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Research Article

EFFECT OF ENDOPARASITES IN THE LENGTH-WEIGHT RELATIONSHIP AND DEVELOPMENTAL STABILITY OF THE THREADFIN BREAM *NEMIPTERUS JAPONICUS* (BLOCH, 1791)

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ABSTRACT

Nemipterus japonicus is one of the most convenient hosts in studying parasites because of its association with polluted marine ecosystems making it as more susceptible to parasitism. Herein, the effect of endoparasites in the length-weight relationship (LWR) and developmental stability of *N. japonicus* were investigated through the use of linear regression analysis and fluctuating asymmetry (FA), respectively. There were 30 infected and 30 non-infected samples of *N. japonicus* collected from the two sites in Mindanao, Philippines then measured morphometrically and examined parasitologically. Results showed that four species of endoparasites were found in the intestine of *N. japonicus*, with *Acanthocephalus* sp. as the most abundant, most prevalent and has the highest mean intensity in Sangali while *Anisakis* sp. in Maluso. Linear regression analysis revealed that all of the samples collected from the two sites exhibit negative allometric growth pattern ($b<3.0$), strongly correlated ($r>0.70$) and found to be highly significant ($P<0.05$), indicating that as the body length of *N. japonicus* increases; its body weight becomes less rotund. The Procrustes Analysis of Variance (ANOVA) showed insignificant levels of FA for non-infected *N. japonicus* while those infected *N. japonicus* yield significant FA value ($p<0.001$) for both sites, implying that the presence of parasites can affect the developmental stability of *N. japonicus*. Findings suggest that parasites can increase the levels of FA due to the stress it induced in the development of the organism and demonstrates that LWR and FA are useful tools in studying the conditions of fish populations in their habitat.

Keywords: Fluctuating asymmetry, Growth pattern, Nemipterids, Parasitism, Water pollution.

INTRODUCTION

Parasitic worms associated with fishes posed a threat to the fish abundance (Amaechi, 2015) for it can considerably affect the physiological, reproductive, physical aspects (Iwanowicz, 2011) and most probably the economic importance of fishes (Rohde, 1993). Fishes are the most convenient biological entity in studying parasites as they are hosts for direct and complex life cycles of the parasites both as final and intermediate hosts. For instance, the threadfin bream *Nemipterus japonicus* or locally known as "Kulisi", is an economically important Nemipterid fish in the Philippines. It is characterized by having an elongated compressed pinkish color body with eleven to twelve pale golden-yellow stripes along its body (ElHaweet, 2013). Since Nemipterids are bottom dwellers, they are mostly affected by the polluted environment where they inhabit in marine ecosystems (Diana & Manjulatha, 2012). The bottom dwelling behavior of *N. japonicus* could be a major contributing factor making them as more susceptible to physical abnormalities and diseases that appears to be associated with parasitism (Diana & Manjulatha, 2012). Fish infections caused by parasites tend to increase with increased level of pollution (Palm, 2011) by increasing the number of secondary hosts and vectors. The increased level of pollution may result to fish distress, a condition that occurs when fish cannot adapt well to their surrounding

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habitat (Ihwan, 2016), resulting to poor immune systems (Binuramesh *et al*., 2005), low fitness performance and destabilize fish development (Almeida *et al*., 2008).

The length-weight relationship (LWR) is important in studying fish biology for stock assessment models and estimation of biomass from length observation of its populations (Abdurahiman *et al*., 2004). It can also estimate the rate of fish feeding and the maturity of fishes. On the other hand, developmental stability is the ability of an organism to buffer its development against genetic or environmental disturbances encountered during development, making it as a fundamental characteristic of development (Pojas & Tabugo, 2015). The most convenient tool used to study the developmental stability of an organism is the fluctuating asymmetry (FA) which measures the variations from symmetry of a symmetrical structure whose sides are said to be genetically identical and experiencing the same environment (Pojas & Tabugo, 2015). LWR provides valuable information in modeling the marine ecosystems where the fish inhabit (Pauly, 1984) while FA has been used as a potential indicator of ecological stress in populations (Trono, 2015) and with these, both are useful tools that can be used to study the conditions of fish populations.

Studies about the effects of parasitic infections on the growth and development of fishes are in scarce (Diana & Manjulatha, 2012) and as added by Pereira, (2015) there should be enough information on the occurrence of parasites associated with marine fishes in their natural habitats especially its effect on the growth and development of fishes. Thus, this study was conceptualized to investigate the probable relations of the presence of parasite and its effect in the LWR and developmental stability of *N. japonicus* FA levels through the use of linear regression analysis and FA, respectively.

