Prevalence of ectoparasites in milkfish (Chanos chanos) from nursery and rearing ponds

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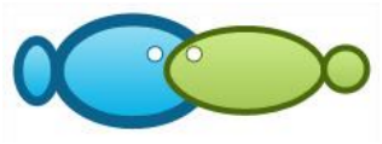

Prevalence of ectoparasites in milkfish (*Chanos chanos*) from nursery and rearing ponds

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Abstract. Milkfish (*Chanos chanos*) cultivation is inseparable from the disease problems caused by viruses, bacteria, fungi, and parasites. *Dactylogyrus* sp. often affects the heart organ of *C. chanos*. Young specimens in nursery ponds are more susceptible to the related diseases than adults in enlarged ponds. The current research compared the prevalence of ectoparasites infesting *C. chanos* in the nursery and rearing ponds, in order to prevent parasite infestation. An experimental study was conducted by using survey methods. Simple random sampling used to select 300 samples from nursery and rearing ponds in 3 different cultivation locations. The main investigated parameter in this study was the prevalence of ectoparasites infesting *C. chanos* in nursery and rearing ponds. Besides, water quality parameters were also determined, including temperature, salinity, pH, brightness, dissolved oxygen levels, and ammonia. The study identified *Dactylogyrus vastator* as the ectoparasites that infested *C. chanos* in the nursery and rearing ponds. The average prevalence of ectoparasites in the nursery pond was of 5.33%, while in the rearing pond it was of 2.0%. The Chi-Square test showed a significance value of 0.293 in both, nursery and rearing ponds. The water quality measurements of both ponds resulted an optimum value in temperature (26-28°C), salinity (17-20 ppt), brightness (25-28 cm), pH (7-8), and ammonia levels (0.01-0.05 mg L^{-1}). The *D. vastator* infestation in *C. chanos* in the nursery and rearing ponds was at an occasional level and did not show significant differences in the prevalence values between the two ecosystem pools.

Key Words: fish disease, *Dactylogyrus vastator,* nursery pond, rearing pond, animal parasites.

Introduction. Fish is an important commodity, especially in terms of supplying high protein content. Milkfish (*Chanos chanos*) is classified as high protein fish (20 to 24%) and low fat content (0.7 to 0.8%) (Hafiludin 2015). *C. chanos* is rich in Omega 3 fatty acid, which can prevent blood clots, reduce cholesterol levels, increase endurance and play a role in brain development in the fetus.

In some developing countries such as Indonesia, Philippines, and Malaysia, fisheries production is a source of income for the country, due to the export via the foreign exchange. Milkfish production progressed by 421,757 tons in 2010, 467,449 tons in 2011, 518,939 tons in 2012, 627,332 tons in 2013, 631,125 tons in 2014 and 905,408 tons in 2015, at a rate ranging between 10 and 45% $year^{-1}$ (Direktorat Jenderal Perikanan dan Budidaya and Menteri Kelautan dan Perikanan 2016). Disease issues caused by viruses, bacteria, fungi, and parasites are inseparable from *C. chanos* cultivation. Emerging diseases are related to environmental damage and stress factors (Lin & Liao 2008; Echem et al 2018; Hanke et al 2019). Ectoparasitic infestation can be a way for infection of other pathogenic organisms (Thilakaratne et al 2003). Dried *C. chanos* from several regions in Taiwan contain of high histamine levels that cause digestive diseases (Hsu et al 2009).

