



Research Article

## Infestation of isopod *Norileca indica* in big-eye scad *Selar crumenophthalmus* off Panay Gulf, Philippines

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### Abstract

The big-eye scad *Selar crumenophthalmus* is a commercially important fish species in the Philippines and is not exempted from parasite infestation. Thus, study aims to determine the prevalence and intensity of isopod infestation in fish, percentage reduction of respiratory surface area of gill filaments and condition factor of the fish. A total of 274 fish samples (mean FL  $142 \pm 5.88$  mm) were collected and examined from Panay Gulf, Philippines. The prevalence and mean intensity of isopod, *Norileca indica* is 9.49 % and two isopods per fish (mean FL  $142 \pm 5.88$  mm), respectively. In fish samples with infestation in both pair of gills, the percentage reduction in gill area decreases in line with increasing fish length. However, there is no significant difference between the reductions of gill area of uninfested and infested fish ( $p > 0.05$ ). Moreover, there was also no clear trend and no significant difference in the condition factors of infested and uninfested fish ( $p > 0.05$ ). Presence of the parasite may hinder in the food intake of fish and may prevent supply of blood to other parts of fish body which may led to stunted growth of host fish. However, fishes infested with parasite were not significantly considered as less healthy as cause of the infestation of isopod without considering other factors that would affect the fish overall state.

### 1. Introduction

Big-eyed scad (*Selar crumenophthalmus*) of the family Carangidae is a small coastal pelagic fish that is abundantly found in the coastal waters (Fadzly et al., 2017). It is a commercially important fish species in the Philippines. Based on the Philippine Fisheries Profile, *S. crumenophthalmus* ranked 3<sup>rd</sup> in the municipal catch by species contributing 6.8 % (76, 693. 27 MT) in the total production and ranked 4<sup>th</sup> in the commercial fisheries, with 36, 050. 64 MT (4.18 %) production (BFAR, 2022).

*Selar crumenophthalmus*, like any other fish species, is not exempted from parasite infestation as reported by many studies. In the Upper Gulf of Thailand, one species of isopod, *Norileca indica*, was found in the branchial cavity of *S. crumenophthalmus* with prevalence and intensity of species infestation at 31.7 % and 2.4 isopods per fish, respectively (Intamong & Kaewviyudth, 2014). While off Mumbai coast in India, the prevalence, mean intensity and mean abundance of infestation with *N. indica* were found to be 37.94 %, 1.38 isopod per fish and 0.51, respectively (Neeraja et al., 2014). In Panay Gulf, Philippines, the prevalence and mean

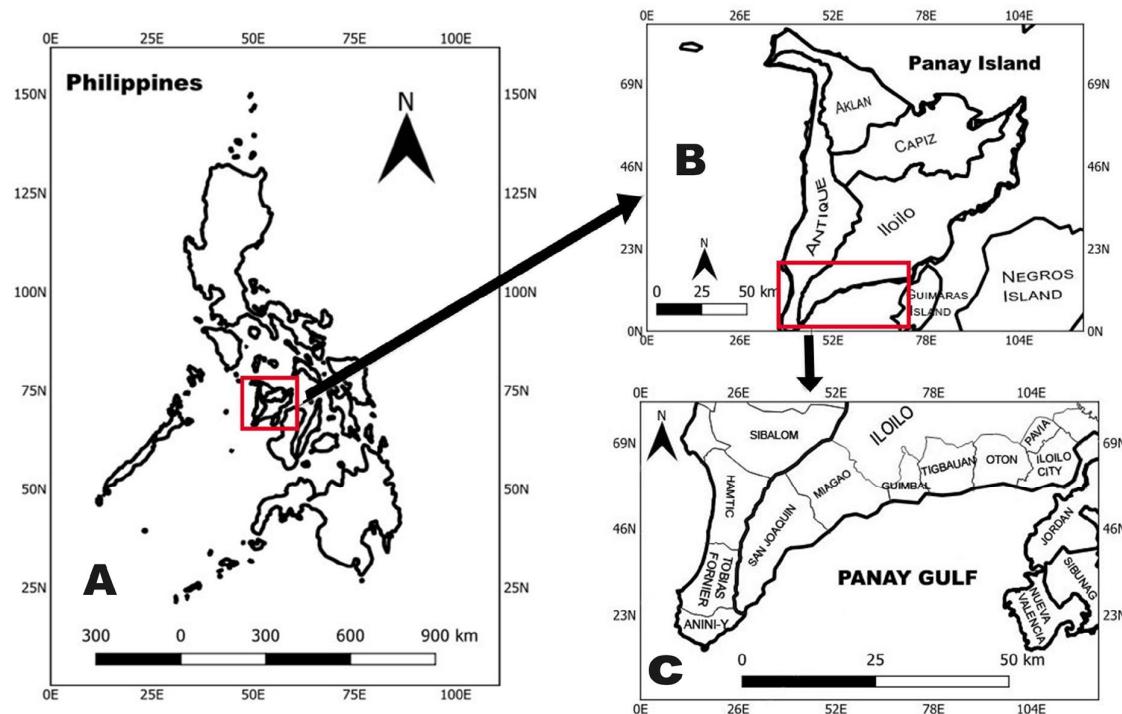
intensity of *N. indica* were 40.7 % and 1.05 isopod per fish for *S. crumenophthalmus* (Cruz-Lacierda & Nagasawa, 2017).

