The prevalence and intensity of gastrointestinal endoparasite worms of cantang grouper (Epinephelus fuscoguttatus - lanceolatus) on floating net cages at Lamong Bay Surabaya, Indonesia

by L D Agustina

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Abstract. Cantang groupers (*Epinephelus fuscoguttatus-lanceolatus*) is a hybridized grouper fish of Brackishculture Center, Situbondo. In Indonesia, currently information about the parasite infection in cantang groupers is still few. This study aims to determine the prevalence and intensity of endoparasite worms that infect the gastrointestinal of cantang groupers (*E. fuscoguttatus-lanceolatus*) on the floating net cages at Lamong Bay, Surabaya. The method used in this study is survey method and analyzed descriptively. The endoparasite worms found in the gastrointestinal of cantang groupers were *Anisakis physeteris* and *Neoechinorhynchus longnucleanus*. The highest prevalence is single infection of *Neoechinorhynchus longnucleanus* was 3 % (occasionally) with intensity of 1 individual/fish and the lowest prevalence was single infection of *Anisakis physeteris* is 1 % (occasionally) with intensity of 1 individual/fish.

1. Introduction

Groupers are one of leading Indonesian export commodities widely cultivated. The production levels of groupers from 2009 to 2013 in Indonesia were 8.791 tons, 10.398 tons, 10.580 tons, 11.950 tons and 18.864 tons respectively [1]. Cultivation of groupers in Surabaya is located in Lamong Bay uses a floating net cage system. Cantang grouper (*Epinephelus fuscoguttatus-lanceolatus*) is hybridized a grouper of female tiger groupers (*E. fuscoguttatus*) with male kertang groupers (*E. lanceolatus*) [2].

In 2014, mass mortality occurred in cantang groupers cultivated on floating net cages at Lamong Bay. The body of the dead fish has a lot of mucus and there were wounds on some parts of the body. In addition, the growth of the fish during cultivation became slower. The mortality in the cantang groupers was suspected to be related to infectious diseases caused by parasites. One of the effects of the spread of endoparasites in the fish is the presence of invertebrates around the floating net cages that act as intermediate hosts of some endoparasite species [3]. Therefore, carnivorous fish including groupers are more likely to be infected by endoparasite worms than herbivores and omnivorous fish [4].

The endoparasite worms found in the gastrointestine of several species of groupers are: *Prosorhynchus lafii* [5], *Prosorhynchus maternus* [6], *Callitetrarhynchus gracilis* [7], *Echinostoma* [8], *Camallanus carangis*, *Procamallanus variolae* [9], *Capillaria plectropomy* [10], *Neoechinorhynchus* sp. and *Serrasentis sagittifer* [11].

The results of sea water monitoring in Lamong Bay Surabaya, Indonesia in 2014 showed that the sea water quality is below the seawater quality standard for biota; it also showed the existence of pollution [12]. Decreased water quality can be a trigger of infectious diseasees in fish. The development of endoparasites in the fish body can be influenced by environmental factors, such as temperature and chemicals in waters [13]. Research on the prevalence and intensity of endoparasite worms in the gastrointestine of cantang groupers (*Epinephelus fuscoguttatus-lanceolatus*) is important to determine the prevalence and intensity of endoparasite worms infecting cantang groupers.

2. Methodology

Life specimens of *E. fuscoguttatus-lanceolatus* were taken from floating net cages at Lamong Bay Surabaya, Indonesia on March to April 2016. The samples used in this study were 100 groupers (three months old with a length of 10-20 cm). This research used survey method and the data were analyzed descriptively. The fish were examined for endoparasite under a dissecting microscope. The staining of endoparasite used Semichen-acetic Carmine method, which refers to Kuhlmann's modification [14]. The illustrations used a lucida camera. The key identifications of endoparasite worms are Amin et al. [15], Chen and Shih [16], Grabda [17], Hoffman [18], Kabata [19] and Pavlovskaya [20]. The prevalence of endoparasite worms infecting the gastrointestines of cantang groupers was calculated based on Bush et al. [21] and the intensity was examined according to Dogiel et al. The category of prevalence and intensity refers to Williams and Williams [23].

