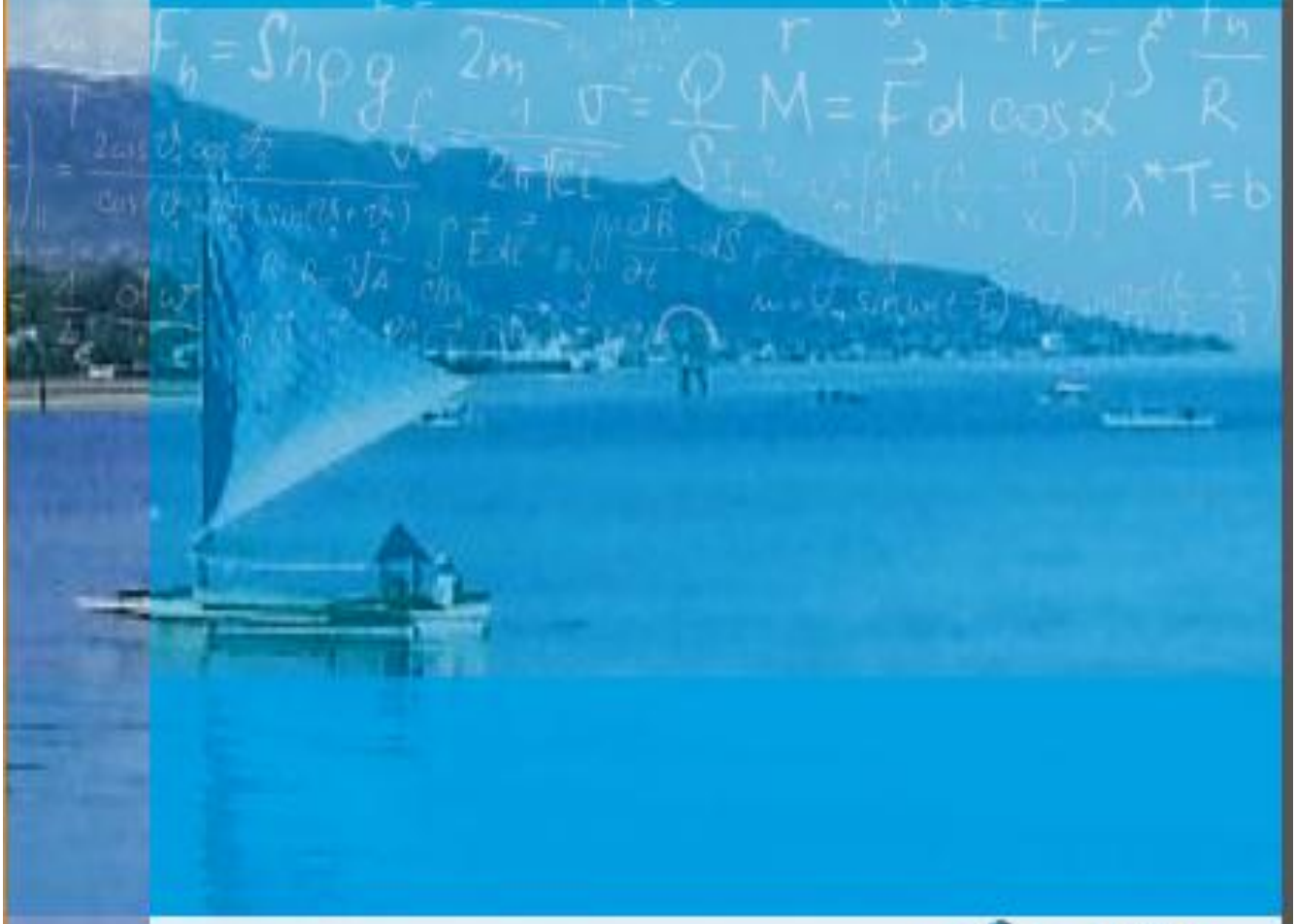
Basic Science
for Sustainable Marine Development

PROCEEDING
INTERNATIONAL SEMINAR 2015

Ambon, 3-4 June 2015



Organized by
Faculty of Mathematics and Natural Sciences
Pattimura University

PROCEEDINGS

1st International Seminar of Basic Science, FMIPA Unpatti - Ambon
June, 3rd – 4th 2015

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Welcoming Address by The Organizing Committee

The honorable, the rector of Pattimura University

The honorable, the vice rector of academic affair, Pattimura University

The honorable, the vice rector of administration and financial affair, Pattimura University

The honorable, the vice rector of planning, cooperation and information affair, Pattimura University

The honorable, all the deans in Pattimura University

The honorable, the key note speakers and other guests.