Dinoflagellate parasite infestation, *Amyloodinium ocellatum* (Dinoflagellida) often occurs in the fins and skin of *C. chanos* that are kept in fish hatcheries in the Philippines (Dequito et al 2015; Virgula et al 2017). In Lizard Island, Moreton Bay, and Wangetti Beach, three Aporocotylid species infected *C. chanos* heart tissue (Yong et al 2016). Four different species of endoparasites were identified in *C. chanos* through microscopic

examination, namely: *Ichthyobodo* sp., *Trichodina* sp., *Acanthocephala* spp. and *Diphyllobothrium latum*, in the Mindanao, Philipine (Echem et al 2018). *Dactylogyrus* was also found in India, occurring in *Garra gotyla* fish species, with a prevalence value of 16.59 (Ahmed & Sharma 2016). Besides, the result showed that prevalence of *Dactylogyrus* sp. on the gill of juvenile *C. chanos* in two different villages of Indonesia was of 53.33% and 86.67% (Mas'ud 2011). In addition, the *Dactylogyrus* parasite also infested the gills of red tilapia fish commodities from Indonesian waters, with a prevalence value of 90% (Irwandi et al 2017).

Dactylogyrus, commonly called gill worm is included in the category of monogeneans, an ectoparasites group, because it has a direct life cycle without requiring an intermediate host. *C. chanos* affected by *Dactylogyrus* are found in Indian waters (Bhuiyan & Musa 2008; Peninal et al 2014; Ahmed & Sharma 2016). The other characteristics of *Dactylogyrus* are the presence of two pairs of eye spots between the anterior parts of pharynx and the body (Ling et al 2016). The infestation is followed by secondary infection with viruses, bacteria, and fungi growing in wounds that continue to expand, thus it can result in death. Therefore, it is very important for farmers to know the prevalence value of *C. chanos* ectoparasites in each aquaculture pond, in order to prevent outbreaks of parasite infestation and disease in fish. Hence, this study was aimed to find out the comparison between the prevalence of ectoparasites which infested *C. chanos* on nursery and rearing ponds.

Material and Method

The selection and sampling of C. chanos. This study was conducted experimentally using a survey method, from December 2016 to January 2017. The study used *C. chanos* obtained from fish farming ponds in the central area of Manyar District, Gresik Regency, Indonesia. This study used purposive sampling method to select the samples. The criteria for the juvenile *C. chanos* specimen selection were a size of about 5 to 10 cm and a nursing pond origin. However, the criteria for adult *C. chanos* were a size of 30 to 35 cm and rearing ponds origin. A rearing pond is a place for juvenile *C. chanos* growing to adult stage. *C. chanos* were captured from three different logging ponds with the same plot area of 0.5 ha. The three plots were situated in the villages of Betoyoguci, Betoyokauman, and Banyuwangi (Figure 1). Fish sampling from rearing ponds used the same method and area. Each sample of *C. chanos* counted 50 fish per plot. Thus, the number of fish samples captured from the two pond types amounted to 300 fish.

Figure 1. The originate place of nursery and rearing ponds. (a) The location of Gresik City in East Java, Indonesia. (b) The Manyar District of Gresik City. (c) The 3 Sub-districts of nursery and rearing ponds.

After obtaining the appropriate number of *C. chanos* samples, these were packaged with plastic and Styrofoam. The sample examination was conducted for one month in the Education Laboratory of the Faculty of Fisheries and Maritime Affairs, Airlangga University, Surabaya, Indonesia.

Sample inspection and calculation of prevalence of C. chanos ectoparasites. First, *C. chanos* samples' length and body weight were measured. The method used in the examination of the ectoparasite in the samples was scrapping, done on both sides of the body surface, gills, and fish fins. Scrapping results were natively observed under a microscope at magnifications of 100x and 400x. Staining techniques were applied on samples affected by ectoparasites to enhance the observations. Ectoparasite infestation prevalence rate was calculated by the ratio of the number of infested fish against the number of examined *C. chanos* samples. In this study, there were used ten range categories for the ectoparasite prevalence values classification, as shown in Table 1. Chi-square analysis revealed different infestation frequency distributions between the sample groups originating from the two aquaculture ecosystems, namely nursery ponds and rearing ponds. Besides, this study also observed the water quality parameters of both ponds: temperature, salinity, pH, dissolved oxygen (DO), brightness, and ammonia levels.