MATERIALS AND METHODS

Description of the sampling sites

The Zamboanga Fish Port Complex (ZFPC) located in Barangay Sangali, Zamboanga City is situated at approximately 7°4'13" North and 122°12'43" East while Municipal Fish Port in Maluso (MFPM) located in the Municipality of Maluso in Basilan Province is situated at approximately 6°33'0" North and 121°52'48" East. ZFPC is considered as the largest marine products processing center in Zamboanga Peninsula and MFPM is located just off the southern coast of Zamboanga Peninsula and considered as one the largest landing centers in Sulu archipelago. The diverse and high quality marine products that are being produced in these ports led to the strengthening of the trade links in Mindanao. Figure 1 shows the map with the actual sites of the two fish port landing centers in Mindanao, Philippines.

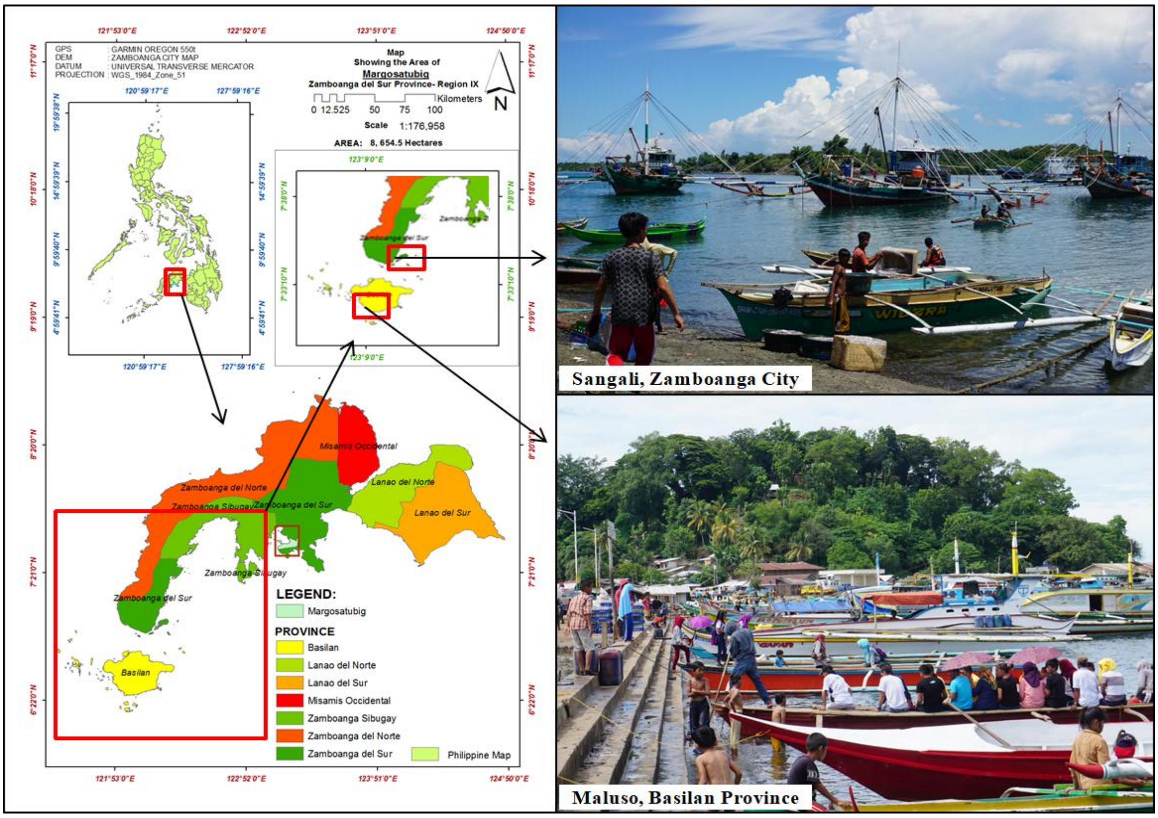

Figure 1. Map showing the geographical location and the actual sites of the two fish port landing centers in Mindanao, Philippines: Zamboanga Fish Port Complex (ZFPC) in Sangali and Municipal Fish Port in Maluso (MFPM).