We have to thank The Almighty God for the blessings that allow this International seminar can be held today. This is the first seminar about MIPA Science in which the Faculty of MIPA Pattimura University becomes the host. The seminar under the title Basic Science for Sustainable Marine Development will be carried out on 3 June 2015 at Rectorate Building, the second floor. There are 250 participants from lecturers, research institute, students, and also there are 34 papers will be presented.

This International seminar is supported by the amazing people who always give financial as well as moral supports. My special thanks refer to the rector of Pattimura University, Prof. Dr. Thomas Pentury, M.Si, and the Dean of MIPA Faculty, Prof. Dr. Pieter Kakissina, M. Si. I also would like to express my deepest gratitude to Dr. Kotaro Ichikawa, the director of CSEAS Kyoto University, Prof. Bohari M. Yamin, University of Kebangsaan Malaysia, Prof. Dr. Budi Nurani Ruchjana (Prisident of Indonesian Mathematical Society/Indo-MS), Dr. Ir. A. Syailatua, M.Sc (Director of LIPI Ambon), and Hendry Ishak Elim, PhD as the key note speakers. We expect that this international seminar can give valuable information and contribution especially in developing basic science for sustainable marine development in the future.

Last but not least, we realize that as human we have weaknesses in holding this seminar, but personally I believe that there are pearls behind this seminar. Thank you very much.

Chairman

Dr. Netty Siahaya, M.Si.

Opening Remarks
By Dean of Mathematic and Natural Science Faculty

I express my deepest gratitude to The Almighty God for every single blessing He provides us especially in the process of holding the seminar until publishing the proceeding of International Seminar in celebrating the 17th anniversary of MIPA Faculty, Pattimura University. The theme of the anniversary is under the title Basic Science for Sustainable Marine Development. The reason of choosing this theme is that Maluku is one of five areas in Techno Park Marine in Indonesia. Furthermore, it is expected that this development can be means where the process of innovation, it is the conversion of science and technology into economic value can be worthwhile for public welfare especially coastal communities.

Having the second big variety of biological resources in the world, Indonesia is rich of its marine flora and fauna. These potential resources can be treated as high value products that demand by international market. Basic science of MIPA plays important role in developing the management of sustainable marine biological resources.

The scientific articles in this proceeding are the results of research and they are analyzed scientifically. It is expected that this proceeding can be valuable information in terms of developing science and technology for public welfare, especially people in Maluku.

My special thanks refer to all researchers and reviewers for your brilliant ideas in completing and publishing this proceeding. I also would like to express my gratefulness to the dies committee-anniversary of MIPA Faculty for your creativity and hard working in finishing this proceeding, God Bless you all.

Dean of Mathematic and Natural Science Faculty

Prof. Dr. Pieter Kakisina, M.Si.

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Histological of haemocyte infiltration during pearl sac formation in *Pinctada maxima* oysters implanted in the intestine, anus and gonad

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ABSTRACT

The experiment was conducted to study histological of haemocyte infiltration during pearl sac formation in *Pinctada maxima* oysters implanted nuclei in different sites. The first factor was the site of nucleus implantation consisted of 3 levels i.e., intestine, anus and ventral gonad. The second factor was time after implantation with 4 levels i.e., 1, 2, 3 and 4 weeks. The saibo used in the experiment was taken from normal *Pinctada maxima* oyster aged 28 months. Selection of *Pinctada maxima* oyster as a donor oyster was based on the same criteria used in selecting the host oyster. Haemocyte infiltration during pearl sac formation in *Pinctada maxima* oysters no different. Four weeks after implantation, there was no haemocyte and inflammatory cell was found.

