

COPEPOD AND NEMATODE PARASITES OF THE THREE-SPOTTED DAMSELFISH *DASCYLLUS TRIMACULATUS* FROM BIDONG ISLAND, TERENGGANU

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Abstract: This study focuses on the parasites in *Dascyllus trimaculatus* fish in Bidong Island. Though *D. trimaculatus* is a common reef fish, there is lack on parasites studies of this fish in Malaysia. The objectives of this study are to identify metazoan parasites and analyse the prevalence and mean intensity the parasites in *D. trimaculatus*. Sampling was randomly conducted on a coral colony of 100 meters within a 15m depth at Pantai Pasir Cina, Bidong Island. This research resulted in the identification of a copepod *Lernaocera branchialis* and a nematode from the family Camallanidae. The copepods collected in this study infected 20 out of 42 *D. trimaculatus* specimens, whereas the nematode-like parasites collected infected 18 of out of 42 *D. trimaculatus*. All copepods found in the gills of *D. trimaculatus* had a prevalence of 4.95 and mean intensity of 47.62%, while the nematode-like parasite, which were found in the brain, had 2.72 prevalence and 42.86% mean intensity. This elucidates that both parasites are categorised as having light level but common frequency of infection, and the current IUCN status of *D. trimaculatus* has not been reported to be harmed by parasites. Though the mean intensity of both parasites is not harmful, the prevalence are concerning and might increase in the future, with further incorporation and monitoring of climate change factors that may affect the damselfish.

Keywords: Three-spotted damsel, nematode parasite, *Lernaocera branchialis*, prevalence, mean intensity.

Introduction

Bidong Island is located in the eastern side of the Peninsular Malaysia and forms part of the Bidong Archipelago. Its locality at the South China Sea (SCS) nurtures high coral reef cover, resulting in a diversified coral ecosystem. Despite its high diversity, there is still a lot of the archipelago that requires much needed study. For one, coral health monitoring should be constantly executed, as poor environmental conditions can encourage excessive algal growth and colonisation of algal turfs (McCook *et al.*, 2001). To date, the coral ecosystem of Bidong Island is composed of myriad of fish communities of various trophic levels to cater to a “check and balance” of the ecosystem health. Of the many contributors, the damselfish (which are planktivorous fish) plays an important role in the coral

reef ecosystem (Gladstone, 2007), as they often feed on epilithic algae, which grows on coral rubble (Wilson & Bellwood, 1997).

Dascyllus are famous as aquarium fish, therefore the high demand results in overharvesting, which is destructive to the fish and the coral ecosystem (Sin *et al.*, 1994). The excessive harvest of coral reef fishes could cause unrecoverable of population (Sin *et al.*, 1994). In Malaysia, the damselfish is endangered because of illegal harvest and habitat destruction (Sin *et al.*, 1994; Allen *et al.*, 2010; Robertson *et al.*, 2010; Jenkins *et al.*, 2012; Allen & Robertson, 2015; Jenkins *et al.*, 2017). The fishes were seen in lower populations on dead coral as compared to live coral (Sin *et al.*, 1994). In IUCN Red List, there are 128 species of damselfish are listed. Among 28 species of damselfish, 17 species are

data deficient and least concern, 2 species are near threatened (Allen & Robertson, 2015; Robertson *et al.*, 2010), 7 species are vulnerable (Jenkins *et al.*, 2017; Allen *et al.*, 2010), 1 species is endangered (Jenkins *et al.*, 2012), and one species is critically endangered (Allen *et al.*, 2010).

In Bidong Island, damselfish ranks as one of the highest percentage composition of fish population (Rumeaida *et al.*, 2014). They feed on small crustaceans and plankton on top of the known epilithic algae (Gladstone, 2007). Damselfish can prove to be important algal regulators by feeding on algae mat that causes poor survivorship and altered growth of *Acropora* (Wilson & Bellwood, 1997; Potts, 1977). It has been known that algae could overgrow and suffocate coral, causing coral death and directly dwindling the number of damselfish communities in the reef, as they are seen to be more abundant at live coral areas (Sin *et al.*, 1994). Among the top 5 most abundant damselfish species in Bidong Island, two *Dascyllus* fish species are present in the waters: *Dascyllus reticulatus* and *Dascyllus trimaculatus* of which the latter is the study subject for parasites.

