Biofouling and attachment of black-lip pearl oyster spat (*Pinctada margaritifera*) in Tanah Merah waters, Kupang Bay, Indonesia

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Abstract. This study examines the fouling and sticking of pearl oyster spat *Pinctada margaritifera* (Linnaeus, 1758) in Tanah Merah Village waters, Kupang Bay, Indonesia. This research is quantitative research using survey methods. The variables observed were the composition, and density of pearl oyster spat each month. Time series data from each variable will be analyzed using multiple linear regression analysis and Spearman correlation. Furthermore, the data will be presented in the form of graphs. The results showed that the correlation (R) between the independent variable (biofouling attachment) and the dependent variable (spat sticking) was 0.8%. The significance level is 0.787 > 0.05, with the regression equation Y = 0.514 + 0.013X. The regression coefficient is positive so it can be said that the direction of influence of variable X (biofouling attachment) on variable Y (spat sticking) is positive. This means that the pearl oyster spat sticking season coincides with the biofouling season. However, the significance value is 0.787 > 0.05 which indicates that variable X does not affect variable Y. However, pearl oyster spat (*Pinctada margaritifera*) can be found in waters throughout the year, but there are 2 (two) periods where the spat attachment is found in higher quantities, namely the period between December and January and the period between June and July. Biofouling shows that the highest abundance occurs in May – July. There is a difference between the peak attachment season of pearl oyster spat and the peak season of biofouling, although both can be found in cultches throughout the year, where the peak of fouling occurs in the rainy season until the end of the rainy season.
Key Words: cultch, composition, correlation, density, season.

Introduction. Pearl oyster cultivation in Indonesia has long been developing with the main cultivated species being *Pinctada maxima* (Jameson, 1901) which produces round pearls (Sahat 2013). Generally, this business is run by large investors both from within and outside the country with the main aim of meeting export demand. Another pearl oyster that lives in Indonesian waters is *Pinctada margaritifera* (Linnaeus, 1758), which produces mabe pearls (blister/half to three-quarters round) in addition to round pearls (Hamzah & Nababan 2009; Tomatala 2015). One of the locations that has natural potential for *Pinctada margaritifera* oysters is Tanah Merah Village waters, Central Kupang District, Kupang Regency, Indonesia.

The production of round pearls has its level of difficulty and has a greater risk of failure (Crusot et al 2021; Aideed et al 2014). In many companies, pearl core installation work is generally done by special staff and generally foreign workers. Unlike pearls, mabe pearls are easier to obtain, so it is more applicable if the cultivation is to be developed based on folk industry. The installation of the mabe pearl core is simpler so that it can be done by the community and the success rate is higher (Aji 2011; Ky et al 2019). Although the price of mabe pearls is lower, it has its market in Indonesia.

The development of pearl cultivation on a people's industrial scale needs to be supported by the availability of a database on business feasibility related to oyster production management (Ky et al 2019; Kishore & Southgate 2016). This includes information on the attachment of biofouling and the natural spat of pearl oysters. The presence of biofouling in pearl oyster cultivation greatly interferes with the growth and life of pearl oysters (Lacoste & Gaertner-Mazouni 2014; Fitridge et al 2012). Therefore, it is important to know the data on the type and percentage of biofouling throughout the year. Likewise, knowledge of pearl oysters' natural spat season is essential when the cultivation to be carried out relies on the supply of seeds from nature (Hwang 2007). However, natural seeds have several advantages, including durability and faster growth than hatchery seeds, in addition to being cheaper when viewed from their operational costs.

With the above considerations, a study has been conducted on biofouling and attachment of pearl oyster spat (*Pinctada margaritifera*) in Tanah Merah waters. This research is expected to provide important scientific information about biometrics and growth of pearl oysters which is important for the development of people-based pearl oyster cultivation business in the future.

Material and Method

Description of the study sites. The research was conducted for a year, from September 2023 to August 2024, in the Coastal Waters of Tanah Merah Village, Central Kupang District, Kupang Regency, Indonesia. The research location is the natural habitat of pearl oysters (*Pinctada margaritifera*) where many spat and oyster saplings are found in this location.