*Norelica indica* was accounted as a common parasite of *S. crumenophthalmus* infesting its branchial cavities (Nagasawa & Petchsupa, 2009). *Norileca indica* is a Malacostraca species belonging to Family Cymothoidae having an expanded mandible palp article 3, pleopods 3 - 4 without folds on endopods, as well as an absence of branchiated pleopod peduncles (Bruce, 1990). In general, the attachment of a parasite to fish can influence the individual host survival and reproduction, as they can change fish behaviour and migration patterns, regulate fish populations, and affect fish community structure (Barber & Poulin, 2002). However, studies on the effects of *N. indica* on *S. crumenophthalmus* in the Philippines is limited. Thus, this study determined the effects of infestation of *N. indica* to the gill structure and physiological state of *S. crumenophthalmus*. Specifically, it aimed to determine the prevalence and intensity of isopod infestation in fish, percentage reduction of respiratory surface area of gill filaments and condition factor of the fish.

## 2. Materials and methods

### 2.1 Fishing ground

Panay Gulf, an extension of Sulu Sea, is located in the central Philippines (**Figure 1**). The expanse of the gulf reached between the islands of Panay and Negros. The gulf is surrounded by the island-province of Guimaras and extends into the Santa Anna Bay between Panay and Guimaras and into Guimaras Strait, between Guimaras and Negros. Panay Gulf is connected to another fishing ground, Visayan Sea through Guimaras Strait. The Port of Iloilo which is located within the gulf is considered as the busiest port in the area. The port is a major route used by various boats navigating between the towns of Iloilo City, Bacolod City and Zamboanga.

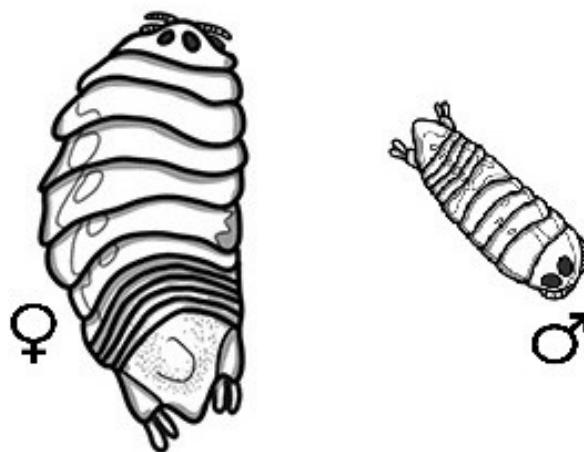


**Figure 1** Study site at Panay Gulf, Philippines. A. Map of the Philippines. B. Map of Panay Island. C. Stretch of Panay Gulf

