

Crustaceans ectoparasite of *Trachinotus blochii* cultivation in hatcheries tub and floating net cages

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ABSTRACT

The aquaculture activity of *Trachinotus blochii* has a high economic value. One of the problems that is commonly found in fish cultivation is ectoparasite crustaceans. It is considered ectoparasites because crustacean may cause bacteria and virus secondary infections. The purpose of this study is to identify the genus of ectoparasite crustaceans that infest *T. blochii* which are cultivated in hatcheries and floating net cages. Observation of *T. blochii* samples from cultivation in hatcheries and floating net cages on the body surface and gill examination using the qualitative method. Scrapping samples that have signs of being infected with ectoparasite crustaceans. Identification of ectoparasite crustaceans by a microscope at 100x and 400x magnifications. There are ectoparasite crustaceans found infecting *T. blochii*, the crustacean that belongs to genus *Canuella*. *Canuella* prevalence value is 3.3% in hatcheries tubs and 3.65% in floating net cages. There is one genus of ectoparasite crustaceans found, namely the genus *Canuella* which infects *T. blochii* that is cultivated in hatcheries tub and floating net cages.

Key words : Hatcheries tub, Ectoparasite crustacean, Floating net cages, *T. blochii*

Introduction

Fishing methods that are not environmental friendly may endanger the sustainability of *T. blochii* population, and cause environmental damage. Hence, a method of fish cultivation is required considering the high opportunities of domestic and export markets, including hatchery and growth (Dharma *et al.*, 2014). To date, cultivation of *T. blochii* can be done by means of a floating net cage system in the sea (Retnani and Abdulgani, 2013). The biggest obstacle faced during fish farming is the outgrowing disease, particularly the ones caused by fish parasites (Bunga, 2008). Although diseases caused by parasites are relatively lower when compared to diseases caused by bacteria and viruses. However, infections of parasites can cause secondary infections by bacteria and viruses that will ag-

gravate the condition of the fish and increase the occurrence of death (Handayani and Bambang, 1999).

Based on the location of the organ infected by the parasite, it can be divided into two different types, namely ectoparasites and endoparasites (Kabata, 1985). Ectoparasites are parasites that persist on the body surface of fish such as skin, fins, tail, and gills of fish, while endoparasites are parasites that infect the inside of the host's body (Indahsari *et al.*, 2019). For example in the digestive, circulatory, or other internal organs. In the farming of marine and brackish water fish, ectoparasite crustaceans may lead to another disease because it can cause fish to experience respiratory disorder or anoxia if it persists in the gills. In addition, ectoparasite crustaceans can cause bleeding on the surface of the body, which becomes port the entry for secondary infection by

bacteria or viruses, thus, reduce the market selling price (Misganaw and Getu, 2016).

Ectoparasite crustaceans that have been found to infest marine fish cultivated in western Indonesia are *Caligus* (Novriadi *et al.*, 2014), *Ergasilus* with a prevalence of 85.7%, *Caligusepidemicus* with prevalence reaching 4.3%, *Cymothoa* with prevalence reaching 14, 3% and *Lernanthropus* with prevalence reaching 88% and *Nerolicaindicus* with prevalence reaching 87.7% (Waluyo, 2014; Yuniar *et al.*, 2007). Based on the above problems, it is necessary to do research on crustacean ectoparasites in *T. blochii* as an effort to map, prevent and control the spread of parasites.

Materials and Methods

This research was conducted in March-April 2017 at the Microbiology Laboratory of the Faculty of Fisheries and Marine Airlangga University.

Tools and materials

The equipment used in this study includes necropsy devices, glass objects, glass covers, pipettes, cameras, Petri dishes, plastic fish packing, pipette drops, seser, Lucida cameras, and phase-contrast microscopes.

The research materials used included samples of star pomfret (*T. blochii*) obtained from hatcheries and floating net cages of the Marine Aquaculture Center, Lombok, Indonesia as the location for sampling, while the materials used for identifying ectoparasite crustaceans were 70% ethanol and Physiological NaCl.