With regards to parasites, though naturally known for its detrimental effects, they play an important role as regulators (Marcogliese, 2002). Parasites could also alter the food chain, by changing in their

prevalence and density in long term and reduce host numbers (Marcogliese, 2002). The studies of parasites on *Dascyllus* fishes are not common in Malaysia. Of the known listed literature from Malaysia (see Sin, *et al.*, 1994; Andersson, 2002; Chen *et al.*, 2004; Rumeaida *et al.*, 2014; Arai, 2015), most pertain on the diversity, disturbance, and conservation measures of the population.

This study focuses on the identification of parasites' species on *D. trimaculatus* and their prevalence and mean intensity. As the two known *Dascyllus* species in Bidong Island are abundant and to date least concerned according to IUCN, there are other facets that needs enlightenment to ensure the holistic biology and sustainability of the *Dascyllus* population.

Materials and Methods

Sampling Area

The sampling for this study was conducted at Bidong Island, which is a research station acquired by Universiti Malaysia Terengganu. The island is located at South China Sea, Malaysia (Figure 1). The South China Sea is noted for its highly productive ecosystem and rich in diversity (Matsunuma *et al.*, 2011). The sampling area was on the coral reef area in front of Pantai Pasir Cina. The fish specimens were collected within 10 meters depth for safety purposes.

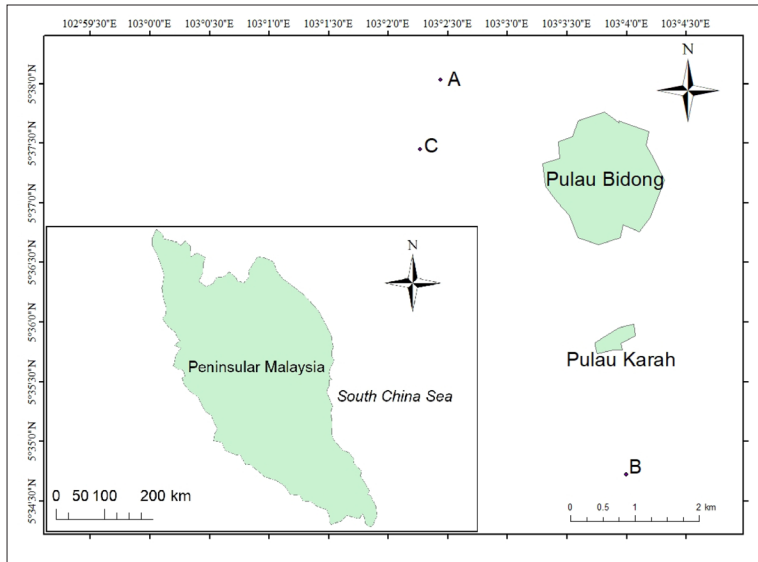


Figure 1: Bidong Island Marine Research Station, South China Sea, Malaysia (5.62°N, 103.07°E). The red circled area indicates the sampling done offshore Pantai Pasir Cina

Sampling and Fish Collection

Divers equipped with scuba diving gears collected the fish host specimens. Clove oil extract was administered via close exposure of the fishes near the coral colonies and worked as a sedative to collect the damselfish. The sedated fishes were collected using 2 feet long scoop net. The sampled fishes were being kept in ice chest with ice and salt to euthanise them humanely. A total of 42 *D. trimaculatus* were collected. The identification of fish was confirmed based on Matsunuma et al (2010). Fish length and weight measurement were recorded. Each fish was placed into separate labelled zipper bags to prevent any cross-contamination of individual hosts.

