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Submission date: 13-Mar-2023 02:22PM (UTC+0700)
Submission ID: 2035998171
File name: 26914-76992-1-SM.docx (997.56K)
Word count: 5066
Character count: 27967

Ectoparasite Prevalences of Cantik Grouper (*E. fuscogutatus x E. polyphekadion*) Cultured in The Floating Net Cages

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Abstract. Monitoring the health of the reared fish in a floating net cage (FNC) is often conducted by examining their ectoparasites. This study therefore aims to determine the ectoparasite prevalences of cantik grouper (*Epinephelus fuscogutatus x E. polyphekadion*). It was conducted in the waters surrounding Pongok Island, South Bangka Regency on the geographical location at 02°53'00" S and 107°01'00" E. The health examination of both fish and ectoparasites was carried out by applying simple random sampling method on three periods, November 2016, February 2017 and May 2017. However, the hydro-oceanography surveys were taken every month during the three years. The grouper checkings (I, II and III) indicated the prevalences of ectoparasites in these periods were 43.3%, 8.8% and 13.5% respectively. Fish death highly occurred in the period I, and the grouper survival rate at harvesting time was only 70% from initial seed stocking of 1,500 fish. Besides, the ectoparasite intensities during the research periods were 1.6, 1.6 and 1.5 respectively. This condition implies that every 10 groupers in the floating cage would potentially be attacked by at least 15-16 ectoparasites. The parasite existence is very harmful for the fish rearing using FNC system. Furthermore, a slow sea water current flow triggered the quick uplifting of the ectoparasites from the seafloor. Moreover, a low temperature prompted energy use in the poikilothermal of aquatic biota and thus they easily got hungry and started looking for food, including the ectoparasites of the host biota. The water condition at the FNC location in 2011 and 2017 was still under the quality standard, indicating that the water body is still able to accommodate chemical substances such as Ammonia, Nitrate, Ortho Phosphate, Nitrite, and Lead. The ectoparasites were supposedly coming from the surrounding environment of the FNC location and were not carried away from the hatchery. It is inevitable that the fish culture management should focus on cleansing the ectoparasites attaching on the groupers, dusting the net cage clean, and arranging the harvest pattern on a particular month following the surrounding environmental condition to prevent ectoparasite attacks.

Keywords: culture, ectoparasite, floating net cage, grouper, prevalence

Introduction. The current issues regarding mariculture in the floating net cage (FNC) are related to the aspects of location preparedness, production of fish and environment. A proper area of rearing groupers using the FNC system in the research location, the waters of Pongok Island, covers 3,474.66 Ha (Adibrata *et al* 2013) while the existing location for running the FNC system is only ±1 Ha until the beginning of 2019. This situation presents a business opportunity of rearing fish in small islands. Additionally, the fish production aspect linked to fish culture trend aims to improve the food security and incomes of fishermen, to ensure natural fish stock sustainably, and to preserve the

biodiversity by focusing on the nutrition, survival rate, reproduction and mortality of fish (Dias *et al* 2012). The environment aspect which is highly correlated to the water quality reduction has the reciprocal relationship with cultured fish health, food stock and disease or fish parasite. Degradating quality of environment becomes one of the obstacle factors in guaranteeing the sustainability of cultured fish production (Karakassis 2001). The impacts of environmental factors for mariculture are highly varied depending on species, rearing method, stock abundance, types of food, oceanographic condition and practices of culture system (Wu 1995). The dynamics of hydro-oceanography is a given environmental factor; however, other factors can be designed to boost the fish culture business. Evaluating the obstacle factors through monitoring the water quality and fish health status is able to decrease the culture problems in order to devise the best strategy based on the environmental condition in the research location.