## 2.2 Test Animal *Selar crumenophthalmus*

A total of 274 fish samples were collected from one fishing trip of a commercial fishing vessel operating with purse seine in Panay Gulf, Philippines in March 2019. All samples collected comprising more than 10 % of the total catch of the fishing vessel were gathered and analyzed. The fish samples were already dead when collected from the fishing vessel thus there is no need for Animal Ethics Clearance (IACUC). The collected fish were kept with ice in a chest and brought to the laboratory. However, only 267 samples were further used for gill filament analysis due to some damaged specimen.

Fork lengths (FL) were measured to the nearest millimeter using a measuring board and weights were obtained to the nearest gram using digital balance. The branchial cavities of fish samples were examined for the presence of isopods. Isopod discovered was removed, identified, and weighed to the nearest gram. Sexes of isopod were determined based on Cruz- Lacierda and Nagasawa (2017) and van der Wal et al. (2017). Female *N. indica* is positioned ventrally in the branchial cavity with the cephalon directed toward the anterior side of the fish and with the body twisted to the left when it was attached in the right branchial cavity and vice versa when attached to the left branchial cavity (Cruz-Lacierda & Nagasawa, 2017) (Figure 2). Its cephalon is 1.1 times longer than width, one eye 0.3 times the length of the cephalon, pereonite extending to middle of cephalon, pereopod 7 basis 0.6 times as long as greatest width, pleopods without setae and uropod more than half the length of pleotelson (van der Wal et al., 2017). Whereas, male bodies were not twisted compared to female and were found along with their female counterparts and occupied the opposite gill cavity (Cruz-Lacierda & Nagasawa, 2017). Its cephalon is 0.79 times longer than wide, one eye 0.6 times length of cephalon, pereonite extending past the posterior margin of eyes, pereopod 7 basis 1.6 times as long as greatest width, uropod same length as pleotelson and has prominent penes (van der Wal et al., 2017). On the other hand, fish gills were taken out using forceps and scissors. The fish gills and isopods were preserved immediately with 10 % buffered formalin.



**Figure 2** *Norelica indica* found in the branchial cavity of *Selar crumenophthalmus*.

## 2.3 Measurement of Gill Filament Reduction

Preserved fish gills were blotted in tissue paper to remove excess moisture. The first pair of gill arches (with gill rakers and filaments) of infested and uninfested fishes were carefully removed. To conveniently outline the gill filaments, the gill rakers were cut from the gill arches using scissors. During gill extraction, some gills were damaged and these were disregarded during analysis.

The influence of infestation on surface area of gill filaments from 267 fish samples were determined according to the method of Ravichandran (2007). The gills were placed in graph paper and the area of the gill filaments with gill arches were outlined using pencil. The imprint drawings of each gill filaments on millimeter graph paper were used to calculate the surface area of gill filaments with gill arches. Surface area of each tracing were determined by counting the number of small squares and the total area obtained. Shrinkage in gill specimen may occur due to formalin; however, these changes were not significant (Parker, 1963; Greszkiewicz & Fey, 2018; Larochelle et al., 2016). On the other hand, fixed isopods were identified to the species level based on their morphological features as recorded by Brusca (1981), Bruce (1987), Bruce (2004) and van der Wal et al. (2017). Individual weights of the isopod were also measured.

## 2.4 Data analysis

The condition factor of the fish was calculated using (Sutton et al., 2000).

$$K = \frac{W \times 100}{L^3} \quad (1)$$

where W is fish weight in grams and L is fish total length in centimeter. While the prevalence (number of infested hosts/number of examined hosts) and the mean intensity (total number of parasites/number of infested hosts) was calculated according to Bush et al. (1997). The surface area of the gills and condition factor of both infested and uninfested fishes were statistically compared using *t*- test. While the difference area was considered as the reduction of respiratory area due to infestation.