Research procedure. This study used cultch which is usually used to collect pearl oyster spat as a medium to observe macrofouling and attachment of spat. The clutch used consists of a rectangular-shaped frame made of galvanized rods measuring 40 x 30 cm, and a string made of braided PE rope fibers is then installed on the clutch frame by tying it using nylon rope on the side opposite to the horizontal direction. This cultch rope is the medium in which pearl oyster spat is attached. Before being placed in the water, the cultch rope is immersed in a treatment bath to remove chemicals contained in the rope fibers. Then the cultch rope is washed and rinsed several times, after which it is ready for use.

As a medium for hanging cultches rafts measuring 2 x 2 m were used. The raft is made of wood tied with PE rope forming a square plane and is equipped with 4 (four) buoys tied to each corner of the raft. The raft is equipped with an anchor so that it is not washed away during heavy currents, and it is also equipped with a marker flag for easy recognition. The raft is placed in water with a depth of at least 2 meters at the lowest tide.

Three cultches were placed in the water by hanging on rafts, and so on each month, three cultches were placed again. Cultch that has been attached after 2 months is taken to observe attachment of pearl oyster spat and biofouling of attachment to cultch. The cultch is removed from its frame, then carefully washed to clean the mud particles attached to the cultch. After that, the cultch was observed in the Fisheries Field Laboratory, UPT. Integrated Field Laboratory of Archipelagic Dryland, Nusa Cendana University.

The biofouling data taken is macrofouling which includes data on the type, amount, and cover of the cultch. The attachment of pearl oyster spat was recorded by counting the number of spats found attached to the cultch plane. The dimension of the spat is measured using a vernier caliper (precision 0.01mm). During the observation period, water quality measurements, namely temperature, dissolved oxygen, pH, salinity, nitrate, phosphate and water currents were assessed. The temperature and DO were measured using DO meter (Lutron™ DO-5519, with DO range: 0 to 20 mg/L x 0.1 mg/L and temperature: 0 to 50 °C). pH was measured using a pH-meter (pH-meter digital ATC™, with range pH: 0.00 – 14.00, resolution: 0.01 accuracy: ± 0.1 (20°C). Salinity was measured using a refractometer (salinity refractometer ATC™, with salinity range 0-100 ppt). Current speed was measured using float tracking method, every month during the study. Nitrate and phosphate levels were analyzed using the spectrophotometric method (Spectrophotometer UV-VIS Hach, DR6000 spectrum wavelength from 190-1100 nm, and spectral bandwidth of 2 nm). Measurement and sampling of water for water quality parameter analysis were carried out at a depth of 1 m, where the cultch was placed.

***Statistical analysis**.* The attachment relationship between macrofouling and spat was analyzed using linear regression analysis. Statistical analysis was conducted with SPSS Statistics software ver. 24.0.

Results

Spat attachment. The results of observations of spat attachment from September to August show that pearl oyster spat (*Pinctada margaritifera*) can be found in waters throughout the year, but there are two periods where spat attachment is found in higher numbers, namely the period between December and January and the period between June and July. The attachment cycle of pearl oysters spat in Tanah Merah Village waters can be seen in the following graph (Figure 1).

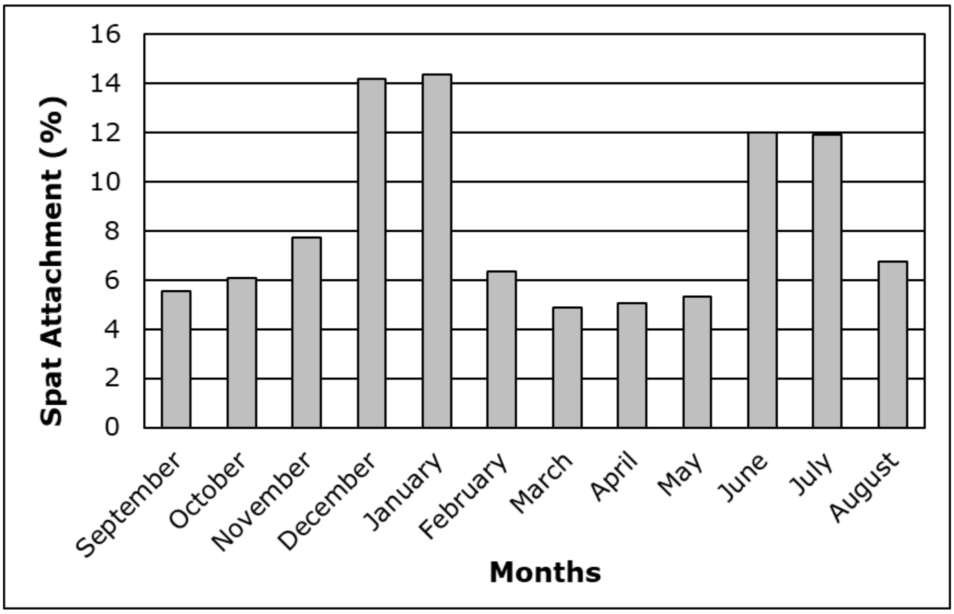


Figure 1. Sticking pearl oyster spat in Tanah Merah Village waters.