Monitoring is undertaken to acquire related information about the fish health status and the eligibility of the environmental condition in protecting and remediating the culture environment (Syakti *et al* 2012). The cultured fish health could be identifed through an agility of movement, no injuring physically, proportional in size and no attacking by disease or parasites. The developed fish culture business using the floating cage system, for instance, some domesticated grouper species is not spared of attacking from parasites. The simplest monitoring on cultured fish is thoroughly checking the ectoparasites directly. The main entrances of this biological contaminating agent are by the environment, culture facility (Humphrey and Langdon 1985; Brown 1993) and fish seed introduction from different hatcheries (Brett *et al* 2005). The management of the rearing fish health and its environment is very crusial to boost the cultured fish production sustainably (Novriadi *et al* 2014).

Some cultured groupers which are able to be produced in hatcheries are such as humpback grouper (*Cromileptes altivelis*), tiger grouper (*Ephinephelus fuscoguttatus*), cantik grouper (*E. fuscoguttatus* x *E. polyphekadion*) and cantang grouper (*Ephinephelus sp,* a hybrid from camouglage grouper and giant grouper). Nevertheless, the mentioned groupers above are still possibly attacked by parasites particularly attaching on their skin and gills. The problems regarding ectoparasites in rearing groupers using FNC system can lower the fish production. The grouper ectoparasites originating from Family Cymothoidae (GBIF Secr 2017) are *Cymothoa rhina* which is well-recognized in Philippines, Guam, Singapore hosting on Lutjanidae (Bruce *et al* 2016), *Ceratothoa imbricata* which is well-known in Australia, New Zealand, Indonesia (Hadfield *et al* 2014) and *Rhexanella sp* which is well-identified in Indonesia and Thailand (Nagasawa and Cruz-Lacierda 2004). The ectoparasites commonly attach on the outer body part or gill of the fish that then inhibit their growth and even until the

death level. This research aims to determine the ectoparasite prevalences on cultured cantik grouper (*E. fuscogutatus x E. polyphekadion*).

Materials and Methods. This research was conducted in the waters surrounding Pongok Island at South Bangka Regency. It was geographically situated on 02°53'00"S and 107°01'00"E (Figure 1). Moreover, it was realized to find out the groupers attacked by ectoparasites in three different periods, namely on November 2016, February 2017 and May 2017. Furthermore, the hydro-oceanography surveys were carried out from November 2016 until October 2017.