## 3. Results

### 3.1 Prevalence and intensity of isopod infestation in fish

A total of 274 fish samples (mean FL 142 mm) were examined in the study. Of 26 fishes infested with *Norileca indica*, 19 were infested with female isopod only and 7 were infested in both pair of gills, where female attached to one side of the branchial cavity and male on the opposite side (**Table 1**). The prevalence and mean intensity of *N. indica* were 9.49 % and two isopods per fish (FL 93- 155 mm). One fish sample had parasite in both gills with 15 offsprings still attached with the gills.

### 3.2 Percentage reduction of respiratory surface area of gill filaments

**Table 1** shows the uninfested and infested gills of *S. crumenophthalmus*. In fish samples with infestation in both pair of gills where female had a counterpart male on the other side of the branchial cavity, the percentage reduction in gill area decreases in line with increasing fish length. The highest percentage reduction of gill area was apparent in fish with FL < 130 mm. However, there is no significant difference in the gill surface area reduction between uninfested gill and gill infested with female isopod ( $p < 0.05$ ). There is also no significant difference in the gill surface area reduction between uninfested gill and gill infested with male isopod ( $p < 0.05$ ).

In fish infested with female isopod only (no infestation or no male isopod on the opposite branchial cavity), there is no clear trend on the reduction of the gill surface area. The highest percentage of gill area reduction can be observed in fish with FL 141- 145 mm. Furthermore, there is no significant difference in the gill surface area reduction between uninfested gill and infested gill ( $p < 0.05$ ). The parasite weight was directly proportional to the fork length of the host fish in this category.

**Table 1** Uninfested and infested gills of *S. crumenophthalmus*.

Fork	Uninfested fish			Infested fish (infested with female and male <i>N. indica</i> )								Infested fish (infested with female <i>N. indica</i> only)					
				Gill infested with Female <i>N. indica</i>				Gill infested with Male <i>N. indica</i>				Uninfested	Gill infested with Female <i>N. indica</i>				
	no. of Gill	no. of fish samples	area (mm <sup>2</sup> ) (U)	Gill area (mm <sup>2</sup> ) (F2)	Difference (mm <sup>2</sup> ) (U-F2)	Gill reduction (%)	Ave wt of parasite (g)	Gill area (mm <sup>2</sup> ) (M2)	Difference (mm <sup>2</sup> ) (U-M2)	Gill reduction (%)	Ave wt of parasite (g)		Gill area (mm <sup>2</sup> ) (U1)	Gill area (mm <sup>2</sup> ) (F1)	Difference (mm <sup>2</sup> ) (U1-F1)	Gill reduction (%)	Ave wt of parasite (g)
Length (mm)	fish samples	area (mm <sup>2</sup> ) (U)															
<130	3	174.50	2	128.50	46.00	26.36	0.32	138.50	36.00	20.63	0.0030	1	193.00	189.00	4.00	2.07	0.11
131 - 135	8	240.44	0	-	-	-	-	-	-	-	-	0	-	-	-	-	-
136 - 140	68	241.63	4	192.50	49.13	20.33	0.52	228.00	13.63	5.64	0.0650	6	263.17	207.17	56.00	21.28	0.43
141 - 145	104	255.37	1	209.00	46.37	18.16	0.43	250.00	5.37	2.10	0.0500	8	272.88	213.38	59.50	21.80	0.51
146 - 150	56	265.38	0	-	-	-	-	-	-	-	-	4	290.75	232.00	58.75	20.21	0.54
> 151	2	312.25	0	-	-	-	-	-	-	-	-	0	-	-	-	-	-

Note: U indicates gill area of uninfested fish; F2 indicates gill area of fish infested by female isopod (with male isopod counterpart on the opposite brachial cavity); M2 indicates gill area of fish infested by male isopod (with female isopod counterpart on the opposite brachial cavity).

U1 indicates the uninfested gill area of fish (with infestation of female isopod on the opposite brachial cavity); F1 indicates gill area of fish infested with female isopod only (no infestation on the opposite brachial cavity).