Working procedures

Sampling was carried out at 3x3x3 m floating net cages with 400 stocking densities for parent size, 3x3x3 m floating net cages with 400 stocking densities for consumption size and concrete tubs with 10,000 stocking densities at seed size. The sample was chosen by means of the purposive sampling method. Purposive sampling is a technique of determining a sample with certain criteria which is based on its research objectives (Sangadji, 2010). The number of *T. blochii* samples taken at the location of floating and cage nets as many as 10 samples from each population consisting of *T. blochii* seeds measuring 2.5 cm long with 30 days maintenance, consumption with a weight size of 300-400 g/fish with a long maintenance of 6-8 months and a parent with a

weight of 0.5-2 kg/head with 1-2 years of maintenance. Sampling for fish health testing was carried out randomly as much as 1% of the population, with a maximum number of 10 samples for both visual and microscopic observations. Handling the sample is done as well and as quickly as possible so that the sample can survive until at the time of the sample inspection at the Fish Health and Environment Laboratory of the Marine Aquaculture Center, Lombok.

Examination of *T. blochii* samples was carried out by means of scrapping and examination of gills using the qualitative method (Hafidloh and Sari, 2019; Noga, 2010; Wijaya *et al.*, 2019). The skin from the head, the operculum to the tail is scraped using a scalpel, so that mucus and parasites are obtained from the surface of the fish's body. The results of scraping the body surface are rubbed on the glass object. The mucus swab is treated with physiological NaCl evenly. Wipe should not be thick and must be bubble free to facilitate the identification process with a microscope at 100x and 400x magnifications (Santoso, 2008).

The gills were examined by isolating it using tweezers and scissors. The filament cartilage is cut with a scalpel and placed on a petri dish. Gill pieces are placed on the glass object. Physiological NaCl drops were evenly distributed and observed under a microscope with a 40x and 100x magnification to examine the presence of ectoparasites crustaceans in the lamella. The ectoparasite crustaceans found were stored in sample bottles containing 70% ethanol for further identification (Aneesh *et al.*, 2014).

The ectoparasite crustaceans found were identified based on its morphological characteristics at the time of observation but were not stained (Yuasa *et al.*, 2003). The ectoparasite crustaceans studied were stored in 70% ethanol. Ectoparasite crustacean was put in object glass to be identified. Identification of ectoparasite crustaceans was carried out under a light microscope with 100x magnification, drawn with the help of Lucida cameras and documented with cameras (Handajani and Samsundari, 2005).

The ectoparasite crustaceans found were identified based on specific morphological characteristics during observation. Identification of ectoparasite crustaceans based on identification keys (Coull, 1977). Parasite identification was carried out at the Microbiology Laboratory of the Faculty of Fisheries and Marine Airlangga University.

Intensity and prevalence are calculated using the

following formulas:

$$\text{Prevalence} = \frac{\text{Number of fish that infested}}{\text{Total Number of samples that will be observed}} \times 100 \%$$

Fish infected with parasites with a rate of 90-100% categorized as always, while fish with quite severe parasites infection (98-99%) categorized as almost always. Fish with 70-89% rate of infection categorized as usual, and 50-69% categorized as often infest fish. The fish categorized as commonly infest fish as the infection rate is 30-49%, while rate infection of 10-29% belongs to frequent category. Occasionally category consist of fish with 1-9% rate of infection, and rarely infest fish consist of fish with 0.1-1 <1% infection. Fish with infection rat of less than <0.01% belongs to the almost never category (Dogel' *et al.*, 1961).

Data analysis

Data analysis was performed using descriptive analysis, which describes the facts as they are (Sangadji, 2010). Data from the identification of ectoparasite crustaceans found are presented in the form of images, while prevalence data are tabulated.

Results

The results of identification showed that 112 *T. blochii* were infested by ectoparasites crustaceans. The results of the identification of ectoparasite crustaceans are in accordance with the identification key of parasites by Coull (1977) can be seen in Table 1.

The results of identification of ectoparasite crustaceans on the body surface and Silver pompano (*T. blochii*) gills cultivated in tubs and floating net cages were found in one type of ectoparasite crustaceans originating from the *Copepod* sub-class namely *Canuella*. *Canuella* was found to infect the body surface of *T. blochii*.