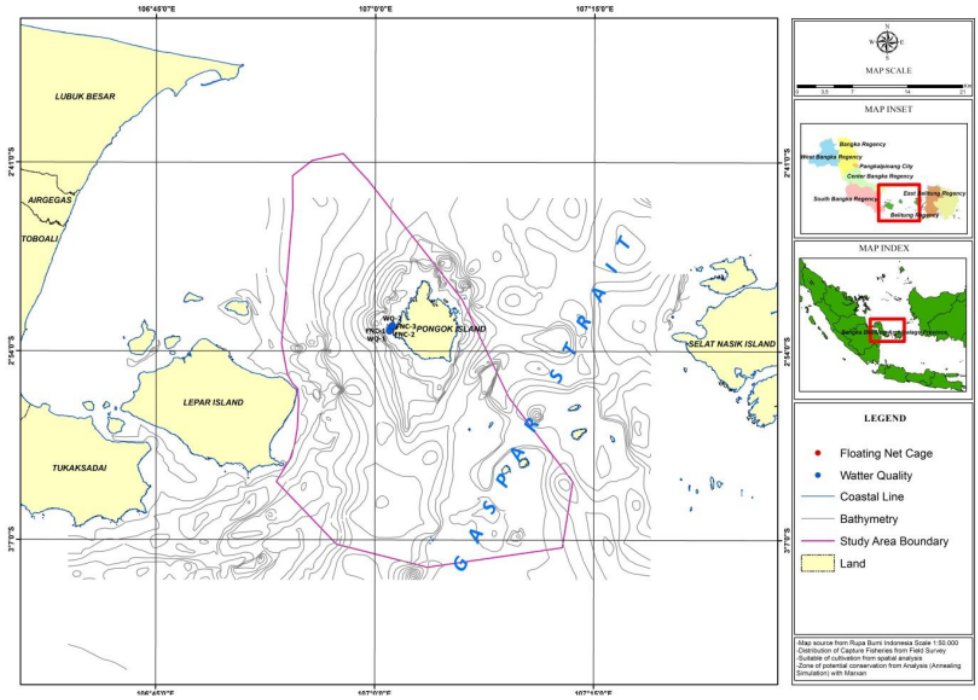


Figure 1. Location of Fish FNC

The fish used in this research were the cantik grouper (*E. fuscogutatus x E. polyphekadion)* in the floating net cage system. Inventorying fish samples surmisedly attacked by ectoparasites utilized simple random sampling method where these groupers were only in one stretch of FNC (Fig. 1), while the hydro-oceanography survey was taken *insitu* as well as the water quality parameter was analyzed in the Proling Laboratory of IPB University. Additional information was collected by interviews and literature study. Tools used to collect the ectoparasites of the grouper were a plastic tank, fresh water, hand gloves, syringe, drugs, handline hook, a set of the stationary, ruler and digital camera. Besides, to measure hydro-oceanographical and water quality

some tools were also utilized, namely current meter, DO-meter, hand refractor-salinometer, secchi disk, a boat with the capacity of ±5 GT equipped with GPS map 585 Garmin (defining time, coordinate, temperature and depth of water) and sampling bottles to store the water containing ammonia, nitrate, ortho phosphate, nitrite and leads.

The samples collected in this research followed a formula as follows (Krejcie dan Morgan 1970):

$$n = \frac{\chi^2.N.P(1-P)}{(N-1).d^2 + \chi^2.P(1-P)}$$

where n is a measure of the sample, N is a number of population, χ^2 is a value of chi-square, P is a proportion of the population and d is an estimated error.

The analysis for ectoparasites used a following formula.

Prevalence (%) = Number of nested fish sample / Number of checked sampling fish x 100%

Intensity = Number of ectoparasite A that infects / Number of the sampled fish attacked by ectoparasite A

If there were more than one species of ectoparasites, than the calculation of dominance was proceeded.

Dominance(%) = Number of ectoparasite species infecting sampled fish / Total ectoparasite infecting the sampling fish x 100%

Results and Discussion. The population of groupers in this research was previously counted before the sampling was taken by interviewing the owner of the floating net cage each time of fish checking on the three different times of observation, namely on November 2016 (Checking I), February 2017 (Checking II) and May 2017 (Checking III). According to the information obtained from the interview of the fish culture owners, the fish stock of groupers on the first period of checking (July 2016) was only relied on supplying coming from collectors in Belitung Regency. Seedlings of this grouper were not always available every time, but adjusting on the existing stock in this regency which they originated from either Bali or East Java. The size of seedlings of groupers to be stocked for rearing in the FNC was ±10 cm of length. The first period of checking (Checking I), the total population of remain fish seedlings was 1,220 individuals (divided into four FNC) due to the death of 280 individuals in which the initial total stock of seedlings was 1,500 individuals. According to the calculation, the values of χ^2, level of confidence, degree of freedom, p-value and error are 3.841, 95%, 0.05, 1, 0.5, 0.05 or 5% respectively. The ectoparasite attaching on both skin and gills of the grouper was identified only one species. The results of fish checkings are shown in Table 1 and Figure 2. Geographically, the FNC location are situated in one area surrounding Pongok Island on the coordinates of 02° 52' 36.4"S, 107° 00' 58.8"E; 02° 52' 33.2"S, 107° 01'

04.4"E; and 02° 52' 15.7"S, 107° 01' 10.7"E. The analysis results of acquired data are presented in Figure 3.