### 3.3 Condition factor of the fish

**Table 2** shows the condition factor of infested and uninfested *S. crumenophthalmus*. The highest condition factor in the infested fish can be observed in fish with FL < 130 mm. In contrast, lowest condition factor in the uninfested fish can be also noted in fish with FL < 130 mm. However, there is no significant difference between the condition factor of fish infested and uninfested with isopod ( $p < 0.05$ ).

**Table 2** Condition factor of infested and uninfested *S. crumenophthalmus*.

Fork length (mm)	Uninfested fish		Condition factor	Infested fish	Condition factor	<i>t</i> - test
	Condition factor	Condition factor				
< 130	1.80 ± 0.042			1.96 ± 0.022		ns
131 - 135	1.94 ± 0.032			-		-
136 - 140	1.93 ± 0.010			1.92 ± 0.030		ns
141 - 145	1.89 ± 0.008			1.84 ± 0.037		ns
146 - 150	1.85 ± 0.012			1.87 ± 0.042		ns
> 151	1.90 ± 0.049			-		-

#### 4. Discussion

This study found that only one species of parasitic isopod, *Norileca indica* infested the pelagic fish *Selar crumenophthalmus* off Panay Gulf, Philippines. Occurrence of *N. indica* for *S. crumenophthalmus* was already documented in the Philippines (Cruz-Lacierda & Nagasawa, 2017; Muji et al., 2021). In this study, the prevalence and mean intensity of *N. indica* are 9.49 % and two isopods per fish (FL 93 - 155 mm), respectively. Compared with the study of Cruz-Lacierda and Nagasawa (2017) in Panay Gulf, the prevalence of infestation in this study is lower however the intensity is higher. The prevalence and mean intensity of *N. indica* in the study of Cruz-Lacierda and Nagasawa (2017) were 40.7 % and 1.05 for *S. crumenophthalmus* ( $n = 81$ , 128 - 228 mm TL). Similarly, in the upper gulf of Thailand, only one species of isopod, *N. indica*, was found in the branchial cavity of *S. crumenophthalmus*. The prevalence and intensity of this species infestation were 31.7 % and 2.4, respectively (Intamong & Kaewviyudth, 2014). Likewise, *S. crumenophthalmus* collected off Mumbai coast (south-west coast), Arabian Sea, India found to be also infested with the cymothoid isopod parasite *N. indica* where the overall prevalence, mean intensity and mean abundance of infestation with *N. indica* were found to be 37.94 %, 1.38 and 0.51, respectively (Neeraja et al., 2014).

The samples in this study was collected in the month of March and some studies showed higher prevalence in this period. A study conducted along the Malabar Coast of India recorded a maximum prevalence of *N. indica* in *Rastrelliger kanagurta* in March and minimum prevalence in September (Kottarathil et al., 2019). In the same way, highest prevalence of the parasite was also observed during March (50 %) and the least in September (20 %) in Thiruvananthapuram coast, South India (Suresh et al., 2021). However, there were also sites that showed high prevalence in the month of August. Months of August and September recorded significant ( $p < 0.05$ ) higher prevalence and abundance of this parasite in *Secutor insidiator* in Visakhapatnam waters along northwest Bay of Bengal (Behera et al., 2016). Moreover, highest prevalence of infestation, mean intensity and abundance in *R. kanagurta* were recorded in the month of August 2018 in Southwest coast of India (Job et al., 2020).

The cyclic pattern of change in prevalence with the seasons indicates that seasonal environmental parameters apparently have a role in the obligatory ectoparasitic life in the host fish (Kottarathil et al., 2019). The prevalence of isopod could be dependent on environmental parameters like rainfall, salinity, and temperature (Suresh et al., 2021). Rains and flooding can both influence depth and movements of water, hence can indirectly affect parasite prevalence (Muji et al., 2021). Salinity indicates the rate of dilution of seawater by land water discharges (Zyadah et al., 2004). The low level of the prevalence during the monsoon and post monsoon months is likely induced by low salinity due to heavy rainfall and gradual increase in salinity until the end of the post- monsoon and winter months (October to January) seems facilitate its gradual parasitic infection (Kottarathil et al., 2019).

