beach areas where there are many coconut shells. Species that have a natural container habitat are *Aedes sp, Anopheles sp* (Rattanarithikul and Harisson, 2005).

Table 4. Anopheles larvae density

Larva density	Sohuwe	Lumalata	Wimital	Kairatu	Piru	Ds Talaga
	8.33-9.00	6.00 - 7.33	6.00-7.00	5.007,33	6.00- 7.50	6.00 - 7.50
Remarks	High	Medium	Medium	Medium	Medium	Medium

Table 5. Anopheles mosquito density

Mosquito density	Sohuwe	Lumalata	Wimital	Kairatu	Piru	Ds Talaga
	7.00- 9.85	5.89 - 6.82	5.09- 6.50	6.00-7,00	6.50- 7.00	6.08 - 7.80
Remarks	High	Medium	Medium	Medium	Medium	Medium

Number of malaria patients (prevalence)

1. Prevalence Rate (PR)Sohuwe Village $= \frac{127}{909}$ x 100% = 13,9
2. Prevalence Rate (PR)Lumalatal Village $= \frac{68}{570}$ x 100% =11,92
3. Prevalence Rate (PR)Wamital Village $= \frac{170}{1.886}$ x 100% = 9,01
4. Prevalence Rate (PR)Kairatu Village $= \frac{130}{1.260}$ x 100% = 10,32
5. Prevalence Rate (PR)Piru Village $= \frac{126}{1.996}$ x 100% = 6,32
6. Prevalence Rate (PR)Talaga Orchard $= \frac{85}{760}$ x 100% = 11,18

Different species of *Anopheles sp* often exhibit different behaviors and the ability to transmit different diseases. Therefore, the type of *Anopheles sp* mosquitoes that transmit the disease in one area is often different with *Anopheles sp* that transmit malaria or *chikungunya* disease in other areas (Sembel, 2009).

Malaria death rate (CFR)

1. CFR Sohuwe Village $= \frac{10}{127}$ x 100% = 7,87
2. CFR Lumalatal Village $= \frac{5}{68}$ x 100% =7,35
3. CFR Wamital Village $= \frac{8}{170}$ x 100% = 4,70
4. CFR Kairatu Village $= \frac{9}{130}$ x 100% = 6,92
5. CFR Piru Village $= \frac{6}{126}$ x 100% = 4,76
6. CFR Talaga Orchard $= \frac{5}{85}$ x 100% = 5,88

CONCLUSION

The behavior of the people towards the use of malaria medicines (Klorokuin, promakuin and traditional medicine), the use of mosquito repellent (*Bakar and baigon*), the habit of hanging clothes and traveling outdoors by using the Likhert scale to 180 respondents in 6 villages of West Seram Regency strongly agreed. Bionomics related to human biting behavior, at dusk and at night 16.00-24.00 peak: 02.00-03.00. Breeding behavior from anopheles mosquitoes by laying eggs in water ponds, boats. water basins, coconuts, wells, water barrels,

swamps, and bathtubs. While the resting/sleeping behavior either oudoor / indoor and on the clothes hanger. Average of Anopheles larvae density was 5.00, 9.00 and Average density of *Anopheles* mosquitoes was 5.09 - 9.85. Prevalence of malaria disease in West Seram Regency was between 6.32-13.9 and CFR value was 4.76-7.87. It is advisable for researchers to conduct follow-up studies related to the spread of anopheles mosquitoes in mountainous areas and other diseases caused by anopheles mosquitoes.

REFERENCES

Azwar, S. (1999). *Sikap manusia, teori dan pengukurannya* [*Human attitudes, theories and measurements*]. Yogyakarta: Pustaka Pelajar

Chandra, B. (1995). *Pengantar statistik kesehatan* [*Introduction to health statistics*]. Jakarta: Buku Kedokteran EGC

Depkes. (1987). *Ekologi vektor dan beberapa aspek perilaku* [*Vector ecology and several aspects of behavior*]. Jakarta.

Fahmi, U. (2004). Malaria situation in Indonesia (police) [Malaria situation in Indonesia (police)]. *Prosiding Symposium of Malaria Control in Indonesia*, TDC, Nopember 29-30.