Table 1. The cantik grouper and ectoparasite

No	Annotation	Innitial and final population	Fish Checking		
			I	II	III
1	Stocking the cantik grouper seeds on July 2016	1,500			
2	Letal		280	55	85
3	Number of populations		1,220	1,165	1,080
4	Population/FNC		305	292	270
5	Samples		684	668	668
6	Attacked fish by ectoparasite		296	59	90
7	Total ectoparasites		472	97	131
8	Average prevalence (%)		43.3	8.8	13.5
9	Average intensity		1.6	1.6	1.5
10	Harvesting the cantik grouper on 2017	±70%			

Source: Data processed, 2017

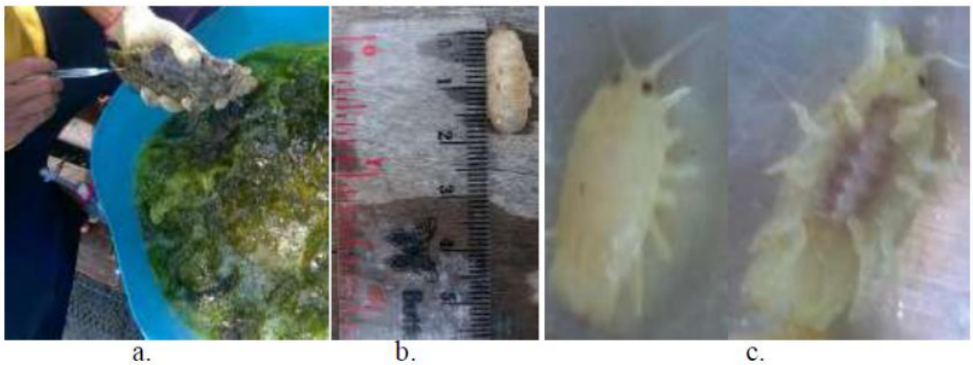
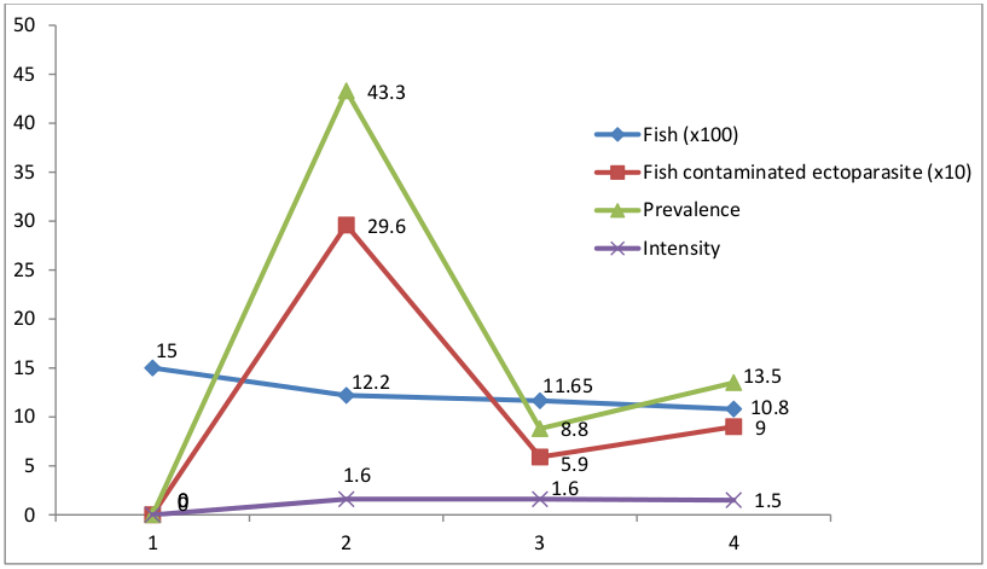


Figure 2. The cantik grouper and its ectoparasite

Annotation:

a. Cantik grouper *(E. fuscogutatus x E. polyphekadion)*;
b. Ectoparasite in a real size;
c. Ectoparasite on dorsal and ventral looks.