The prevalence was significantly highest during the pre- monsoon/ summer (February - May) reaching a maximum in March (Kottarathil et al., 2019). Isopod prevalence vary seasonally especially during warmer months (Garcia-Guerrero & Hendrickx, 2003) since water temperature showed a significant positive correlation with the isopod infestation rate (Mahmoud et al., 2019). Higher prevalence in pre- monsoon (February - May) may be due to the effect of increased salinity (Suresh et al., 2021). Salinity usually peaks in the summer (February - May), which might be favorable for the parasite to infect its host fish (Aneesh et al., 2013; Kottarathil et al., 2019). The levels of Oxidizable Organic Matter (OOM) were extremely high in spring and summer seasons (15 to 20 mg O<sub>2</sub>/L, respectively) (Mahmoud et al., 2019). This high load condition of organic matter provides food sources to the most of cymothoid isopod parasites resulting for parasites to become 2 - 3 times more efficient during this period (Danovaro, 2003). The suitable environment conditions of high salinity and abundance of food, represented in fish, give rise to the overall high prevalence (Ali & Aboyadak, 2018).

The parasite- host interactions among isopods and marine fishes have shown high parasitic prevalence in association with high abundance of the host fish (de Carvalho-Souza et al., 2009). This help parasites to find the host easily due to higher host availability (Job et al., 2020). The period of sample collection in this study coincide with the results of Dalzell and Penaflor (1989) where minor peak of production of *S. crumenophthalmus* caught by ring nets in the Moro Gulf, Philippines occurred between March and April. However, other fishing ground in the Philippines such as Camotes Sea showed pronounced peak of catch per effort during November to January (Dalzell & Penaflor, 1989). This trend is also parallel to that for *S. crumenophthalmus* landed into Navotas from all parts of the Philippines (Dalzell & Ganaden, 1987).

Parasitic infestation of finfishes also depends upon host factors such as age, size, sex, maturity stage, behavior, feeding and breeding (Behera et al., 2016; Vinoth et al., 2010; Ravi & Rajkumar, 2007). However, there is no apparent trend on the relationship of fork length of host fish and number of isopod parasite in this study. This supports the results of Neeraja et al. (2014) which indicated that there is no significant difference was observed in the prevalence, mean intensity and abundance of *N. indica* infestation on different length classes of *S. crumenophthalmus* and this can be attributed to more similarity in physiological (matured) condition of the hosts observed, which is more or less equally preferred by *N. indica*.

Other studies reported that there was a parasitic preference towards certain body sizes which varies depending upon the species of the host (Romestand et al., 1977). Results of Muji et al. (2021) indicates that as the length of fish increases, the length of the isopod increases at an exponentially decreasing rate up to a certain extent. There is a positive relationship between parasite size and host size because large bodies are correlated with high fecundity and therefore a large body size is selected for when larger host are present (Moranda & Sorcib, 1998). Furthermore, the positive correlation between the body length of non-ovigerous female and male isopods and their host total length suggests that infection may occur when fish are still small and that the parasite grows with their host (Cruz-Lacierda & Nagasawa, 2017). On the contrary, Behera et al. (2016) suggested that *N. indica* infestation were higher in relatively smaller fishes of *R. kanagurta* and *Nemipterus randalli* because of the physiological condition of the smaller fishes which were preferred by *N. indica*.

Aside from host size preference, parasitic infestation may also be influenced by the breeding season of the host. Cymothoid populations are variable, breeding seasons of host fish were found to be consistent (Muji et al., 2021) and isopod prevalence rates are affected by breeding seasons of the host (Jemi et al., 2020). The spawning season of *S. crumenophthalmus* in Hawaii was found to occur between April and September (Clark & Privitera, 1995). In the study of Muji et al. (2021) in Batangas, Philippines, the highest number of fish infected with isopods was the sample collection in September. Since spawning season had already taken place during the time of the study, the increased host populations in September most likely provided the isopods greater chance to infect more fish (Muji et al., 2021). It was also observed that higher prevalence and intensity of parasitic infestation was monitored in female hosts than that in male hosts (Ravichandran et al., 2009; Behera et al., 2016; Neeraja et al., 2014). This could be due to the lowering of estrogen levels in female fishes during breeding season which makes them more susceptible to parasitic infections (Thomas, 1964).