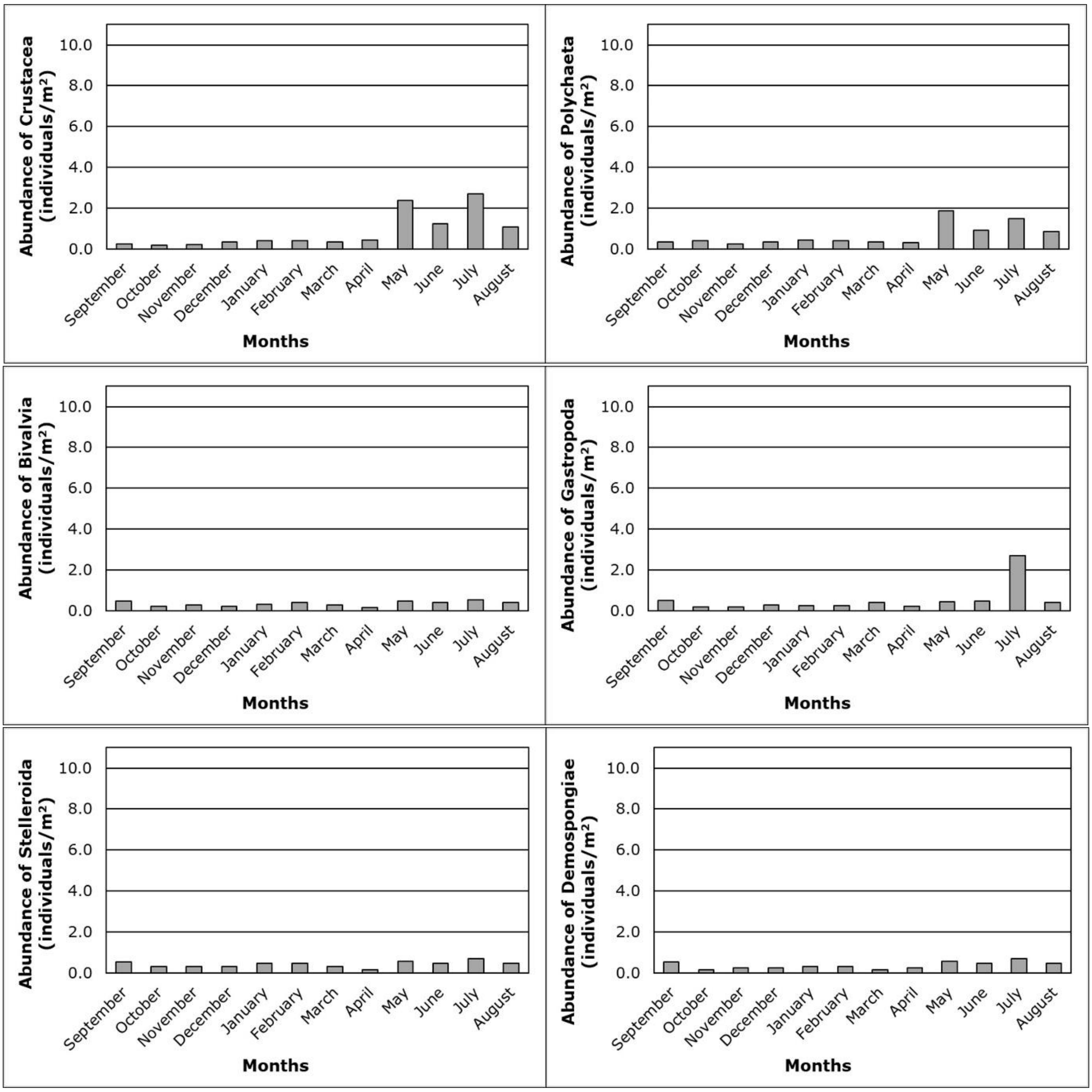
Spat sticking that occurs in Tanah Merah Village waters shows that the spat attachment season naturally occurs in two periods of the year. Thus, if the pearl oyster cultivation efforts to be developed rely on natural seed/spat supply, the right time to harvest spat in the waters of Tanah Merah Village is in both periods. Pearl oyster seeds/spat in this location are in abundant condition, therefore it is necessary to maintain environmental sustainability in this location, because environmental changes, especially those related to aquatic fertility and pollution, can interfere with the natural production of oyster seeds.