Source: Data processed, 2017

Figure 3. Number of fish and its ectoparasites

The hydro-oceanographic condition in the research location surrounding Pongok Island is presented in Table 2. The depth of the existing FNC is about 10 meters located between Pongok Island and Celangen Island. The total area of these FNCs is approximately one hectare dividing into three blocks and situated in one location.

Table 2. The hydro-oceanographic condition of waters surrounding Pongok Island

No	Month	Blowing Wind	Seawater current condition*)	Seawater Temperature
1	Jul 2016	Southeast (transition)	Two-way (switchover)	28.4
2	Aug 2016	Southeast	Low	28.1
3	Sep 2016	Southeast	Low	28.8
4	Oct 2016	Southwest (transition)	Normal	28.7
5	Nov 2016	Southwest	Normal	29.3
6	Dec 2016	Southwest	Strong	28.7
7	Jan 2017	West	Strong	28.1
8	Feb 2017	West	Strong	28.0
9	Mar 2017	Northwest	Normal	28.1
10	Apr 2017	North	Normal	28.7
11	May 2017	North	Low	29.4
12	Jun 2017	North (transition)	Two-way (switchover)	29.2

Source: Data gathered from survey, interviews and analysis, 2017

Note: *) speed of wind blowing: low (<0.25 m/s), normal (0.25-0.3 m/s), strong (>0.3 m/s)

The condition of seawater quality parameters in the floating net cage consisting of ammonium, nitrate, ortho phosphate, nitrate, and lead, is listed in Table 3 as follows.

Table 3. The condition of seawater quality parameters in the FNC

No	Parameter	During high tide (mg/l)	During low tide (mg/l)	BM*)	Method
	Nov 23, 2017*)				
1	Ammonia (NH3-N)	0.144	0.155	0.3	APHA
2	Nitrate (NO3-N)	0.089	0.150	-	APHA
3	Ortho Phosphate (PO4-P)	0.004	0.005	0.015	APHA
4	Nitrite (NO2-N)	0.020	0.067	0.5	APHA
5	Lead (Pb)	0.024	0.027	0.008	APHA
	Apr16, 2011*)				
6	Ammonia (NH3-N)	0.081	0.058 – 0,132	0.3	APHA
7	Nitrate (NO3-N)	0.031	0.011 – 0.012	0.008	APHA
8	Ortho Phospate(PO4-P)	<0.005	<0.005	-	APHA
9	Nitrite (NO2-N)	<0.002	<0.002	-	APHA
10	Lead (Pb)	0.028	0.019 – 0.031	0.05	APHA

Source: Data from survey and analysis, 2017; Adibrata *et al* 2013
Note:
*) : The water quality standard was based on the Ministerial Decree of Environment Number 51 of 2004 Appendix III (for Marine Biotas). The coordinate location of FNC on November 23, 2017 was 02° 52' 36,4" S 107° 00' 58,8" E; and on April 16, 2011 was 02° 52' 15,7" S 107° 01' 10,7" E

The district of Pongok Islands possesses an area of 89.67 km^2 (BPS of South Bangka Regency, 2017). The use of Indonesian small islands is intended to prioritize the purpose of conservation, education and training, research and development, marine cultivation, tourism, marine and fish industry, and sustainable fishery industry, organic agriculture, stock raising and/or state defence and security (Constitution Number 27 of 2007 jo Constitution Number 1 of 2014). This district is determined as one of the central areas for catch and culture fishery in South Bangka Regency (Local Government Regulation of South Bangka Regency Number 6 of 2014) that needs support from all stakeholders in order that the management of these small islands could be optimized and raising the enterpreneurs in the small scale fisheries.