Results of this study revealed that fishes parasitized by both male and female isopods showed that as the fish fork length increases, the percent reduction of gill surface area decreases. The reduction in the surface area of gill is due to several factors such as mode of contact, size, movement and duration of stay of the parasites (Ravichandran, 2007). Fish with highest percent reduction of gill area was apparent in fish with  $FL < 130$  mm. This may be due to nutritional drain by the parasite that led to retarded growth of the host fish (Ravichandran et al., 2009; Sethi, 2012; Ravi & Rajkumar, 2007). Although, the infestation did not cause immediate death, it had affected the normal growth of the host fish (Intamong & Kaewviyudth, 2014). The female isopods was deeply pressed against the gill filaments of first gill arch and occupied two third surface of the first gill (Neeraja et al., 2014) or even filled the entire branchial chamber of the host which may produce pressure on the gill

surface (Intamong & Kaewviyudth, 2014). Thus, causing pressure atrophy, necrosis and stunted growth of corresponding gill filaments (Neeraja et al., 2014). Its histological changes were characterized by damage in the epithelial cells and/or hyperplasia, lamellar swelling, telangiectasis and aneurysm (Rameshkumar & Ravichandran, 2013). Cymothoid is one of the largest fish parasites responsible for obvious gill tissue destruction (Rameshkumar & Ravichandran, 2013). Damage of the gill filaments had led to reduction in respiratory surface area of the first gill (Neeraja, 2014). The parasite also prevents blood supply to reach the majority of gill filament leading to atrophy and necrosis followed by the sloughing of the affected part (Ali & Aboyadak, 2018). Although gill surface area reduction in fishes was observed in this study, the condition factor of infested and uninfested fish was not significantly different ( $p > 0.05$ ).

Results also agree with the study of Ridanovic et al. (2015) which revealed that there was no statistical significance in condition factor parameter between the number of protozoa parasite infected and non-infected fish individuals ( $p < 0.05$ ). Trilles (1979) also found that several species of European cymothoids were capable of slowing down the growth in their hosts, although they did not affect weight-size ratios of the fish. It can be inferred that the rate of growth by weight and average size was not affected much due to infestation (Ravi & Rajkumar, 2007). Since the assigned size groups in this study have only slight difference (5 mm only), it is expected that condition factor may not also vary much. Furthermore, other researchers stated that individual fish infected with single parasite were not significantly “less healthy” than non-parasitized fish, and only in cases of multiple infections or stress, would an effect on condition of hosts be relied (Brusca, 1981). Some authors also stated that the condition factor of fish populations and health state of the populations represents basic parameters for measuring impact of humans on ecosystems and biotops (Treer et al., 2000). The unfavorable environment circumstances deteriorate the physiological condition of fish lowering their immune defense mechanisms, therefore, rendering fish susceptible to numerous opportunistic infections (Elgendi et al., 2017).

## 5. Conclusions

*Norileca indica* was observed to infest *Selar crumenophthalmus* off Panay Gulf, Philippines at 9.49 % overall prevalence and intensity of two isopods per fish. The sampling period (month of March) of the study was found to be favourable for isopod to thrive due to increased in temperature and subsequently increased in water salinity and high level of organic matter at this time. Organic matter served as food source of the isopod so it can grow and reproduce. Furthermore, availability of host was also high at this period since it corresponds to the minor peak production of *S. crumenophthalmus* in the Philippines. Nevertheless, the infestation of the isopod did not affect the well-being of the fish since the condition factor is not only caused by a single factor such as parasite but a combination of multiple stressors including unfavourable ecosystem.

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