Keywords: Nucleus implantation, Anus, Intestine, Ventral gonad, Haemocyte, *Pinctada maxima.*

INTRODUCTION

In general, implantation of nucleus in culture pearl production is conducted by inserting the nucleus in the gonad. However, implantation of nucleus in the other parts of the oyster body is possible. In the process of blister pearl production, which is mostly produced by the fresh water oysters, the nucleus is implanted into the shell wall. The use of gonad as a site of nucleus implantation is probably based on the success and the quality of pearl produced. Culture pearl production with different locations of the nucleus and saibo implantation in *Pinctada fucata* oyster was reported (Alagarswami 1974) and the color, shape and quality of the pearl produced were affected by the different sites of nucleus implantation (Chellam *et al.* 1991).

The pearl can be produced more than one in four areas in the body of the freshwater mussels (Chatchavalvanich *et al.* 2010). However, the basic biological data of host oysters during pearl sac formation in different sites of nucleus implantation are not available. These

data and information are essential in understanding the biological basis of nucleus implantation and pearl sac formation in the host oysters implanted in different sites of the body. This information could be used to develop multiple implantations in host oysters such as *Pinctada maxima* to increase culture pearl production.

Histological changes in the host oysters after implantation and during pearl sac formation in pearl oysters *in vivo* and *in vitro* were studied in *Pinctada fucata* (Velayudhan *et al.* 1994; Awaji & Machii 2011) and in *Pinctada margaritifera* oysters (Cochennec-Laureau *et al.* 2010) and histochemistry study of mantle and pearl sac secretory cells in *Pinctada maxima* (Dix 1972). The observation on histological changes in the host oysters during pearl sac formation in the *Pinctada maxima* host oysters (Scoones 1996; Mamangkey & Southgate 2009) is limited in the oysters implanted in the gonad.

These data and information are essential in understanding the histological basis of nucleus implantation and pearl sac formation in the host oysters implanted in different sites of the body. This research was designed to provide basic histological haemocyte infiltration data of host oysters implanted in different locations in the abdomen during pearl sac formation to improve the understanding of pearl sac formation and the probability of multiple implantations in the *Pinctada maxima* host oysters.

MATERIALS AND METHODS

Experimental materials

This study was conducted from September 2011 to February 2012 at the commercial pearl farm of CV. Aru Duta Indah in the Garaga, Obi Island (01°25'S, 127°20'E) North Mollucas Province, Indonesia. Host and donor oysters used in this experiment were *Pinctada maxima* oysters that were cultured by the commercial pearl farm. The first factor was the location or site of nucleus implantation consisted of 3 levels i.e., in the intestine, anus and ventral gonad. The second factor was week of measurement after implantation of nucleus consisted of 4 levels i.e., 1, 2, 3 and 4 weeks. The parameters measured histological of haemocyte infiltration during pearl sac formation in *Pinctada maxima* oysters implanted nuclei in different sites.

The other thirty six (36) oysters succeeded in implantation were used for measurement of histological haemocyte infiltration during pearl sac development. The saibo used in the experiment was taken from normal *Pinctada maxima* oyster aged 28 months. During implantation, the host oysters were placed in a standing position so that the oysters experienced oxygen deficiency that stimulated the opening of the oyster shells. When the shells were open, the spatula was used to separate the gill covering the gonads. The locations of nucleus implantation in the abdomen were ventral gonad, intestine and anus. After making a small incision (6.6 mm) in the sites of implantation (ventral gonad, intestine and anus), the nucleus that was attached with a saibo was inserted. All this process was done carefully so that the oysters did not experience stress. For histological observation of pearl sac development, the abdomen of the oyster succeeded to form pearl sac was cut and isolated and were saved in buffer normal formalin (BNF) for future histological preparation in the laboratory.

Parameters measured

The organs used for nucleus implantation (intestine, anus, and ventral gonad) were isolated for histological preparation. The histological preparation of the haemocyte infiltration

during pearl sac developing pearl sac used the haematoxcillin eosin staining (HE) technique. Data analysis was done descriptively.