Table 1. Results of identification of crustacean ectoparasites infesting *T. blochii* which are cultivated in hatcheries and floating net cages

Crustacean ectoparasites	Predilection
<i>Canuella</i>	Body Surface

Canuella's morphology has a dorsoventral flat body with a body length of 0.6–0.7 mm like the general *Herpaticoida* order. The body is divided into 10 segments consisting of a *cephalothorax*, metasome, urosome, and caudal hemp. The *cephalothorax* is

shaped like a shield with a slightly protruding part of the rostrum. In the cephalosome, there are no eye-spots. The *Antennule* is divided into six segments and the exopod antenna is divided into two segments. The first leg endopod is divided into two segments of the same size, the second segment exopod does not have setae, the first segment exopod has setae and the second segment exopod and endopod do not have hair. The second leg endopod is divided into two segments, the second endopod segment is longer than the first segment, the first segment has setae. The third leg endopod is divided into two segments. The fourth leg endopod is divided into two segments, the first segment has one setae, the second segment is also set. The fifth leg is divided into two parts, namely basoendopod and exopod and smaller in size as female characteristics, in the basoendopod and exopod parts there are no slabs. Caudal flax does not have flame-shaped setae.

Based on the results of measurements of several organs in three *Canuella* individuals found to infest *T. blochii* that cultured in hatcheries tubs and floating net cages data obtained were the average size of each organ such as antenna, antennule, cephalothorax, first to the fifth leg and caudal hemp presented in Table 2.

Based on Table 2, it can be seen that *Canuella* found has H and average body width of 0.65 and 0.15 mm. The length and width of cephalothorax are 0.15 and 0.12 mm, length and width of metasomes are 0.95 and 0.15 mm. The length and width of the urosome are 0.19 and 0.08 mm. The length and width of the antennule are 0.27 and 0.35 mm. Antenna length and width 0.17 and 0.25 mm. First to fifth leg length averages <0.20 and width <0.09 mm. Caudal flax has a length and width of 0.18 and 0.17 mm. The prevalence of crustacean ectoparasites infesting fish in hatcheries tub and floating net cages is presented in Table 3.

The prevalence of *Canuella* infestations in hatcheries tubs was 3.3% and 3.65% in floating net cages. The prevalence value by *Canuella* is categorized as an occasional category where parasites sometimes infest *T. blochii*. The results of observations of water quality in the hatcheries tub and floating net cages are presented in Table 4.

Based on Table 4, it is known that water quality that meets the standards for cultivation is 30.73 °C, DO is 6.42 ppm, pH is 7.92, salinity is 30.4 ppt and ammonia is 0.082 ppm which is still in the range of

Table 2. Morphometry *Canuella* that infesting on silver pompano (*T. blochii*) in hatcheries tub and floating net cages

Parameters		Size (mm)	
		Range	Mean
Habitus	Length	0.6 – 0.7	0.65
	Width	0.1 – 0.2	0.15
Cephalothorax	Length	0.1 – 0.2	0.15
	Width	0.1 – 0.13	0.12
Metasom	Length	0.09 – 0.1	0.95
	Width	0.1 – 0.19	0.15
Urosom	Length	0.18 – 0.2	0.19
	Width	0.07 – 0.09	0.08
Antennule	Length	0.25 – 0.3	0.27
	Width	0.03 – 0.04	0.35
Antennae	Length	0.16 – 0.19	0.17
	Width	0.02 – 0.03	0.25
First leg	Length	0.18 – 0.2	0.19
	Width	0.07 – 0.09	0.08
Second leg	Length	0.17 – 0.2	0.18
	Width	0.06 – 0.08	0.07
Third leg	Length	0.19 – 0.22	0.2
	Width	0.08 – 0.1	0.09
Fourth leg	Length	0.18 – 0.21	0.19
	Width	0.07 – 0.09	0.08
Fifth leg	Length	0.16 – 0.7	0.16
	Width	0.07 – 0.09	0.08
Caudal rami	Length	0.17 – 0.19	0.18
	Width	0.17 – 0.18	0.17

quality standards for cultivation.

Discussion

Based on the results of examinations carried out on the body surface and gills of *T. blochii*, crustacean ectoparasite found infested *T. blochii* in a hatcheries

tub was *Canuella* in the copepodid stage. *Canuella* found on the surface of the body *T. blochii*. *Canuella* is thought to be parasitic because it was found on the surface of the body of the fish when scrapping, although it is known that *T. blochii* is a fast swimmer fish. *Canuella* can attach to *T. blochii* using its antennule which is commonly used for copulation. The antennule is an organ shaped like a hook used to grip (Coull, 1977).