A prevalence is a number of diseases or injury cases presenting in a certain population during a point of time (Kent 2016). In this research, the prevalence was calculated by comparing the attacked fish of ectoparasites and the total checked fish of sampling where these ectoparasites attaching on both skin and gills. In addition, the failure of culturing fish utilizing the FNC in the waters surrounding Pongok Island was usually related to several factors, namely parasites, fish tired during a transportation process, unpredictable pollutants, virus detected when the fish have grown up, cannibalism if lack of feed or difference in size, weather and extreme environmental factor. For the fishermen of the FNC system, a routine checking of the cultured groupers aimed to discharge the ectoparasites, to provide vitamin injections, and to short the fish

(Figure 2). The ectoparasites of the groupers identified in this research are originating from Family Cymothoidae (GBIF Secr 2017) with their classification as follows.

Kingdom: Animalia
Phylum: Arthropoda
Class: Malacostraca
Ordo: Isopoda
Family: Cymothoidae
Genus: Cymothoa
Species: *Cymothoarhina* (Schioedte & Meinert, 1884);
Ceratothoa imbricata (Fabricius, 1775)
Genus: Rhexanella
Species: *Rhexanella sp* (Stebbing, 1911).

Based on Table 1, the total seedling stock of groupers in all FNC was 1,500 individuals with each size of ±10 cm/fish in length. The feed in form of drowned pellets were routinely given for two months, and then the cutting fish in pieces and cleaning from spines were administered for the transition period, and eventually the trash fish were being these groupers' feed. At the fifth month, the reared fish were ready to be checked for attacking parasites by simply checking their skin and gills. When an ectoparasite was found attaching, it was repealed and killed using hands. The attacked fish were then isolated in a tank containing fresh water which had been mixed with a cefotaxime medicine. Another way which has been successfully recognized in controlling the parasites of Goliath grouper (*Epinephelusitajara*) is by bathing the fish using fresh water over five minutes (Silva *et al* 2014). The attacked fish were injected prior before it was released back into the FNC in order to be getting healthy again, no limping, and its appetite increased. Only remained 1,220 groupers in total four FNCs after five months of rearing which were about 305 individuals in each FNC.

The sampled groupers indicate that there was about 43.3% of the fish (Checking I) in November 2016 (Figure 3). It means that there are 43 groupers were attacked by the ectoparasites in a 100 individuals of population. This prevalence of ectoparasite is harmly impacted for the fish culture which becomes one of the fish death sources. It was depicted during five months of rearing. There were 280 grouper seedlings death with wounds on skin and gills as well as the injured fish were difficult to eat. Further, the prevalence decreased in the next checking period (Checking II) on February 2017 which was only 8.8% that was probably due to the three prior months, those fish had been cleaned from the ectoparasites. However, there were still a number of ectoparasites originating from remaining unchecked fish and coming up the sea bottom below the FNC which presumably function as the ectoparasite habitat and hideout. Due

to this attack, there were 55 grouper seedlings death during this period. This prevalence increased on the last checking (Checking III), namely becoming 13.5% which killed 85 fish. This is supposedly a number of ectoparasites coming from most unchecked fish before and/or swimming up from the seafloor surrounding environment due to an effect of hydro-oceanographic condition.

