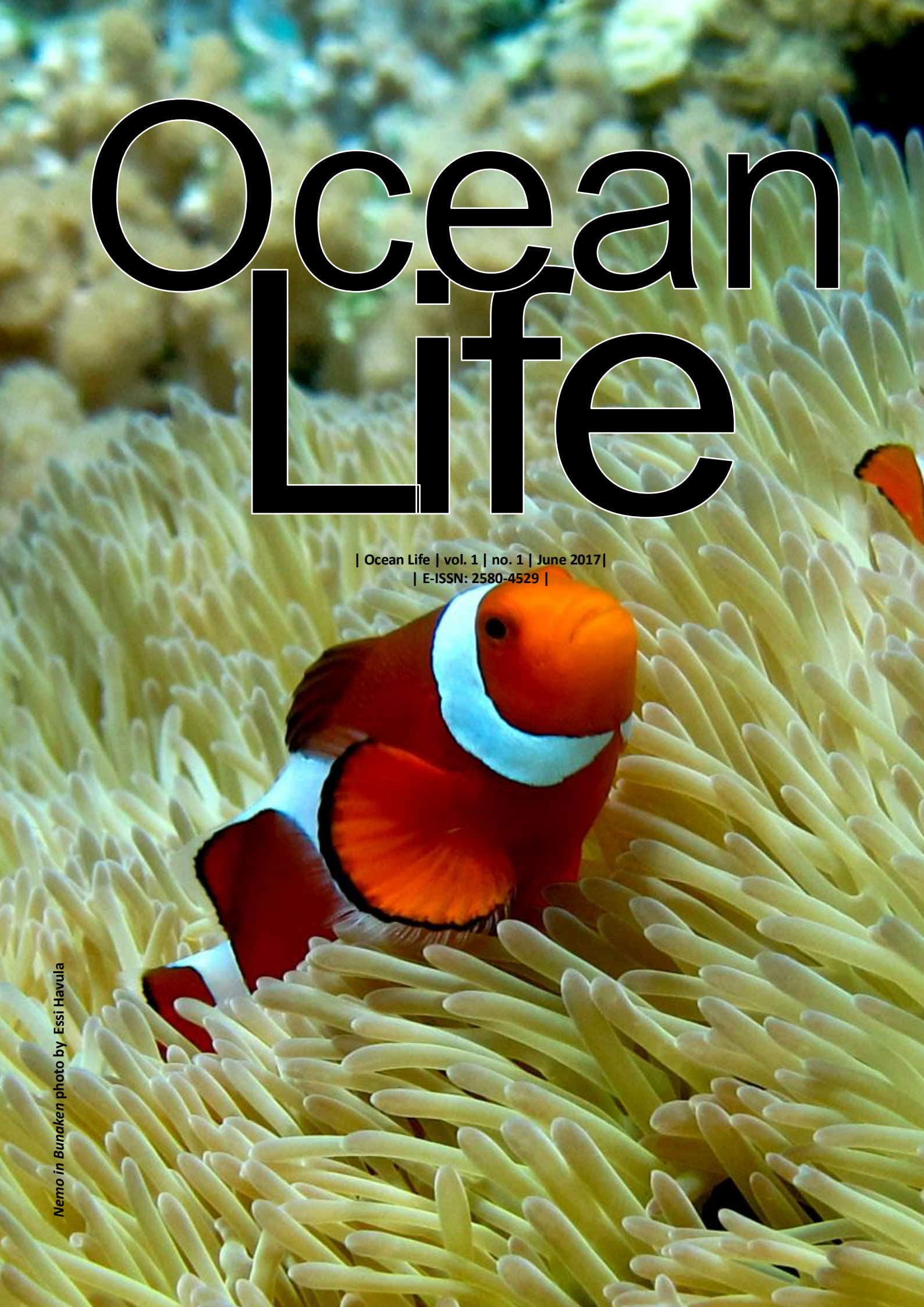
Ocean Life

| Ocean Life | vol. 1 | no. 1 | June 2017|
| E-ISSN: 2580-4529 |



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Ocean Life

| Ocean Life | vol. 1 | no. 1 | June 2017| E-ISSN: 2580-4529|

Published semiannually **PRINTED IN INDONESIA**

E-ISSN: 2580-4529

Ocean Life

| Ocean Life | vol. 1 | no. 1 | June 2017 |

ONLINE
http://smujo.id/ol

e-ISSN
2580-4529

PUBLISHER
Society for Indonesian Biodiversity

CO-PUBLISHER
Universitas Papua, Manokwari, Indonesia

OFFICE ADDRESS
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PERIOD OF ISSUANCE
June, December

Society for Indonesian Biodiversity

Universitas Papua, Manokwari, Indonesia

Published by Smujo International for The Society for Indonesian Biodiversity and Universitas Papua Manokwari, Indonesia

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Aims and Scope: ***Ocean Life*** (abbreviated as **Ocean Life**) encourages submission of manuscripts dealing with all aspects of maritime and marine resources in estuaries, coastal zones, continental shelf, the seas and oceans, including marine biodiversity and fisheries resources, biochemistry, physiology, behaviour, and genetics of marine life, socio-economic and cultural aspects, conservation and management, as well as biogeochemistry, marine pollution, and climate change.

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Title of the article should be written in compact, clear, and informative sentence, preferably not more than 20 words. Name of author(s) should be completely written. **Name and institution** address should also be completely written with street name and number (location), postal code, telephone number, facsimile number, and email address. Manuscript written by a group, author for correspondence along with address is required. First page of the manuscript is used for writing above information.

Abstract should not be more than 200 words. **Keywords** is about five words, covering scientific and local name (if any), research theme, and special methods which used; and sorted from A to Z. All important **abbreviations** must be defined at their first mention. **Running title** is about five words. **Introduction** is about 400-600 words, covering the background and aims of the research. **Materials and Methods** should emphasize on the procedures and data analysis. **Results and Discussion** should be written as a series of connecting sentences, however, for manuscript with long discussion should be divided into subtitles. Thorough discussion represents the causal effect mainly explains for why and how the results of the research were taken place, and do not only re-express the mentioned results in the form of sentences. **Concluding** sentence should be given at the end of the discussion. **Acknowledgments** are expressed in a brief; all sources of institutional, private and corporate financial support for the work must be fully acknowledged, and any potential conflicts of interest are noted.

Figures and Tables of maximum of three pages should be clearly presented. Title of a picture is written down below the picture, while title of a table is written above the table. Colored figures can only be accepted if the information in the manuscript can lose without those images; chart is preferred to use black and white images. Author could consign any picture or photo for the front cover, although it does not print in the manuscript. All images property of others should be mentioned source. **There is no appendix**, all data or data analysis are incorporated into Results and Discussions. For broad data, it can be displayed on the website as a supplement.

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Journal:

Saharjo BH, Nurhayati AD. 2006. Domination and composition structure change at hemic peat natural regeneration following burning; a case study in Pelalawan, Riau Province. Biodiversitas 7: 154-158.

Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta***.** 50th Annual Symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.) Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. www.molecularsystemsbiology.com. DOI:10.1038/msb.2008.24

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OCEAN LIFE
Volume 1, Number 1, June 2017
Pages: 1-10

E-ISSN: 2580-4529
DOI: 10.13057/oceanlife/o010101

Review: Biology of the commercially used sea urchin *Tripneustes gratilla* (Linnaeus, 1758) (Echinoidea: Echinodermata)

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Manuscript received: 5 January 2017. Revision accepted: 1 June 2017.

Abstract. *Toha AHA, Sumitro SB, Hakim L, Widodo N, Binur R, Suhaemi, Anggoro AW. 2017. Review: Biology of the commercially used sea urchin* Tripneustes gratilla *(Linnaeus, 1758) (Echinoidea: Echinodermata). Ocean Life 1: 1-10. Tripneustes gratilla* is a species of sea urchin in shallow tropical waters. The species is economically and commercially important, has ecological value, and has prospects as a biological control agent. It is considered the commercially traded sea urchin. Overexploitation has caused a sharp decline in *T. gratilla* populations. Understanding the biological aspects of *T. gratilla* is critical to the future sustainable use of this resource.

Keywords: Economically important, sea urchin *Tripneustes gratilla*

INTRODUCTION

*Tripneustes gratilla*is sea urchin is classified into kingdom Animalia, phylum Echinodermata, subphylum Echinozoa, class Echinoidea, subclass Euechinoidea, infraclass Carinacea, superordo Echinacea, ordo Camarodonta, infraordo Echinidae, superfamily Odontophora, family Toxopneustidae and genus *Tripneustes* (L. Agassiz 1841) (Kroh 2013). This species has distinct morphological characteristics (Toha et al., 2012). Various colors of the spine and tube feet (Toha et al. 2015).*T. gratilla* has been categorized as a primary herbivore (Lawrence and Agatsuma 2013, Unsworth et al. 2010), and its distribution is spread throughout the tropical waters of the Pacific and Indian Oceans (Kroh 2013).

Tripneustes gratilla (Linnaeus 1758) is economically important due to its value in supporting small-scale fisheries and commercial trade and is important ecologically (Williams 2002; Juinio-Meñes et al. 2001; Toha and Zain 2003; Toha 2006; Rahman et al. 2009; Toha et al. 2013). The sea urchin is also reported to own a prospect as a biological control agent (Stimson et al. 2007). In addition, it contains bioactive compounds useful for drug discoveries and pharmacological research (Takei et al., 1991; Nakagawa et al., 2003).

This paper covers comprehensive information on this species' morphology, ecology, genetics, and conservation. That we hope can provide important information to improve human welfare through the advancement of science development, technology, and environmental science.

MORPHOLOGY

Tripneustes gratilla is a round-shaped sea urchin with different morphological characteristics. Its body surface has a colorful short spine and tube feet (Toha et al. 2015) which can be moved for defense and locomotion. Its body (corona or test) is divided into an aboral and oral surface. Surfaces are separated by the ambitus (horizontal circle with a large diameter). Each surface is ended with a circular opening covered by flat structures. There are two major openings in the corona, the peristome and periproct.T. gratilla is enclosed within a testis-like structure, consisting of unified plates forming a container where the species conduct its activities. Its testis is a body part that determines its general morphology.

Tripneustes gratilla has different diameters and heights, which are influenced by age and maturity. Some of them have 16.5-94.5 mm (Eklöf et al. 2009), 90 mm (Dafni and Tobol 1986), 97.9 mm (Fouda and Hellal 1990, and 120 mm (Coleman 1991) in diameter. And some of them have 155 mm (Baker 1968) heights. Maximum size of 160 mm test diameter is reported by Rahman et al. (2014). Test diameter observed in Indonesian waters ranges from 82.1 mm (Darsono and Sukarno 1993) and 100 mm (Radjab 1997). While Toha et al. (2012) recorded different sizes around Papua; Manokwari 56.97-77.92 mm; Saubeba 76-90.37 mm; Wasior 62.1-93.46 mm; Biak 50-87.5 mm and Serui 58-77 mm.

HABITAT

Tripneustes gratilla is known to inhabit different habitats (Lyimo et al. 2011), including: seagrass (Traer 1980, Calvin et al. 1985; Sammarco 1987; Sumitro et al. 1992; Aziz 1994; Susetiono 2004; Lyimo et al. 2011), algae, microalgae and macroalgae (Ogden et al. 1989, Lyimo et al. 2011), sand with coral rubble, coral reef with reef flat (Lyimo et al. 2011; Lawrence and Agatsuma 2013).

However, in particular parts of the world, *T. gratilla* thrives in typically similar habitats. In Japan, *T. gratilla* is observed in coral reef intertidal and subtidal zones (Shigei 1970). In Okinawa, Japan, this species is found in a sandy reef, seaweed, and algae areas on a reef flat (Shimabukuro 1991). In Papua New Guinea, *T. gratilla* is observed in *Thalassia hemprichii* fields (Nojima and Mukai 1985, Mukai et al. 1987), while in the Philippines, *T. gratilla* lives in seagrass areas dominated by *Thalassia* and *Enhalus* species and *Sargassum* sp. (Regalado et al. 2010). In Indonesia, this species lives in sandy and muddy bottom reef areas as well as seagrass and algae flourishing habitats (Toha et al. 2012). *T. gratilla* is also occasionally found in sandy and muddy sand covered by seagrass at 0.5-20 meters depth (Radjab 2004).

The *Tripneustes gratilla* species could be observed at a depth of 75 m (Lawrence and Agatsuma 2013). Still, according to Lawrence (2007), their most common habitat is in very shallow water on various hard substrates between depths of 2 and 30 meters. Ogden et al. (1989) spotted this species along Hawaii, living in coral reef areas with sandy lagoons covered with seagrass and algae. In general, *T. gratilla* is found in Japan's intertidal and littoral zone of coral reef ecosystems, with sea temperatures ranging from 23.6-26.8°C (Shigei 1970). In Ambon, Indonesia, *T. gratilla* lives within 23.3-26.2˚C (Silahooy et al. 2013), and Madagascar lives in 26-32˚C ranges. However, Toha et al. (2012) reported that this species is found within 30-31˚C, 30-32‰ salinity, and pH 7.3-8, respectively, and Dafni (1992) reported survival below 15˚C. A report from Aqaba, Jordan, showed that this species could not move when transposed from 27 to 22˚C (Lawrence 1973). That could indicate an inactivity period during summer due to temperatures from 21-27˚C (Dafni 1992). The highest latitude this species can be found is on South East Easter Island (Fell 1974), where sea temperature ranges from 17.5-24˚C (DiSalvo et al. 1988).

DISTRIBUTION

Tripneustes gratilla is spread out throughout Western Pacific; Eastern Africa (the Red Sea throughout Natal), Southern Islands (from Norfolk and the Kermadec Islands through Marquesas and Hawaii), Australia (from Port Jackson on the eastern coast through Shark Gulf on the western coast) and Southern Japan (including Bonin Islands) (Mortensen 1943). Lessios et al. (2003) and Lawrence and Agatsuma (2013) also reported that *T. gratilla* also spread extensively from Central Pacific through African Coast in the Indian Ocean. According to Shokita et al. (1991), *T. gratilla* is a sea urchin species found extensively along the Indian Ocean, Pacific and Indo Pacific, Indo Malaya, including Australia, Japan, the Eastern Coast of Africa, and the Eastern part of Hawaii (Kroh 2013).

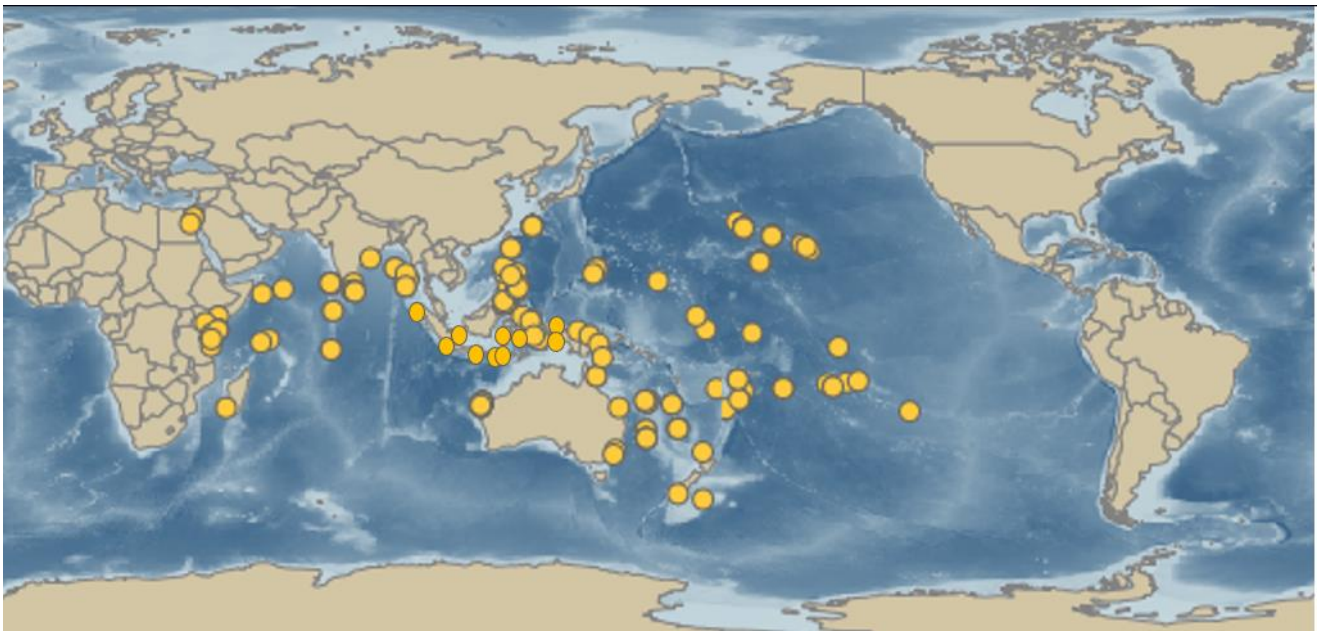

Figure 1. Distribution of sea urchin *Tripneustes gratilla*. Yellow dots indicate the locations of *T. gratilla* (Modified from http://iobis.org/mapper/?taxon=*Tripneustes*%20gratilla)

In Indonesia, *T. gratilla* spreads throughout all oceans, including the eastern part of Indonesia (Aziz 1993) except the Arafura Sea (Clark 1946). *T. gratilla* is also found in Kema (Supono and Arbi 2010),Merah Cape (Lembeh Strait) (Susetiono 2004), Kapoposan Islands (Tuwo 1995), Spermonde Islands in Southern Sulawesi (Tuwo and Tresnati 1995), Morotai Beach in Northern Halmahera (Yusron 2006), Osi Islands in Western Seram Northern Maluku (Syam et al. 2002), Banda Neira (Andamari et al.1994), Tamedan in South East Maluku (Radjab 1997), Ambon (Silahooy et al. 2013), Buton in South East Sulawesi (Kasim 2009), Southern Lombok Beach (Aziz 1994), Nusa Dua Bali (Darsono and Sukarno 1993) and Balekambang-southern Malang (Sumitro et al. 1992). In addition, *T. gratilla* was also found in Padaido-Papua (Radjab 2004) and other Papua region such as Saubeba, Rendani Beach, Tanjung Pepaya, Nabire, Wasior, Biak (Toha and Fadli 2008; Toha et al. 2012). In addition, *T. gratilla* is spread out throughout all Papua-Indonesia with different local names (Toha and Zain 2003; Toha et al. 2014). *T. gratilla* was also found in Sanur-Bali, Tomia (Southeast Celebes), Jayapura (Papua), Palu, Donggala, Togian (middle Celebes).

DENSITIES

The density of *T. gratilla* varies over time and location. The lowest density was found in February 2006 and increased after two years (Edgar et al. 2009, Valentine and Edgar 2010). While in habitat, covered sea grass and non-seagrass density vary from 0.18±0.16 ind./m^2 to 0.54±0.21 ind./m^2 (Lyimo et al. 2011).