Canuella is a copepod from the order Herpaticoida that is eurythermal and holoeuryhalin which can greatly survive in various environment and mostly lives in brackish water and sandy or muddy seas (Noodt, 1957). *Canuella* is a fast swimmer organism and likes to melt against the substrate. *Canuella* used to eat macroalgae using the mandible, coxa, and spina, while *Canuella*'s nauplius used both antennas. The mandible is an organ that connects the anterior part of the posterior cephalosome. The mandible is composed of praecoxa (with one cutting tooth at the edge), one coxa-base, one endopod, and exopod. *Canuella* has six nauplius stages and six copepodid stages where on the fifth and sixth instars the copepodid is the *Canuella* adult stage (Vincx and Heip, 1979).

The prevalence of *Canuella* infestations in hatcheries tubs was 3.3% and 3.65% which belongs to occasional categories of parasites (Williams and Bunkley-Williams, 1996). *Canuella* infestation has a relatively low intensity and does not cause clinical symptoms in infected fish, this condition falls into the light intensity category. This is allegedly due to the quality of water that has met the standards for cultivation such as temperatures of 30.73 °C, DO 6.42 ppm, pH 7.92, the salinity of 30.4 ppt and am-

Table 3. Prevalence of ectoparasitic infestations in *T. blochii* in hatcheries tub and floating net cages

Fish Habitat	Crustacean Ectoparasite			Infested Fish (samples)	Total Samples (Samples)	Prevalence (%)
	Genus	Stadia	Predilection			
Hatcheries- Tub	<i>Canuella</i>	Copepodid	Body Surface	1	30	3.3%
Floating-Net Cages				3	82	3.65%

Table 4. Results of observations of water quality in tubs for hatcheries tub and floating net cages

Parameters	Hatcheries tub	Floating Net Cages	Quality standards
Temperatures (°C)	30.73	30.35	29 – 32
DO (mg/l)	6.42	7.54	>5
pH	7.92	6.45	7 - 8.5
Salinity (ppt)	30.4	30.45	32
NH3 (mg/l)	0.08	0.03	0.3

monia of 0.082 ppm which is still in the range of quality standards for cultivation. In addition, the relatively small scales of *T. blochii* and high fish swimming speeds are thought to complicate the infestation of ectoparasites crustaceans. Severe infestations by parasites are supported by host health conditions, a supportive environment for parasitic life and infectious pathogenic agents. Ammonia levels above the quality standard are due to the large amount of organic matter in the waters that comes from feed waste and other organic materials. The water conditions of floating net cages in the sea always naturally stirred so that the water quality of the water is always homogeneous, besides that the relative condition of the waters is still clean because there is no disposal of waste and garbage thrown directly into the sea. The quality of water in the tub is also still in the range of quality standards to minimize the presence of environmental stressors, considering the seeds still have lower immunity than fish in floating net cages.

In this study, there was no infestation by crustacean ectoparasites such as *Caligus*, *Lernanthropus*, and *Cymothoa* in the adult stage to be identified. This is influenced by host factors, pathogen agents and the environment. Environmental factors such as season, temperature, salinity, pH and dissolved oxygen are factors that determine the parasite infestation in the host (MacColl, 2009; Poulin, 2004). *T. blochii* which is in floating net cages is at a higher risk to be infested by *Canuella* than those in hatcheries, due to the longer the fish cultivated, the size of the fish will increase, as well as the surface for *Canuella* to infest. Parasitic infestations increase along with the cultivation time, since the accumulation of parasitic infestations from the environment (Poulin, 2000; Wootten *et al.*, 1982).

Caligus is an ectoparasite crustacean that infects much fish that are cultivated in the sea and brackish water rather than in fresh water. Only one species of *Caligus* infest freshwater fish, namely *C. lacustris*. There are seven species of *Caligus* spread to infest fish in the Asian region. *C. epidemics* is the *Caligus* species that considered as the most dangerous parasite in marine aquaculture because it can infest all types of fish. *C. epidemic* has also been found to infest fish that are cultivated in Indonesian marine with a prevalence of 88% which is included in the category that is usually the parasite that infects fish (Hasmi and Helmi, 2013).