The grouper seedlings attacked by the ectoparasites in the FNC were physically seen moving unnormally (the fish moved sideways), weak, and their skin, fins, and gills were blistered, as well as they breathed on the seawater surface, or lean (possessing a big head and slim body). Furthermore, the parasite prevalence is varied on groupers, for all leopard coral grouper (*Plectropomusleopardus*) is 40% (Sauyai *et al* 2014), and the highest happened on common pandora fish (*Pagellus erythrinus*) with a size range of 16-18 cm in the coastal areas of El Kala, Aljazair in a summer (Gasmi *et al* 2017). The highest prevalence occured on cantang grouper (*Epinephelus fuscoguttatus-lanceolatus*) in eastern coast of Pangandaran, Indonesia, which is about 66.6% (ectoparasite: *Diplectanum*) and in the traditional FNC is approximately 44.4% (*Diplectanum*) (Rostika *et al* 2018). Moreover, the prevalence of ectoparasites also happened on fish with sizes in a range of 15-27.5 cm in the Mariculture Center of Lombok, West Nusa Tenggara, Indonesia, reaches 27.5% (ectoparasite: *Monogenea* with *Neobenedenia girellae* species with a size of 3.8-5.4 x 1.5-2.5 mm in length) attacking the surface of body or skin, eyes, dorsal and ventral fins (Dewi *et al* 2018). The fish attacked by parasite *Benedenia acanthopagri* is about 25.78% causing an increasing respiratory disorder on opercular and surface movement, gulping atmosphere air, propelling mouth open, bleeding on the base of fin, peeling off on tail, tail fin, and dorsal area; demonstrating different ulcers on body parts, emerging gill blockage (Hassan *et al* 2015). The results of this research inform an evidence that the prevalence of parasites in a range of 25.78-66.6% can disrupt enough the cultured fish in the floating net cages and eventually until to be a death level of the fish.

The intensities of ectoparasites during the three checking periods were 1.6, 1.6 and 1.5 respectively. This means that every 10 seedlings of groupers in the FNC is attacked by at least 15-16 ectoparasites. The existence of ectoparasites is very detrimental on fish cultured in the FNC system. Interviewing to the fishermen in the research location (fish raisers) implies that the ectoparasites are still there every year eventhough their emersion are fluctuated, sometimes in a large quantity and in other time is lower that attack the reared fish. The highest intensity of ectoparasites occurred on the Cantang grouper (*Epinephelus fuscoguttatus-lanceolatus*) in eastern coast of Pangandaran, Indonesia, which is about 2.9 (Ectoparasite: *Diplectanum*) and in the traditional FNC is about 2 individuals (Ectoparasite: *Diplectanum*) (Rostika *et al* 2018). The highest intensity of ectoparasite attack on Cantang grouper (*Epinephelus*

fuscoguttatus-lanceolatus) occurred in the Lombok Mariculture Center, West Nusa Tenggara – Indonesia, namely approximately 2.75 (Ectoparasite: *Monogenea* with species *Neobenedeni agirellae*) (Dewi *et al* 2018). This research points out that the intensity of ectoparasites during this research is lower than other locations in Indonesia, however, an intensity of 1.5 is enough to disturb the cultured grouper activity causing many cultured fish died.

Monitoring the hydro-oceanographic condition associated with the presence of ectoparasites is gained from the seawater temperature and seawater current condition. On November 2016, considerable ectoparasite prevalence appeared. This condition was started by a low seawater current flow (<0.25 m/s) occurring mainly on August and September 2016, and a low seawater temperature happened especially on August and October 2016 (Table 2). The low seawater current brought about moving up ectoparasites from the seafloor freely without any obstacles to attack the rearing fish. Conversely, a strong seawater current (>0.3 m/s) was able to inhibit the ectoparasites gliding for attaching on the fish. Furthermore, the cold seawater temperature can hasten the metabolism of biota body to equalize its body temperature and the surrounding environment. The aquatic biota body is going to burn quickly the energy which makes it easily get hungry and has to look for a food source. Due to this condition, the ectoparasites enable to look for a host of aquatic biota as their food source. The marine biotas are poikilotherm in where the water temperature is very influential (Singh *et al* 2013; Lall and Tibbetts 2009) on their bodies and escalating their metabolism. The temperature of the environment affects the energy-releasing and nutrition intaking. On a low temperature, fish efficiently utilize their protein and lipid for energy than the carbohydrate (Lall and Tibbetts 2009). On February 2017, there was low ectoparasite prevalence. This condition was initiated by a strong seawater current flow and a low seawater temperature on the previous month. It is presumed that the ectoparasites locating on the sea bottom are difficult to gliding up attaching on the fish in the FNC. Unfortunately, in May 2017, the ectoparasite prevalence increased. This condition emerged because of a normal and low seawater current flow and a low seawater temperature in March. It is assumed that the ectoparasites on the sea bottom come up more freely to attach on the groupers in the FNC.