Mukai et al. (1987) observed the highest density of *T. gratilla* in less than one-meter depth in PNG, where seagrass was found covering 80% of the observed areas. Alcoverro and Mariani (2002) observed an average density of 1.5 ind./m^2 in Mombasa, Kenya. Uy et al. (2000) also found the same quantity in the Philippines. In southern Guimaras, Philippines, density ranges from 0.06-0.58 ind./m^2. A very high density is observed at Lucero, Bolinao, Pangasinan (Philippines) reaching 4.6 ind./m^2 (Juinio-Meñes et al. 2008a). However, a density of more than 4 ind./m^2 is also reported in a few places in Lord Howe Island Marine Park (Valentine and Edgar 2010). In Hawaii and the Red Sea, *T. gratilla* density ranges from 2.9-4.4/m^2 (Ogden et al.1989) and 50 ind./m^2 (Dafni and Tobol 1986) respectively.

In Indonesia average density of this species varies in different locations. For example, in the area around Osi Island, the density is approximately0.754±0.152 ind/m^2 (Syam et al. 2002), while around Nusa Dua, Bali, Darsono and Sukarno (1993) reported the density was around 0.278 ind/m^2, in Banda Neira around 2.83 ind/m^2 (Andamari et al.1994), and Kema 0.84 ind/m^2 (Supono and Arbi 2010).

Dotan (1990) stated that *T. gratilla* distribution around Aqaba Gulf follows no specific patterns time-wise, which was not directly correlated with coverage and with high variation from time to time. Therefore, the high density of *T. gratilla* potentially affects the abundance and distribution of macroalgae (Stimson 2007).

BEHAVIOR

Tripneustes gratilla typically lives in a group with patchy distribution patterns (Aziz 1993; Syam et al. 2002; Toha and Fadli 2008) to increase fertilization success (Levitan 2004). However, observations in Indonesia showed species distributions overlapping in one place with *Toxopneustes pileolus*, *Mespilia globules*, *Temnotrema toreumaticus*, and *Pseudoboletia maculata* (Aziz 1993) though with a tendency to live separately from other species.

This distribution, however, remains inconsistent; some individuals may be found to live separately from other individuals. For example, Nojima and Mukai (1985) observed that some of *T. gratilla* tend to live in couples, while populations that showed no individual-to-individual body contact were observed, even with high density (Shimabukuro 1991). In addition, despite some observations in Madagascar finding that this species lives without a specific distribution pattern (Maharavo et al. 1994), Lawrence and Agatsuma (2013) reported that *T. gratilla* is often found in groups of three or four, often touching and even overlapping.

Maharavo et al. (1994) also reported that this species has a strong tendency to consume seagrass in one area before moving to another with a high seagrass density. Nojima and Mukai (1985) found that these species move 1.3 meters/day haphazardly in seagrass beds. Another observation showed that *T. gratilla* aggregated in groups of 10-20 individuals in Madagascar; some were dominated by *Thalassodendronciliatum* (Alcoverro and Mariani 2002). *T. gratilla* has variable feeding habits, grazing nocturnally on Reunion Island (Lison deLoma et al. 1999) and diurnally in the Aqaba Gulf (Schumacher 1974).

Tripneustes gratilla exhibits cryptic covering behaviors, which serve as protection from predators, light exposure, and strong currents (Park and Cruz 1994). Ziegenhorn (2016) found that *T. gratilla* partially underneath rocks covered more and with more algae than urchins totally underneath rocks. Ziegenhorn (2016) also found that *T. gratilla* preferred a cover that best protects them from UV radiation. Spine loss did not significantly affect urchins' ability to cover, and urchins with removed spines still preferred opaque cover.

DIET

According to Rahim and Nurhasan (2016), seagrass and seaweed are the main diets for most sea urchins, and *T. gratilla* is the most well-known seagrass grazer (Lyimo et al., 2011). Therefore, information on feeding preference is important to study the effect of sea urchins on seagrass beds, which could also contribute important information to sustain the management of seagrass ecosystems (Eklöf et

al. 2008). Seagrass is mostly an herbivore though some species also consume animal-related materials (Lawrence 1975). According to de Loma et al. (2002), sea urchins feed on detritus material from seagrass as well as epiphytic and epibenthic micro and macroalgae, depending on variable factors including food availability and food preference (Lyimo et al. 2011).

The diet of T. gratilla varies depending on habitat (Lawrence and Agatsuma 2013) and development stage. The larval stage requires an external food source to allow juvenile benthic development. Pre-feeding embryos cannot directly eat phytoplankton and will grow epithelial cells on their surface that can detect food particles and decompose them into organic materials (Miner 2007). *Early stage T. gratilla* eat sessile diatoms while bigger individuals consume macroalgae (*Sargassum* spp., seaweed, and microflora) (Shimabukuro 1991).

Tripneustes gratilla is an omnivore that generally lives around the substrate and eats various algae, periphyton, and seagrass (Eklöf et al. 2008; Stimson et al. 2007; Tomascik 1997) as well as some crustaceans and mollusks (Radjab 1997). *T. gratilla* mostly consumes seagrass of different types, including *Thalassodendron ciliatum*, *T. hemprichii*, *E. acoroides*, *Syringodim isoetifolium*, *Cymodocea rotundata*, and other types of algae (Alcoverro and Mariani 2002; Kasim 2009; Lyimo et al. 2011). Laboratory scale experiment showed that *T. gratilla* preferred fresh brown algae *Eckloniaradiata* and disliked dry *Ecklonia radiata*, *Sargassum linearifolium*, and *Ulva lactuca* (Dworjanyn et al. 2007; Cyrus et al. 2015).

Tripneustes gratilla in Hawaii was observed to consume macroalgae and sand containing macroflora (Ogden et al. 1989). At the same time, a further investigation in the Philippines showed the remnants of *T. hemprichii*, *Halimeda*, *S. isoetifolium*, and *rubles* in their intestine, which shows that this may be their main source of food (Klumpp et al. 1993). However, the most dominant seaweed in Madagascar in their intestine was *S. isoetifolium* (Vaïtilingon et al. 2003). In Papua New Guinea, *T. gratilla* consumes *Cymodocea* spp., *E. acoroides*, *Halophila ovalis*, *S. isoetifolium*, and *Thalassia hemprichii* (Hattori et al. 1985). While in Kenya and Tanzanian, it consumes *T. ciliatum* (Alcoverro and Mariani 2002) and mixes with *S. isoetifolium*, *C. rotundata*, *Halodule uninervis*, and *T. hemprichii* (Lyimo et al. 2011), respectively.

In Indonesia, *T. gratilla* eats a variety of diets. In Bali, it eats cuts of *Ulva* sp. and seagrass leaves (Darsono and Sukarno 1993). In Southeast Maluku, it dominantly consumes *E. acoroides*, *T. hemprichii* and *Caulerpa* sp., *Padina* sp., *Sargassum* sp. *T. gratilla* was also reported to consume crustaceans (Copepoda, Amphipoda), mollusks (Gastropoda, Bivalvia) (Radjab 1997).

REPRODUCTIVE CYCLE

Tripneustes gratilla was observed to exhibit an annual reproductive cycle with variable intensities. *T. gratilla* has an annual reproduction cycle influenced by various parameters, including water temperature, day length, and feeding activities (Vaïtilingon et al. 2005). Both males and females have a reproductive system divided into five gonads. Despite being sexually dimorphic, it is difficult to distinguish males and females externally except if the gonad is in a mature stage. The mature female's gonad is bright orange, while the male's cement is bright yellow when mature. Gonad, called ROE, is not only where egg or sperm production takes place but also the main food storage chamber (Bruce 1988).

Maximum gonad size can reach 10-15% of its net body weight (Fouda and Hellal 1990). Individual weight is 25-89g, while gonad weight varies between 0.203-1.925g, equal to 0.003-0.042% of the total body weight (Radjab et al. 2010). Gonad production increases when its size reaches 70 mm and has shown no observed decreasing pattern even when it reaches 100 mm. Muthiga (2005) reported that there is no significant correlation between gonad index and shell diameter, while a significant correlation is found between gonad weight and shell diameter.

Gonad production is influenced by diet. Male and female gonad production is slightly different in output quantity (Lawrence 1987). However, this has never been thoroughly observed in the species. *T. gratilla* in sandy areas produces less sperm and egg than those living in seagrass (Jafari and Mahasneh 1984). Seasonally, most gonad peak production happens in spring (Tuason and Gomes 1979).

Gonad development occurs when its diameter reaches 50 mm in less than one year (Dafni and Tobol 1986; Juinio-Menez et al. 1998). Mortensen (1943) reports that the gonophores open at an earlier stage in some regions (gonads will already be present in those specimens). Gonad index increases at 70 mm in size and remains at this index even when its diameter reaches 100 mm. Gonad maturity levels can be explained through a few steps (Radjab 1997), and these are 0 (neutral), 1 (initiation), 2 (developed), 3 (early maturity), 4 (mature), 5 (spawning), and may vary depending on the time of observation (Radjab 1997).

On a laboratory scale, early maturity occurs when the diameter reaches 40 mm (Radjab et al., 2010). The first maturity occurs at 1.5 years old when the shell diameter reaches 60 mm (Trinidad-Roa 1989). Observation in Tamendan and Japan shows that gonads mature and spawn the whole year (Radjab 1997) and in the summer and fall, respectively. In Bali gonad matures at diameter of 40 mm (Darsono and Sukarno 1993).

The spawning season varies in *T. gratilla*. In Taiwan, it happens in fall (Chen and Chang 1981), in the northern Red Sea (Pearse 1974) and Gulf of Aqaba (Kidron et al. 1972) in spring, summer through fall in Kenya (Muthiga 2005), and in fall at the Philippines (Chen and Chang 1981). While in Tamendan, South East Maluku, Indonesia, spawning seasons were predicted between August and September and continue through mid-October (Radjab 1997). Other observations in the Philippines also show that *T. gratilla* may spawn throughout the year Tuason and Gomez (1979)

Eggs and sperms mature throughout the year in Solitary Island, with peak production occurring in fall and winter.

However, *T. gratilla* can only induce fertilization in fall (O'Connor et al. 1978). The gonad development phase is correlated with granule accumulation that starts from March through June and declines in summer in Okinawa. This mature gonad is packed with eggs and sperm in September (Shimabukuro 1991). Gametogenic activities occur throughout the year.

Pearse (1974) concluded that reproductive effort in *T. gratilla* doesn't correlate with temperature. Contrary to this, Chen and Chang (1981) stated that spawning-related geographical variations correlate with temperature. Another observation reported that *T. gratilla* had mature and spawning phases each month, but an increased percentage of spawning phases was observed in August and September (Radjab 1997).

LIFE CYCLE AND GROWTH

Sea urchins can live up to 100 years. Maximum age estimates acquired through the growth zone in tiny skeleton bones in *Lytechinus variegatus* show that this species can live to maximum 4 years (Beddingfield and McClintock 2000), while the same method of measurement used for *Strongylocentrotus intermedius* resulted in a maximum age estimate of 10 years (Agatsuma 2001). Another method using C^{14} showed that *S. franciscanus* could live more than 100 years (Ebert and Southon 2003). *T. gratilla, however,* has been found to live relatively in a short period. According to Lawrence (2001) and Shimabukuro (1991), *T. gratilla* can not live more than two years. Ebert (1982) also reported that *T. gratilla* could only live for a maximum of 1 year.

Tripneustes gratilla experiences few growth cycles in its life, with egg fertilization through to adulthood consisting of two cycles which include pre-adult (embryonic phase, larvae, and juvenile) and the adult phase (Shokita et al. 1991). *T. gratilla* takes about two to five years to become a reproducing adult.

Generally, sea urchins are single-sex with prevalence in spawning areas and are able to disengage their sperms and eggs in the water column for fertilization (Levitan 2005, 2006; Rogers-Bennett 2007; Byrne et al. 2010). *T. gratilla* spawns once a year. Fertilized eggs will then turn into blastula and gastrula and subsequently grow into a planktonic prismatic stage. In line with skeleton growth, the prismatic stage develops into larvae (echinopluteus or pluteus), which have several cilia, which may vary between 4 and 8 depending on the development stage (Byrne et al. 2008a, 2008b).

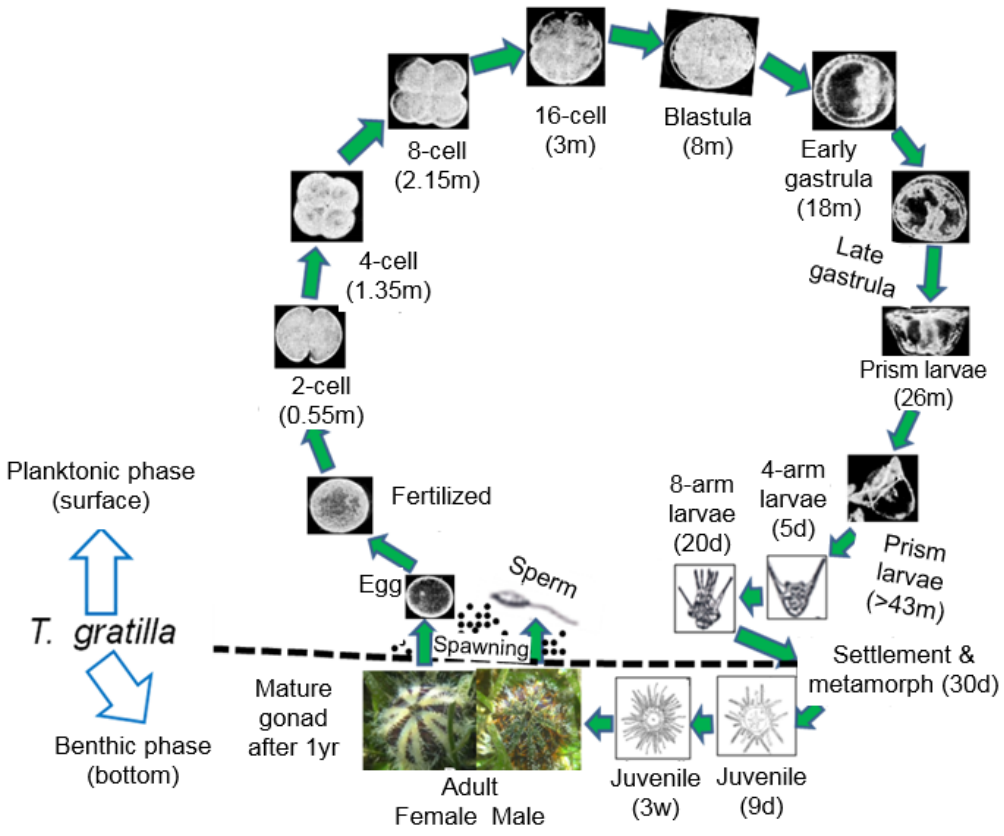

Figure 2. Life cycle of *Tripneustes gratilla*

Laboratory scale observations in Japan show that larvae *T. gratilla* can develop completely within 20-30 days, depending on temperature and food supplies (Shokita et al. 1991). However, an experiment in the Philippines showed that the average larval duration was 42-52 days (Junio-Menez et al. 1998). Shokita et al. (1991) also reported that the larval cycle (prismatic larvae, 4 and 8 arms larvae) in wild environments could persist for up to 25 days.

Laboratory observation shows that this species can attain complete metamorphosis within 18 days, while similar observations in Taiwan show this duration can take up to 30 days (Chen and Run 1988). Shimabukuro (1991) reported that diameter growth in *T. gratilla* could reach 60-70 mm. Similar observations in the Philippines (Bacolod and Dy 1986) and the Aqaba Gulf (Dafni 1992) reported that *T. gratilla* grew to 60 mm within five months of observation. On average, shell diameter growth in Tamedan, South East Maluku, was 0.05 mm/day, while growth in weight can reach 0.10 g/day (Radjab 1997).

Tripneustes gratilla found living in Southern Guimaras, and the Philippines can reach 60 mm within 8 months (Regalado et al. 2010). However, similar studies in the same place a year before showed that the same diameter was reached within 10 months (Beldia et al. 2003). The difference in growth parameters shows that habitat conditions and diet may affect growth level and maximum size in *T. gratilla* (Regalado et al. 2010, Juinio-Meñes et al. 2008). Shimabukuro (1991) also reported that growth could slow down when this species experiences stress. Furthermore, it was reported that the growth could escalate when this species changes its diet from sessile diatoms to macroalgae. Individual diameter can also reach 10 mm in June and 60-70 mm in November.

RECRUITMENT

According to Ebert (1982), recruitment in this species can be patchy due to the random movement of this species throughout the area it inhabits. This finding is supported by Dafni and Tobol (1986), who found random recruitment areas in the Red Sea. The random areas' recruitment success is related to their ability to live in different habitats, including sand and sea grass beds, lagoon, coral reefs, and the intertidal zone. According to Juinio-Meñes et al. (2008b), recruitment success on the farming scale is influenced by biophysical and local management intervention.

Recruitment peaked in November in the Philippines, with a smaller peak in March (Bacolod and Dy 1986). Another observation in the same place also shows that this species mainly recruits in April and May. Observations from Papua New Guinea suggest that recruitment only happens once a year (Mukai et al. 1987). However, the same observations during 1980-1981 by (Dafni 1992; Dafni and Tobol, 1986) showed that *T. gratilla* with less than 10 mm in size are found in large quantities in January through February, May through June, and in August (Dafni 1992; Dafni and Tobol 1986).

MORTALITIES

The Pacific and Atlantic populations of Tripneustes have shown a high mortality and fecundity rate with fast individual and population growth (Lawrence and Agatsuma 2013). Mortality in *T. gratilla* is influenced by several factors, including seasonal rains and bad weather (Vaïtilingon et al. 2005), decreased recovery (Ebert 1982), succession (Dafni 1992; Dafni and Tobol 1986), entrapment in shallow water (Shimabukuro 1991), habitat alteration and predation (Regalado et al. (2010), natural death (Juinio-Menez 2008) and many other unknown factors (Eklöf et al. 2009).

Regalado et al. (2010) reported that mortality in *T. gratilla* can reach 99.3%. Juinio-Menez (2008) also reported that natural mortality in different sites in Luzon Northwestern and Central Philippines varies from 91-96% and 99%, respectively. High mortality in Hawaii, Kenya, Seychelles, and Israel can be caused by low recovery capacity after one year (Ebert 1982). Dafni (1992) and Dafni and Tobol (1986) also reported that mass mortality in Eilat typically happens twice during winter succession. However, low mortality is observed during rainy seasons on shallow reef flats in Okinawa (Shimabukuro 1991).

PREDATORS

Tripneustes gratilla is hunted by humans for its delicious gonads, and other predators include parrotfish, triggerfish, and pufferfish (Mahon and Parker 1999), which also consume its gonads and shell contents. Specifically, in seagrass areas, *T. gratilla* is hunted by *Cassis*sp (Tertsching 1989). However, the main predators of T. gratilla in protected areas and hard, rough substrates are sea stars (*Protoreaster linki*) (Shears and Babcock 2002, Bonaviri et al. 2009; Eklöf et al. 2009). Eklöf et al. (2009) also found that *T. gratilla* has many other predators, including Asteroidea, Gastropoda, Balistidae, and Labridae.