3. Results and discussion

From the identification of endoparasite on gastrointestines of cantang groupers (*Epinephelus fuscoguttatus-lanceolatus*) on floating net cages at Lamong Bay Surabaya, Indonesia, two species of endoparasite worms attached to the intestinal wall were found, which are third stadia larvae of *Anisakis physeteris* and adult worm of *Neoechinorhynchus longnucleanus*. The two species of endoparasite worms can be seen in figure 1 to figure 4.

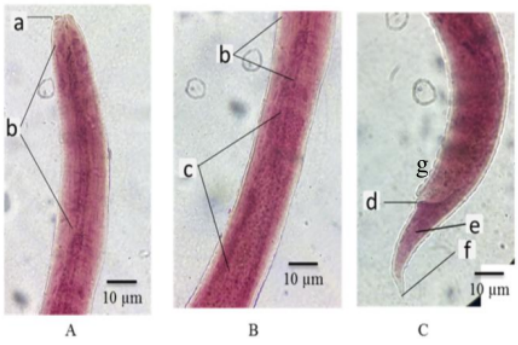

Figure 1. Third Stadia Larvae of *Anisakis physeteris*, scale bar = 10 μm. Coloring images based on binoculars microscope magnification 400x. Description; (A) Anterior part, (B) Mid body part, (C) Posterior part, (a) Booring tooth, (b) Esophagus, (c) Ventriculus, (d) Anal, (e) Appendix, (f) Tail, (g) Vulva.

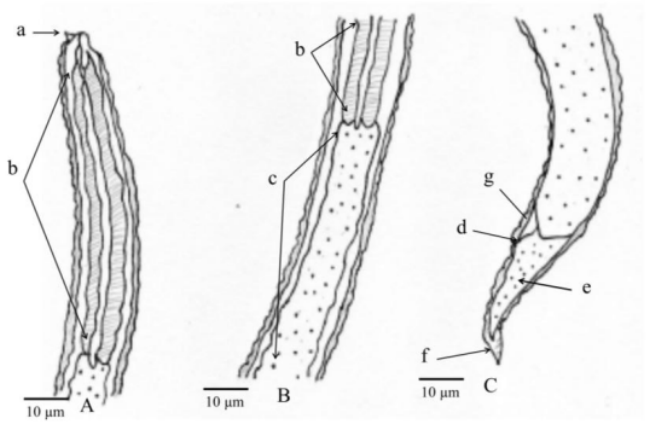

Figure 2. Third Stadia Larvae of *Anisakis physeteris*, scale bar = 10 μm. Images with binocular microscope equipped with camera lucida. Description; (A) Anterior part, (B) Mid body part, (C) Posterior part, (a) Booring tooth, (b) Esophagus, (c) Ventriculus, (d) Anal, (e) Appendix, (f) Tail, (g) Vulva.

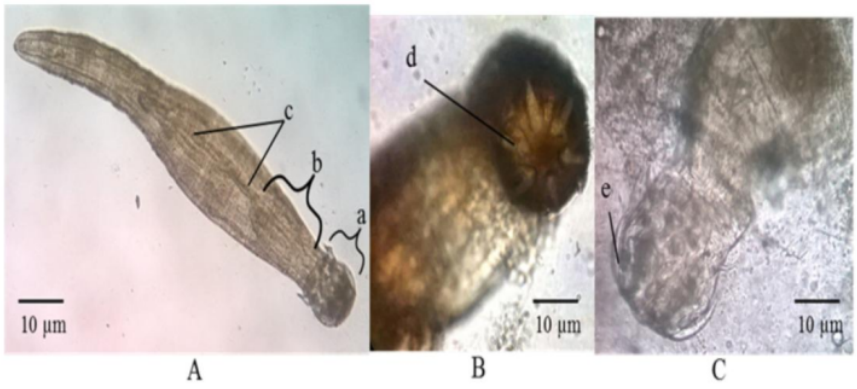

Figure 3. Adult worm of *Neoechinorhynchus longnucleanus*, scale bar = 10 μm. Native on a magnification binocular microscope magnification 100x (in figure A) and magnification 400x (in figures B and C). Description; (A) whole body, (B) Anterior end portion, (C) Proboscis, (a) Proboscis, (b) Proboscis receptacle, (c) Leminisci, (d) Hook the first line, (e) Hook.