Table 1

Explanation of ectoparasite prevalence value and range

Category	*Value range (%)*
Almost never	<0.01
Very rare	0.01-0.1
Rare	0.1-1
Occasional	1-9
Often	10-29
Common	30-49
Frequent	50-69
Usual	70-89
Almost always	90-98
Always	99-100

Results. In the collected *C. chanos* samples, the average length of specimens obtained from the mating ponds in the villages of Betoyoguci, Betoyokauman and Banyuwangi was of 7.48 cm, 7.97 cm and 8.27 cm, and the average weight was 5.07 g, 4.70 g, and 5.34 g, respectively. A specimen from the mating ponds is represented in Figure 2 (a).

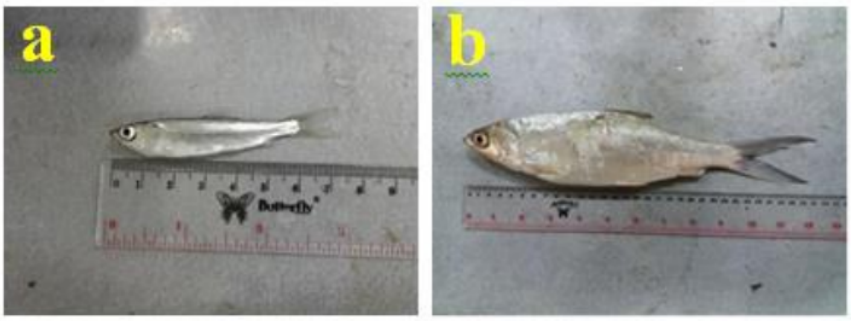

Figure 2. (a) Samples of *Chanos chanos* from a nursery pond. (b) Samples of *Chanos chanos* from a rearing pond (original).

From the harvest areas, i.e. the rearing ponds in the villages of Betoyoguci, Betoyokauman and Banyuwangi, the average length was 32.65 cm, 32.86 cm, and 33.27 cm, respectively, and the weight was 266.46 g, 267.3 g and 272.7 g, respectively. A specimen from the rearing ponds is represented in Figure 2 (b).

Sample inspection and calculation of prevalence of C. chanos ectoparasites. The ectoparasites identified in *C. chanos* samples, through the microscopic examination, were of the genus *Dactylogyrus* (Figure 3a). *Dactylogyrus* ectoparasites were found in all of the nursery pond test areas with an average prevalence value of 5.33% (Table 2), corresponding to the occasional category.

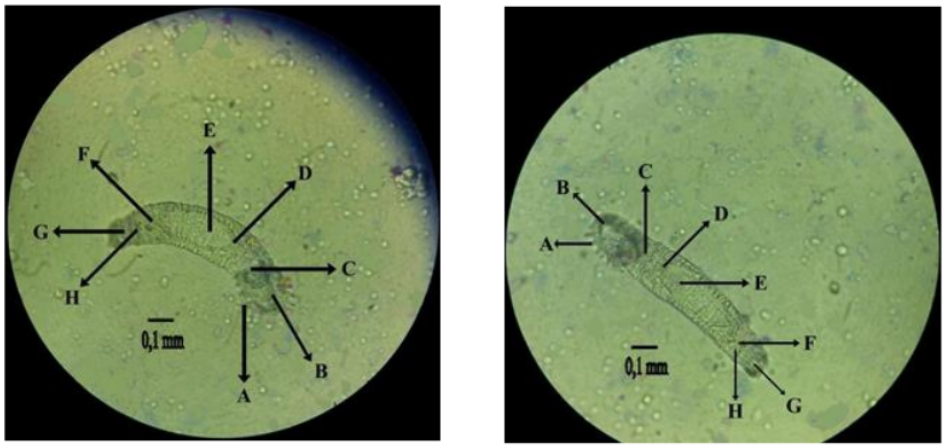

Figure 3. (a) *Dactylogyrus* on nursery ponds (100x magnification); (b) *Dactylogyrus* on an enlarged pond (100x magnification). A. Edge hooks, B. Anchors, C. Vitelline bags, D. Testicles, E. Ovaries, F. Pharynx, G. Cephalic glands, H. Eye spots.