COMMUNITIES

According to Valentine and Edgar (2010), *T. gratilla* has a specific role as an ecosystem engineer. High density can cause a significant decrease in algal coverage, including red and brown algae. Encrusting coralline algae coverage increases with higher densities of *T. gratilla* (Bacolod and Dy 1986). *T. gratilla* is also a pest in seagrass farming; the recovery process is not entirely affected. Hyper-abundant populations of *T. gratilla* have been observed to overgraze complete seagrass beds of primarily *Thalassodendron ciliatum* on the Kenyan coast (Alcoverro and Mariani 2002; Zanre and Kithi 2004; Uku and Björk 2005).

GENETIC

More than 274 nucleotide sequences for *T. gratilla* are available in the NCBI database (http: //www.ncbi.nlm.nih.gov/nuccore). Including the full *T. gratilla* mitochondrial genome ranging from 15720-15725 bp. Available sequences include many samples from COI partial group (more than 220 partial sequences). There are various uses for nucleotide sequences, such as for morphological and molecular phylogeny (Littlewood and Smith 1995), genetic patterns (Liggins et al. 2014), marker organization (Carlon and Lippe 2007), preliminary design for barcoding (Hoareau and Boissin 2010), phylogeny and expression (Gibbons et al. 1994), mtDNA phylogeny (Zigler and Lessios 2003), retrovirus-like element (Springer et al. 1991), the evolutionary history of larval skeleton morphology (Kinjo et al. 2008), and others.

Studies on the molecular genetics of the *T. gratilla* show that high gene flow up to more than hundreds of kilometers is a standard characteristic of all sea urchin species possessing a planktonic larval stage (Lessios et al., 2001, Liggins et al., 2014). Similar patterns are found in *T. gratilla* in Indonesian waters (Toha et al. 2014).

CONSERVATION

Tripneustes gratilla has not yet been assessed for the IUCN Red List. However, *like* other sea urchins, it has biological characteristics that make it susceptible to overexploitation. In general, sea urchins mostly prefer particular habitats, and their slow movement restricts their distribution and may cause them to be vulnerable to local extinction. In general, sea urchin fisheries do not have any management system or local restrictions. Sea urchins are also a target for biotechnology, and various biologically active compounds have been isolated from the sea urchin species. However, the target species for these purposes is not specific, and local communities have some financial benefits. Therefore, it is necessary to regulate this industry to maintain viable populations sustainably (Micael et al. 2009).

Conservation is important to protect *T. gratilla* stocks. Several steps conducted for echinoid conservation in France (Mediterranean and Atlantic), Ireland, Iceland, South Korea, the Philippines, and China are: entry limitation (moratorium) followed by active programs to reduce latent businesses, resources survey at various complexity levels, to use an annual total fishing permit based on resources analysis, zonal and regional management towards rotational harvest points, and to use a minimum legal size (Williams 2002).

In the Philippines, Marine Science Institute has opened a *T. granilla* hatchery at the reef flats of Bolinao, Pangasinan. South Korea does this on a coastal scale through village collaborations that decide how many people have access and when to do it. Vessel permit restrictions are also a sea urchin fisheries management strategy in South Korea. Russia's surface fisheries are set by regulating total allowable catch based on fisheries free-survey and fisheries information analysis. Minimum size permits and fishing seasons are also implemented to protect their spawning.

In New Zealand, the sea urchin fishery is a non-quota fishery managed through various permits, seasons, and fishing ground closures. On the other hand, the Tasmanian sea urchin fishery was regulated through a moratorium strategy for new entrants in the 1990s, and the permit cannot be moved. In addition, in 2002, Tasmania conducted a competition for a total allowable catch, in which it was divided into zones, and the total allowable catch was used to encourage management and regulation. In New South Wales, the sea urchin fishery used entry limitations through removable permits. In addition, area closures are applied to restrict access.

The present conservation mechanism should be integrated and consistently set up globally. Furthermore, sea urchin fisheries need an ecosystem approach through increased information exchanges between government institutions, NGOs, and academicians, and inter-stakeholder dialogues, including industries and sea urchin resources communities. Developing and integrating several suggestions for size and spatial scale difference considerations (local, regional and global) will allow sustainability in using sea urchin species as resources. There is also a clear necessity to raise our biological knowledge of the target species to promote that group diversity is maintained (Micael et al. 2009).

ACKNOWLEDGEMENTS

The authors thank the University of Papua and Higher Education of Republic Indonesia for funding the National Strategic Research (No. 235/H42/KU/2009). In addition, the authors thank the anonymous reviewers for their comments which improved the manuscript.

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OCEAN LIFE
Volume 1, Number 1, June 2017
Pages: 11-13

E-ISSN: 2580-4529
DOI: 10.13057/oceanlife/o010102

Short Communication: New record of Indian oil sardine *Sardinella longiceps* from the coastal region of Bangladesh

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Manuscript received: 10 May 2017. Revision accepted: 5 June 2017.

Abstract. *Shamsunnahar, Baki MA, Sarker A, Hasina Begum M, Zafaria M, Islam NN, Ahmed MdS. 2017. New record of Indian oil sardine* Sardinella longiceps *from the coastal region of Bangladesh. Ocean Life 1: 11-13.* We report the first record of Indian oil sardine *Sardinella longiceps* from the southern coast of the Bay of Bengal, Bangladesh. The sample specimens were collected from Pathorghata, Barguna, Bangladesh, on 25 October 2015. Morphometric and meristic studies were performed for taxonomic identification. Genomic DNA was extracted from tissue samples, and the mitochondrial Cytochrome Oxidase Subunit I (COI) gene was amplified for molecular characterization of this species. The morphometric and meristic data and DNA barcoding confirm the presence of *S. longiceps* in Bangladesh. This report updates this species's geographical distribution, confirming its presence in the coastal region of Bangladesh, extending the number of marine fish known from the area.

Keywords: *Sardinella longiceps* Bangladesh, COI gene, DNA barcoding

Abbreviations: BLAST = Basic Local Alignment Search Tool, COI = Cytochrome c oxidase subunit I gene, DNA = Deoxyribonucleic acid, FAO = Food and Agricultural Organization

INTRODUCTION

Indian oil sardine (*S. longiceps*) is a commercially important clupeid fish worldwide. The species is a strongly migratory coastal pelagic, with a geographical distribution limited to Arabia, Mombasa, Seychelles, Iran, Pakistan, India, Ceylon, Java, Bali straits, North Borneo, and the Philippines (Nair 1972; Mohanty 2004). According to the FAO, this species is confined to the Indian Ocean (Northern and Western parts only), the Gulf of Aden, and the Gulf of Oman, but not the Red Sea or the "Gulf" eastward to the southern or Eastern coasts of India.

This study was conducted to resolve some questionable identification of sardine species found on the southern coast of the Bay of Bengal. After surveying different coastal areas of the country, the presence of *Sardinella longiceps* was confirmed based on morphometric analysis and DNA barcoding of the sample collected from Pathorghata, Barguna.

MATERIALS AND METHODS

Samples were collected on 25 October 2015 from Pathorghata, Barguna (22°19'37.24"N 91°50'12.14"E). They were preserved in ice soon after collection and then kept frozen in a laboratory at -18 °C until further use. Morphometric and meristic characteristics were recorded using an FAO species identification sheet. The length was measured on a cm scale. A portion of tissue was taken from the sample fish for genomic DNA extraction. DNA was extracted using the standard method (Ward et al. 2005) with a little modification (Chowdhury et al. 2016). Universal fish primer (FishF1 and FishR1) was used (Ward et al. 2005) to amplify the COI gene. Amplified DNA was sequenced, and BLAST was used to confirm the species.

The original voucher specimen DUZM 073 of *S. longiceps* is kept at the Museum of Department of Zoology, University of Dhaka, and has public access. In addition, the DNA barcoding data can be retrieved from the NCBI GenBank;
https://www.ncbi.nlm.nih.gov/nuccore/KX988263.

RESULTS AND DISCUSSION

Description

Morphometric analysis showed a body depth of 4.0-4.4 cm and a head length of 2.9-3.1 cm in standard lengths. Snouts were longer than the diameter of the eye, which was positioned 5.5-5.9 cm in length of head; maxillary extended to below anterior part or near the eye's middle. 188-214 gill rakers were positioned on the lower part of the anterior arch. Ventral scutes were sharply keeled, 26-29 in number.

There were 16-18 dorsal fin rays, 14-16 anal fin rays, and 9 pelvic fin rays below or behind the middle of the dorsal fin. A dark spot occurs at the edge of the operculum (Figure 1).

Morphometric and meristic characteristics are given in table1 and compared with other published reports (Raja 1968; Nair 1973).

Remarks

The Morphomeristic characteristics of the specimens from the Bay of Bengal fall within the range limit of other specimens reported from the Indian Ocean (Nair 1970; Froese & Pauly2016; Shah 2014). Meristic counts also fall within the range of previous work (Day 1865; Whitehead 1965; Nair 1972; Raja 1967; Froese & Pauly 2016; Shah, 2014). The description of these specimens agrees with the work of Nair (1970) (Table 1).

The amplified COI gene was sequenced, and BLAST (Basic Local Alignment Search Tool) with sequence deposited in the GenBank showed 99% matching with sequence number JF4369381. Therefore, the submitted sequence was assigned GenBank accession number KX988263, confirming this region's molecular identification of Sardinella longiceps.

Table. 1. Morphometric and meristic characteristics of *Sardinella longiceps*

Characteristics	**Present study (n=22)**	**Raja (1968)**	**Nair (1973)**
Total length (cm)	17.67±0.65	-	-
Standard length (% of TL)	14.00±0.59 (79.25)	-	-
Fork length (% of TL)	14.84±2.22 (84)	-	-
Head length (% of SL)	4.75±0.33 (33.93)	-	-
Eye diameter (% of HL)	0.83±0.08 (17.41)	-	-
Body weight	1.71±0.15	-	-
Body depth (% of SL)	3.4±0.25 (24.29)	-	-
Meristic characteristics			
Dorsal fin ray	16-18	15-17	16-18
Pectoral fin ray	15-17	15-17	-
Pelvic fin ray	9	9	9
Anal fin ray	14-18	13-16	14-16
Scute	26-29	-	31-36

Figure 1. *Sardinella longiceps* (Lateral view)

In conclusion, the nearest species distribution is reported from the Eastern coast of India (Froese & Pauly, 2016). No previous occurrence is documented for this species from the Bay of Bengal area. The present study, therefore, extends the known geographical distribution of *S. longiceps*. In this area, the species will have been unknowingly commercially exploited along with other closely related species. This new taxonomic identification will be helpful for the proper management and conservation of this species in the future.

ACKNOWLEDGEMENTS

We greatly acknowledge the partial financial support from the Ministry of Science and Technology, Government of Bangladesh as a form of NST Fellowship to the first author, Grants No. 39.00.0000.012.004.16-11(107). Authors' contributions: SN & MSA: Molecular identification and write-up; MAB, AS & AMBZ: Specimen collection and morphometric identification; NNI & MHB: Experimental, Sequence editing, and submission.

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OCEAN LIFE
Volume 1, Number 1, June 2017 E-ISSN: 2580-4529
Pages: 14-19 DOI: 10.13057/oceanlife/o010103

Ecotourism development to preserve mangrove conservation effort: Case study in Margasari Village, District of East Lampung, Indonesia

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Manuscript received: 25 April 2017. Revision accepted: 7 June 2017.

Abstract. *Setiawan W, Harianto SP, Rommy Qurniati R. 2017. Ecotourism development to preserve mangrove conservation effort: Case study in Margasari Village, District of East Lampung, Indonesia. Ocean Life 1: 14-19.* The conservation efforts by the Margasari community have enhanced mangrove forest areas. However, the utilization of mangrove forests by people around was low. To make the conservation effort sustainable, needs to develop mangrove ecotourism so they can utilize mangrove forest existence. This research intended to study the conservation and ecotourism effort in Margasari Village and the community perception of ecotourism development. This research was conducted in March-April 2017 in Margasari Village by interviewing 96 respondents. The data was collected through field observation, key informant interviews, and structured questionnaires. The result showed that the conservation effort was protecting, preserving, and utilizing mangroves such as mangrove nurseries, mangrove plantations, using *jeruju* and *pedada* fruit as food, and counseling the villagers not to cut mangroves and enter the mangrove forest. The ecotourism activities included boating around the mangrove, planting tourism, and bird watching. The conservation and ecotourism efforts were conducted by the people who joined community groups. But it was dominated by the group manager. So the financial benefit from ecotourism had limited for a few people. Nevertheless, the Margasari community agreed with the ecotourism development and was willing to participate in developing ecotourism.

Keywords: Conservation effort, ecotourism, mangrove forest, community

INTRODUCTION

The existence of mangrove forests has an important role in the coastal area; mangrove vegetation has the ability to equalize the environment and neutralize the pollutant materials (Rochana 2011). Mangrove forests that have 200 m of mangrove thickness from the coastline with 30 trees/100 m of tree density and 15 cm of trunk diameter could muffle about 50% tsunami wave powers (Rusdianti 2012). Dense mangrove forests offer protection of coastal areas from tsunami waves. Mangrove protect coastline, enrich coastal waters, support coastal fisheries, yield beneficial forest products, serve as habitat for various kinds of fauna, and as sites for burgeoning ecotourism industry (Kusmana 2015). Sustainable management of mangrove forests will ensure these benefits for surrounding communities. The management challenge and forest protection in Indonesia often came from the local community around the forest. Magdalena (2013) reported that the protection by Sasak (Nusa Tenggara Barat) and Dayak Kenyah (Kalimantan Timur) communities have supported the sustainable forest management.

Most of the mangrove forest areas which grow along the coast are protected areas which inclined open for everyone who entered (open access) (Kustanti et al 2014). Lampung province had 1,105 km of the total coastline which was planted with mangroves as long as 896 km (Purnobaski 2001). Nowadays the mangrove forest ecosystem is threatened, and the extent of the damage to mangrove ecosystems is a cause for concern (Nugraha et al 2015). But mangrove forest area in Margasari has enhanced 117.59 ha during 2010-2013 (Cesario et al 2015). This expansion happened due to the emerging land and conservation effort through planting activity by community in Margasari and other parties outside the village (Monografi Desa Margasari 2012).

The utilization of mangrove forest by Margasari villagers is very limited. According to Ariftia et al. (2014), the direct utilization value of mangrove forest in Margasari is only 18% of the total economic value. The low use of mangroves could be a threat as Qurniati et al (2017a) revealed community surrounding the forest have limited capital, it could decrease the conservation effort by the community. The mangrove conservation effort should be equal to the sustainable utilization through developing mangrove-focused ecotourism. The ecotourism activities appreciated the local resource potential and based on community so it prevented the change of land ownership, social and cultural community because the community acted as subject and main beneficiaries, besides ecotourism also supported the effort of the sustainable economic development because of giving an alternative livelihood as income resources (Rizky et al. 2016). For a reason, this research intends to study the conservation and ecotourism effort by Margasari community and the social perception of ecotourism based on mangrove development.

MATERIALS AND METHODS

Study area

The research was conducted in March and April 2017 in Margasari Village, Sub-district of Labuhan Maringgai, District of East Lampung, Lampung, Indonesia (Figure 1). This location was chosen because the surrounding community has an already established conservation effort which has resulted in the increase of mangrove forest area.

Data were collected using observation, structured and in-depth interview and literature study. The observation and in-depth interview were used to collect the data about the conservation efforts of the community. The structured interview used a questionnaire, to investigate community perception of ecotourism development. The literature study was used to collect data about the general description of Margasari Village and surrounding mangrove forest. There were six key individuals of samples in conservation effort who determined by using snowball sampling. Snowball sampling is a non-probability sampling technique in which a researcher begins with a small population of known individuals and expand the sample by asking those initial participants to identify others that should participate in the study. The respondents of ecotourism development were 90 respondents who measured by Slovin's formula. Slovin's Formula was used to determine the sample size of a population of 989 people for an interview by a questionnaire. The conservation effort was analyzed descriptively based on Indonesia Government Rule No 5 in 1990 and No 32 in 2004 regarding conservation effort. The questions of community perception of ecotourism development were categorized by scoring method to identify the level of community perception in ecotourism development based on mangrove.

RESULTS AND DISCUSSION

Based on the Indonesia Government Rule No 5 in 1990, conservation effort is managing and utilizing natural resources wisely to ensure the existence of it in this time and the future. This includes three key activities: protection, preservation and sustainable utilization of natural resources.

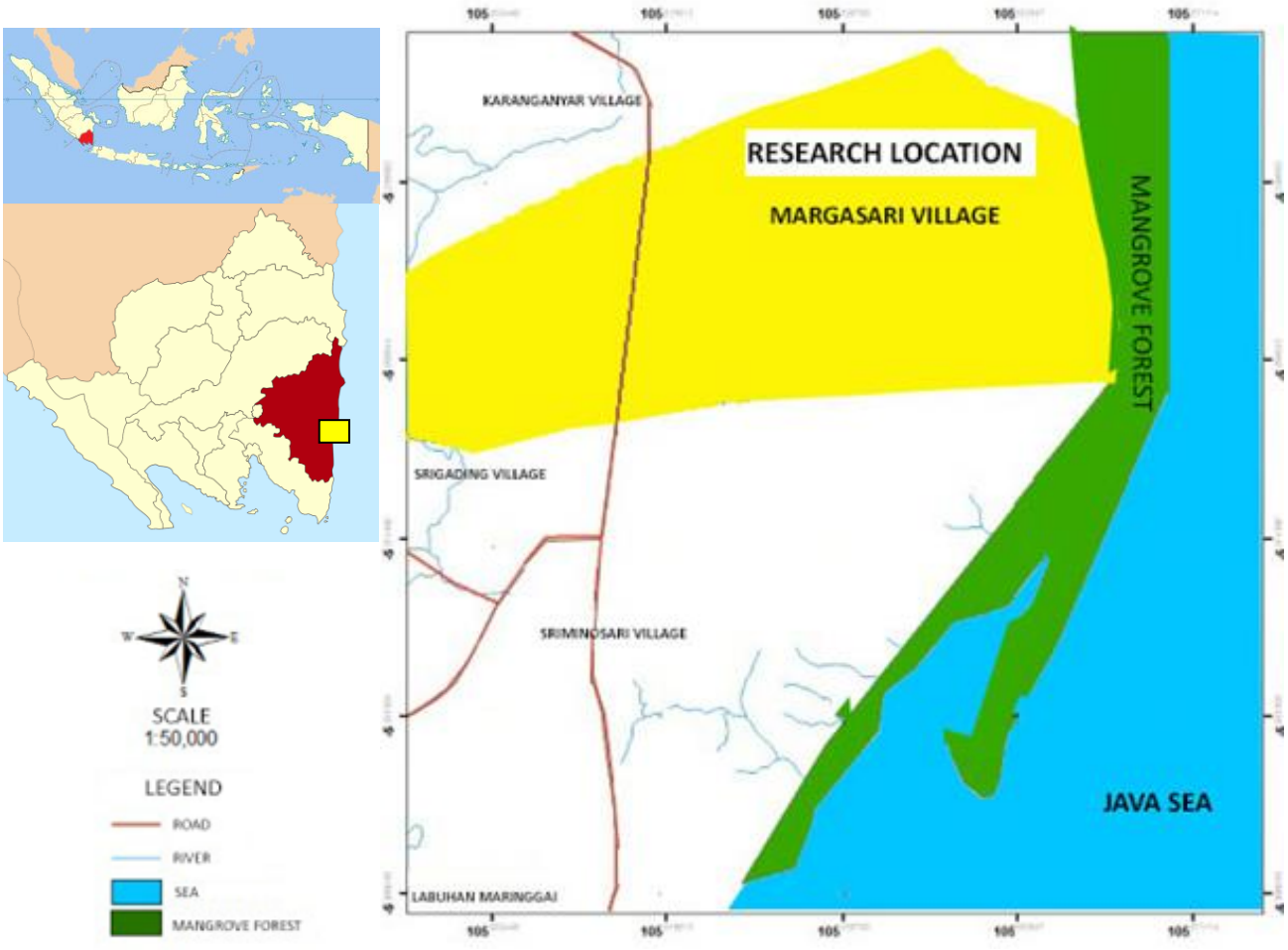

Figure 1. Map of study area showing mangrove forest and Margasari Village, Sub-district of Labuhan Maringgai, District of East Lampung, Lampung, Indonesia

Protection of mangrove forest

Mangrove protection effort by Margasari community was to prohibit people to enter mangrove forest area and cut trees down. This conservation effort, as described in Laila (2014) is included in public environmentalist category, are the residents who attempt to improve the condition of the environment directly through their actions and behavior. In Margasari, the forest protection was conducted by Margajaya Group and Wanita Cinta Bahari (WCB) Group. The leader of Margajaya Group and all the group members appealed to the wider community through village events such as *kumpulan kampung* (village gathering), *hajatan* (party), or *yasinan* (prayers meeting). While the leader of WCB group gave a warning directly to the people who entered the mangrove forest and appealed to the community through *pengajian* (religious meeting) in order to not damage the mangrove. Reports were given directly to the village chief if there were people who damaged the mangrove forest. These protection activities were based on the responsibility to keep the mangrove forest sustainable. The others people in Margasari protect the mangrove forest by following the rules of mangrove so the mangrove could grow well without any disturbance from human.