Each *Caligus* species has a tolerance to salinity,

temperature, and concentration of different organic materials that affect the distribution of *Caligus* in the sea. *Caligus* can infest quickly at temperatures of 16-23 °C and maximum salinity of 32 ppt so 128 and 188 *Caligus* is found to infest two cultured fish (Nagasawa, 2004). This can be the cause of *Caligus* sometimes infesting fish in floating net cages or in tubs. *Caligus* can grow bigger and live longer at low temperatures. *Caligus* has a short life cycle at high temperatures so that in tropical waters that have a temperature of ± 30 it will rarely be seen *Caligus* infestation in cultivated *T. blochii* (Hasmi and Helmi, 2013).

Lernanthropus is an ectoparasite crustacean which infests the gills of large marine fish and can be seen directly with the eye when compared to other copepod parasites. *Lernanthropus* eats gill tissue and fish blood so that it can cause hyperplasia, erosion, anoxia, and hemorrhagic. The impact of infestation will not be seen if the degree of infection is low but severe infestation, as well as secondary infection by bacteria or viruses, may cause fish death (Chu *et al.*, 2012). Fish infected with *Lernanthropus* will experience respiratory problems, excess mucous secretion until death. This is caused by damage around the parasite attachment area to the gill lamella with the organ of the foot, antenna, maxilla and maxilliped (Chu *et al.*, 2012; Jithendran *et al.*, 2008). *Lernanthropus* attaches to the gill filaments using the antenna organ and maxilliped, while the female *Lernanthropus* is assisted with modified leg organs to attach to the host gill filaments (Manera and Dezfuli, 2003).

Lernanthropus uses antennas to enter gill filaments during the copepodid stage and settles into adulthood. *Lernanthropus* is a parasite in fish that live in warm water because at low temperatures and waters that have high water movement will cause the population of *Lernanthropus* to decline. Waters that are in areas that have spring and fall are waters that are suitable for the development of eggs and *Lernanthropus* infective stages (Ho *et al.*, 2011; Kabata, 1979). This is thought to be the cause of *Lernanthropus* not found in the research environment because it has a temperature of 30.35 °C salinity of 30.54 ppt so that it is not an appropriate place for the distribution and proliferation of *Lernanthropus*.

Cymothoa is a common ectoparasite found infesting the surface of the body and the oral cavity of the host. Two *Cymothoa* individuals can be found infest-

ing the host's mouth with the female at the bottom of the mouth while the male on the other side. The size of the female is larger than the female that inhabits the host's mouth (Rameshkumar *et al.*, 2016). *Cymothoa* is holoxenic or only lives in one host. The host infested with *Cymothoa* is not specific. *Cymothoa* eats mucus, epithelium, and tissue in the host's body. Fish that are infected with *Cymothoa* will experience physiological, behavioral and morphological disorders. *Cymothoa* population explosion can occur in parasitic infestations in the host for a long time (Bharadhirajan *et al.*, 2014). *Cymothoa* is more commonly found in shallow waters than deep waters (<100 m) *Cymothoa* can be used as a water bio-indicator because these parasites like water that have a high content of contaminated organic matter (Sidabalok, 2013). The polluted aquatic environment can be a food source of *Cymothoa* when it is free-swimming and can affect host immunity (Khan and Thulin, 1991). The absence of *Cymothoa* on *T. blochii* in this study was due to the relatively optimal aquatic environment for aquaculture because the ammonia content was still below the standard quality, which meant that the organic matter and pollutants were relatively low.

For the future study, it is necessary to further identify *Canuella* ectoparasites by Scanning Electron Microscope (SEM) so that morphology of the ectoparasites which infest *T. blochii* can be easily identified and further research is needed to prove *Canuella*'s relationship with the host.

Conclusion

The conclusion of this study is that ectoparasites which infect *T. blochii* which are cultivated in hatcheries tub and floating net cages are *Canuella* at the copepodid stage. *Canuella* prevalence value 3.3% in hatcheries tubs and 3.65% in floating net cages.

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