When viewed from the current conditions in Tanah Merah Village there is a mangrove ecosystem that supports the natural productivity in the water, and besides that domestic activity in this location is still relatively low because housing adjacent to the coast is rare. However, fishing activities that are not environmentally friendly, such as catching fish using poison/electricity, certainly require supervision and regulation from the authorities.

After all, the natural spat attachment of pearl oysters is related to spawning events in the waters. Thus, it can be suspected that naturally pearl oysters experience two spawning seasons in nature, namely December to January and June to July, or simultaneously with the period of changing seasons, namely at the change of the dry season to the rainy season and at the change of the rainy season to the dry season.

Biofouling. The observations showed that the highest abundance of biofouling occurred in May – July (Figure 2). This shows that there is a difference between the peak attaching season of pearl oyster spat and the peak season of biofouling, although both can be found in cultches throughout the year. The peak of biofouling actually occurs during the rainy season until the end of the rainy season. This is likely related to the dynamics of inorganic nutrients in water that increase during the rainy season, where in the rainy season water runoff from land carries many inorganic nutrients from land both from rock scouring by water flow and dissolved organic matter loads eroded by water flow from land.

However, most biofouling organisms are primary producers in aquatic environments, which require the presence of dissolved nutrients in the water, especially phosphates and nitrates. Phosphate in the form of orthophosphate and nitrogen in the form of nitrate are forms that can be utilized by primary producers in the process of photosynthesis. The development of primary producers such as algae will certainly increase the presence of biofouling organisms at trophic levels above them such as crustaceans and polychaetes. However, the presence of biofuling in waters shows that natural aquatic ecosystems are still in good condition and shows that waters are also still suitable for the growth of pearl oysters.