Collection of samples

In each sampling site, 30 samples of infected *N. japonicus* and 30 samples of non-infected *N. japonicus* were randomly chosen as representative of each population, having a total of 120 specimens. The sexes of the fish samples were not identified. The samples were placed in an ice chest with proper labels then placed in a container filled with ice. Samples were brought in a laboratory room of the Department of Biological Sciences, College of Science and Mathematics, Western Mindanao State University, Zamboanga City, Philippines for further examinations.

Morphometric measurements

The total body length of infected and non-infected samples of *N. japonicus* was determined by measuring the distance from the tip of the nose to the longest ray in the caudal fin of *N. japonicus* to the nearest 1 cm using a ruler. The fresh weight of *N. japonicus* was weighed to the nearest 0.1 g using a Digital Balance. Figure 2 shows the body length measurement for *N. japonicus*.

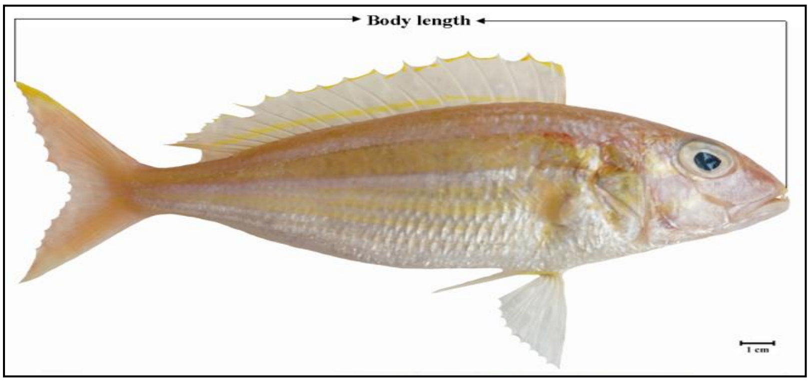

Figure 2. *N. japonicus* showing the measurements of its body length.

Parasitological examinations

The fish samples were examined for the detection of endoparasites. Fish samples without endoparasites were marked as non-infected and for those with endoparasites, samples were marked as infected. Each fish samples were opened up dorso-ventrally and the internal organs were examined. The entire digestive systems were removed and placed in a Petri dish with saline water. The stomach and intestine were washed with tap water after collecting endoparasites. The inner linings were scraped with scalpel to examine for microscopic parasites. The scraping was mixed with 10 drops of 0.8% saline water in a petri dish. A drop of a mixture was placed in a slide for microscopic examination. Slide was examined under stereomicroscope (Aloo-Obudho *et al*., 2004). For the preservation of endoparasites, samples were preserved in alcohol formalin acetic acid (AFA). AFA was prepared by adding 5 ml glacial acetic acid and 5 ml formalin to 90 ml of 70% ethyl alcohol (Echem *et al*., 2018).

Identification of endoparasites

Images of endoparasites were photo-documented using ILCE SONY α2000 23.4 Megapixel prior for identification. All parasites were identified based on the comparison of distinctive body shapes and the morphological features those described in literature. The identification guide by Pouder (2014) was also used for proper identification of the major taxa of adult parasites of fish. Lastly, all of the identified endoparasites were validated by an expert to the lowest possible taxon.

Assessment of infected samples

For the assessment of infected samples, the method of (Margolis *et al*., 1982) was used for the computation of the percentage abundance, prevalence and mean intensity of parasites. The formula for calculation can be referred as follows:

$$Percentage\ abundance = \frac{Number\ of\ individuals\ of\ a\ specific\ parasite}{Total\ number\ of\ individuals} \times 100$$

$$Prevalence = \frac{Number\ of\ infected\ fish}{Number\ of\ examined\ fish} \times 100$$

$$Mean\ intensity = \frac{Number\ of\ parasites\ found}{Number\ of\ infected\ fish}$$

Regression analysis

Linear Regression Analysis was used to determine the relationship of the length and weight of *N. japonicus* using Paleontological Statistics version 3.0 (PAST 3.0). The relationship between the length and weight of a fish is usually expressed by the equation *W=aLb* (Siuashanthini, 2012) where, *W* is the weight of the fish, *L* is the total length, "*a*" is the intercept and "*b*" is the slope.