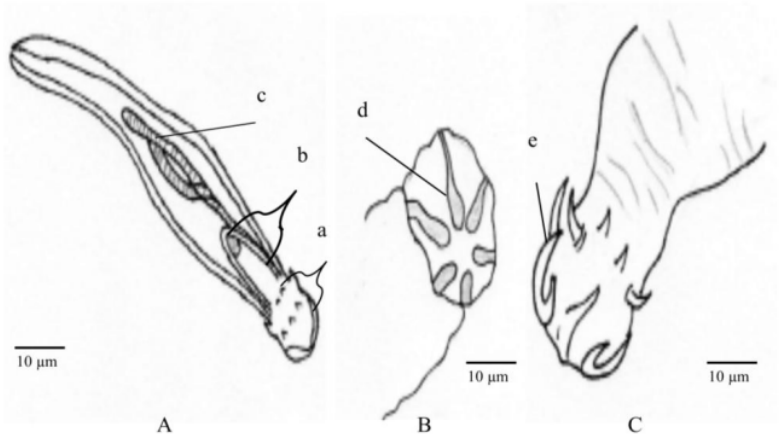

Figure 4. Adult worm of *Neoechinorhynchus longnucleanus*, scale bar = 10 μm. Images with binocular microscope equipped with camera lucida. Description; (A) whole body, (B) Anterior end portion, (C) Proboscis, (a) Proboscis, (b) Proboscis receptacle, (c) leminisci, (d) Hook the first line, (e) Hook.

Anisakis physeteris are included in Phylum Nemathelminthes, Class Nematoda, Order Ascaridida, Suborder Ascaridata, Family Anisakidae, and Genus *Anisakis* [12]. This worm has an elongated cylindrical body, with a total length of 4.113 mm and width of 0.13 mm. There were cuticle, booring tooth (larval tooth), esophageal with a length of 0.63 mm, ventriculus with a length of 0.13 mm, intestine, conical tail and on the tail, there was no mucron. This is in accordance with the statement of Anshary [24] and Hoffman [18] that the worm *Anisakis* sp. could be distinguished from other Anisakidae parasites by looking at the anterior end (booring tooth) and the ventricular shape that looks elongated and looks like white spots when observed with a binocular microscope. *Anisakis physeteris* worms were included in *Anisakis* type II which has a conical tail without mucron, according to [24, 16, 17]. *Anisakis physeteris* found was female larvae of third stadia larvae characterized by the presence of vulva in the posterior body. The life cycle of *Anisakis* starts from eggs which develop to second stadia larvae that infect the first intermediate host (small crustaceans), then ingested by the fish, developed into third stadia larvae in the fish body and adult in marine mammals. Nematode worms usually infect the gastrointestine of fish whereas larvae was found in almost every organ of fish [24, 25, 18]. The study of Chen and Shih [16] found third stadia larvae of *Anisakis physeteris* infecting the gastrointestine of *Scomber australasicus*.

Neoechinorhynchus longnucleanus belongs to the Phylum of Acanthocephala, Class of Eoachanthocephala, Order of Neoechinorhynchida, Family of Neoechinorhynchidae and Genus of *Neoechinorhynchus* [15]. This worm has a short round proboscis equipped with three row hooks; each line has six hooks. The hook on the first row is the largest compared to those on the second and third rows (fig 3B,C; 4B,C). According to Hoffman's ,the genus *Neoechinorhynchus* has a small, bowed or straight, short proboscis body equipped with three rows of hooks with each row of six hooks; the anterior hook is longer and greater than the others. *Neoechinorhynchus longnucleanus* has an anterior hook with a simple root shape, two leminisci of different lengths of 0.81 mm and 0.67 mm (fig 3A,C; 4A,C). This is in line with the statement of Amin et al. that *Neoechinorhynchus longnucleanus* has an anterior hook root with a simple shape and has two different lengths of leminisci and a large nuclei. The worm was an adult *Neoechinorhynchus longnucleanus*. The life cycle of the genus *Neoechinorhynchus* developed into adulthood in marine and freshwater fish, frogs and turtles; larvae developed in the crustacea. Adult worms of *Neoechinorhynchus* were attached to the small intestine and some of them in the peritoneal cavity [18,26]. Amin et al. found *Neoechinorhynchus*