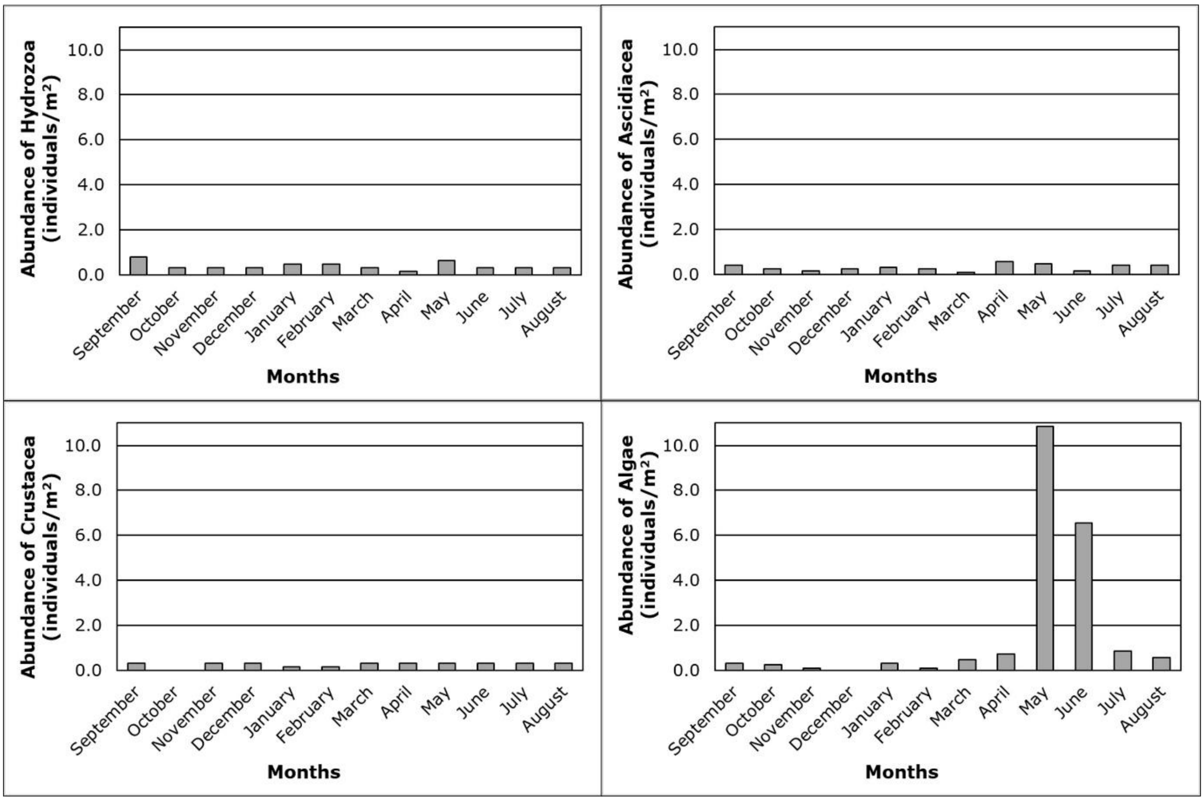


Figure 2. Abundance of biofouling during a year on pearl oyster cultch.

Another phenomenon also shows that cultch parts that have been attached by biofouling are not affixed by pearl oyster spat, however, placing cultch spat in nature outside its spawning season is also not effective due to the relatively low attachment of spat. Therefore, the placement of pearl oyster cultch will be more effective when done in the pearl oyster spat season of occurrence, so that the chance of cultch to be attached to pearl oyster spat will be higher and the event of attachment and biofouling will be earlier. The most commonly found biofouling organisms are the Crustacea class consisting of 7 genera, namely: *Balanus* spp., *Sesarma* spp., *Amphipoda* spp., *Urothoe* spp., *Penaeus* spp., *Episanthus* spp. and *Pilumnus* spp. The next most numerous genera are from Class Polychaeta and Gastropoda. The genera of Polychaeta found are *Eunice* spp., *Hydroides* spp., *Nereis* spp., and *Polychaeta* spp., while the genera of Gastropoda found are *Clypeomorus* spp., *Littorina* spp., *Monodonta* spp., and *Turritella* spp.

Environmental conditions. The condition of the parameters of the aquatic environment throughout the year is related to spat attachment and biofouling in cultch. Therefore, in this study, a water quality analysis was also carried out to get an overview of the dynamics of water quality throughout the year.

Temperature in Tanah Merah waters throughout the year ranges between 24-29°C with an average value of 26°C. The highest temperature occurs in August to October then rises again in the period from March to May, but the increase in temperature in that month is not too high. On average, the water temperature in the location is relatively stable and supports the life of aquatic organisms.

The salinity of Tanah Merah waters throughout the year ranges between 26-35 ppt with an average value of 30.19 ppt. The highest salinity occurs in August to October then decreases slightly and increases slightly in the period from March to May, but the increase in salinity in that period of the month is not too high. On average, the salinity of waters in the location is relatively stable and supports the life of aquatic organisms.

Dissolved oxygen in Tanah Merah waters throughout the year ranges between 4.72-5.46 mg/L with an average value of 5.06 mg/L. The highest dissolved oxygen concentration occurs in December then decreases slightly and increases again in the period from June to July, but the increase in dissolved oxygen in that month is not too high. On average, dissolved oxygen values at the location are relatively stable and support the life of aquatic organisms.