Measurement of fluctuating asymmetry

In coordinate acquisition, each fish sample from each population was photographed on its both sides using ILCE SONY α2000 23.4 Megapixel where images were oriented in the same position. A total of 23 landmark points were digitized using the tpsDig software. The location of the landmarks and the anatomical descriptions of each are presented in Figure 3 and Table 1, respectively.

Symmetry and Asymmetry in Geometric Data (SAGE) program version 1.0 was used to evaluate the FA levels of *x* and *y* coordinates of the landmarks per individual for both sides of the body of *N. japonicus*. Procrustes superimposition analysis was performed with the original and mirrored configurations of the right and left sides, simultaneously. Procrustes ANOVA tests were performed to assess the significance of Symmetry (individuals), Sides (directional asymmetry; DA), Individuals × Sides (fluctuating asymmetry; FA), and their respective error were also included as effects. In addition, Principal Component Analysis (PCA) was performed to detect the components of variances and deviations for the samples to carry out an interpolation based on a thin-plate spline and then visualize shape changes as landmark displacement in the deformation grid (Marquez, 2006).

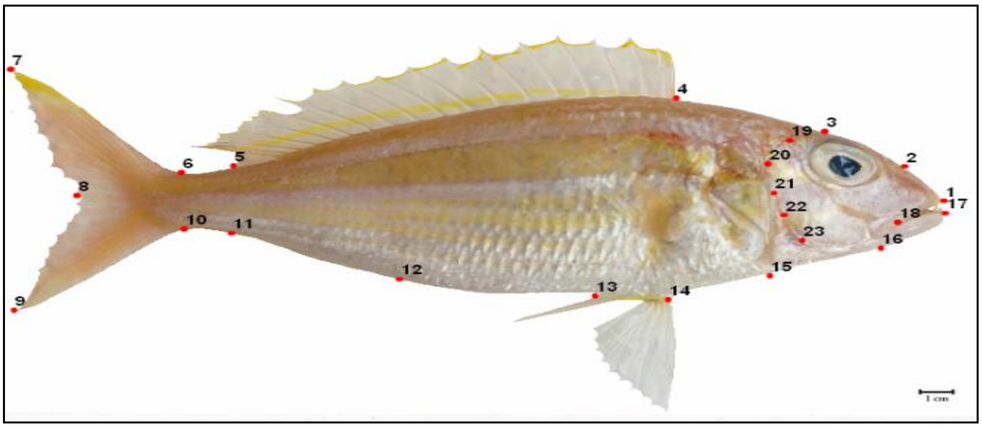

Figure 3. Location of 23 landmark points on *N. japonicus*.

Table 1. Description of landmark points in the body shape of *N. japonicus*.

Landmark points	Descriptions	Landmark points	Descriptions
1	Anterior tip of the premaxilla	13	Posterior insertion of the pelvic fin
2	Anterior tip of the snout	14	Anterior insertion of the pelvic fin
3	Dorsal margin of the body, point superior to the eye	15	Lower beginning of the operculum
4	Anterior insertion of the first dorsal fin	16	Ventral margin of the body, point inferior to the eye
5	Posterior insertion of the first dorsal fin	17	Anterior tip of the lower jaw
6	Dorsal insertion of the caudal fin	18	Point of convergence between the dorsal and the ventral mandible
7	Postero-dorsal tip of the caudal fin	19	Point below the upper beginning of the operculum
8	Distal tip of the caudal fin's middle fin ray	20	Curvature located inferior to the upper beginning of the operculum
9	Postero-ventral tip of the caudal fin	21	Point above the black spot located at the edge of the opercle
10	Postero-ventral insertion of the caudal fin	22	Antero-dorsal insertion of the pectoral fin
11	Posterior insertion of the second anal fin	23	Superior starting point of the curvature located superior to the lower beginning of the operculum
12	Anterior insertion of the second anal fin		

RESULTS AND DISCUSSION

Morphometric studies are necessary to determine the growth form and growth rate of a fish for the proper management of its populations (Simon *et al*., 2010). Larger body length and heavier body weight are reflected in the samples collected in Maluso both for infected and non-infected samples (Table 2). Fish samples collected from both sites have not reached the sexual maturity as their total body length ranged only from 15 cm to 19 cm. Accordingly, *N. japonicus* attained sexual maturity as their body measured a total length of 24 cm. However, the timing for the collection of samples in the sampling site could be one of the factors for these differences (Kalhoro *et al*., 2014).