Parasitological Examination

The fishes' external anatomies were examined, including skin, operculum, gills, eyes and mouth cavity while the fish samples were still fresh. Dissection of fish was initiated by cutting open the anus to the operculum, cutting upward from anus

to the operculum to expose the organs. The muscle, which connects operculum, was removed (Gupta & Mullins, 2010). Internal organs removed and placed on a petri dish. The fish muscle was cut open to examine the muscles under dissecting microscope Olympus SZX7 and Carl Zeiss Stemi DV4 for any apparent cyst or moving parasites and compound microscope Olympus SZX7 with an attached camera Lucida for observing other smaller parasites (e.g., nematodes). The brain mass with diluted with distilled water on pressed on a glass slide using a cover slide. Every parasite sample obtained was preserved in 70% ethanol and labelled separately according to Berland (1984). The calculation of prevalence and mean intensity follows Bush et al. (1997). Images of the parasites were captured using Dino-Eye C piece camera mounted on the above-mentioned dissecting microscopes. For identification of various parasitic groups in damselfish, the authors referred to Lo (1999), Lo and Morand (2000), Nagel and Grutter (2007), Gunter and Adlard (2008), Sasal and Gadenne (2017) and, Santos and Sikke (2017).

Results and Discussion

Fish Collection Data

A total of 42 *D. trimaculatus* were sampled at Pantai Pasir Cina, Bidong Island. Table 1 shows the weight and length data of *D.*

trimaculatus sampled. The mean weight was 5.30 grams and the mean length was 4.87 cm. The *Dascyllus* sampled were fairly small, in comparison to the 11 cm standard length recorded in Matsunuma et al., (2011).

Table 1: Total number, minimum, maximum, mean, standard deviation of weight and length of *D. trimaculatus*

Dascyllus trimaculatus	N	Minimum	Maximum	Mean	Standard Deviation
Weight (g)	42	0.10	43.80	5.30	7.48
Length (cm)	42	2.40	12.20	4.87	2.05

Nematode parasite from the family Camallanidae (Railliet & Henry, 1915)

Phylum: Nematoda
 Class: Chromadorea
 Order: Rhabditida
 Family: Camallanidae

Site of infection: Brain

Prevalence of infection: 42.86% (18 out of 42 fishes were infected)

Mean intensity: 2.72

Diagnostic Remarks:

Fourty nine nematode specimens from the family Camallanidae were found specifically in the brain (Figures 2 and 3).

Species identification posed a challenge due to the lack of obvious external morphology characteristics present such as lip region, eosophageal lumen, intestine, rectum, excretory pore, and tail. The nematodes were small even under microscope ranging 0.075-0.120 mm. The nematodes had a distinct split-end (as seen in Figure 3), suggesting that it belongs to the family Camallanidae (order Rhabditida). Males from the family Camallanidae are known to have two spicules, with some species being hardly visible and could be absent (Santos & Moravec, 2009). Camallanid nematodes are parasites that mainly infects the stomach and intestines (Anderson, 2000), thus finding these nematode parasites in the brain of *D. trimaculatus* is highly unusual.



Figure 2: A nematode parasite likely from the family Camallanidae under microscope magnification 10X



Figure 3: The nematode parasite has two spicules at tail, the parasites found has one split end, which is similar to the description of Camallanidae (scale bar 0.05mm)

Copepod parasites *Lernaeocera branchialis*
(Linnaeus, 1767)

Phylum: Arthropoda
Class: Hexanauplia
Order: Siphonostomatoida
Family: Pennellidae
Species: *Lernaeocera branchialis*

Site of infection: Gills

Prevalence of infection: 47.62% (20 out of 42 fishes were infected)

Mean intensity: 4.95

Diagnostic Remarks:

Ninety-nine *Lernaeocera branchialis* specimens (Figure 4) were collected from

the host's gills. The species is readily diagnosed according to the following features: the cephalothorax has a pair of antennules, which the antennules are four-segmented; a pair of antennae present, at ventrally and posterior of the antennules; the buccal tube is present posterior to the antennae. The copepodid has a frontal filament, which is different compared to other siphonostomatid parasites. Its frontal filament diverges into two when penetrates the host's tissue. While the body of *L. branchialis* consists of four pairs of swimming legs, six thoracic somite, included genital segment, and lastly its caudal rami (Brooker, 2007).