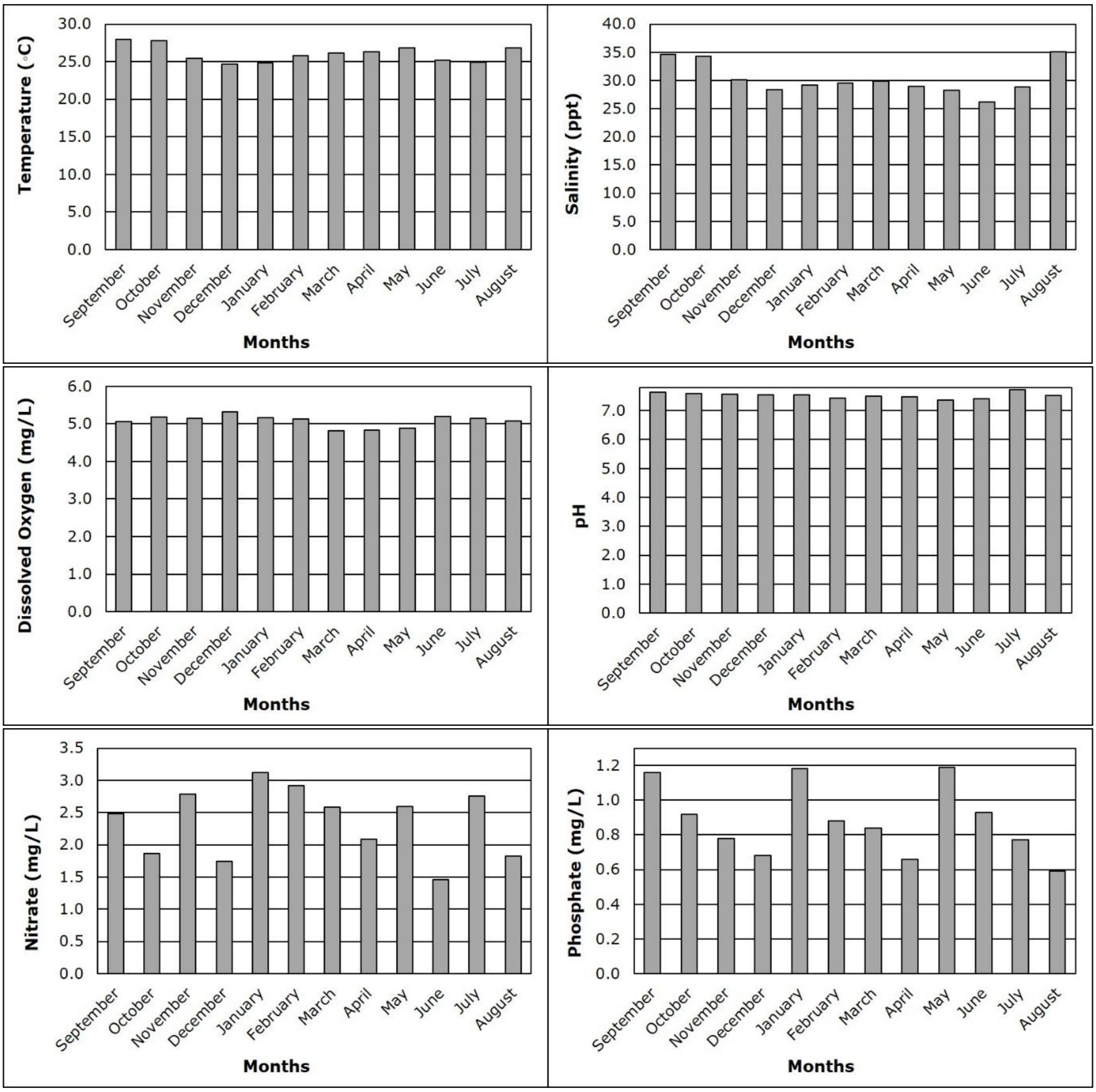


Figure 3. Dynamics of water quality parameters in Tanah Merah Waters.

The pH of Tanah Merah waters throughout the year ranges between 7.2-7.8 with an average value of 7.53. The pH is highest in July then slightly decreased and rose again in the period from August to October, but the pH increase in that period of the month was not too high. On average, the pH of the waters in the location is relatively stable and supports the life of aquatic organisms.

Phosphate levels of Tanah Merah waters throughout the year range between 0.48-1.34 mg/L with an average value of 0.87 mg/L. Nitrate values of Tanah Merah Waters throughout the year range between 1.72-3.54 mg/L with an average value of 2.35 mg/L. Nitrate and phosphate values show a relatively similar pattern of fluctuations where the highest occurs in December then slightly decreases and increases again in September, January and May and is slightly lower in other months (Figure 3).

The amount of correlation/relationship (R) between biofouling attachment and pearl oyster spat attachment is 0.087. The effect of the independent variable (biofouling attachment) on the bound variable (spat attachment) is 0.8%. $F_{calculated}$ value = 0.77 with a significance level of 0.787 > 0.05, so there is no influence of the biofouling attachment variable (X) on the spat attachment variable (Y). The constant value (a) is 0.514 while the regression coefficient (b) value is 0.013. So, the regression equation is Y = 0.514 + 0.013X. Adding 1% of the biofouling sticking value, the spat sticking value will increase by 0.013. The regression coefficient is positive so it can be said that the direction of influence of variable X on variable Y is positive. This means that in Tanah Merah waters, the pearl oyster spat sticking season coincides with the biofouling season. However, the significance value is 0.787 > 0.05 which indicates that variable X (biofouling attachment) does not affect variable Y (spat sticking). Thus, while operating the cultch in natural waters, it does not require biofouling cleaning, but the most important thing is that the placement of cultch should be in the spat attachment season so that the results are optimal. However, the part of the cultch field that has been biofouled will no longer be affixed by the pearl oyster spat.