The occurrence of the parasite infection in the wild habitats is a normal phenomenon but the increasing number of the parasites can occur by some reasons such as water pollution (Iwanowicz, 2011). Parasitological examinations revealed that there are four identified endoparasite species present in the intestines of infected *N. japonicus* collected from the two sites in Mindanao, Philippines. One species belongs to Phylum Acanthocephala while three species belong to Phylum Nematoda. Figure 4 shows the morphological features of the four identified endoparasite species found in the intestine of *N. japonicus* viewed under stereomicroscope.

A noticeable characteristic of *Acantho cephalus* sp. is its proboscis armed with a hook, used for the attachment to the wall of the intestine of the fish host (Figure 4A). *Anisakis* sp. has a thin, cylindrical and characterized by its distinctive watch-spring coiled unsegmented body shape (Figure 4B). *Capillaria* sp. is characterized by its long and slender body shape and it has no segmentation (Figure 4C). *Eustrongyloides* sp. has a long, thick and unsegmented slender body shape (Figure 4D). These endoparasites species are found in the intestine of *N. japonicus*, this could be due to digestion process of *N. japonicus* and can cause damage in the intestine of the fish (Echem *et al*., 2018).

Among the four identified endoparasite species, *Acanthocephalus* sp. and *Anisakis* sp. are only found from the fish samples collected in Maluso and Sangali, respectively. These two endoparasite species are the most abundant, most prevalent and has the highest mean intensity found from the two respective sites, while, *Capillaria* sp. is the only endoparasite species that is common to all sites and exhibits the least abundant, least prevalent and has the lowest mean intensity (Table 3). The prevalence and intensity of endoparasites can be influenced by the life cycle of parasite species, its host and its feeding habits. The hygienic condition of the water bodies are also important to be considered in keeping the marine habitat free from introduction in any parasitic contamination (Hussen *et al*., 2012). Accordingly, if the value of b is equal to 3.0, it indicates that the growth of fish is isometric, meaning shape of fish does not change as it grow, while if the value of b is greater than 3.0, it indicates that the growth of fish is positive allometric, meaning as the length of fish increases, its weight becomes heavier and if the value of b is less than 3.0, it indicates that the growth of fish is negative allometric, meaning as the length of fish increases, its weight becomes less rotund (Zubia *et al*.,2014).

Herein, all of the samples collected from the two sites exhibit negative allometric where $b<3.0$, which suggest that as the body length of *N. japonicus* increases, its body weight becomes less rotund. This is due to the fact that as the fish grows, the amount of food it consumes which includes the different stages of parasites also increases, thus affecting its body weight (Aloo-Obudho et al., 2004). However, the value of b depends on several factors such as the body shape, maturity stage and the amount of fats present inside the body of fish (Moutopoulos & Stergiou, 2002) and vary seasonally depending on the habitat of organism (Özaydin & Taskavak, 2006). On the other hand, correlation coefficient (r) value for all the samples are greater than 0.70 in all instances, indicating a strong correlation of the LWR of *N. japonicus* and found to be highly significant where $P<0.05$. Results of the LWR of *N. japonicus* are shown in Table 4 and the plots of regression analysis are presented in Figure 5 showing the distribution of variables.

Table 2. Mean and standard error data of the morphometrics of infected *N. japonicus* collected from the two sites in Mindanao, Philippines.

Samples	Body length (cm) (Mean±SE)	Body weight (g) (Mean±SE)
Sangali infected	16.053 ± 0.166	56.097 ± 1.853
Sangali non-infected	15.617±0.766	53.783±1.877
Maluso infected*	19.277 ± 0.236	93.523 ± 3.687
Maluso non-infected*	19.623±0.253	95.764±3.724

Table 3. Summarized data for the percentage abundance, prevalence and mean intensity of the identified endoparasites found in the infected *N. japonicus* collected from Sangali, Zamboanga City (N=30) and Maluso, Basilan Province (N=30).

Endoprasites	Number of fish affected	Number counted	Percentage abundance (%)	Prevalence (%)	Mean intensity
Sangali, Zamboanga City					
Anisakis sp.	15	17	53.125	50	0.57
Capillaria sp.	3	3	9.375	10	0.10
Eustrongyloides sp.	12	12	37.500	40	0.40
Total	30	32	-	-	-
Maluso, Basilan					
Acanthocephalus sp.	24	30	81.081	80	1
Capillaria sp.	6	7	18.919	20	0.23
Total	30	37	-	-	-

Table 4. Length-weight relationship of infected and non-infected *N. japonicus* samples collected from the two sites in Mindanao, Philippines.