The condition of seawater quality parameters in the FNC in both periods of 2011 and 2017 was under the quality standard based on the Ministerial Decree of Environment No. 51 of 2004 in Appendix III (for marine biotas) signifying that the water body is able to tolerate inputs of Ammonia, Nitrate, Ortho Phosphate, Nitrite and Lead. This is probably due to the existing condition of FNC is still a little small with a total size only ±1 Ha. Based on the water quality data, the chemical elements are still far below the threshold and do not disturb yet the FNC of culture activity in the surrounding

waters of Pongok Island. The water quality parameters as the mentioned elements above are not the factors inhibiting the growth and development of ectoparasites.

Based on the field survey, the ectoparasites are supposedly coming from the surrounding environment of the FNC and they do not host on seedlings originating from hatcheries. This information was attested by the ectoparasites attaching on the fishing baits during the fishermen and the researcher conducted a line fishing at nights in the waters nearby location of the FNC on condition of low seawater current and cold temperature. The baits were not eaten by fish but they were swarmed over by the ectoparasites which were identified similarly with species in the FNC. The schooling grouper in the FNC became the target of ectoparasites for candidate hosts. The more FNC contains groupers, the more ectoparasite will increase both attaching on culture fish and the seafloor beneath the cages. The management of fish culture was carried out by cleaning and revoking the ectoparasites attaching on the skin and gills of the reared groupers, cleansing the net cages, and setting the pattern of stocking and harvesting time of the fish on particular months and following the environmental condition as the prevention to the ectoparasites appearing. These techniques are believed to inhibit the ectoparasites which are potentially harming the cultured groupers in the FNC.

Conclusions. The checking of the cantik grouper in three different periods demonstrates the ectoparasites prevalences are 43.3%, 8.8% and 13.5% respectively. This signifies that the highest death of the cultured fish occurs during on checking I and the survival rate of the cultured fish in a year in the FNC is 70% of total initial stocking seedlings of groupers, namely 1,500 individuals. The intensities of ectoparasites in checking I, II and III are 1.6, 1.6 and 1.5, showing that every 10 groupers in the floating net cage is pottentially attacked by at least 15-16 ectoparasites. The existence of ectoparasites is harmful for culturing fish in the FNC system. The monitoring results of hydro-oceanographic condition point out that the ectoparasite prevalence occurs on two situations, namely during low seawater current flow and low temperature of the seawater. The low seawater current flow triggers the ectoparasites on the seafloor freely coming up to attack the groupers, and the cold seawater temperature is able to escalate the energy use of aquatic biota getting easily hungry. The aquatic biota is poikilothermic and going to be easily hungry in this condition so they have to look for food sources including the ectoparasites. The condition of seawater quality parameter in the location of FNC in both years 2016 and 2017 periods is under the quality standard based on the Ministerial Decree of the Environment No. 51 of 2004 in Appendix III (for marine biota). This exhibits that the water body is still able to tolerate the inputs of elements such as Ammonia, Nitrate, Ortho Phosphate, Nitrite and Lead. The ectoparasites are supposedly originating from the surrounding environment of the FNC and are not hosted on the

cultured fish coming from hatcheries. The management of cultured groupers is undertaken by cleaning and revoking the ectoparasites that are being attached on the skin and gills of the cantik grouper, cleansing the cage net, and adjusting the stocking and harvesting patterns on certain month following the surrounding water condition as the prevention to hamper the ectoparasites attacking the cultured groupers in the FNC.

Acknowledgments. Thanks to Mr. Anton as the fisherman of groupers in Pongok Island - South Bangka Regency who facilitated and supported the field data collection, as well as assisted during conducting this research.

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