Ectoparasites of the genus *Dactylogyrus* were found also in the samples captured in the rearing ponds (Figure 3b), with an average prevalence of 2% (Table 2), corresponding to the occasional category. The study concludes that ectoparasites present in the ponds only occasionally infested the *C. chanos* living in the aquaculture ecosystems.

Analysis of the prevalence of ectoparasites in *C. chanos* from nursery and rearing ponds was done by non-parametric statistical tests. The result of the Chi-Square test was a significant value (Asymp.sig.2-sided) of 0.293. This test had a degree of freedom value of 5, which showed significant value (11.07) in the probability value (x2) of 0.05. Besides, it also showed significant value (15.09) in the probability value (x2) of 0.01. These results indicated that there was no significant difference of ectoparasites prevalence in *C. chanos* from nursery and rearing ponds.

In addition, this study also produced data on the water quality measurement of each aquaculture pond, indicating that nursery and rearing ponds showed variable water parameters values (Table 3). Average arameter values were: a temperature of 27°C, a salinity of 19 p17 a pH of 7-8, a brightness of 26.5 cm, a DO level of 4.5 ppm, and an ammonia level of 0.03 mg L-1. In this study, brightness was used as an indicator of light intensity in the water, which shows the fish feasibility.

Table 2

Prevalence calculation results of ectoparasites in *Chanos chanos* in nursery and rearing ponds

District	*Nursery ponds*				*Rearing Ponds*			
	Sample (tails)	*Infected fish (tails)*	*Prevalence (%)*	*Category*	*Sample (tails)*	*Infected fish (tails)*	*Prevalence (%)*	*Category*
Betoyoguci	50	3	6	Occasional	50	1	2	Occasional
Betoyokauman	50	4	8	Occasional	50	2	4	Occasional
Banyuwangi	50	1	2	Occasional	50	0	0	Almost never
Total	150	8	5.33	Occasional	150	3	2	Occasional

Table 3

Observation results of nursery and rearing ponds water quality observations

Parameter	*District*					
	Betoyoguci		*Betoyokauman*		*Banyuwangi*	
	Nursery ponds	*Rearing ponds*	*Nursery ponds*	*Rearing ponds*	*Nursery ponds*	*Rearing ponds*
Temperature (^{0}C)	27	27	28	27	26	26
Salinity (ppt)	18	19	17	18	20	20
pH	8	7	7	7	8	8
Brightness (cm)	28	26	28	27	26	25
DO (ppm)	4	5	3	4	5	6
Ammonia (mg L^{-1})	0.04	0.02	0.05	0.02	0.02	0.01

Discussion. This study aimed to compare the prevalence of ectoparasites which infest *C. chanos* in the mating ponds and enlarged ponds, in order to prevent parasite infestation in both *C. chanos* ponds. Based on the results of the study, it was found that ectoparasites found in *C. chanos* samples from the nursery and rearing ponds were the genus *Dactylogyrus*. *Dactylogyrus* parasites are often found in fish species in the waters of Indonesia, India, Philippines, and several other areas. This parasite is noted to also infect *G. gotyla* fish in Indian waters (Ahmed & Sharma 2016). *Dactylogyrus* attaches to the gills using two anchors and fourteen edge hooks, which cause bleeding and determines pathological changes in the blood, threatening the lives of infested fish (Chaundhary et al 2013). Gills infested with *Dactylogyrus* will have a pale color. *Dactylogyrus* appears like a white nodule which causes the gill cover to always be open (Koyun 2011). The condition of the gills becomes severe if there is necrosis in the tissues, eventually causing the fish death due to the impairment of respiratory function (Kumar & Kumar 2013). Severe lesions of the tissue, under the skin and gills, cause a slow fish movement, a dark body color and alterations of the nervous system (Turgut et al 2012).