Preservation of mangrove forest resource

The Margasari community preserves the mangrove forest by developing a nursery and planting mangroves. The nursery activities are conducted by Margajaya Group, which has ten members. The Margajaya Group took the seeds of mangrove fruit from inside mangrove forest area and planted them on the nursery site. The Margajaya Group sold the seeds for IDR 1.300/seed (USD 0.1/seed). Unfortunately, the nursery project was suffering because the Margajaya Group was unable to compete with the group from Purworejo Village that had a seed certification. Purworejo village is approximately 20 km from Margasari Village and also have mangrove forest adjacent to Margasari mangrove forest.

The people who were not a member of any community groups participated in the mangrove planting in 2006-2007, and in December 2016 a government-initiated planting program took place. At present, the number of participatory community members has declined, only the members of Margajaya Group continue to plant the mangroves. Otherwise, Aflaha (2013) found that all people supported the mangrove planting activities as well as participated in the activity of sustaining the mangrove forest area.

The incentive factor for willingness to plant and develop mangrove nurseries in Margasari is economic. Community members received wages of IDR 50,000/day (USD 3.85). This wage was paid by the event organizer (outside parties), usually the local government (forestry service). But many mangrove programs from government were project oriented so the people who participated were low (Qurniati et al. 2017b).

Utilization of mangrove forest

The utilization of mangrove forest resources is limited to production of *terasi* (shrimp paste), jeruru (*acanthus ilicifolius*) leafs, pedada (*sonneratia caseolaris*) fruits and ecotourism activity. *Terasi* is paste for cook seasoning made from fermented baby shrimp. *Terasi* produces 2-3 times per week by Pengolah Terasi Groups. The average *terasi* production is 100kg/month with the price is IDR 30000/kg (USD 2.3/kg). *Terasi* is sold to local markets and visitors in mangrove.

WCB Group produces chips from jeruru (*acanthus ilicifolius*) leafs and syrup from pedada (*sonneratia caseolaris*) fruits. This group utilized *jeruju* and *pedada* from mangrove forest area in Margasari. Initially, the syrup product was packaged in plastic bottles but glass bottles are now used to maintain the quality of syrup (Herwanti 2015). The productions of *jeruju* and *pedada* fruits were to order. In the local market, the consumers of these products are visitors. Whereas, Sabana (2014) said that mangrove syrup production had good prospects to be developed from the production and marketing sides.

Every month 2,200 kg of chips and 1,000 bottles of syrup are produced. This number was different in 2014, Ariftia et al. (2014) found the production number was 2,280 kg of chips and the syrup was 2,280 bottles monthly. This means the production has declined because of limited orders and the number of participant members who made it. The members had another activity like managing the household and helping their husband to manage ponds. According to their leader, the number of WCB Group members has decreased from 32 to 9 active members who managed *pedada* and *jeruju* fruits.

Besides *terasi*, *jeruju* and *pedada* products, Margasari community has been developing ecotourism since 2006 as one of economic utilization of the mangrove forest. Ecotourism activities include mangrove walking tracks, bird watching, reading house, boat trips through the mangroves, planting packages, and shrimp pond tours. The visitors were typical students, lecturers and researchers, on average ten people visited per month.

Not all the community participated in ecotourism management in Margasari. Only the community who join the group in Margasari participated in ecotourism. Ecotourism was managed by the village groups; Nelayan Group, Margajaya, PLH, WCB and Pengolah Terasi. WCB and Pengolah Terasi Groups produced some products as souvenir from mangrove. Margajaya group and Nelayan Group provided services as tour guide and transportation by boat around the mangrove forest. PLH Group provided homestays for the visitors, who were most commonly researchers. There were ten members of Nelayan Group who were actively involved in ecotourism, while in PLH Group, only the leader was active. The limited community who joined, in line with Soedigdo and Prino (2013) stated that the community had not joined maximally in providing tourism services. Although, the active participation in ecotourism management had important because the natural knowledge and culture have high value as the ecotourism interest (Hijriati and Mardiana 2014).

The ecotourism development in Margasari is not currently running well because many facilities have been damaged. The conditions of these facilities have affected visitor interest. As Suchaina (2014) said, if ecotourism place had facilities and infrastructure that are below standard, it could decrease the interest to visit the tourist sites. The facilities which have been damaged are the bird watching tower and mangrove track. The access to the bird watching tower has been closed by the shrimp pond fence and the tower is lower than the mangrove stands. The low maintenance also caused damage to facilities. As stated by Johan (2016) ecotourism in Indonesia has not been developed optimally.

Community perception of ecotourism development

The community perception of the ecotourism development is divided into several aspects including community knowledge of ecotourism, willingness to participate in ecotourism development, economic benefits of ecotourism for the community, damage caused by visitors and the sustainability of the mangrove ecotourism development in Margasari. The community knowledge of ecotourism was measured based on the community's understanding of the difference between ecotourism and regular tourism. Ecotourism focuses on the concept of nature-based elements in travel activities to enhance visitor experiences, as well as an increased desire to minimize adverse impacts of tourism to the environment and it considers the wellbeing of local people and including educational components in travel activities (Bandara and Vlosky 2016) different from regular tourism (mass tourism) commonly is artificial tourist attraction and it is not empowering local people (Basyuni et al. 2016).

More than 90% of Margasari community did not understand the concept of ecotourism. People thought that ecotourism was the same as common tourism. Community members who understood the concept of ecotourism were people who participated in ecotourism management and had the knowledge about ecotourism from the extension program and being active in community group activities. These findings are in line with the research of Kurniawan (2015) who found that member of the Rendoli community thought the ecotourism is a usual tourism tour.

After determining the level of community knowledge, five aspects of ecotourism development were measured. These five perception aspects about the ecotourism development are shown in Table 1.

The willingness of Margasari community to participate in the ecotourism development was high. This willingness is based on a belief that ecotourism activities could increase the community income besides their main livelihood. Margasari community would participate in management planning, counseling of ecotourism development, ecotourism guide, providing homestay, selling and produce souvenir for the visitors. Through the high level of community participation, the ecotourism development in Margasari would be sustainable. As stated by Aflaha (2013), the ecotourism development would run well if the society participation are high and continue to be active.

Table 1. Community perception of ecotourism development

Behavior	Agree/ willing	Less agree/ willing	Not agree/ willing
Willingness to participate in ecotourism development			
Planning activities	89	2	8
Tour guide activities	65	12	23
Souvenir activities	97	2	1
Providing homestay	70	1	17
Counselling about ecotourism	97	2	1
Community perception of ecotourism group			
Group formation	98	2	0
The willingness to join group	88	6	5
Government intervention in Margasari ecotourism			
Partial intervene	98	0	2
Full intervene	70	10	20
Ecotourism impact on economic benefits			
Increase economic benefit for community in Margasari	11	11	78
Economic benefit only for some participants	57	6	3
Visitors effect the environment damage			
Garbage in forest area	10	1	89
Garbage in village	3	86	11
Damaging the mangrove trees	3	1	96
Damaging the ecotourism facilities	1	3	86
Willingness to develop sustainable mangrove ecotourism	99	0	1

An ecotourism group is a group that cared about ecotourism and were willing to participate in the ecotourism activities. At this time Margasari has some groups which manage ecotourism, but the management was disorganized. They work individually without organizing by the group. They used the group name to participate in ecotourism because the groups have network to visitor. Outside parties (government, NGOs, universities) that would visit the mangrove area would contact the groups. This limits the participation of other people who were not members of the group. For this reason, it is necessary to develop a new group for tourism purposes to act as s coordinating institution for all community members who are willing to participate in developing mangrove-based ecotourism. The majority of the Margasari community (88%) agreed to develop ecotourism group and showed a willingness to join. The participation of local community in ecotourism activities was very important, because they would provide most of the attractions and also determine the quality of the tourism product (Rizky et al. 2016). Besides, according to Pamungkas (2013), the local community could influence the development pattern and the ecotourism policy with the significant diversity in globally.

Mangrove ecosystem has the potential to improve the community prosperity because it has unique and special characteristics, for that the mangrove ecosystem needs to be developed as alternative ecotourism destination (Agussalim and Hartoni 2014; Saputra and Setiawan 2014). The current ecotourism that has been developed in Margasari has not successfully increased the income of the community. Only 12% of community members had received additional income from mangrove ecotourism. Whereas, the objective of ecotourism is developed prosperity of community which would provide additional livelihood and it increase family income (Hijriati and Mardiana 2014; Manahampi et al. 2015; Sari 2015; Rizky et al. 2016).

Besides community prosperity, one of the ecotourism development goals is to decrease pressure on the forest as a resource (Flamin and Asnaryati 2013). Base on that mangrove ecotourism development needs to consider the possibility of disturbance from visitor. Currently, the disturbance caused by visitors such as environmental damage in mangrove forest ecosystems, ecotourism facilities or the village neighborhood is low. The ecotourism visitors generally are students and researchers both domestic and international with visitor numbers currently at only 5-10 people per month. This number needs to increase so all community in Margasari could participate in mangrove ecotourism.

The ecotourism activities in Margasari have been conducted since 2000 and are well known by the people. Most of Margasari community members (88%) agree with the development of mangrove ecotourism. However, the limitation of facilities, infrastructure and the number of visitors become challenging for sustainable development of mangrove ecotourism. The community needs support and collaboration from government and non-government organizations to develop ecotourism in Margasari. Besides, it needs to increase the community capacity and institution reinforcement to manage the mangrove ecotourism. With increasing economic benefits from mangrove ecotourism management, the community surrounding mangrove forests will participate in the protection and preservation of the mangrove forest ecosystem.

Protection effort in the form of prohibition to enter mangrove areas is effective in keeping mangrove sustainable and it reduces environmental damage. However, the prohibition has been limited utilization effort by the community. Whereas, institutional protection by custom rules should provide greater space for civil society to participate in the efforts to achieve sustainability of function and utilization of forest resources (Hidayat 2017). The low economic benefit of mangrove has caused the community participation in mangrove management to decline. Further development of mangrove-based ecotourism is expected to increase community participation in the management of mangrove forests.

ACKNOWLEDGEMENTS

We would like to thank to Dr. Hj. Bainah Sari Dewi for the suggestion on the manuscript.

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OCEAN LIFE
Volume 1, Number 1, June 2017 E-ISSN: 2580-4529
Pages: 20-25 DOI: 10.13057/oceanlife/o010104

The enhancement in comprehension for the younger generation of school age in conserving coastal biodiversity in Kepulauan Seribu, Indonesia

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Manuscript received: 19 April 2017. Revision accepted: 8 June 2017.

Abstract. *Handayani T, Widiarti R, Soepardjo AH, Afriyani F, Burhanuddin E. 2017. The enhancement in comprehension for the younger generation of school age in conserving coastal biodiversity in Kepulauan Seribu, Indonesia. Ocean Life 1: 20-25.* The exploration and exploitation can lead to the ecosystem and natural resources degradation. Kepulauan Seribu of Special Area of Jakarta has been extensively utilized and developed for tourism areas, which could lead to decreasing environmental conditions. Therefore, the awareness for keeping the ecosystem and marine life should be developed early. The objective of this research is to determine the level of understanding and ability to conserve the marine environment in their neighborhood for the school-age generation. The method used in this study was quantitative with descriptive analysis. Two groups of Junior High School students of Pulau Tidung and Pulau Harapan of the Kepulauan Seribu were provided training on conserving coastal ecosystems. The education and training encompass the observation activity for coral reefs using the Coral Health Chart method, seagrass condition observation through the Seagrass-Watch method, and introduction to the mangrove ecosystem. Based on the study, it can be inferred that the resulting activity indicated the level of understanding of cognitive abilities of two groups of Junior High schools in those two locations has increased. In addition, their affective ability increased sharply toward the students from Pulau Tidung whereas, in Pulau Harapan, a large increase in psychomotor abilities occurred. The conclusion of this program is managed to improve the understanding and ability to act for the younger generation in conserving marine and coastal environments.

Keywords: environmental education, marine conservation, Pulau Seribu, the younger generation

INTRODUCTION

The education and training of conservation encompass an early stage from the basic principle of conservation. Therefore, this matter is good to be performed at an early age. Therefore, giving basic education and training to the younger generation from the Junior High School students in Kepulaun Seribu, especially Pulau Harapan and Pulau Tidung, are necessary to conduct. Therefore, the objective of this research is to determine the level of understanding and ability to conserve the marine environment in their neighborhood for the school-age generation.

Pulau Harapan and Pulau Tidung are part of the National Park of Kepulauan Seribu in Jakarta Bay, Indonesia. Those two islands are included in the residential zone of four zones of the National Park (Estradivariet al., 2007; Farhan and Lim, 2011). However, even though it is included in a residential zone, which has a high human activity, there are also Marine Protected Areas in Pulau Harapan and coral plantation area in Pulau Tidung, which needs to be maintained because the area is filled with many marine biodiversity components, such as mangrove, coral-reef fishes, coral transplantations, even terrestrial biodiversity which live in the coastal area.

Changing how people think about the conservation of species and ecosystem diversity is necessary. In many parts, human activities in small islands exploit marine resources, degrade aquatic, and cause irreversible losses of biological diversity (Petrosillo et al. 2007). Those are some reasons we should educate the young generation in small islands about the environment. By giving the material of coastal ecosystems and their conservation, the young generation who live on the small islands will participate and preserve their neighborhood. The participants also need to continue to monitor the observation site and maintain the environmental condition during training, including the Marine Protected Areas in those islands

MATERIALS AND METHODS

This research was conducted in two locations, namely at Pulau Tidung, a small island close to Jakarta, and Pulau Harapan, which lies off the northern part of the Kepulauan Seribu, Jakarta Bay, Indonesia (Figure 1).

To find out how far the participants' comprehension and improvement of the coastal ecosystem conservation in their neighborhood, we used the questionnaire method before and after training (Figure 2). The questionnaires focused on three specific questions: students' knowledge about their coastal ecosystems (especially mangrove, seagrass, and coral reefs); their awareness of marine conservation on their island; and their understanding of environmental quality and environmental impacts due of marine tourism.

The questionnaires were administered to twenty participants from both islands, Pulau Tidung and Pulau Harapan, before the next activities (in this context, outfield training activities about coastal ecosystems). Then they were going to answer another questionnaire on the same topics in the last part of this training activity.

Ten Junior High School students were selected to be trained as the target group for each island. A training method that is used to give information about conservation on coastal ecosystems consists of mangrove ecosystem observation, which is identified the type of mangrove in the locations and measures the density; seagrass observation by using Seagrass-Watch to learn about seagrass community structure; and also coral observation using Coral Watch method to check the condition of coral health (Figure 3). The result data of seagrass and coral observation will be uploaded to www.seagrasswatch.org and www.coralwatch.org websites so that many stakeholders can use the data in the ocean field, and the participants could also monitor the area more easily (Widiarti and Farid 2007).

The questionnaires' results, compiled from discussion, knowledge, and skills, are necessary to obtain the competency progress in understanding the need for coastal environment and biodiversity conservation.

Figure 1. Location of research area at Pulau Tidung and Pulau Harapan, Kepulauan Seribu, Jakarta Bay, Indonesia

A **B** **C**

Figure 3. Outdoor activities during training at the Pulau Tidung and the Pulau Harapan, Kepulauan Seribu, Jakarta Bay, Indonesia. A. Mangrove observation, B. Seagrass watch, C. Coral watch

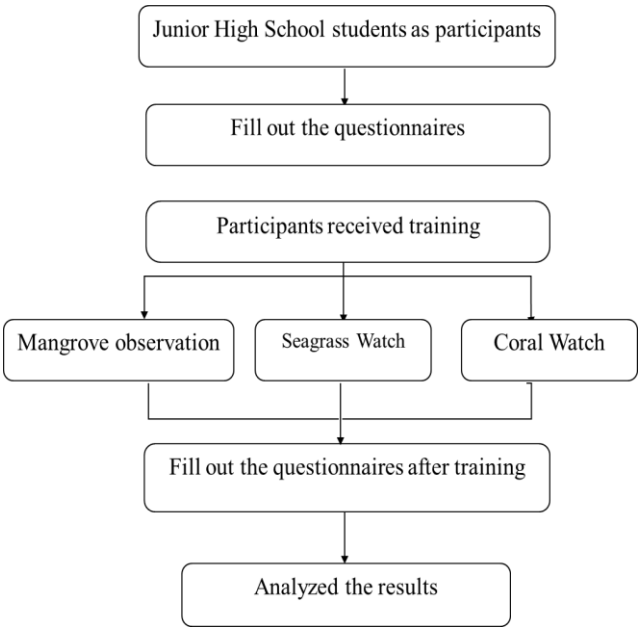

Figure 2. The scheme of questionnaires methods for collecting data

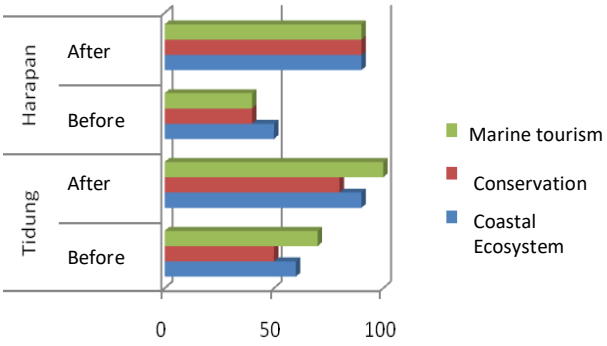

Figure 4. Student comprehension before and after training

RESULTS AND DISCUSSION

Figure 4 shows the students who obtained training on marine ecosystems, coastal ecosystems, conservation, and marine tourism. Their knowledge increased after training, particularly for the Junior High Scholl students of Pulau Harapan.