RESULTS

Histology of haemocyte infiltration during pearl sac development in Pinctada maxima oyster implanted in the intestine

Histology of haemocyte infiltration during pearl sac development in *Pinctada maxima* oyster implanted in the intestine is presented in Fig. 1. In oysters implanted nucleus in the intestine and succeeded in pearl sac formation, the inflammatory cells and haemocytes infiltrations were high 1 week after implantation. Two weeks after implantation, the number of inflammatory cells and haemocytes began to decline. Three weeks after implantation, the number of inflammatory cells and haemocytes were very low and the injury began to recover. At the end of observation, 4 weeks after implantation, no haemocytes and inflammatory cells was found.

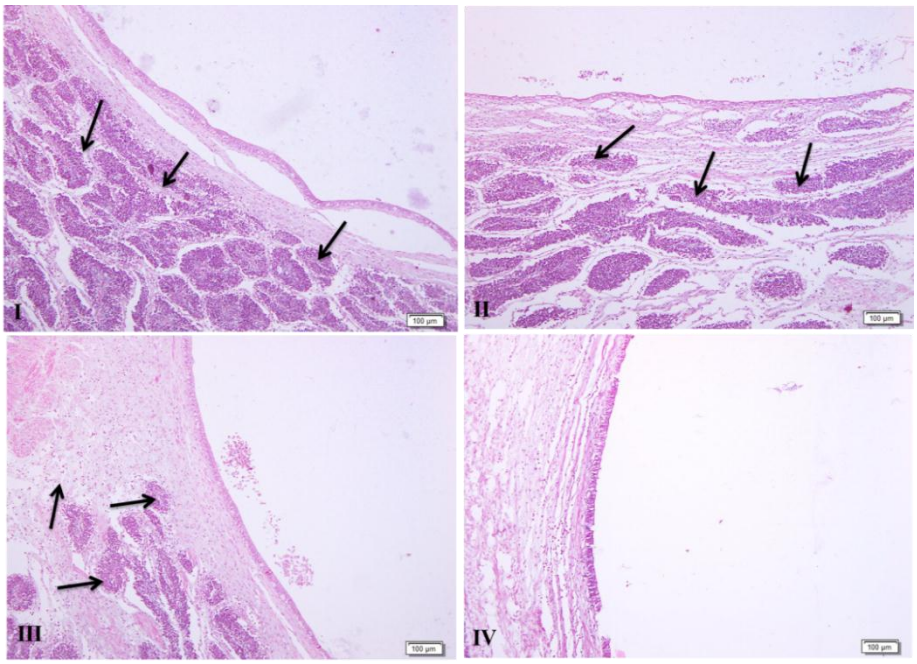

Figure 1. Histology of haemocyte infiltration during pearl sac development in *Pinctada maxima* oyster implanted in the intestine. Arrows indicated haemocytes. One week after implantation (I), the inflammatory cells and haemocyte were high. Two weeks after implantation (II), the number of inflammatory cells and haemocyte began to decline. Three weeks after implantation (III), the number of inflammatory cells and haemocytes were very low and the injury began to recover. Four weeks after implantation (IV), there was no haemocyte and inflammatory cell was found. H&E. x100.

Histology of haemocyte infiltration during pearl sac development in Pinctada maxima oyster implanted in the anus

Histology of haemocyte infiltration during pearl sac development in *Pinctada maxima* oyster implanted in the anus is presented in Fig.2. Oysters implanted nucleus in the anus and

succeeded in pearl sac formation showed the similar pattern of haemocyte infiltration and the recovery of injury as those implanted in the intestine. One week after implantation, inflammatory cells and haemocytes infiltrations were high. Two weeks after implantation, the number of inflammatory cells and haemocytes began to decline and three weeks after implantation, the number of inflammatory cells and haemocytes were very low and the injury began to recover. At the end of observation, 4 weeks after implantation, there was no haemocyte and inflammatory cell was found.