Samples	Intercept (*a*)	Slope (*b*)	Correlation coefficient (*r*)	Permutation (*P*)
Sangali infected	-1.0178	2.2872^{-AM}	0.8449^{SC}	0.0001**
Sangali non-infected	-1.3012	2.5333^{-AM}	0.7065^{SC}	0.0001**
Maluso infected	-1.7896	2.9208^{-AM}	0.8909^{SC}	0.0001**
Maluso non-infected	-1.8858	2.9858^{-AM}	0.9410^{SC}	0.0001**

Legend: IM - Isometric if $b=3.0$; +AM - Positive allometric if $b>3.0$; -AM - Negative allometric if $b<3.0$; SC - Strong correlation when $r>0.70$; MC - Moderate correlation when $r>0.50$; WC - Weak correlation when $r<0.50$; **show highly significant; and *show significant relationships at $\alpha=0.05$.

Figure 4. External morphology of identified endoparasites viewed under stereomicroscope; (A) *Acanthocephalus* sp. (B) *Anisakis* sp. (C) *Capillaria* sp. and (D) *Eustrongylo ides* sp.

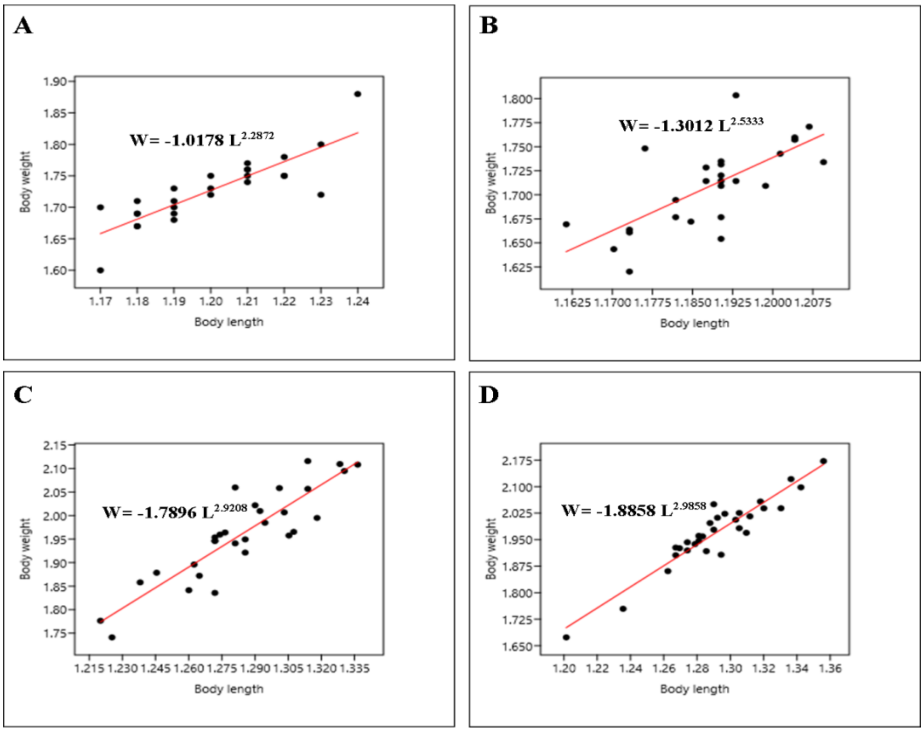

Figure 5. Plot for the length-weight relationships of *N. japonicus*; (A) Sangali infected samples, (B) Sangali non-infected samples, (C) Maluso infected samples, and (D) Maluso non-infected samples.

Table 5. Procrustes Analysis of Variance (ANOVA) results for the infected and non-infected samples of *N. japonicus* from the two sites in Mindanao, Philippines.