Knowledge of coastal ecosystems (especially mangrove, seagrass, and coral reefs)

The survey was conducted using a questionnaire on the coastal ecosystem given to the Junior High School students. There were 7 main questions on mangrove, seagrass, and coral reef to be answered by the students. The main findings are presented in Table 1. Based on Table 1, it can be seen that the knowledge of both groups of students on mangroves, seagrass, and coral reef changed and improved. In addition, their students' awareness of coastal ecosystem conservation would be improved in the future.

Based on 20 participants, the students of the Pulau Harapan and the Pulau Tidung are dissimilar in that the Pulau Harapan students are more concerned with biodiversity and coastal ecosystems than the Pulau Tidung. Since the Marine protected area is located in Pulau Harapan, the Pulau Harapan students' knowledge is higher. There were many coral transplantation sites in that area, mostly branching coral (*Acropora* sp.) which had already been transplanted one year. Some of the participants from Pulau Harapan came from fishermen's family backgrounds. Therefore, they were passionate about keeping their environment healthy by conserving the ecosystems, especially the coral. They knew that a healthy ecosystem could provide many species of fish in large quantities. Douvere (2008) mentioned that the influence of human activities would limit their fishing activity and capacity. The Pulau Harapan is located north of Kepulauan Seribu National Park of Jakarta, where marine tourism activities are still limited (Farhan and Lim 2011). The degradation of coral reefs and seagrass will influence their life. The awareness of coral reef disturbance and seagrass depletion that will reduce the availability of fish has already made

this group more concerned about conservation. The increase in psychomotor abilities is a more visible improvement in the Pulau Harapan target group. The Pulau Tidung Island was dominant in the biodiversity theory, especially the mangrove ecosystem. This matter arose because some parts of the island still have genuine and long, standing mangroves.

Awareness of marine conservation on their island

Using Seagrass-Watch and Coral Health Chart method, two participant groups of the Yunior High School of the Pulau Tidung and the Pulau Harapan practiced identification, learned seagrass community structure, and differentiated the healthy and dead coral (bleached). The student group of both islands also learned how to contribute their data, and everyone can access that through the Internet. Thus, there are differences in collecting the marine ecosystem data, especially concerning coral reefs. The Pulau Harapan target student group was more obvious on doing activities in the field, maybe because they like to help their parents fishing. Meanwhile, the Pulau Tidung student group has been the most partially familiar being with a more comfortable life, less resistant to being on the water, and much slower to absorb the techniques and how to monitor coral reefs. The increase in psychomotor abilities is a more visible improvement in the Pulau Harapan student target group.

There are two types of areas in the Pulau Tidung, the biggest one is an island for residential with so many tourism activities (Tidung Besar Island), and the others are used for mangrove plantation areas (Pulau Tidung Kecil), less human activities (Farhan and Lim 2011). The participants from the Pulau Tidung mostly learned information about conserving their environment after they learned in location. There were many differences in the coastal condition in Tidung Besar (the biggest one) and Tidung Kecil (the small one). They could find healthy mangroves in the research area (Tidung Kecil) like *Rhizophora stylosa*. It lives near the seagrass beds in Tidung Kecil. *Enhalus acoroides* and *Thalassia hemprichii* were also found along its coastal area. They are the most common seagrass species because the substrate firmness is muddy sand, which coalesced with mangrove (Azkab 2006). *Thalassia hemprichii* is the most common seagrass in tropical areas, especially in Indonesia. The rhizome, leaves, and roots are very strong in any substrates (Mckenzie et al. 2000). One seagrass functions as mammals *Dugong dugong*'s food, indicating we need to keep the seagrass ecosystem safe to conserve this biota. These mammals would not come to the area with very high human activities. Seagrass kept dissolved oxygen (DO) in water as a coastal producer. Tidung participants increased their awareness of human activities' effects by monitoring three coastal ecosystems on their island during training. It is good that twenty participants from each island had increased their awareness to keep and conserve coastal ecosystems (mangrove, seagrass, and coral reefs) safe.

Moreover, mangrove in Pulau Harapan was less than in Pulau Tidung, but they began to be planted again recently by the society. Even mangrove conservation has become part of the community and government programs nowadays. So that they assume the mangroves must be maintained. The knowledge of the identified type of mangroves found in their environment has increased after training. They had a deep concern for conserving mangroves. We could find *Rhizophora stylosa* and *Avicennia marina* (juv.) with dominant substrate muddy sand in the Pulau Harapan; there was also *Sonneratia* sp. in the past, but it already was gone. Most of them are the result of cultivation.

Some of the Pulau Harapan target group participants came from fishermen's families. So they had a deep passion for keeping their environment healthy by conserving the ecosystems, especially the coral ecosystem. They knew that a healthy ecosystem could provide many species of fish in large quantities. The awareness of coral reef disturbance and seagrass depletion that will reduce the availability of fish has already made this group more concerned about conservation (Table 2). However, most of the parents of the Pulau Tidung target group are not fishermen. Most of them work in tourism, such as being guides, selling souvenirs, or opening restaurants.

Understanding environmental quality and environmental impacts due to marine tourism

The Pulau Harapan participants who used to refuse input of human activities because it could threaten their environment filled the questionnaires with very open-mindedness about marine tourism. They explained their answers during the discussion after training. They learned that their coastal environment would damage not only because of marine tourism but their awareness about how to keep their environment conserved, especially since there is Marine Protected Area on the island. So they consider their role in the future conserving their environment, especially coastal ecosystems. So if marine tourism is on their island, they will make it marine ecotourism to conserve and save the ecosystems (Table 3).

Another opinion came from the target group in the Pulau Tidung, which is become a tourism region since the early twenty-first century. The condition made most participants lack awareness about the conservation of their environment before they filled out the questionnaires and trained. They knew that high quantities of tourism did not have some effect on their daily life. Tidung Besar has had its coastline reduced lately (Farhan and Lim 2011), and it could not be denied that marine tourism had a role in it. But during the training, we found *Millepora* (fire coral) along the Tidung coastline, and it's not good for tourism activities in that area. Therefore, the participants learned about dangerous biota in coastal ecosystems. *Millepora* is one of the branching corals, which is very dangerous because it could irritate the skin (Barbier et al., 2011).

Table 1. Questionnaire results on coastal ecosystem

Questions	Alternatives answers before training	Awareness	Answer after training	Awareness
What do you know about mangroves?	Nothing	****	Nothing	-
	Know without reason	***	Know without reason	***
	Explaining the question	**	Explaining the question	***
What is the function of mangroves for your neighborhood?	Don't know	***	Don't know	
	Answer with samples	**	Answer with samples	**
	Explaining some samples and reasons	**	Explaining some samples and reasons	***
What do you know about seagrass?	Not answer	****	Not answer	*
	Answer for a simple reason	*	Answer for a simple reason	***
	Explaining reasons		Explaining reasons	**
How important is seagrass to your neighborhood?	Do not know	**	Do not know	
	Important, no reason	****	Important, no reason	**
	Explaining reasons	*	Explaining with reasons	****
What do you know about coral reefs?	Sea rocks	*	Sea rocks	
	Sea plants	****	Sea plants	
	Sea animal	*	Sea animal	****
How important are coral reefs in your neighborhood?	Important without reason	****	Important without reason	**
	Important, explain the reasons	*	Important, explain the reasons	***
What is the effect of human activities on coral reefs?	Hesitate	***	Hesitate	*
	Human activities are not good for coral reefs	***	Human activities are not good for coral reefs	****

Table 2. Questionnaire results on conservation

Questions	Alternatives answers before training	Awareness	Answer after training	Awareness
What do you know about conservation?	Nothing	****	Nothing	
	Know without reason	***	Know without reason	***
	Explaining the question	**	Explaining the question	***
What kind of conservation have you done to your neighborhood?	Not answer	****	Not answer	*
	Answer with simple samples	**	Answer with simple samples	****
	Explaining with samples	*	Explaining with samples	**
How important is conservation for your neighborhood	Do not know	***	Do not know	
	Important	**	Important	****
	Explaining reasons	*	Explaining reasons	****

Table 3. Questionnaire results on marine tourism

Questions	Alternatives answers before training	Awareness	Answer after training	Awareness
What do you know about marine tourism?	Nothing	****	Nothing	-
	Not much	****	Not much	*
	Explaining the question	**	Explaining the question	***
What is the potential for marine tourism in your neighborhood?	Not answer	****	Low	**
	Refuse with reasons	**	Good, they want to develop their island	****
	Marine Protected Area	*	Neutral	*
Would you like to join if there were marine tourism on your island?	Not answer	****	Yes	****
	Hesitate	**	Hesitate	***
	Depend on their parents	**	Depend on their parents	**

The aesthetic appeal of the marine environment, especially coastal ecosystems, could be an exceptional point for small islands to make marine tourism. In addition, public awareness of nature could keep the marine protected area safe. One of the human activities that influence the Marine Protected Area is humans, as top predators are generally removed. Still, they could come back at a great number as visitors. Those are some effects of human activities on marine communities (Milazzo et al. 2002). Those reasons also could be why participants, especially the target group from Harapan Island, hesitated about marine tourism in their neighborhood.

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OCEAN LIFE
Volume 1, Number 1, June 2017
Pages: 26-31

E-ISSN: 2580-4529
DOI: 10.13057/oceanlife/o010105

Threat of blast fishing on coral diversity in Peucang Island, Ujung Kulon National Park, Indonesia

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Manuscript received: 6 December 2016. Revision accepted: 10 June 2017.

Abstract. *Gumbira RWW, Rizkia F, Pribadi TDK, Hidayat MS. 2017. Threat of blast fishing on coral diversity in Peucang Island National Park, Indonesia. Ocean Life 1: 26-31.* As a maritime country with high biodiversity, the health of Indonesian coral reefs is of deep concern. Coral reef ecosystems are brittle, and mortality occurs relatively quickly after disturbance, not only by nature but also by human activities such as fish bombing. Fish bombing is a big issue that has always threatened coral reefs, including on Peucang Island, part of Ujung Kulon National Park. This place is protected by law to preserve its natural diversity. A study on coral coverage was conducted to observe the corals and related biota conditions in a coral reef ecosystem. Data was collected using the Point Intercept Transect method in 2m and 8m depths. The results showed that coral coverage at 8-meter depth is only 44.02 percent of the live coral at 2-meter depth. The reduction in the value of Diversity Indices, Dominance Indices, and the Evenness Index in all study sites led to low diversity, with no dominance by a particular species at any of the study sites. The results also showed that corals are well distributed in both depths. Over the past three years, the damage to corals in Peucang Island has generally increased by 0.7 percent due to fish bombing activities.

Keywords: Blast fishing, coral, diversity, Peucang Island, Ujung Kulon

INTRODUCTION

Coral reefs are one of the most productive and varied ecosystems on earth and provide many ecosystem services. For example, coral reefs provide a place for marine life to live, find food, shelter, and breed and also act as a buffer against wave action to prevent erosion and abrasion (Souter and Linden 2000). Coral reefs consist of colonies that became the structure on the ocean floor in the form of deposits of calcium carbonate ($CaCO_3$) produced by coral animals and belong to the Phylum Coelenterata (hollow animals) or Cnidaria (Papu 2011).

The coral reef ecosystem is friable, and mortality occurs quickly after disturbance (McCormick and Weaver 2012). Coral growth is influenced by human activities and natural factors, such as nutrient availability, predators, and the physicochemical condition of the sea. In certain circumstances, natural conditions will create stable coral reefs. However, human factors, such as fish bombing and anchors, can damage coral reefs (Papu 2011). Burke et al. (2012) estimated that 60 percent of the world's coral reefs are under immediate threat from human activities due to excessive exploitation of marine resources.

Indonesia is situated within the Coral Triangle Zone, which contains the world's highest marine biodiversity, so it should be maintained and preserved (Burke et al., 2012). The Ujung Kulon National Park Authority (2009) reported that the water in Ujung Kulon and the surrounding waters of Panaitan is where fishing using explosives takes place quite often, which causes damage to coral reefs. Using bombs on Peucang Island creates violent shocks in the water, which can virtually destroy the surrounding marine life. Not only due to fish bombing, but other fishing activities also use environmentally damaging methods as well such as Potassium Cyanide (KCN), trapping on reefs, arad nets, and various other gears that can damage the whole environment and make living coral biota, either soft corals or hard corals, fetched (Ujung Kulon National Park 2011, unpublished data).

Based on these facts, information on the coverage of coral reefs is expected to reveal the broader impact of the fish bombing that has caused biodiversity degradation on the ecosystem of coral reefs. For this reason, the study can be used as data to shape policies on coral reefs management in Ujung Kulon National Park.

MATERIALS AND METHODS

Study sites

Data was collected in August 2014 at Peucang Island, Ujung Kulon National Park, Banten, Indonesia (102º02'32 "-105º37'37" E and 06º30'43 "- 06º52'17" S). Survey dives were conducted at six selected locations to represent the condition of coral reef ecosystems in the Western and Eastern parts of Peucang island. The Western study sites consisted of Bonsai, Handarusa, and Karang Copong, while the Eastern sites were Ciapus, Citerjun, and Citerjun 2 (Figure 1).

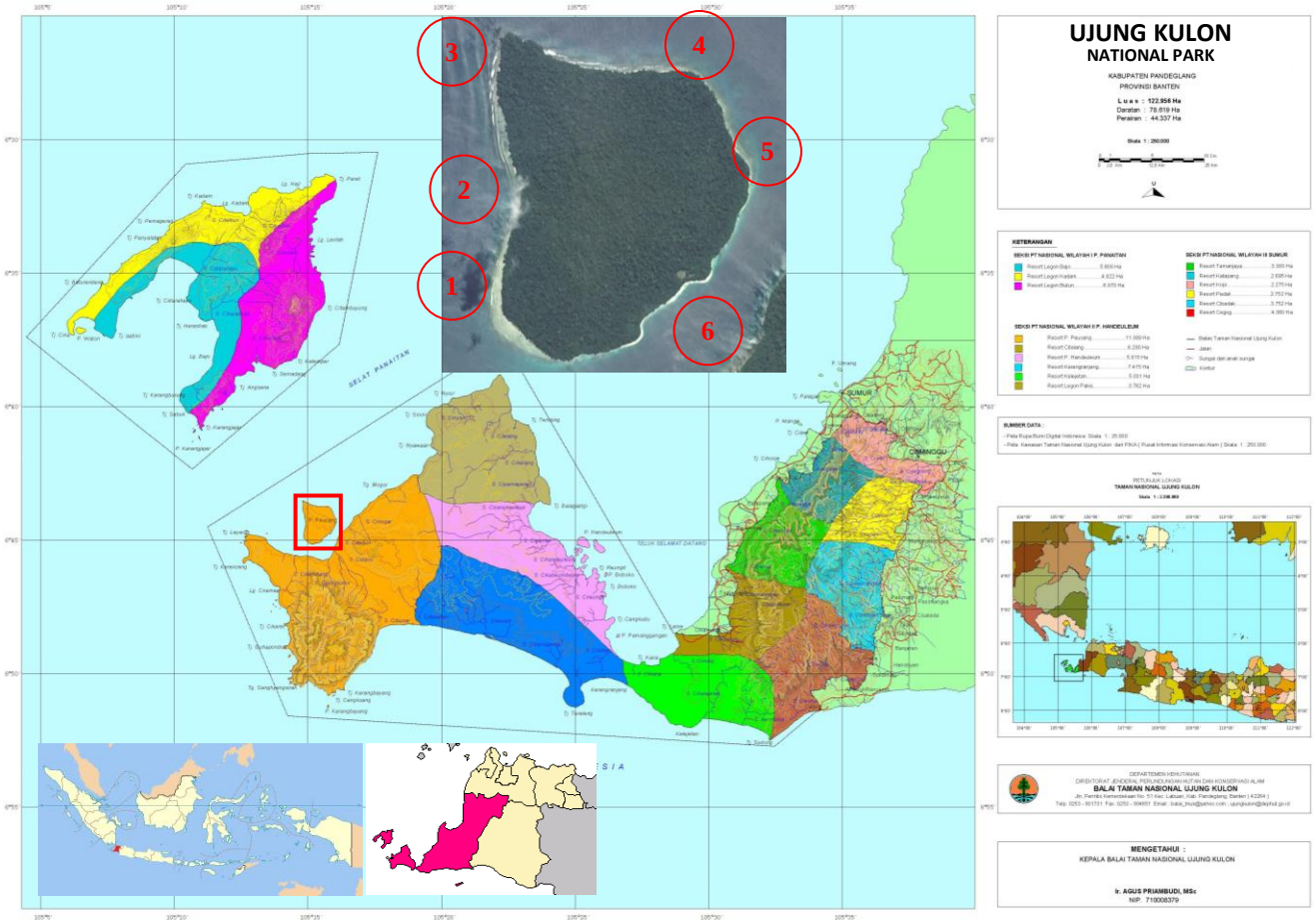

Figure 1. Location of Peucang Island in Ujung Kulon National Park, Pandeglang District, Banten, Indonesia. Spots: 1. Bonsai, 2. Handarusa, 3. Karang Copong, 4. Ciapus, 5. Citerjun, 6. Citerjun 2

Observation of coral reefs

Point Intercept Transects (PIT) were conducted to investigate the category of life forms (Jokiel et al., 2015). The PIT calculates the substrate's percentage cover (% coverage) using a rope marked at every 0.5 meters or a scale tape (Smart meter roll-out). The distance between transects one, two, and three were 5 meters (Manuputty and Djuwariah 2009). Data were collected from two depths, 2 and 8 meters, from the six research sites, using 150 m transects. Substrate, coral lifeform, or type of other organism was recorded at 0.5 m intersects along each transect and calculated into percent coverage for each category. In addition, any additional information, such as the description of the location, was recorded, especially if there was any obvious damage due to previous bomb fishing.

Data analysis

The data were arranged in the form of observational tables to be analyzed using this formula (Manuptty and Djuwariah 2009). Hard coral life forms identified comprised Acropora Branching (ACB), Acropora Submassive (ACS), Acropora Encrusting (ACE), Acropora Tabulate (ACT), Coral Branching (CB), Coral Encrusting (CE), Coral Foliose, (CF), Coral Heliopora (CHE), Coral Massive (CM), Coral Millepora (CME), Coral Mushroom (CMR), Coral Submassive (CS), and Bleached Coral (BC). In addition, dead coral was recorded as Dead Coral (DC). Other biotas recorded were categorized as Halimeda (HA), Hydroids (HY), Macro algae (MA), Other (OT), Soft Coral (SC), and Xenia (XE), and life forms included abiotic (non-living) were categorized as Rock (RCK), Rubble (RB), and Sand (S) (Piquero et al. 2015).