Dinkes Propinsi Maluku. (2005). *Profil Dinas Kesehatan Provinsi Maluku* [Profile of the Maluku Provincial Health Office]. Dinkes, Maluku.

Dinkes Seram Bagian Barat. (2006). *Laporan Kegiatan IRS dan MFS/MBS* [*IRS and MFS / MBS Activity Reports*]. Piru.

Gandahusada. (2000). *Parasitologi Kedokteran* [*Medical Parasitology*]. FK UI. Jakarta.

Harfriani, H. (2012). Efektivitas larvasida ekstrak daun sirsak dalam membunuh jentik nyamuk [The larvicidal effectiveness of soursop leaf extract in killing mosquito larvae]. *Jurnal Kesehatan Masyarakat*, 7(2), 164-16.

Hidajati. (2006). Mewaspadai Re-Emerging Malaria [Beware of Re-Emerging Malaria]. Majalah Kedokteran Tropis Indonesia.

Notoatmodjo (2003). *Pendidikan dan Perilaku Kesehatan* [*Health Behavior and Education*]. Rineka Cipta, Jakarta.

Pulungan, E. S., Santi, D. N., & Chahaya, I. (2012). *Hubungan sanitasi lingkungan perumahan dan perilaku masyarakat dengan kejadian filariasis di Kecamatan Kampung Rakyat Kabupaten Labuhan Batu Selatan* [The relationship between residential environmental sanitation and community behavior with the incidence of filariasis in the Kampung Rakyat Subdistrict, Labuhan Batu Selatan Regency]. Skripsi. FKM USU Medan.

Qomariah, M. (2004). *Survey nyamuk Anopheles yang berpotensi sebagai vektor malaria di bekas penggalian timah Kolong Ijo Kelurahan Bacang Kota Pangkal Pinang* [*Survey of Anopheles mosquitoes which have the potential as malaria vectors in the former excavation of Kolong Ijo tin, Bacang Village, Pangkal Pinang City*]. Disesrtasi. Universitas Diponegoro.

Rattanarithikul., & Horison, B. (2005). Illustrated keystothe musquitoes of Thailand I. Background; Geographic Distribution; Lists of Genera, Subgenera, dan Species; dan a Key tothe Genera. *The South east Asian Journal of Tropical Medicine*, 36(Supplement 1).

Rampengan, T. H., Laurentz, I. R. (1997). *Penyakit infeksi tropik pada anak* [*Tropic infectious disease in children*]. EGC, Jakarta.

Rehena, J. F. (2005). Bionomik nyamuk anopheles dan penentuan vektor malaria di Dusun Uraur Kecamatan Kairatu [Bionomic anopheles mosquito and malaria vector determination in Uraur Hamlet, Kairatu District]. *Media Ilmiah MIPA*, 2(1), 1-12.

Rehena, J. F. (2006). Dampak Lingkungan Terhadap Kepadatan Nyamuk Anopheles, Perilaku dan Prevalensi Malaria di Daerah Pesisir dan Pegunungan Kecamatan Kairatu [Environmental Impact on Anopheles Mosquito Density, Malaria Behavior and Prevalence in Coastal and Mountain Areas of Kairatu District]. *Media Ilmiah MIPA*, 3(2).

Sembel, D. T. (2009). *Entomologi kedokteran* [*Medical entomology*]. Andi, Yogyakarta.

Soedarto. (1995). *Protozoologi kedokteran* [*Medical protozoology*]. Widya Medika, Jakarta.

Sumantri, A., & Rosidati, C. Analisis Kepadatan Nyamuk Anopheles sp di dalam Rumah Berdasarkan Lingkungan di Desa Sidareja, Kecamatan Kaligondang, Kabupaten Purbalingga [Density Analysis of Anopheles sp in the House Based on the Environment in Sidareja Village, Kaligondang District, Purbalingga Regency]. Skripsi UIN Jakarta.

Zein, U. (2005). *Penanganan Terkini Malaria Falciparum* [*Current Management of Falciparum Malaria*]. Fakultas Kedokteran Universitas Sumatera Utara, Medan.