Discussion. The attachment of pearl oyster spat (*Pinctada margaritifera*) in Tanah Merah waters occurs in two sticking periods (attachment season), namely in the period of December-January and the period of May-July. This phenomenon shows that natural pearl oyster spat can be harvested in these two seasons and shows that the natural spawning season occurs in two seasons that coincide with the transition season of the rainy season to the dry season (May-July) and the transition season of the dry season to the rainy season (December-January).

Pinctada margaritifera is a protandry hermaphrodite, with a spawning season of July and November for *Pinctada margaritifera*. This species has two spawning periods a year. The onset of reproduction appears to be regulated by sea-level temperature. Sexual composition in nature shows the number of female oysters is more than the number of male oysters during the spawning season (Hwang 2007). The dominant proportion of gonadal mature oysters in Hadramawt Egyptian waters was recorded in December-March in females and throughout two periods in males, namely January - March and August - October (Aideed et al 2014). Studies in French Polynesia show that black plate cultches are more efficient for *Pinctada margaritifera* spat collections. In addition, this black plate cultch can be reused so that it is more efficient than the shade-mesh plastic cultch commonly used (Crusot et al 2021).

Observations in Tanah Merah waters, Kupang Bay showed that the highest abundance of biofouling occurred in May – July (Figure 3). This shows that there is a difference between the peak attaching season of pearl oyster spat and the peak season of biofouling, although both can be found in cultches throughout the year.

In bivalve cultivation, the dominant biofouling organisms are filter feeders that can compete for food with the bivalves maintained, sometimes causing mortalities or reducing their growth rate (Lacoste et al 2014). Biofouling studies on pearl oysters (*Pinctada margaritifera*) cultivated in the lagoons of French Polynesia show that the presence of biofouling has no effect on the survival index, growth rate, colonization rate, and reproduction of pearl oysters, so it is important to review the need to control biofouling at the pearl oyster production stage before grafting (Lacoste et al 2014).

The study in East Lombok NTB Indonesia found 36 types of biofouling with 21 species from animal groups, namely 6 phyla (Arthropods, Bryozoa, Annelida, Cnidaria, Porifera, and Mollusca), and 15 species from plant groups with two divisions (Spermatophyta and Thallophyta). The number of species in each phylum is 11 species from Arthropoda, 3 species from Bryozoa, 4 species from Annelida, and 1 species each from Cnidaria, Porifera, and Mollusca. From plants, 14 types of Thallophyta and 1 type of Spermatophyta were found (Jefri et al 2017). Studies in Orpheus and Magnetic islands, Australia, on the Akoya pearl oyster *Pinctada fucata* (A. Gould, 1850), showed that the biofouling community was dominated by the hydroid *Obelia* sp., the bryozoan *Parasmittina* sp., the clam *Saccostrea* sp. and the ascidian *Didemnum* sp. (Guenther et al 2006).

Queensland and Western Australia are generally dominated by invertebrates such as barnacles, other bivalves, bryozoans, and tubiculous polychaetes. Ascidians, hydroids, and algae are significant types of soft biofouling. Biofouling can compete with pearl oysters which reduces growth, causes shell deformity, interferes causing core rejection, and in the worst case, results in oyster death. In addition, biofouling of aquaculture equipment such as panels, nets, ropes, and buoys increases the drag on the rope opening and closing valves, causing stress to oysters or interfering with nacre production (De Nys & Ison 2004).

Biofouling increases the operational and economic costs associated with pearl production. Because existing procedures to reduce oyster biofouling can be detrimental to survival and growth and can pollute the surrounding environment, the development of alternative biologically mediated methods could potentially improve production and ecological sustainability (Bertucci et al 2016). Natural cleaning studies of biofouling black-lip pearl oyster, *Pinctada margaritifera*, by butterfly fish (*Chaetodon* spp.) in Rangiroa Atoll, French Polynesia namely *Chaetodon auriga*, *Chaetodon citrinellus*, *Chaetodon ephippium*, *Chaetodon lunulatus*, *Chaetodon trifascialis* and *Chaetodon ulietensis* showed that all *Chaetodon* species cleaned the surface of pearl oysters by removing epibionts, despite differences in cleaning efficiency. *Chaetodon* is an omnivorous fish that has proven to be the most efficient cleaner (% cleaning range: 26-40% of total biomass). In this group, *C. ephippium* removes most of the biomass (41% on average) by targeting algae and anemones. The types of biofouling cleaned by *Chaetodon* spp. are algae, sponge, tunicate, and anemone (Bertucci et al 2016).