The condition of coral reef ecosystems was determined by the percent coverage of life-hard corals with the criteria that can be seen in Table 1 (Manuputty and Djuwariah 2009).

Table 1. Criteria level of percent coverage of life reefs in Peucang Island waters, Ujung Kulon National Park, Banten, Indonesia

Percentage (%)	Criteria
0-24.9	Broken
25-49.9	Moderate
50-74.9	Good
75-100	Very Good

Overview of the continual condition of the community structure of building organisms like coral reefs can be seen from the value of Diversity Indices (H') (Colwell 2008), Evenness Indices (E) (Wilsey and Stirling 2007), and Dominance Indices (C) (Sagar and Sharma 2012). Generally, a stable ecosystem has a high Diversity Indice and Evenness Indices, and Dominance Indices, which have to remain close to zero (Odum 1971).

RESULTS AND DISCUSSION

Condition of the research area

In this research, Peucang was divided into two parts of study areas, western and eastern. The western part consists of Bonsai, Handarusa, and Karang Copong while the eastern part Ciapus, Citerjun, and Citerjun 2. The physical parameters of Peucang Island Water can be seen in Table 2.

Reef ecosystem condition

The observation of coral reef conditions in Peucang Island water can be seen in Figure 2. The highest percentage of hard coral is 61.06% at 2 meters depth, while dead coral at a 2-meter depth is around 21.67%. Other organisms, such as macroalgae and soft corals measured in an 8-meter depth, had a percentage cover of 39.56%. Abiotic (non-living) elements such as sand, rock, and rubble were found at 8-meter depths with a percentage of 16.67% (Figure 2).

There is an obvious difference in hard coral coverage between 2-metre and 8-meter depths. The highest hard coral coverage at 2-meter depth was in Karang Copong locations with 70.33%, while the lowest was in Bonsai with a percentage of 50.67%. At an 8-meter depth, the highest hard coral coverage was in the Citerjun location, with a percentage of 33.00%, while the lowest was in Karang Copong, with a percentage of 20.33% (Figure 3).

At a 2-meter depth, the hard coral coverage was dominated by life forms like ACB of 21.83%, while there was no trace of BC, CHE, or CMR. At an 8-meter depth, 8.06% of the hard coral coverage was dominated by ACB life forms, and the lowest was the CF, with a percentage of 0.42% (Figure 4).

Dead coral coverage at a 2-meter depth was highest at Bonsai with a percentage cover of 33.67%, while the lowest was at Citerjun 2 with a percentage cover of 8%. At an 8-meter depth, dead coral coverage at Karang Copong was observed to be the highest, with a percentage of 34%, while the lowest was 8.33% (Figure 5).

Table 2. Physical parameters of Peucang Island Water, Ujung Kulon National Park, Banten, Indonesia

Parameter	Depth	
	2 m	8 m
Temperature (°C)	29	29
Brightness (m)	14	14
GPS Coordinate	$102^0$2'32" – $105^0$37'37" E, $06^0$30'43" – $06^0$52'17" S	

The percentage cover of other organisms at a 2-meter depth was highest at Citerjun with 19.67%. In contrast, no other organisms were recorded at Coral Copong. At an 8-meter depth, the percentage cover of other organisms was highest at Karang Copong, with a percentage of 44.67%, while the lowest was found in Citerjun with 31% (Figure 6). The percentage cover of other organisms is much higher at 8-metre than 2-metre for all sites.

Abiotic coverage at a 2-meter depth had the highest percentage cover (27.6%) at Handarusa, but none of these could be found at the location of Citerjun 2. At an 8-meter depth, the highest abiotic coverage was found at Bonsai with a percentage of 24%, while the lowest was in Karang Copong with a percentage of 1% (Figure 7). Here again, the difference between both depths was obviously noted.

Community structure of coral reefs

While illustrating the structure of coral reef communities, necessary analyses of data are required, such as Diversity Indices (H '), Evenness Indices (E), and Dominance Indices (C) on every observation location. Diversity (H '), Evenness Indices (E), and Dominance Indices (C) data of the coral reefs are shown in Table 3.

The highest Diversity Index (H') value at a depth of 8 meters could be found in Karang Copong and the lowest in Handarusa. At a depth of 2 meters, the highest value of H' could be found in Ciapus and the lowest in Citerjun 2. The highest Evenness Index (E) value at a depth of 8 meters was in the Karang Copong, and the lowest was in Ciapus. At a depth of 2 meters, the highest value of E could be traced to the Karang Copong and the lowest in Citerjun. The highest Dominance Index (C) value at a depth of 8 meters could be traced in Handarusa and the lowest in Karang Copong. At a depth of 2 meters, the highest value of C was found in Citerjun and the lowest in Karang Copong (Table 3).

Discussion

Environmental conditions

The same temperature was recorded at Western and Eastern sites, 29 °C. According to Hoey et al. (2016), the temperature affects the speed of the metabolism and reproduction of coral. The optimal temperature for coral growth ranges between 23-30°C; the higher the temperature, the higher the coral animal's metabolism, so oxygen solubility is reduced.

Table 3. Diversity Indices (H '), Evenness Indices (E), and the Dominance Indices (C) of the coral reefs at Peucang Island, Ujung Kulon National Park, Banten, Indonesia

Locations	H'		E		C	
	8 m	2 m	8 m	2 m	8 m	2 m
Bonsai	1.98	1.83	0.86	0.83	0.17	0.21
Handarusa	1.34	1.92	0.75	0.87	0.33	0.17
Karang Copong	1.96	1.93	0.89	0.93	0.16	0.16
Ciapus	1.44	1,97	0.69	0.86	0.31	0.19
Citerjun	1.71	1.67	0.74	0.73	0.26	0.29
Citerjun 2	1.74	1.53	0.76	0.79	0.28	0.27

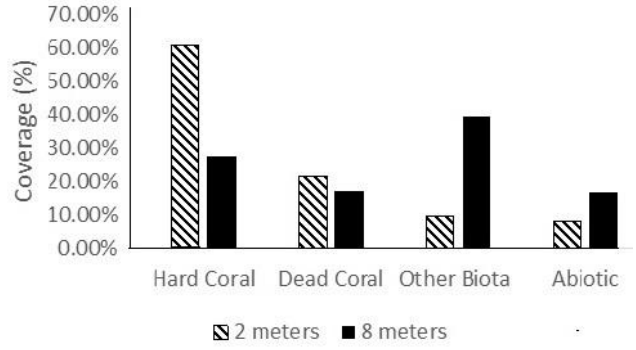

Figure 2. Percentage cover-based clustering of hard coral, dead coral, other biotas, and abiotic at 2 and 8-meter depths at Peucang Island, Ujung Kulon National Park, Banten, Indonesia

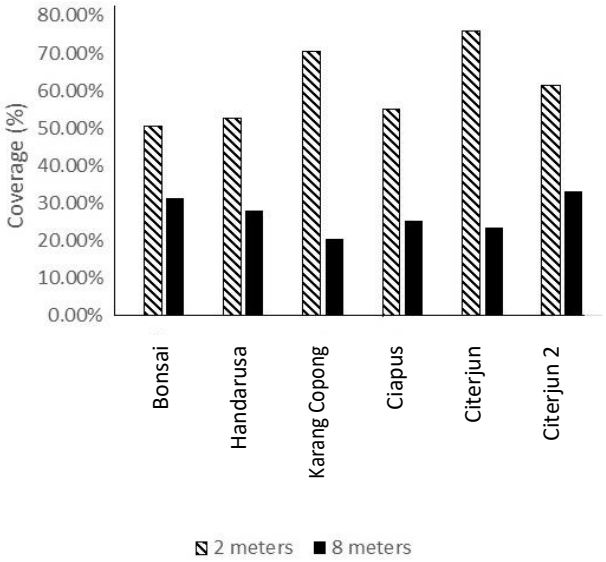

Figure 3. The percentage cover of hard corals at 2 and 8 meters deep at Peucang Island, Ujung Kulon National Park, Banten, Indonesia

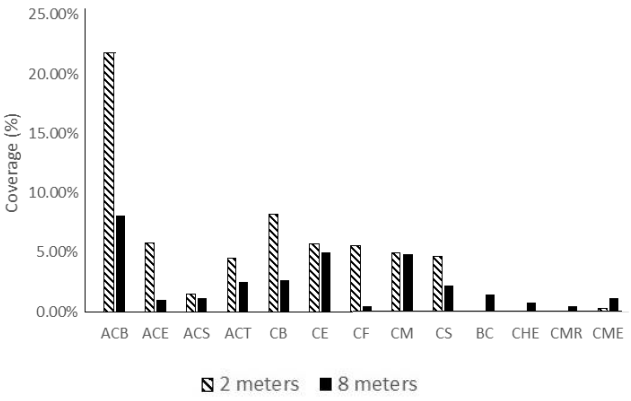

Figure 4. The percentage of hard coral coverage based on life forms at 2 and 8 meters deep at Peucang Island, Ujung Kulon National Park, Banten, Indonesia

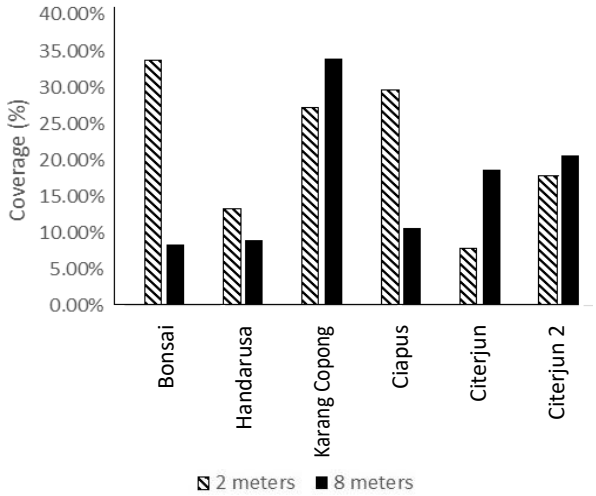

Figure 5. Percentage of dead coral coverage at 2 and 8 meters deep at Peucang Island, Ujung Kulon National Park, Banten, Indonesia

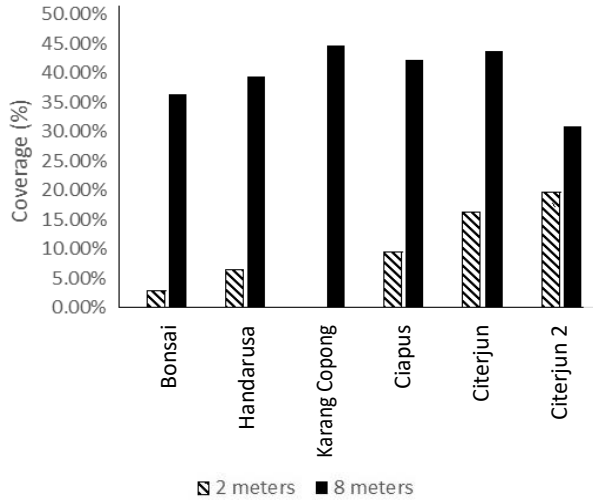

Figure 6. Percentage of other biota coverage at 2 and 8 meters deep at Peucang Island, Ujung Kulon National Park, Banten, Indonesia

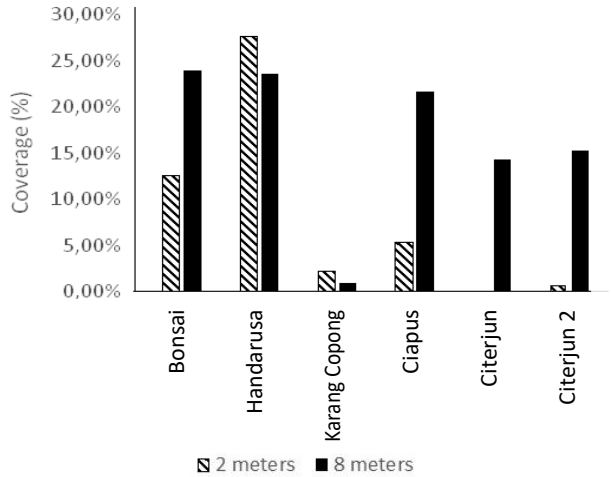

Figure 7. Percentage cover of abiotic (non-living) elements at 2 and 8 meters deep at Peucang Island, Ujung Kulon National Park, Banten, Indonesia

Visibility in Western and Eastern regions was between 10-17 meters. The level of visibility indicates that the water still gets good sunlight during daylight. According to Supriharyono (2007), the coral can grow well at a depth of <20 m so that more light can penetrate the water and be good for coral growth. However, the visibility from the bottom is as far as 10 meters on the sites of Karang Copong and Citerjun, presumably because the research location was in a state of slack tide, resulting in sediment particles getting lifted, and thus the water looks turbid. Relatively, turbid water conditions can also trigger a low percentage of coral coverage (Jones et al., 2015).

Condition of the reef ecosystem

The condition of the coral reef ecosystem is determined by the amount of hard coral coverage (Manuputty and Djuwariah 2009). Hard coral species commonly found in this study were Acropora Branching (ACB) life forms. This hard coral lifeform can grow in water with a strong current, although it can be destroyed if exposed to storms. The atoll has a relatively fast growth rate compared to other types of hard coral. Acropora grows in clean water with high light penetration, free of sedimentation that could impede the penetration of light (Desvianti and Choesin 2015).

The regional management and local fishermen of Peucang Island admitted that many fish bombing activities are used for fisheries, which have caused much damage to coral reefs. As a result, the coral growth rate is not in line with the rate of exploitation. According to Pontoh (2011), the characteristics of an area affected by fish bombing can be seen when diving in the sea. A crater is left by the bomb blast and many areas of coral rubble. At the research location, craters from bomb blasts, evidence of coral bleaching, and areas of coral rubble were observed at depths of 2 and 8 meters.

In addition, other indicative traits of ecosystem degradation can be seen, such as a high percentage of Dead Coral (DC), Rubble (RB), and other organisms such as Soft Coral (SC) (Manuputty and Djuariah 2009). Kilarski and Everson (2008) found that the coral coverage caused by the presence of dead coral fragments (Rubble) and Dead Coral (DC) indicates the process of coral destruction. Severely broken coral fragments indicate using explosives to get the fish on the reefs (Kasnir 2011). Prasetia (2013) said that if most coral reef ecosystems are mostly Dead Coral, it stands to reason that the coral reefs have been destroyed. Habitat destruction will impair the food web and impact fish life.

Dead Coral (DC) is the result of various damage, either from nature itself or humans, which causes the degeneration and loss of the colored zooxanthellae from coral tissue, and the coral won't be able to survive (Yusri 2016). Therefore, the dead Coral coverage percentage of all locations is included in the category of very high (dead coral coverage> 2%) (Mellor 2007), with the most severe locations in Karang Copong Bonsai at a depth of 8 meters and 2 meters.

Rubble (RB), including abiotic categories, is found in all dive sites, and 7 out of 10 dive sites (2 and 8 meters deep) are considered very high because their value of coverage exceeds 10% (Mellor 2007). Five of the seven locations were found at a depth of 8 meters. That suggests that damage to coral reefs from fish bombing is more prevalent at a depth of 8 meters, with the location of Coral Copong as the location with the largest rubble. More coral rubble in the form of fragments would hamper the recruitment of other organisms, and it may take several decades and even centuries for them to recover (Mellor 2007).

Soft Corals (SC) tend to grow more rapidly in areas with low light penetration, many of which can be found at a depth of 8 meters (Desvianti and Choesin 2015). Therefore, the existence of soft corals can be used as an indicator of the condition of hard corals, which can be in a critical state or damaged (Blue and Wijoyo 1999). The location Citerjun at a depth of 8 meters, has the highest frequency of the presence of macroalgae. That is presumably due to macroalgae which outcompete coral re-growth after disturbance. That is related to the Ciapus site conditions with a very high amount of rubble.

Overall, all six areas with a depth of 8 meters on Peucang Island have experienced a higher percentage of damage than those at a depth of 2 meters; this can be seen from the lower percentage cover of hard coral. At a depth of 8 meters, coral reef coverage reaches 26.88%, showing the condition of moderate damage. However, compared with data collected in the past three years (Ujung Kulon National Park Authority 2011, unpublished data), coral reef coverage at a depth of 8 meters has decreased by 16.36%. The decrease in coral reef coverage can result from fishing activity. The closer a fishing boat approaches the land, the noisier it will become, causing the fish to swim deeper, resulting in a more prevalent fish bombing (Fauziyah and Jaya 2010).

At a 2-meter depth, the percentage of live hard coral coverage reaches 61.05%, showing the good condition and an increase of 14.95% compared to the data in 2011. Overall, coral coverage on reefs around Peucang Island has experienced moderate damage, with a percentage cover of 43.96%, showing a 0.7% decrease over the past three years (Ujung Kulon National Park Authority 2011, unpublished data).

Community structure of coral reefs

Diversity Index (H ') at a depth of 8 meters has an average value of 1.7, while the value of 1.81 could be found at a depth of 2 meters. According to Princess et al. (2012), both Indexes are classified in conditions with low diversity and community stability (H '<2). The H value has decreased compared to coral reefs coverage data of Peucang Island in 2011, with the values of 2.11 (8 meters deep) and 2.25 (2 meters deep) categorized as a moderate state of diversity and community stability (2 <H' <3) (Ujung Kulon National Park 2011, unpublished data).

Evenness Index (E) at a depth of 8 meters is worth an average of 0.78, while at a depth of 2 meters, the value is 0.84. Sudiana (2005) said that the Evenness Indices value (E) is close to 1, which indicates the equal distribution of individuals among species. Therefore, the evenness Index (E) of Peucang Island in this study is more stable than the data in 2011 that worth 0.63 (8 meters deep) and 0.73 (2

meters deep) (Ujung Kulon National Park 2011, unpublished data).

Dominance Indices (C) at 8 meters deep are worth an average of 0.25, while it is 0.22 at a depth of 2 meters. Therefore, dominance Index values are nearing 0, indicating no dominant species (Sudiana 2005). Dominance Index (C) obtained in this study was measured lower than the data in 2011, with values of 0.19 (8 meters depth) and 0.13 (2 meters depth) (Ujung Kulon National Park 2011, unpublished data).

In conclusion, the coral reef coverage in Peucang Island, Ujung Kulon National Park, has experienced moderate damage and decreased coral coverage within three years. Evidence of excessive bomb fishing was present at a depth of 8 meters. The reduction in the Diversity Index, Dominance Index, and Evenness Index in all study sites showed the low diversity of species with no domination. The results also showed that corals are well distributed in both depths. However, exploitation and utilization of natural resources around Pulau Peucang have the potential to increase. Without adequate management and control over the nature of fishing practices in this area, coral reefs will experience continued degradation, leading to damaged marine ecosystems.

ACKNOWLEDGEMENTS

This study was supported by the Annual Student Program of Natural Exploration of Department Biology Universitas Padjadjaran and Ujung Kulon National Park, Indonesia.

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OCEAN LIFE
Volume 1, Number 1, June 2017
Pages: 32-40

E-ISSN: 2580-4529
DOI: 10.13057/oceanlife/o010106

Profiling marine bacterial dioxygenase gene involved in Polycyclic Aromatic Hydrocarbon degradation

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Manuscript received: 4 March 2017. Revision accepted: 22 June 2017.