Effects	SS	Df	MS	F	*p*-value	Significance
			Sangali Infected Samples			
Individuals	0.0943	1218	0.0001	1.5153	0.0000**	Highly significant
Sides	0.0212	42	0.0005	9.9011	0.0000**	Highly significant
Individuals × Sides	0.0622	1218	0.0001	1.1066	0.0014*	Significant
Measurement error	0.2327	5040	0.0001	---	---	
			Sangali Non-infected Samples			
Individuals	0.0657	1218	0.0001	1.5733	0.0000**	Highly significant
Sides	0.0305	42	0.0007	21.1936	0.0000**	Highly significant
Individuals × Sides	0.0418	1218	0.0001	0.9814	0.6575	Not significant
Measurement error	0.1761	5040	0.0001	---	---	
			Maluso Infected Samples			
Individuals	0.2087	1218	0.0002	1.5957	0.0000**	Highly significant
Sides	0.1187	42	0.0028	26.3232	0.0000**	Highly significant
Individuals × Sides	0.1308	1218	0.0001	1.3752	0.0000**	Highly significant
Measurement error	0.3934	5040	0.0001	---	---	
			Maluso Non-infected Samples			
Individuals	0.3242	1218	0.0003	3.1599	0.0000**	Highly significant
Sides	0.1085	42	0.0026	30.6518	0.0000**	Highly significant
Individuals × Sides	0.1026	1218	0.0001	0.7491	1	Not significant
Measurement error	0.5668	5040	0.0001	---	---	

Note: Individuals = Shape variation; Sides = Directional asymmetry; Individual × Sides interaction = Fluctuating asymmetry; ***$p<0.0000$* - Highly significant; **$p<0.001$* - Significant; *$p>0.05$* - Not significant; Significance was tested with 99 permutations.

The "Individuals" (I) is considered as the block effect and detects variations among individual genotypes. The "Sides" (S) is the main fixed effect that measures directional asymmetry between two sides. The "Individuals × Sides interaction" is a measure of FA and anti-symmetry or the failure of the effect of individuals to be the same from side to side. An error term (m) represents measurement error (replications within Sides by Individuals) (Pojas & Tabugo, 2015). In this study, the *p*-value of the Individuals × Sides interaction both for Sangali and Maluso infected samples yield significant FA value where $p<0.001$ (Table 5), which suggest that the presence of parasite affects fluctuating asymmetry in *N. japonicus*. The higher the FA (significant FA), the more developmentally unstable the organism and as such, higher FA value was exhibited by Maluso infected samples (SS=0.1308, $p=0.0000$**) compared in Sangali infected samples (SS=0.0622, $p=0.0014$*), signifying that *N. japonicus* samples in Maluso are more developmentally unstable.

Principal Component Analysis (PCA) was derived from Procrustes Analysis that examined the variability of landmark points in tangent space. First principal component depicts vectors at landmarks that show the magnitude and direction in which that landmark is displaced relative to the others, while the second principal component depicts the difference via the thin-plate splines, an interpolation function that models change between landmarks from the data of changes in coordinates of landmarks (Marquez, 2006). PCA that implied deformation for "Individuals × Sides interaction" of infected and non-infected samples from the two sites is shown in Figure 6 where it can be seen that the red dots represent the morphological landmarks used in this study while the blue arrows indicate the direction as well as the magnitude of the fluctuation. The percentage values of PCA represent the level of variability in the data (Pereira, 2015). Based on the percentage of overall variation exhibited by PC1 and PC2, infected samples in Maluso exhibit higher level of variability (PC1 is 33.5065% and PC2 is 29.7102%) compared to infected samples in Sangali (PC1 is 28.5327% and PC2 is 16.6792%). Table 6 shows the level of variability explained by the first two principal components.

Table 6. Variance explained by first two principal components of infected and non-infected samples of *N. japonicus* from the two sites in Mindanao, Philippines.

Samples	PC1 (%)	PC2 (%)
Sangali infected	28.5327	16.6792
Sangali non-infected	42.7145	20.7157
Maluso infected	33.5065	29.7102
Maluso non- infected	20.9016	18.5149