Figure 4: Circled is the frontal filament of *L. branchialis*. Abbreviations: c, cephalothorax; L1, first swimming leg; L2, second swimming leg; L3, third swimming leg; gs, genital segment; cr, caudal rami (scale bar 0.15mm)

Discussion

Parasites of damsel fishes worldwide

Records on parasites of *Dascyllus* fish worldwide are poorly reported (Table 2). Of the many studies, focus mainly pertains to *Dascyllus aruanus* and *D. reticulatus* coming from Australia and French Polynesia, and one report of *D. trimaculatus* from the Philippines. The most reported parasites coming from the host genus *Dascyllus* were monogeneans (family Dactylogyridea), crustacean isopods (family Gnathidae) and one report of myxozoan parasite.

Based on available literature of parasites from *D. trimaculatus*, it was reported that the host collected from the Phillipines (Santos & Sikke, 2017) were parasitised by gnathid crustaceans. In comparison to our study, our hosts were void of these gnathid parasites, thus our findings of the copepod and nematode parasite represent new records of host-parasite association. We further elaborate on the specifics of these parasites under sections "*Nematode parasites of D. trimaculatus from Bidong Island*" and "*Copepod parasites of D. trimaculatus from Bidong Island*".

Table 2: List of studies on *Dascyllus* fish's parasites worldwide and current research

<i>Dascyllus</i> host	Parasites	Infected organs	Location	Reference
<i>D. aruanus</i>	Monogenea Dactylogyridea: Haliotrema	Gills	Mo'orea Island, French Polynesia	Lo, 1999
<i>D. aruanus</i>	Myxozoa Bivalvulida: Myxidiidae	Gall bladder	Great Barrier Reef, Australia	Gunter & Adlard, 2008
<i>D. aruanus</i>	Crustacean Isopod: Gnathiidae	Skin	Great Barrier Reef, Australia	Nagel & Grutter, 2007
<i>D. reticulatus</i>	Monogenea Dactylogyridea: Haliotrema	Gills	Opunohu Bay, Mo'orea Island, French Polynesia	Sasal & Gadenne, 2017
<i>D. aruanus</i>	Monogenea Dactylogyridea: Haliotrema		Mo'orea Island, French Polynesia	Lo & Morand, 2000
<i>D. trimaculatus</i>	Crustacean Isopod: Gnathiidae	Gills	Negros Oriental, Philippines	Santos & Sikke, 2017
<i>D. trimaculatus</i>	Crustacean Copepod: Pennellidae Camallanid nematode	Gills Brain	Bidong Island	Current study

Nematode parasites of Dascyllus trimaculatus from Bidong Island

The family Camallanidae that were found in reef-associated fish were reported to have wide geographical distribution and host species range (Rigby *et al.*, 1997). It is not common for nematode to infest the brain of fish, and Camallanidae does not infest brain of fish. Therefore there is a possibility this case is an accidental parasitism as that of Martin's *et al.* (1970). The author recorded the presence of nematode which infects human has infected the brain of marine mammals. The nematode could not be identified, as a fully mature worm were not available except for unfertilized nematode eggs. Martin *et al.* (1970) suggested that the mammals were infected by ingestion of infected organisms.

Copepod parasites of Dascyllus trimaculatus from Bidong Island

Lernaeocera branchialis has two hosts in its life cycle, an intermediate host and a definitive host. The two nauplius stage are free swimming zooplankton, suggesting that *L. branchialis* is not host specific. Following the nauplius stage is an infective copepodid stage. The copepod will then start to find the intermediate host in their four chalimus stage. The intermediate host for the chalimus stages would be demersal fish and it is usually flounders (Brooker *et al.*, 2007). The copepod will have to find a definitive host to continue its mature adult stage. During the sampling of *D. trimaculatus*, the depth of water was lesser than 15 meters. As mentioned above, intermediate host of *L. branchialis* are demersal fish and the fact

that Pantai Pasir Cina is present in shallow waters, this would allow ease of infestation of *L. branchialis* towards *D. trimaculatus*. Findings of *L. branchialis* being pathogenic on small fish and have been reported to have minimal effect but not depending on the fish length (Knudsen & Sundnes, 1998). *Dascyllus trimaculatus* is small and *L. branchialis* were found on them with light level of infestation and common frequency of infestation.