longnucleanus infecting the intestine of *Strongylura strongylura* and Ruckert et al. found it in the gastrointestine of tiger grouper (*E. fuscoguttatus*).

3.1. Prevalence of endoparasite worms

Prevalence refers to the precentage of fish infected by endoparasites in the population. The prevalence of cantang groupers infected by endoparasite worms could be seen in table 1.

Table 1. Prevalence of cantang groupers infected by endoparasite worms on floating net cages at Lamong Bay Surabaya, Indonesia (100 samples).

No	Species of Endoparasite	Infected fish (individual)	Prevalence (%)	Infection Category (Wiliams and Williams, 1996)	Predilection of endoparasites
1	*Anisakis physeteris*	1	1	*Occasionally*	Intestinal wall
2	*Neoechinorhynchus longnucleanus*	3	3	*Occasionally*	Intestinal wall
Total		4	4		

The total prevalence of endoparasite worms infecting the gastrointestine of cantang groupers was 4%. The highest prevalence was a single infection of *Neoechinorhynchus longnucleanus* with a prevalence rate of 3 % and the lowest prevalence was a single infection of *Anisakis physeteris* with a prevalence rate of 1 %. According to Williams and Williams [23], the prevalence value was included in the occasional category. The prevalence of cantang groupers infected by *Anisakis physeteris* and *Neoechinorhynchus longnucleanus* was low compared with some studies that found some prevalences of *Anisakis* and *Neoechinorhynchus* worm such as third stadia larvae of *Anisakis physeteris* infecting *Strongylura strongylura* from Taiwanese waters with prevalence of 4 % [16], the prevalence of *Anisakis* sp. in gastrointestine of *Lutjanus malabaricus* on the auction of fish at Brondong Lamongan was 67 % for fish with the size of 21-24 cm and 80 % for fish with the size of 25-37 cm [27], while *N. longnucleanus* was found to infect the intestinal wall of cultured *E. fuscoguttatus* in Lampung Bay, which was 2.9 % [11], the prevalence of *N. agilis* in *Cheon labrosus* in Beymelek Lagoon Lake in Atalya, Turkey was 24% for male and 8.3 % for female [28] and the prevalence of *N. villaldoi* in *Austrolebias bellottii* from Punta Indio, Argentina was 80% [19].

Fish could act as a definitive host of *Neoechinorhynchus* [30, 18] and as an intermediate host of *Anisakis* [24, 31, 18]. The presence of *Anisakis physeteris* and *Neoechinorhynchus longnucleanus* infections on the intestinal wall of cantang groupers is due to the eating habit of the fish as predators, such as eating trash fish and small shrimp (which could act as intermediate hosts of the endoparasite worms). The above statement was confirmed by Heemstra and Randall [16] that *Epinephelus* were epibethic predators feeding on macro invertebrates (mainly crustaceans). It was also supported by Ruckert et al. who stated that fish could be attacked by food-borne diseases and the existence of invertebrates around the floating net cage as an intermediate host of some endoparasite worms. Therefore, these may affect the spread in fish. Fidyandini et al. also stated that low prevalence rates were due to parasite adaptation abilities in host body and host compatibility for parasitic survival and environmental quality.

3.2. Intensity of endoparasite worms

Intensity refers to the endoparasites that infect each individual. The intensity of cantang groupers infected by *A. physeteris* and *N. longnucleanus* could be seen in table 2.

Table 2. Intensity of cantang groupers infected by *A. physeteris* and *N. longnucleanus* on floating net cages at Lamong Bay Surabaya, Indonesia.