Abstract. *Mishra M, Das S. 2017. Profiling marine bacterial dioxygenase gene involved in Polycyclic Aromatic Hydrocarbon degradation. Ocean Life 1: 32-40.* The degradation of polycyclic aromatic hydrocarbons (PAHs) by bacteria has been widely reported. While many pure cultures have been isolated and characterized for their ability to grow on PAHs, little is known regarding the diversity of microorganism involved in PAH degradation in the environment. The aim of this study was characterization of the gene for enzymes involve in PAHs Degradation, and to make a bank collection of strains for further screening research**.** Pure bacterial cultures were sampled from a highly enriched consortium for biodegradation analysis. Bacterial strains capable of degrading pyrene and anthracene were isolated from Paradeep estuary (Odisha, India) water sample by selective enrichment. Five strains of pyrene and anthracene degrading pure cultures were collected, named as MP-1, MP-4, MP-9, MP-14, MP-18. Isolates were characterized by gram staining, utilization of citrate, sugar fermentation, swimming and swarming motility and antibiotic sensitivity test. In seven days isolates MP-1,MP-4,MP-9,MP-14,MP-18 shown to degrade 58.4%, 42.1%, 29.1%, 31.7%, 31.8% of pyrene at a concentration 100 mg/l and 56.3%, 46%, 44.6%, 30.4%, 48.2% of anthracene at a concentration100mg/l. Polymerase chain reaction (PCR) with PAH-specific primers successfully amplifies a dioxygenase gene in MP-4. The presence of dioxygenase gene may lead to unraveling the underlying mechanism on how bacteria develop the abilities to degrade high-molecular-weight PAH. The understanding to profile not only the bacterial community but also the dioxygenases which they encode provides a powerful way for both assessing bioremediation potential in the environment and the discovery of novel dioxygenase genes.

Keywords: Anthracene, biodegradation, dioxygenase gene, marine bacteria, pyrene

INTRODUCTION

Biodegradation is a viable bioremediation technology for organic contaminants. The purpose of bioremediation is to transform organic pollutants into harmless metabolites or mineralize the contaminants into carbon dioxide and water (Alexander 1985). A possible remedial technology requires microorganisms being capable of quick adaptation to and efficient uses of pollutants of interest in a particular case in a relevant period. Microorganisms can utilize pollutants as substrates or metabolize them under various factors. Hence, understanding the catabolic pathway, mechanisms, and responsible enzymes is an effective means to define essential factors for efficient cleanup of pollutants. Biodegradation involves uses of a wide range of microorganisms to break chemical bonds and has been well reviewed (Klein 2000). Nevertheless, biodegradation is a very active field, and new findings are rapidly contributed to the literature.

PAHs (Polycyclic aromatic hydrocarbon) are known as aromatic hydrocarbons with fused benzene rings, typically arranged of two or more rings. PAHs are generated during the thermal breakdown of organic molecules and their succeeding recombination, such as partial combustion at high temperature (500-800 °C) or subjection of organic material at low temperature (100-300 °C) for extended periods leads to PAH production. They appear as colorless, whitish, yellow solids with low solubility in water, high melting, high boiling points, and low vapor pressure. PAHs are the environmental pollutant that is found naturally or in several polluted soils as a result of industrial activities, as well those of creosote wood-treatment facilities (Mueller et al. 1989). PAHs have involved considerable attention due to their potential toxicity for higher organisms and resistance to microbial degradation (Kanaly and Harayama 2000). A wide range of microorganisms have been discovered that can degrade very stable, deadly organic compounds, e.g., polycyclic and aliphatic hydrocarbons (Kanaly and Harayama 2000; Habe and Omori 2003; Van Hamme et al. 2003). Among these microorganisms, numerous Arthrobacter species can degrade PAHs (Grifoll et al. 1992; Seo et al. 2006). The PAHs have gathered significant environmental concern due to their occurrence, recalcitrance, bioaccumulation potential, and carcinogenic activity.

Microbial degradation is the primary degradation process of PAHs (Bumpus 1989; Yuan et al. 2001). The process depends on the environmental conditions, number, and type of the microorganisms, nature and chemical structure of the chemical compound being degraded. PAHs are biodegraded into less complex metabolites, and through mineralization into inorganic minerals, H_2O, CO_2 (aerobic) or CH_4 (anaerobic). The rate of biodegradation depends on microbial population, oxygen, pH, temperature, accessibility of nutrients, the chemical structure of the

compound, chemical partition in growth medium, and cellular transportation properties (Singh and Ward 2004).

The majority of information on PAHs degradation pathways derived from studies on gram-negative bacteria especially the genus Pseudomonas (Simon et al. 1993; Bosch et al. 1999). Under aerobic conditions, bacterial degradation of PAHsbegins with the incorporation of both molecules of molecular oxygen to the aromatic ring by a dioxygenase. The case of naphthalene (smaller molecular weight PAHs) degradation by cultured microorganisms has been studied extensively (Cerniglia 1992). Microbial degradation of PAHs is an important decomposition process for these contaminants in nature, thereby, represents a potential solution to the environmental problems posed by these chemicals. In recent years, PAHs biodegradation studies have received much attention and many related reports have documented that PAH dioxygenase and catechol 2,3-oxygenase are identified as two key PAH-degrading-related enzymes (Resnick et al. 1996; Meyer et al. 1999).

Some recent studies on genetic diversity have been mainly conducted with microbial populations or many bacteria. It focuses on analyzing the variety of dioxygenase genes of a bacterium. *Pseudomonas rhodesiae* KK1 of utilize a wide range of monoaromatic compounds including polycyclic and heterocyclic aromatic hydrocarbons such as carbazole and naphthalene previously mentioned (Kahng 2002). Various types of heterocycles comprising oxygen, sulfur, and nitrogen are detected in the environment which derived from instinctive or anthropogenic sources. Dibenzofurans, dibenzodioxins, and dibenzothiophene are within the most critical environmental pollutants and well reviewed by Klein, 2000. Thereby, only aerobic bacterial degradation of non-halogenated heterocyclic aromatics is briefly discussed in this to make a general relation with PAH degradation.

However, recent studies on cloned dioxygenase from *Norcardioides aromaticivorans* IC177 demonstrated that some dioxygenase could catalyze both reactions. PAH dioxygenases can catalyze several responses, including reduction, mono-and de-oxygenation (Resnick et al. 1996). In addition to the multiple responses with specific dioxygenases, current genomic or proteomic search with several PAH-degrading bacteria (e.g., *Burkholderia* spp. and *Mycobacterium* spp.) exposed that multiple dioxygenases exist in a single bacterium, probably playing different roles in PAH degradation (Resnick et al. 1996; Liang 2006).

Generally, methyl-ethyl-naphthalenes and phenanthrenes are common contaminants in the environment and, however, a limited amount of studies have been done about bacterial degradation. Alkyl PAHs catabolism in aerobic bacteria suggests a wide diversity of enzymes involved. These include oxidation of methyl group to an alcohol, aldehyde, carboxylic acid, decarboxylation, demethylation, and deoxygenation. Even so, production of alkyl salicylate or alkyl phthalate suggests that the reaction may prefer non-substituted PAH systems. Many anaerobes can transform Nevertheless, PAHs and their alkyl derivatives through novel catabolic pathways. Proteomics has been recently employed in studies of environmental microbiology and has shown their vast impact on the field of biodegradation and bioremediation.

Majority of the studies on the microbial metabolism of PAHs have been carried out with strains that use the compound under investigation as a growth substrate (Cerniglia 1992; Kanaly and Harayama 2000). However, some degrading bacteria act on a variety of compounds that do not support their growth and secreted partially oxidized products (Grifoll et al. 1995). This versatility is partly due to the broad substrate specificity of the degradative enzymes, as has been widely established for naphthalene and toluene dioxygenases (Wackett et al. 1988; Gibson et al. 1995). The primary objective of present work is the localization of dioxygenase gene cluster, for the enzyme involved for degradation of PAHs by potential marine bacterial isolates.

The objective on this research was to (i) isolate and screen pyrene and anthracene degrading Bacteria, (ii) to study the degradation of pyrene and anthracene by isolates, (iii) amplify dioxygenase gene locus, (iv) characterize the strains.

MATERIALS AND METHODS

Isolation

The samples were obtained from Paradeep (N 20º 17.542’ & E 86º 42.996’), Odisha. Water is kept in sterilized falcon tubes, stored in ice and transferred to the laboratory as soon as possible. One ml of sample was suspended into Basal Minimal media (BMM) broth (Dipotassium phosphate -7g/L, Monopotassium phosphate-2g/L, Magnesium sulphate-0.10g/L, Ammonium sulphate -1g/L, Sodium citrate -0.50g/L) (Sambrook et al. 1989) supplemented with 100 mg/L of pyrene and anthracene, as the sole carbon and energy source for growth and then it was kept at 37ºC/160 rpm for 15 days. After 15 days of incubation, the inoculum was diluted and inoculated on Sea Water Nutrient Agar plates. The isolated colonies from these plates were selected for further screening.

Screening

Each BMM agar plates was incorporated with pyrene and anthracene. The pyrene and anthracene stock solution (5mg/mL) was made in Hexane, and it was spread on prepared BMM agar plates. The plates were dried in laminar for few minutes so that hexane will evaporate. Next, the isolated colonies were streaked over the pyrene and anthracene. The same colonies were also inoculated in Minimal broth with 100mg/L and kept in the incubator shaker for seven days to monitor the growth and disappearance of PAH visually, which is further maintained for degradation studies.

Carbon source utilization

The strains were inoculated in freshly prepared LB broth and incubated at 37ºC for 24 hours. Following this step, the cell mass was collected and centrifuged at 4 ºC/7000 rpm for 10 minutes. Then, the supernatant was

discarded, and the pellet was resuspended in 1ml of BBM. 100 µL of the suspended cell was moved to 5ml BMM tubes with 100 mg/l of pyrene and anthracene separately as a sole carbon source. BMM supplemented with 1% glucose without any PAHs was kept as a control for growth. After that, these tubes were held at 37ºC/180 rpm for seven days. To monitor the growth, OD_{595} was taken at a regular time interval, i.e., 0 days, one day, three days, five days and seven days.

Standard curve of pyrene and anthracene

The stock solution of pyrene and anthracene was prepared in 5mg/mL of hexane. 5ml of pyrene and anthracene from the stock solution working solution in hexane of (0.5µg/mL, 1µg/mL, 10µg/mL, 50µg/mL and 100µg/mL) was prepared in triplicate for the standard curve. It was scanned to get the λ_{max} value and absorbance between 200-400nm using UV-Visible Spectrophotometer.

Degradation study

Degradation was performed by inoculating strains into test tubes containing 5 ml of Minimal Medium broth supplemented with 100mg/L of PAH (Pyrene and anthracene) (Kiyohara et al. 1982). PAH was delivered to media from the stock solution (5mg/mL) in hexane and kept for few hours to remove hexane from the media entirely. Thereafter, the BMM-PAH tubes were subsequently incubated at 37ºC/180rpm for seven days. After seven days, the cell was harvested by centrifugation, and it was again transferred to 200mg/L of BMM-PAH tubes (Pyrene and anthracene). The tubes were incubated at 37ºC/180 rpm for another seven days.

After seven days, BMM-PAH tubes were centrifuged min at 6000 rpm for 10 min. The supernatant was removed, while the pellet was rinsed with saline water two times. To above pellet, sterile basal minimal media was added without any carbon source. Then the OD was read at 595 nm and adjusted to 0.1. From 0.1 OD modified culture, 500 µL was transferred to BMM media with the addition of 100mg/l of PAH and incubated at 37ºC/160 rpm for seven days. After seven days the whole media was used for extraction and quantification of residual PAH (Pyrene and anthracene).

Extraction and quantification

Residual PAH was extracted from 5 ml of culture with an equal volume of Dichloromethane (DCM). For equal extraction volume of DCM was added to the degradation setup. The tubes were then vortex for 10 min and kept for another 10 min to separate the aqueous and organic phase. The upper organic layer was extracted and dried over sodium sulfate. Thereafter organic phase was pipette out and kept for drying overnight. The residual was resuspended in the same volume of n-hexane. The extracted pyrene and anthracene was diluted ten times in n-hexane. The residual pyrene and anthracene concentration was calculated from the standard curve of respective PAH. The absorbance of pyrene and anthracene was taken at 335nm and 254nm respectively.

Characterization of the isolates

Gram staining: gram reaction

The bacterial culture was smeared on a clean grease free slide with a sterile loop. The smear was air-dried and then fixed by heating method. Then, it was subjected to the following staining reagents: Flooded with Crystal violet (1 min), followed by washing with running distilled water. Next, flooded with Gram's Iodine (1 min), and washed with running distilled water. Then the slide was flooded with Gram's decolorizer (30 sec). After that, the slide was counterstain with Safranine (30 sec) and rinsed with running distilled water. The slide was air dried, and the cell morphology was checked under the microscope.

Biochemical tests: sugar fermentation tests

The sugar test was performed by using Bacillus Hi-media biochemical kit which contains different sugars. Kit A consists of Lactose, Maltose, Dextrose, Fructose, Raffinose, Xylose, Trehalose, Galactose, Melibiose, Sucrose, L-Arabinose, Mannose. Kit B includes Inulin, Sodium gluconate, Dulcitol, Glycerol, Inositol, Salicin, Sorbitol, Mannitol, Adonitol, Arabitol, α-methyl-D-glucoside, Erythritol. Kit C consists of Rhamnose, α-methyl-D-mannoside, Melezitose, Cellobiose, Xylitol, ONPG, Esculin hydrolysis, D-Arabinose, Citrate utilization, Malonate utilization, Sorbose. Sugar fermentation test was used to detect bacteria's ability to ferment sugar and produce gas and acid end product.

Swimming and Swarming motility tests

Swimming and swarming motilities are a rapid and a coordinated movement of a bacterial population across solid or semi-solid surfaces. This sort of motility is an example of bacterial multicellularity, an emerging concept in microbiology. Swarming motility was first described by (Henrichsen 1972) and has been mostly studied in genus *Serratia* (Alberti and Harshey 1990), *Salmonella* (Harshey 1994), *Escherichia* (Harshey and Matsuyama 1994), *Yersinia* (Young et al. 1999), *Aeromonas* (Kirov et al. 2002), *Bacillus* (Kearns and Losick 2004), *Pseudomonas* (Daziel et al. 2003*), Proteus* (Caiazza et al. 2005), *and Vibrio* (Rather 2005). This multicellular behavior is commonly observed in controlled laboratory conditions and depends on two critical elements: (i) the nutrient composition and (ii) viscosity of culture medium (i.e., the percentage of agar). One specific feature of this type of bacterial motility is the formation of dendritic fractal-like patterns generated by migrating swarms moving away from an initial position.

For swimming motility test, a lower agar nutrient concentration (0.2%) was prepared, and for swarming motility test, the higher content was used (0.5%). The agar was then poured plates in semi-solid condition, and ten µL of culture was inoculated at the center of the dishes, then the plates were kept in the incubator for 24 hrs at 37ºC.

Antibiogram

All the strains were examined for antimicrobial resistance by the method of Bauer et al. 1966 with Hi-Media, an antibiotic-impregnated media. All isolated

culture was swabbed in the MHA plates. Following this step, the antibiotics disc were kept over it. The following discs with concentration of the antibiotics as stated in the parenthesis were used, Gen: 10µg, AZM: 30µg, C: 50µg, AM: 30µg, E: 15 µg, NX: 10µg, S: 10µg, VA: 30 µg, MET: 5µg, T: 30µg, K: 30µg, AC: 10µg. Based on the diameter of the inhibition zones around the disc, the strains were characterized as susceptible or resistant based on diameter of the inhibition.

Preparation of lysates

Fresh cultures were transferred to 2 ml of LB medium and incubated at 37ºC for overnight. 1 ml of the suspension was centrifuged at 7500 rpm for 10 min at 4ºC. The supernatant was discarded, and the rest of the culture was transferred to the pellets again centrifuged at 7500 rpm for 10 min at 4ºC. The supernatant was discarded, and washed with 200 µL of sterile MQ water was added and mixed adequately by vortexing. This mixture was then centrifuged for 10 min at 4ºC at 7500 rpm, and the supernatant was discarded, followed by an additional washing step using sterile MQ as mentioned above. The tube was kept in a water bath at 100ºC for 10 min, and after that tube was kept on ice for 5 min, then centrifuged at 10,000 rpm for 5 min at 4ºC. The supernatant was transferred to a new fresh tand ube and used as the template for PCR or stored at -20ºC if not used immediately.

Amplification of pahs dioxygenase locus

The DNA sample was resuspended in sterilized distilled water to get the final concentration of 100mg/ µL. The primers for the amplification of the DNA were as follows: Forward primer-(GAG ATG CAT ACC ACG TKG GTT GGA); Reverse primer -(AGC TGT TGT TCG GGA AGA YWG TGC MGT T) (Cebron et al. 2008). 10X buffer contained 50Mm KC_2L, 1.5 Mm MgCl2, 20 Mm Tris-Cl (pH 8) and gelatine. The dNTP mixture was prepared with 2.5 Mm each of dATP, dTTP, dGTP, and dCTP to make a final concentration of 100 µL and kept at -20ºC. Taq DNA polymerase was stored buffer containing 50% glycerol. It was available at a concentration of 3 µL from the manufacturer.

Methodology

Genomic DNA was amplified in sterile PCR tubes with reaction volume of 25 µL containing the following components-10X assay buffer-2.5 µL, dNTP -0.5 µL, MgCl2 -1.5 µL, Forward primer -0.5 µL, Reverse primer-0.5 µL, Template -4 µL, Taq DNA polymerase-1 µL, autoclaved MQ water was added to make up the volume to 25 µL. The amplified PCR products were then stored at -20ºC for further use. Table 1 shows the PCR conditions.

Table 1. PCR conditions

		Temperature	Time
	Initial denaturation	95ºC	5 minutes
30 cycles	Denaturation	95ºC	30 seconds
	Annealing	57ºC	30 seconds
	Extension	72ºC	30 seconds
	Final extension	72ºC	7 minute

RESULTS AND DISCUSSION

Isolation and screening

The growth of isolates on Sea water nutrient is shown in Table 2. Twef inty-one isolated colonies were observed with distinct colony morphologies. These isolates were further screened for their potential to degrade PAHs. Preliminary screening was done merely by continuously monitoring the growth of strains on BMM-PAH agar plates (Table 3) and BMM-PAH broth for seven days (Figure 1). After seven days, among 21 isolates, continuous growth was observed in 5 strains (MP-1, MP-4, MP-9, MP-14, MP-18) and OD readings were found to be more than 0.5 at 595 nm.