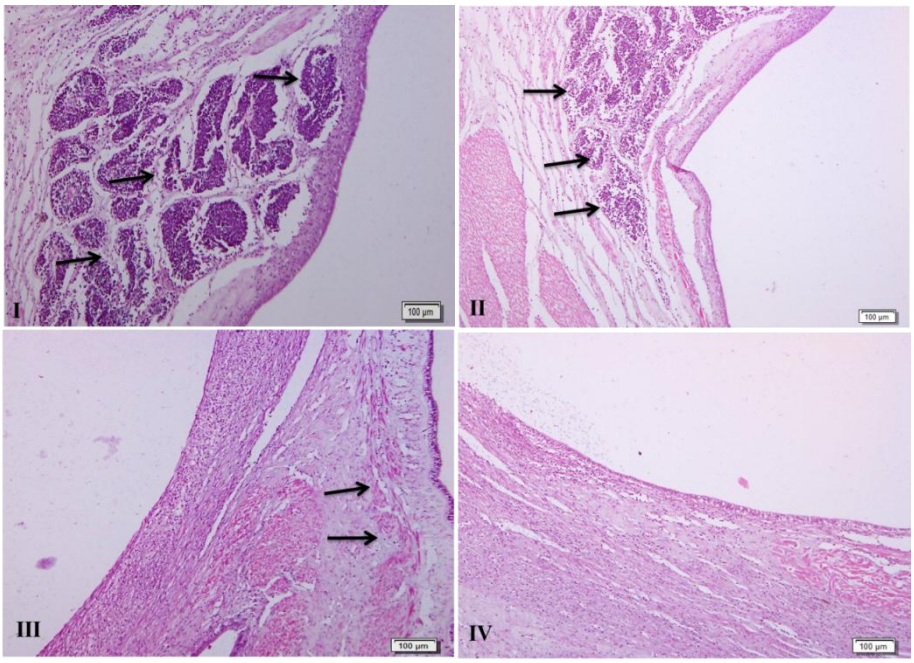

Figure 2. Histology of haemocyte infiltration during pearl sac development in *Pinctada maxima* oyster implanted in the anus. Arrows indicated haemocytes. One week after implantation (I), the inflammatory cells and haemocytes were high. Two weeks after implantation (II), the number of inflammatory cells and haemocytes began to decline. Three weeks after implantation (III), the number of inflammatory cells and haemocytes were very low and the injury began to recover. Four weeks after implantation (IV), there was no haemocyte and inflammatory cell was found. H&E. x100.

Histology of haemocyte infiltration during pearl sac development in Pinctada maxima oyster implanted in the ventral gonad

Histology of haemocyte infiltration during pearl sac development in the *Pinctada maxima* oyster implanted in the ventral gonad as a standard site of nucleus implantation is presented in Fig. 3. Oysters implanted nucleus in the ventral gonad and succeeded in pearl sac formation showed the similar pattern of haemocyte infiltration and the recovery of injury as those implanted in the intestine and anus. One week after implantation, the inflammatory cells and haemocytes were high. Two weeks after implantation, the number of inflammatory cells and haemocytes began to decline, followed by the recovery of injury with a very low inflammatory cells and haemocytes 3 weeks after implantation. The same results were found in the oysters implanted nucleus in the intestine and anus. There was no haemocyte and inflammatory cell was found 4 weeks after implantation.