No.	Species of Endoparasite Worm	Number of Infected fish (%)	Number of Endoparasite Worm	Intensity (Individual/fish)
1.	*A. physeteris*	1	1	1
2.	*N. longnucleanus*	3	3	1
Total		4	4	1

The total intensity of endoparasite worms found in the gastrointestinal of cantang groupers was 1 individual/fish, that was regarded as mild infection [23]. The intensity of cantang groupers infected by *Anisakis physeteris* and *Neoechinorhynchus longnucleanus* was low compared with some research that found some intensity of *Anisakis* and *Neoechinorhynchus* worms such as; the intensity of *Anisakis* sp. in the gastrointestine of *Lutjanus malabaricus* on the auction of fish at Brondong Lamongan was 5 individual/fish (fish size 21-24 cm) and 18 individual/fish (fish size 25-37 cm) [27]. The intensity of *Anisakis* sp. in *Chanos chanos* on the ponds of Ketapang Village, Mauk, Tangerang District, Province of Banten was 4 individual/fish [20], while the intensity of *N. agilis* in *Cheon labrosus* in Beymelek Lagoon Lake in Atalya, Turkey was 2 individual/fish (male) and 4 individual/fish (female) [28] and the mean intensity of *N. villaldoi* in *Austrolebias bellottii* from Punta Indio, Argentina was 3.13 individual/fish [29].

The intensity value was also influenced by the endoparasite egg survival factor [35, 36]. This study found that endoparasite worms only infected the intestinal wall of cantang groupers. This is in line with the statement of Murata et al. that the small intestine and lumen of the small intestine provide nutrients. The structure and physiology of the intestine could be parasitic microhabitat affecting the presence of parasites. The seawater quality at Lamong Bay was not much different from the standard of seawater quality for biota (based on the Decree of the Minister of Environment No. 51 of 2004 on the Quality Standard of Sea Water). The pH of 7.85 is within the normal range (pH 7-8.5) and the temperature of 29.1^{0}C is in the normal range of sea water temperature (28-30^{0}C). Meanwhile, the salinity was 29.3 ppt, less than the normal of sea salinity of 33-34 ppt. The low salinity of this study is due to seasonal changes. Komarawidjaja explained that water quality degradation can be a driving force for the development of pathogens in fish.

4. Conclusions

The endoparasite worms infecting cantang groupers were *Anisakis physeteris* and *Neoechinorhynchus longnucleanus*. The total prevalence of endoparasite worms infecting the gastrointestine of cantang groupers was 4% with total intensity of 1 individual/fish. The predilection of both species of worms was on the intestinal wall of cantang groupers.

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GRADEMARK REPORT

FINAL GRADE

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GENERAL COMMENTS

Instructor

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RUBRIC: 6TH-8TH SCIENCE ARGUMENT (CER)

CLAIM

Take an arguable position on the scientific topic and develop the essay around that stance.

ADVANCED	The essay introduces a precise, qualitative and/or quantitative claim based on the scientific topic or text(s), regarding the relationship between dependent and independent variables. The essay develops the claim and counterclaim fairly, distinguishing the claim from alternate or opposing claims.
PROFICIENT	The essay introduces a clear, qualitative and/or quantitative claim based on the scientific topic or text(s), regarding the relationship between dependent and independent variables. The essay effectively acknowledges and distinguishes the claim from alternate or opposing claims.
DEVELOPING	The essay attempts to introduce a qualitative and/or quantitative claim, based on the scientific topic or text(s), but it may be somewhat unclear or not maintained throughout the essay. The essay may not clearly acknowledge or distinguish the claim from alternate or opposing claims.
EMERGING	The essay does not clearly make a claim based on the scientific topic or text(s), or the claim is overly simplistic or vague. The essay does not acknowledge or distinguish counterclaims.

EVIDENCE

Include relevant facts, definitions, and examples to back up the claim.