It is suggested that *D. trimaculatus* is not the definitive host. This is because there is no egg bearing of female of *L. branchialis* found. The mature female *L. branchialis* would not undergo metamorphosis into adult stage of gravid form unless it is in the definitive host (Brooker *et al.*, 2007). The definitive host is usually gadoid fish (Boxshall, 1974). There were many studies on *L. branchialis*, which appeared to commonly infest gadoid fish (Kabata, 1961; Brooker, 2007; Brooker *et al.*, 2007). These studies mostly took place in Atlantic waters, where gadoid fish geographically occur due to temperate waters. Malaysia being in tropical waters are void of these gadoid fish. Furthermore, although *Lernaeocera* were found more prevalent in inshore waters, they appear to be limited in distribution in warmer waters, preferring temperatures below 20 (Brooker *et al.*, 2007).

In an interesting case study in the tropical waters of Bali, Indonesia, Kleinertz (2014) recorded the presence of a pennelidae copepod on the host of *Epinephelus areolatus*, a reef-associated grouper. Matsunuma (2011) has recorded *Epinephelus areolatus*, a reef-associated grouper in waters of Terengganu. We could contemplate that *Lernaeocera* could use *Epinephelus* as a definitive host (in comparison to gadoid fishes of the temperate waters) since Bali and Terengganu occur in tropical waters and both geographical regions are present of the *Epinephelus* species. It would garner much interest to further investigate on the above

hypothetical approach of the life cycle of *Lernaeocera* and its use of various hosts.

Preliminary evidence observation of host size association with parasite

The ectoparasite copepods in this study have noticeably higher prevalence and mean intensity as compared to the nematode endoparasite. This comes to no surprise since ectoparasites live in the outer region of host, and have higher chances of area availability to infest, thus the parasite size correlates positively with prevalence and mean intensity (Poulin, 1999). Large endoparasites are expected to have low mean intensity as they have limited space to grow and reproduce (Poulin, 1999). Both prevalence and mean intensity of ectoparasites could be expected to correlate with the increase in size of host. If the space availability for attachment is larger, the intensity can increase as there is more space to infest, thus the prevalence could be affected as well (Brooker, 2007). However, the copepod in our study were not affected by the increase of size and weight of the fish. Most of the infested fishes were small to average-sized, ranging between 2.4 to 6.4 grams. Poulin (1999) recorded in Sasal *et al.* (in press) stated there is no relationship between ectoparasite body size and prevalence in fish.

The nematode parasite number did not increase with the fish size and weight. Poulin (1999) stated that large nematode should be correlating positively with prevalence but negatively with intensity of infection. They infest higher number of fishes but low numbers per host, as they can produce more eggs. On the other hand, small nematodes could have high intensity in host (Poulin, 1999). The nematodes were mostly found on the smaller fish, but none infects big fishes. Our evidence is in agreement to Poulin's (1999) hypothesis, where ectoparasites were predicted to have low intensity but high prevalence.

Conclusion

The research of parasites on *Dascyllus* fishes in Malaysia needs to be looked into more thoroughly as parasites studies on coral fish in general are still very limited, and to overrule any possibilities of parasites affecting these fragile communities. Most of the previous studies on parasites on *Dascyllus* fishes worldwide resulted in many of the parasites infecting the gills and skin of *Dascyllus* fish. As show in this study and previous records, there are most likely more ectoparasites on coral reef fish compared to endoparasite due to the sheer size of the host.

This study's finding is concerning, as the frequency of prevalence of *L. branchialis* and nematode from the family Camallanidae is at a common stage although the level of infection is light, in comparison to previous studies where only isopod crustaceans and monogeneans were found on *Dascyllus*. However, incorporating molecular tools for identification will further elucidate the species of parasites we are dealing with specifically, and will portray better means of the vulnerability of the *Dascyllus* fishes's biology and means for conservation.

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