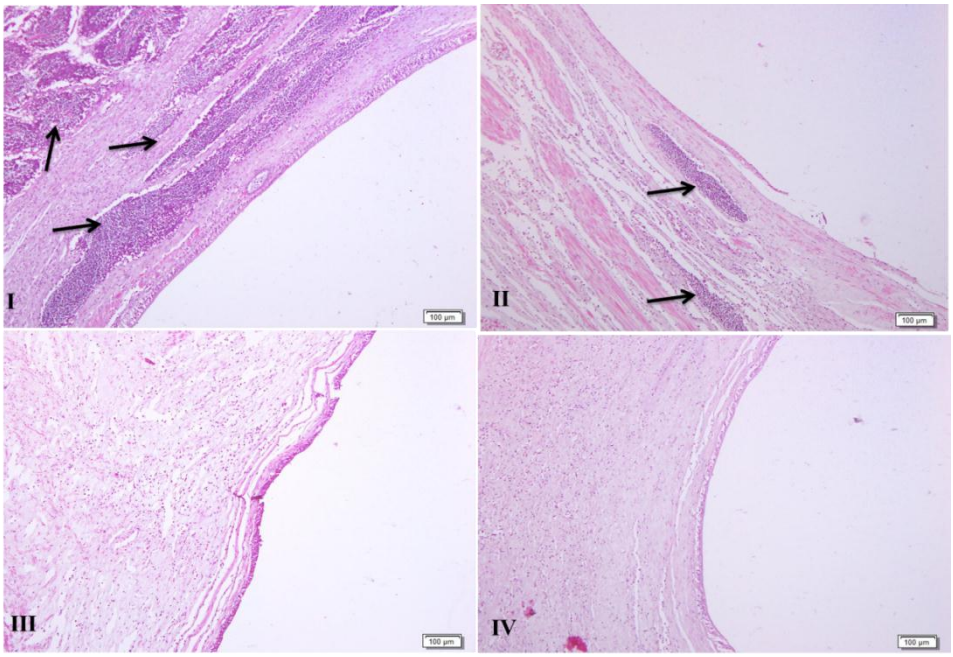

Figure 3.Histology of haemocyte infiltration during pearl sac development in the *Pinctada maxima* oyster implanted in the ventral gonad. Arrows indicated haemocytes. One week after implantation (I), inflammatory cells and haemocytes were high. Two weeks after implantation (II), the number of inflammatory cells and haemocytes began to decline. Three weeks after implantation (III), the number of inflammatory cells and haemocytes was very low and the injury began to recover. Four weeks after implantation (IV), there was no haemocyte and inflammatory cell was found. H&E. x100.

DISCUSSION

Histology of haemocyte infiltration during pearl sac development in the *Pinctada maxima* oyster implanted in the ventral gonad similar pattern of haemocyte infiltration and the recovery of injury as those implanted in the intestine and anus. The same results were found in the oysters implanted nucleus in the intestine and anus. There was no haemocyte and inflammatory cell was found 4 weeks after implantations. This result indicated implantation in intestine and anus can be used as alternative locations of nucleus implantation in pearl culture in *Pinctada maxima* for multiple implantations.

Operation and incision process in the intestine, anus, and ventral gonad during nucleus implantation caused the oyster stress. Stress increases the total haemocytes on the *Crassostrea gigas* oyster. Stress affects several hormones activities such as CRH (corticotrophin releasing hormone), ACTH (adrenocorticotrophic hormone), cytokines, noradrenaline, adrenaline, dopamine, and cortisol (Lacoste *et al.* 2002). Stress activates the endocrine system such as corticotrophin releasing hormone (CRH), which stimulates the release of adrenocorticotrophic hormone (ACTH). The presence of ACTH stimulates the release of biogenic amino acids, which eventually lead to secondary effects on oysters (Hooper *et al.* 2007).

During stress, cortisol was reported to increase (Hooper *et al.* 2007), that was associated with the increased glucose concentrations. Increased stress during early implantation increased haemocyte infiltration and haemolymph glucose concentration. When the implantation injury was cured, haemocytes was low and haemolymph glucose

concentration reached the lowest levels. The decreased haemolymph glucose concentration with the advance of pearl sac growth after implantation could indicate the possibility of increased glucose uptake without increased in glucose mobilization or uptake to the haemolymph. Glucose are required for energy source for basal metabolism and for supporting synthetic activities as well as for synthesis of material build up from glucose, such as conchiolin. Conchiolin is organic in nature and consists of mucopolysaccarides (Chellam *et al.* 1991).

CONCLUSIONS

Haemocyte infiltration during pearl sac formation in *Pinctada maxima* oysters no different.The intestine and anus can be used as alternative locations of nucleus implantation in pearl culture in *Pinctada maxima* for multiple implantations.

ACKNOWLEDGEMENTS

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