ADVANCED	The essay supplies sufficient relevant, accurate qualitative and/or quantitative data and evidence related to the scientific topic or text(s) to support its claim and counterclaim.
PROFICIENT	The essay supplies relevant, accurate qualitative and/or quantitative data and evidence related to the scientific topic or text(s) to support its claim and counterclaim.
DEVELOPING	The essay supplies some qualitative and/or quantitative data and evidence, but it may not be closely related to the scientific topic or text(s), or the support that is offered relies mostly on summary of the source(s), thereby not effectively supporting the essay's claim and counterclaim.
EMERGING	The essay supplies very little or no data and evidence to support its claim and counterclaim, or the evidence that is provided is not clear or relevant.

REASONING

Explain how or why each piece of evidence supports the claim.

ADVANCED	The essay effectively applies scientific ideas and principles in order to explain how or why the cited evidence supports the claim. The essay demonstrates consistently logical reasoning and understanding of the scientific topic and/or text(s). The essay's explanations anticipate the audience's knowledge level and concerns about this scientific topic.

PROFICIENT	The essay applies scientific reasoning in order to explain how or why the cited evidence supports the claim. The essay demonstrates logical reasoning and understanding of the scientific topic and/or text(s). The essay's explanations attempt to anticipate the audience's knowledge level and concerns about this scientific topic.
DEVELOPING	The essay includes some reasoning and understanding of the scientific topic and/or text(s), but it does not effectively apply scientific ideas or principles to explain how or why the evidence supports the claim.
EMERGING	The essay does not demonstrate clear or relevant reasoning to support the claim or to demonstrate an understanding of the scientific topic and/or text(s).

FOCUS

Focus your writing on the prompt and task.

ADVANCED	The essay maintains strong focus on the purpose and task, using the whole essay to support and develop the claim and counterclaims evenly while thoroughly addressing the demands of the prompt.
PROFICIENT	The essay addresses the demands of the prompt and is mostly focused on the purpose and task. The essay may not acknowledge the claim and counterclaims evenly throughout.
DEVELOPING	The essay may not fully address the demands of the prompt or stay focused on the purpose and task. The writing may stray significantly off topic at times, and introduce the writer's bias occasionally, making it difficult to follow the central claim at times.
EMERGING	The essay does not maintain focus on purpose or task.

ORGANIZATION

Organize your writing in a logical sequence.

ADVANCED	The essay incorporates an organizational structure throughout that establishes clear relationships among the claim(s), counterclaims, reasons, and evidence. Effective transitional words and phrases are included to clarify the relationships between and among ideas (i.e. claim and reasons, reasons and evidence, claim and counterclaim) in a way that strengthens the argument. The essay includes an introduction and conclusion that effectively follows from and supports the argument presented.
PROFICIENT	The essay incorporates an organizational structure with clear transitional words and phrases that show the relationship between and among ideas. The essay includes a progression of ideas from beginning to end, including an introduction and concluding statement or section that follows from and supports the argument presented.
DEVELOPING	The essay uses a basic organizational structure and minimal transitional words and phrases, though relationships between and among ideas are not consistently

	clear. The essay moves from beginning to end; however, an introduction and/or conclusion may not be clearly evident.
EMERGING	The essay does not have an organizational structure and may simply offer a series of ideas without any clear transitions or connections. An introduction and conclusion are not evident.

LANGUAGE

Pay close attention to your tone, style, word choice, and sentence structure when writing.

ADVANCED	The essay effectively establishes and maintains a formal style and objective tone and incorporates language that anticipates the reader's knowledge level and concerns. The essay consistently demonstrates a clear command of conventions, while also employing discipline-specific word choices and varied sentence structure.
PROFICIENT	The essay generally establishes and maintains a formal style with few possible exceptions and incorporates language that anticipates the reader's knowledge level and concerns. The essay demonstrates a general command of conventions, while also employing discipline-specific word choices and some variety in sentence structure.
DEVELOPING	The essay does not maintain a formal style consistently and incorporates language that may not show an awareness of the reader's knowledge or concerns. The essay may contain errors in conventions that interfere with meaning. Some attempts at discipline-specific word choices are made, and sentence structure may not vary often.
EMERGING	The essay employs language that is inappropriate for the audience and is not formal in style. The essay may contain pervasive errors in conventions that interfere with meaning, word choice is not discipline-specific, and sentence structures are simplistic and unvaried.