An experiment was conducted in north Queensland, Australia to assess the effect of biofouling and predation cleaning methods on the growth and survival of black-lipped pearl oyster juveniles (*P. margaritifera*). The results showed that the way biofouling cleaning affects the growth and survival of juvenile *P. margaritifera.* The predators of *P. margaritifera* in northern Australia include crabs, stomatopods, flatworms, gastropods, and fish. The stomatopod, Gonodactylus falcatus, is the most destructive predator with individuals consuming more than 20 juvenile pearl oysters per week. *Paramonacanthus japonicus* does not kill pearl oysters but trims the edges of oyster shells. Eradicating predators every month affects the growth of pearl oysters, but monthly inspection of aquaculture containers does not significantly improve oyster survival (Pit & Southgate 2003).

The pearl oyster industry in Indonesia generally relies on natural spat collection where spat is abundant during the summer (Aji 2011). The results of this study showed that biofouling sticking did not affect spat sticking. Another point shows that in Tanah Merah waters the attachment season of pearl oyster spat coincides with the biofouling attachment season. In addition, it is recommended that during operating the cultch in natural waters, it does not require cleaning biofouling, but the most important thing is that the placement of cultch should be in the spat attachment season so that the results are optimal. However, the part of the cultch field that has been biofouled will no longer be affixed by the pearl oyster spat.

Bivalve cultivation around the world is affected by the growth of biofouling. Submerged infrastructure and shells of preserved species constitute new substrates for various epibionts, consisting primarily of suspension feeders (Lacoste & Gaertner-Mazouni 2014). Handling biofouling requires significant operational costs for pearl cultivation (Guenther et al 2006). Biofouling in mariculture is a specific problem where cultivated species and/or infrastructure are exposed to a wide variety of biofouling, leading to significant production impacts. In shellfish aquaculture, the main impact is the attachment of direct biofouling to stocks causing physical damage, mechanical disturbances, biological competition, and environmental modification, in addition, infrastructure is also affected (Fitridge et al 2012).

The development of biofouling is generally regarded as an outbreak for shellfish aquaculture, and its control results in additional costs that can reach up to 30% of the industry's total operating costs. Epibionts not only affect their host species (basibionts), but also disrupt the ecological function of the ecosystem in which they live. The cleaning impact of biofouling can be stressful and damaging to cultivated species. Control of biofouling should be carefully reconsidered, based on a holistic approach considering: the interaction between the epibiont and its basibiont; its impact on the final product; and its contribution to ecosystem sustainability (Lacoste & Gaertner-Mazouni 2014). Conservative estimates of biofouling cleaning consistently range from 5–10% of production costs. Control of biofouling in aquaculture is achieved through avoidance of natural recruitment, physical removal, and use of antifoulants. The ever-increasing growth and expansion of the aquaculture industry and increasingly stringent regulations on biocides in food production require the development of innovative antifouling strategies. It must meet environmental, social, and economic standards while effectively preventing the settlement and growth of resilient multi-species biofouling consortia (Fitridge et al 2012). However, the collection of natural black-lip oyster spat in Tanah Merah waters should be done in the peak spat seasons in December - January or June - July. In addition, cultch placement should be done in the peak season of pearl oyster spat, because outside the peak season, the incidence of biofouling attachment is higher. In addition, cultches that have been affixed by biofouling, cannot be affixed by pearl oyster spat.

Conclusions. The results showed that pearl oyster spat (*Pinctada margaritifera*) can be found in waters throughout the year. Still, there are two periods where spat attachment is found in higher quantities, namely the period between December and January and the period between June and July. Biofouling shows that the highest abundance occurs in May – July. There is a difference between the peak attachment season of pearl oyster spat and the peak season of biofouling, although both can be found in collectors all year round, where the peak of fouling occurs in the rainy season until the end of the rainy season.

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Conflict of interest. The authors declare that there is no conflict of interest.

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