Overall, the prevalence of ectoparasites in nursery and rearing ponds is still classified as occasional (sometimes), which is still not a major threat for aquaculture, due to a sampling conducted during the rainy season, when the frequency of *Dactylogyrus* ectoparasites infestations is low. *Dactylogyrus* ectoparasites population density increases in the summer (dry season). The temperature is relatively stable compared to summer and winter, since there were only few temperature fluctuations during the rainy season (Hossain et al 2008). This affects the life cycle of *Dactylogyrus* where eggs hatching depends on the temperature and time of breeding of the parasite: 27 to 28 days at 8°C, 10 to 15 days at 12°C, 3 to 5 days at 20°C, and 2 to 3 days at 24-28°C (Soylu et al 2010). Thus, the results of this study indicate that there is no significant difference in prevalence between ectoparasites in *C. chanos* in nursery and rearing ponds.

In the current study, *Dactylogyrus* ectoparasites were found in the gills of *C. chanos*, a predilection also observed by Mas'ud (2011), because *Dactylogyrus* infestation occurs in host fish during feed ingestion and respiration (Özer & Öztürk 2005). The prevalence of *C. chanos* ectoparasites taken from the sampling ponds is greater than in the specimens captured from the enlarged ponds. This happens because the *C. chanos* in the nursery pond are smaller and younger so that the immune system is not fully developed.

C. chanos seeds move slower than an adult milkfish, therefore it is easier for ectoparasites to infest the host (Villaluz et al 1983). Environmental factors such as water temperature and other physical physical-chemical factors tend to influence the parasite's fluctuation cycle (Modu & Shaharom2014). *C. chanos* and mullet (Mugilidae) had significantly higher growth rates during the dry season, compared to the wet season (Mirera 2011).

Other ectoparasites such as *Chilodonella* and *Trichodina* were not found in this study, due To their relatively short life cycle and to the sub-optimal living conditions. The life cycle of *Chilodonella* lasts from 12 hours to 24 hours at a water temperature of 5°C to 20°C (Bellec et al 2014). *Protozoa* ectoparasites can arise due to poor water quality conditions and poor treatment of fish (Martins et al 2015). *Lernaea* ectoparasites were not found in this study because *C. chanos* are fast swimmers. Hence, *Lernaea* ectoparasites will have difficulty infesting the *C. chanos* body using its holdfast (Stavrescu et al 2014). Also, *Lernaea* usually grow at temperatures of 20 to 25^{0}C, which are are relatively lower than the temperature at the time of sampling (Marina et al 2008).

Water quality in all sampling sites, both in the nursery and in the rearing ponds, was optimal for aquaculture, as indicated by the small prevalence of ectoparasites that infested both *C. chanos* in nursery and rearing ponds. A low DO content affects the oxygen supply in fish, thus the respiration process is disrupted, causing stress. DO measurement results produce an optimal value from 3 to 6 ppm. This study provides information to entrepreneurs, *C. chanos* farmers and scientists, in order to improve the culture management and the understanding of the *C. chanos* health issues. Hence,

preventive activities can be deployed in order to avoid *C. chanos* infestation with several types of ectoparasites living in the *C. chanos* ecosystem.

Conclusions. In the nursery ponds and in the rearing ponds, *Dactylogyrus* occasionally infested *C. chanos*, with an average prevalence value of 5.33% and 2%, respectively. Both aquaculture ponds had no significant differences in the prevalence values (for a significance value of 0.293) and water quality parameters (temperature, pH, brightness, oxygen levels and ammonia) indicated optimal conditions.

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Prevalence of ectoparasites in milkfish (Chanos chanos) from nursery and rearing ponds

GRADEMARK REPORT

FINAL GRADE

/0

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.