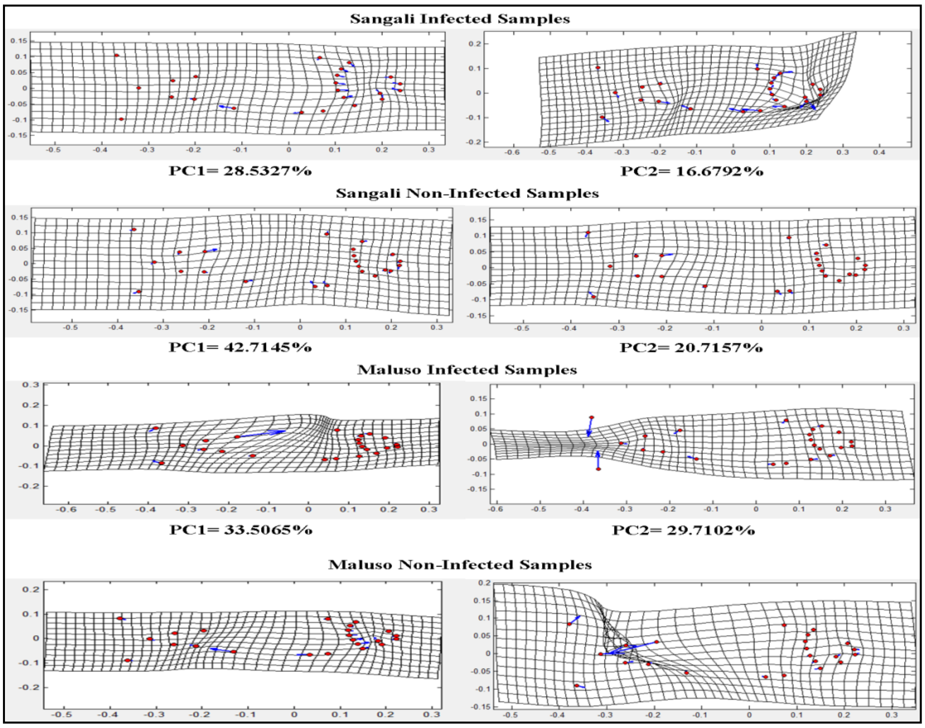

Figure 6. Principal Component Analysis (PCA) implied deformation for "Individuals × Sides interaction" (fluctuating asymmetry) of infected and non-infected samples from the two sites in Mindanao, Philippines.

The mechanisms by which parasites cause increased asymmetry in fishes and for *N. japonicus* in particular are still subject to further studies though there were some acceptable ideas that could explain the effect of parasites in their hosts. One of the plausible explanations for this is that parasites can limit host nutrient availability by reducing host food intake, digestion, absorption and nutrient assimilation, resulting to nutritional deprivation of various forms, destabilizes host development and eventually elevates the levels of fluctuating asymmetry (Polak, 1994). In addition, parasites compete with their hosts for resources, and therefore invade upon host metabolism that can reduce the growth and development of its host species (Goater *et al*., 1993).

Both ectoparasites and endoparasites are common in fishes but endoparasites are more dangerous and cause extensive damage to their hosts, however, migration of nematodes such as *Anisakis* sp. and *Capillaria* sp. which is found in *N. japonicus* during this study may cause the tissue damage in its internal parts specifically in the intestine (Echem *et al*., 2018). Most of the commonly encountered fish parasites were Acanthocephalans and Nematodes. In this case, there is high parasitic load in *N. japonicus* samples examined that might lead to nutritional deprivation which in turn caused increased FA. The costs of parasitism could result in increased physiological stress during development and thus, directly induce high levels of asymmetry. These variations in asymmetry of *N. japonicus* could be an indication of adaptive strategy for their survival pattern (Sepe, 2019).

Overall, findings of the study suggest that occurrence of endoparasites thus affect the growth and development of *N. japonicus* as reflected in the results of linear regression analysis and measurement of FA. The results of this study can be used for additional information for future studies in relation to the topic, however, experimental studies are recommended to further elucidate the effects of parasitism in other biological aspects of fish. Herein, the potential of LWR and FA as useful tools in studying the conditions of fish populations was demonstrated.

CONCLUSION

The results of the study revealed that as the body length of *N. japonicus* increases, its body weight becomes less rotund whether it is infected or not with endoparasites as reflected to be a negative allometric growth pattern. Four species of endoparasites were found in the intestine of infected *N. japonicus* with *Acanthocephalus* sp. which belongs to Phylum Acanthocephala as the most abundant, most prevalent and has the highest mean intensity in Sangali while *Anisakis* sp. which belongs to Phylum Nematoda in Maluso. The FA is a bioindicator of stress and developmental stability for individual adaptation and in a population. Results showed insignificant levels of FA for non-infected *N. japonicus* while those infected *N. japonicus* yield significant FA for both sites. This indicates that the presence of parasites can affect the developmental stability of *N. japonicus*. Findings suggest that parasites can increase the levels of FA due to the stress it induced in the development of the organism. Herein, this study demonstrates that LWR and FA are useful tools in studying the conditions of fish populations in their habitat.

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