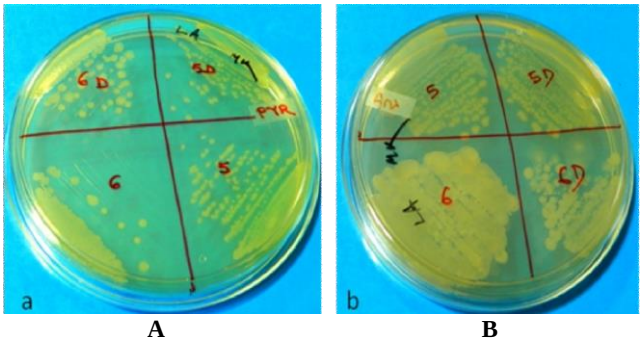
Carbon source utilization

To monitor the growth, OD_{595} was taken at a regular time interval, i.e., 0 days, one day, three days, five days and seven days by ELISA plate reader (Figure 2). The growth pattern of isolates in term of OD_{595} in different carbon source is presented in Table 4-5.

Table 2. Growth of isolates on Sea water nutrient agar plates.

Isolates	Growth on sea water nutrient agar plates
MP-1	+++
MP-2	+
MP-3	±
MP-4	+++
MP-5	–
MP-6	++
MP-7	–
MP-8	±
MP-9	+++
MP-10	++
MP-11	+
MP-12	–
MP-13	–
MP-14	+++
MP-15	++
MP-16	–
MP-17	+
MP-18	+++
MP-19	+
MP-20	++
MP-21	±

Note: +++ : excellent, ++ : good, + : satisfactory, ± : variable, -: negative



A **B**

Figure 1. Isolated colonies on basal minimal media. A. pyrene, B. anthracene

Figure 2. 96 well plate used to monitor the growth

Table 3. Growth of isolates on BMM-PAH agar plates

Isolates	Pyrene	Anthracene
MP-1	+	+
MP-4	+	+
MP-9	+	+
MP-14	+	+
MP-18	+	+

Table 4. OD_{595} for pyrene

Isolates	0 day	1 day	3 days	5 days	7 days
MP-1	0.011	0.104	0.229	0.339	0.5
MP-4	0.02	0.2	0.389	0.415	0.499
MP-9	0.022	0.197	0.303	0.415	0.495
MP-14	0.01	0.221	0.287	0.346	0.4
MP-18	0.012	0.2	0.299	0.395	0.487

Table 5 .OD_{595} for anthracene

Isolates	0 day	1 day	3 days	5 days	7 days
MP-1	0.03	0.24	0.47	0.64	0.82
MP-4	0.024	0.2	0.46	0.58	0.67
MP-9	0.021	0.34	0.5	0.66	0.84
MP-14	0.03	0.2	0.53	0.66	0.86
MP-18	0.03	0.29	0.46	0.65	0.79

Table 6. Standard curve of pyrene and anthracene at different concentration

Concentration	Pyrene (A_{335})	Anthracene (A_{254})
0.5 µg	0.0001	0.0001
1 µg	0.0004	0.0002
10 µg	0.064	0.0741
50 µg	0.306	0.323
100 µg	0.586	0.592

Standard curve of pyrene and anthracene

For anthracene and pyrene, λ was found to be 254nm and 335nm respectively, and the absorbance at this λ was considered to prepare the standard curve. λ_{max} and a standard curve of pyrene and anthracene are shown in Figure 3-4. Table 6 shows the absorbance value for different concentration.

Extraction and quantification

The results of degradation studies of these isolates were illustrated under quantification and extraction method. Before quantification and extraction, the prepared stock at different concentration was scanned within 200 to 400 nm in UV-Visible Spectrophotometer. pyrene shows its peak at 335 nm (Figure 3), and anthracene shows its peaks at 254 nm (Figure 4). Quantification and extraction were done using Dichloro-methane, then it was resuspended with 1mg/mL of hexane. After extraction OD was taken at 334nm for pyrene and 252 nm for anthracene for each sample (MP-1, MP-4, MP-9, MP-14, and MP-18). The results of degradation studies of these isolates in pyrene and anthracene were shown in (Table 7-8). The remaining concentration of pyrene and anthracene was calculated from the standard graph of the compound.

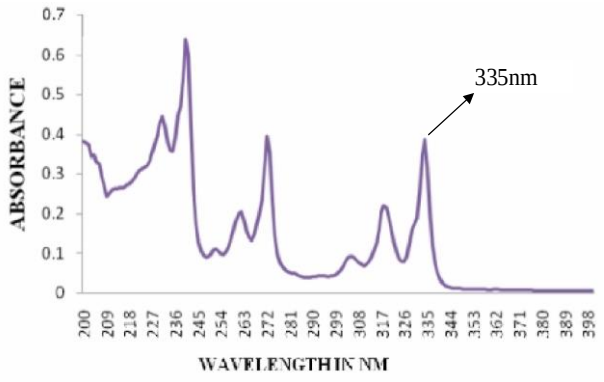


Figure 3. γ_{max} for pyrene (335nm)

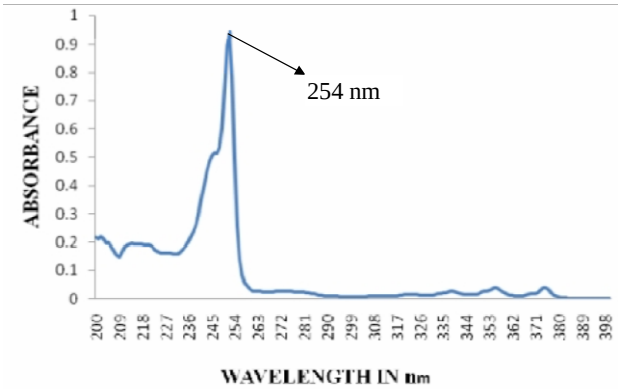


Figure 4. γ_{max} for anthracene (254nm)

Table 7. Percentage degradation of pyrene after 7 days.

Isolates	Initial Conc. (I) mg/L	Conc. (C) mg/L after 7 days	%Degradation =C/I*100
Control	100	0.997	0.997
MP-1	100	41.5366	58.4633
MP-4	100	57.8139	42.1860
MP-9	100	70.8106	29.1893
MP-14	100	68.2300	31.7691
MP-18	100	68.1499	31.8500

Characterization of isolates

All the five strains (MP-1, MP-4, MP-9, MP-14, MP-18) were found to be Gram-negative (Figure 5), motile and show positive Citrate Utilization test. The results of the characterization of these isolates were illustrated in Table 9-10.

Carbohydrate utilization

Sugar fermentation test was used to detect bacteria's ability to ferment sugar and produce gas and acid end product. The result of the carbohydrate utilization pattern was given in Table 11. Most of the strains varied in the pattern of utilization of 34 different sugars. Most of the isolates varied in the pattern of utilization of 34 different sugars.

Table 8. Percentage degradation of anthracene after 7 days

Isolates	Initial Conc. (I) mg/L	Conc. (C) mg/L after 7 days	%Degradation =C/I*100
Control	100	0.994	0.994
MP-1	100	43.6358	56.3641
MP-4	100	53.9531	46.0468
MP-9	100	55.3651	44.6348
MP-14	100	69.5418	30.4581
MP-18	100	51.7863	48.2136

Table 9. Characterization of isolates

Characteristic	MP-1	MP-4	MP-9	MP-14	MP-18
Gram stain	-	-	-	-	-
Colour of colonies	Cream	White	White	Cream	White
Motility	+	+	+	+	+
Citrate utilization test	+	+	+	+	+

Note: +: Positive, -: Negative

Table 10. Swimming and Swarming Motility Tests

Motility test	MP-1	MP-4	MP-9	MP-14	MP-18
Swimming(0.2%)	+	+	+	+	+
Swarming(0.5%)	+	+	+	+	+

Note: +: Positive

Table 11. Sugar Fermentation Tests

Sugars	MP-1	MP-4	MP-9	MP-14	MP-18
Lactose	-	-	-	-	-
Xylose	-	-	-	-	-
Maltose	-	-	-	-	-
Fructose	-	-	-	-	-
Dextrose	-	-	-	-	-
Galactose	-	-	-	-	-
Raffinose	-	-	-	-	-
Trehalose	-	-	-	-	-
Melibiose	-	-	-	-	-
Sucrose	-	-	-	-	-
L-Arabinose	-	-	-	-	-
Mannose	-	-	-	-	-
Inulin	-	-	-	-	-
Glycerol	-	-	-	-	-
Sodium gluconate	-	+	-	-	-
Salicin	-	-	-	-	-
Dulcitol	-	-	+	-	-
Inositol	-	-	-	-	-
Sorbitol	-	-	-	-	-
Mannitol	-	-	-	-	-
Adonitol	-	+	-	-	-
Arabitol	-	-	-	-	-
Erythritol	-	-	-	-	+
α-Methyl-D-glucoside	-	+	-	-	-
Rhamnose	-	-	-	+	-
Cellobiose	-	-	-	-	-
Melezitose	-	-	+	-	-
α-Methyl-D-Mannoside	-	-	-	-	-
Xylitol	-	-	-	-	-
ONPG	-	-	-	-	-
Esculin hydrolysis	+	+	+	+	+
D-arabinose	-	-	-	-	-
Malonate utilization	+	+	+	+	+
Sorbose	-	-	-	-	-

Note: +: Positive, -: Negative

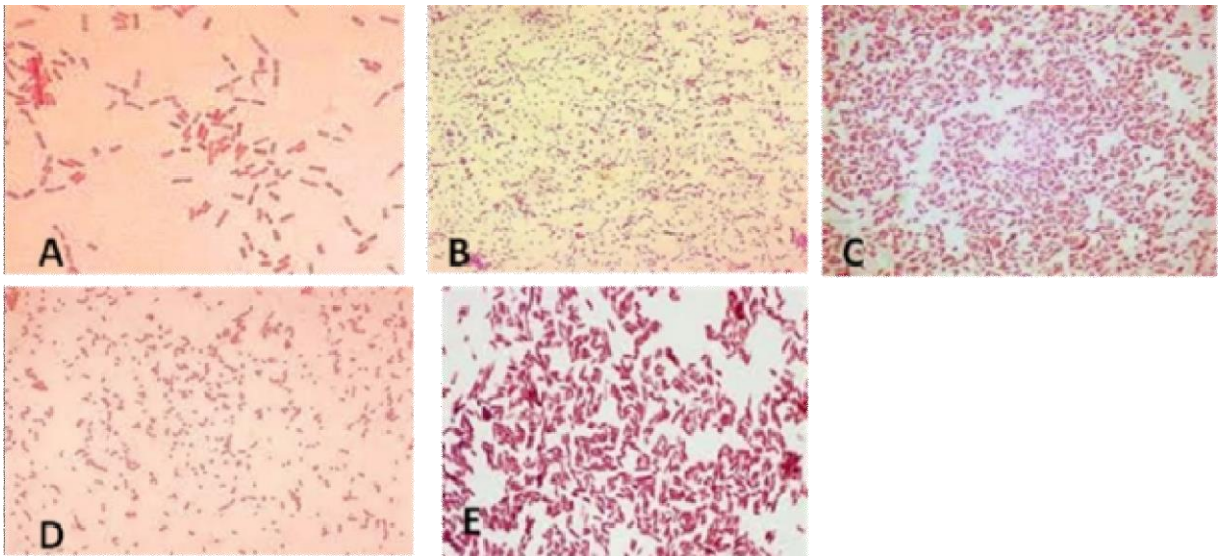


Figure 5. Gram Staining of five Isolates. A. MP-1, B. MP-4, C. MP-9, D. MP-14, E. MP-19

Antibiogram

All the five isolates were examined for antimicrobial resistance by the method of Bauer et al. 1966 with antibiotic-impregnated discs (Hi-Media). The strains were characterized as resistant or susceptible resistant based on the diameter of the inhibition zones around the disc (Fig .6). Gen10, C 50, E 15, S 10, AZM 30, T 30 and K 30 were sensitive to all five isolates but AM 30, and AC 10 is resistant in MP-1 and MP-14, VA 30 is resistant in MP-1, MP-4 and MP-9, NX 10 is resistant in MP-1 and all 4 isolates were sensitive, MET 5 are resistant in all the five isolates (Table 12 and 13).

Amplification of pahs dioxygenase locus

Polymerase chain reaction (PCR) amplification using PAH-specific primers revealed the presence of a dioxygenase gene in MP-1 (Figure 7). The size of the product was found to be 300 bp.

Discussion

Our study focused on the isolation and screening of potential pyrene and anthracene degrading bacteria from the marine source. Marine water and sediments receive waste from all terrestrial, atmospheric and freshwater source and being polluted by a variety of organic and inorganic pollutants. A total of 21 bacteria were isolated from the enriched contaminated water samples, mainly by the formation of inhibition zones on BMM with sprayed pyrene and anthracene as the sole carbon source. Five PAH-degrading bacteria from were further analyzed phenotypically for their ability to degrade PAH and whether they contain dioxygenase locus or gene which plays a vital role in the degradation of PAH. The analysis was designed to screen PAH-degrading isolates as potential sources for degradation of PAH, i.e., pyrene and anthracene.

After selective enrichment of PAHs degrading bacteria, five isolates named as MP-1, MP-4, MP-9, MP-14, MP-19 were studied for their degradation potential. In 7 days of observation, MP-1, MP-4, MP-9, MP-14 and MP-19, found to degrade 58.4%, 42.1%, 29.1%, 31.7%, and 31.8% of pyrene and 56.3%, 46%, 44.6%, 30.4% and 48.2% of anthracene respectively. The results obtained with the above test demonstrated that these particular isolates degrade more potentially pyrene than anthracene.

All isolates proceeded to PAH spray plate method, degradation, quantification. Physical and biochemical characterization was done through Gram staining, citrate utilization test, sugar utilization test, swimming and swarming motility test, antibiotic sensitivity test. All the isolates show different morphological aspects regarding Gram staining, the color of colonies, motility and citrate utilization. Among all the strains only MP-1 is Gram-positive and rest were Gram-negative. This particular isolate is highly motile. All the above strains are showing a positive result in Swimming and Swarming motility test.

Table 12. Zone (mm) of inhibition of antibiotics against five isolates.

Isolates	GEN	AM	AZM	C50	E 15	VA	NX	S	MET	T	K	AC
MP-1	16	0	18	17	19	0	0	12	0	28	19	0
MP-4	29	9	29	26	28	0	24	17	0	19	24	13
MP-9	28	9	3	19	24	0	25	16	0	19	24	13
MP-14	23	0	3	21	22	9	32	20	0	22	19	0
MP-18	21	19	24	19	15	16	16	19	0	24	14	22

Note: Gen 10-Gentamicin, Amp 30-Amphicillin, Azi 30-Azithromycin, Chl 50-Chloramphenicol, Ery 15-Erythromycin, Van 30-Vancomycin, Nor 10-Norfloxacin, Str 10-Streptomycin, Met 5-Methicillin, Tet 30-Tetracyclin, Kan 30-Kanamycin, Ac 10-Ac

Table 13. Antibiotic resistance pattern

Strain No.	Strain name	Antibiotic resistance pattern
1	MP-1	AC^R,MET^R,NX^R,VA^R,AM^R
2	MP-4	MET^R,VA^R
3	MP-9	MET^R,VA^R
4	MP-14	AM^R,MET^R,AC^R
5	MP-18	MET^R

Note: R: Resistant

Figure 6. Inhibition of bacterial growth on MHA by antibiotic discs in disc diffusion technique

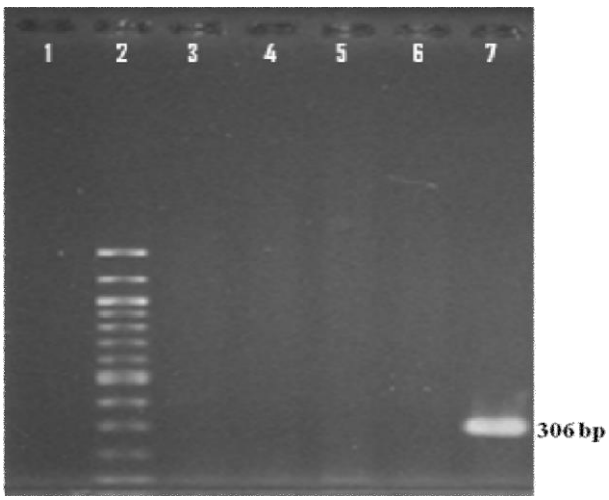


Figure 7. Gel Photograph showing amplification of *dioxygenase* gene. Lane 1: -ve Control, Lane 2: 100bp ladder, Lane 3-7: MP-4, MP-9, MP-14, MP-18 and MP-1

All the isolates show a negative result in the carbohydrate utilization test. They were subjected to the carbon source utilization process; degradation and quantification which displayed different types of effects, i.e., all strains were differentially potentially to degrade polycyclic Aromatic Hydrocarbons. The standard curve of the certain PAH, i.e., pyrene and anthracene show maximum absorbance at 335 and 254nm respectively. The results from degradation and quantification study suggested strong evidence about the percentage of degradation of isolates in PAHs. Among all the strains MP-1 is most potential, showing 58.4% degradation of pyrene and 56.3% degradation of anthracene. In the previous report, the degradation rate of anthracene was studied in four strains (*Escherichia coli*, a soil bacterium, *Alcaligenes* sp. and *Thiobacter subterraneus*). The mean degradation of anthracene demonstrated by these four bacterial strains was found to be 28.57%, 30.19%, 26.58% and 32.11% (Kampfer and Dott, 1988). Both *Pseudomonas* and *Alcaligenes sp.* are the most common bacteria occurring at polluted sites due to enhanced selection by a high concentration of organic xenobiotics (Abd-Elsalam et al. 2006).

The result of the present study confirmed that many of bacterial strains, especially Gram-negative bacteria were found capable of degrading PAH compound at various extents (Kiyohara et al. 1982), thus, indicates that most efficient of the PAH-degrading bacteria belong to the genus *Pseudomonas.* The previous report described that the pyrene induced pdoA1 and pdoB1 genes from *Mycobacterium sp*. is closely homolog to the nidA and nidB genes (Krivobok et al. 2003). These genes encode the terminal oxygenase component of the initial aromatic ring dioxygenase, nidA and nidB, whose products catalyze the conversion of pyrene to 4,5-dihydroxy-4,5-dihydropyrene, have been cloned and sequenced from *Mycobacterium vanbaalenii* (Khan et al. 2001). These results strongly suggest that not only the dioxygenase gene but also nidA and nidB genes are widely distributed among pyrene utilizing *Mycobacteria* which play an essential role in the degradation of PAH.

The result of the antibiotic sensitivity test revealed that all isolates were resistant towards methicillin. However, these strains exhibited different inhibition zone pattern towards different antibiotics. The PAH compound used for the studies were pyrene and anthracene, the metabolic pathway of which is essential for degradation study. These two compounds show different degradation pathways by forming different intermediates.

PCR amplification with PAH-specific primers identified the presence of a dioxygenase gene in MP-1 at 306bp of the product size306bp. There are many specific PCR primers designed directly on the nucleotide sequence, with either no or low degeneracy, with specific target for each type of PAH-dioxygenase genes (Laurie and Lloyd 1999; Lloyd Jones et al. 1999; Wilson et al. 1999; Ferrero et al. 2002;Widada et al. 2002; Baldwin et al. 2003; Brezna et al. 2003; Dionisi et al. 2004; Johnsen et al. 2006). Accordingly, there has been numerous PAH-degrading bacteria isolated from water sediments, to quantify the percent of degradation in the presence of the dioxygenase gene, followed by a trial before use.

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