DISTRIBUTION PATTERN OF *Acanthogyrus* SP. (ACANTHOCEPHALA: QUADRIGYRIDAE) IN NILE TILAPIA (*Oreochromis niloticus* L.) FROM SAMPALOC LAKE, PHILIPPINES

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ABSTRACT – Studies on acanthocephalans underscore many aspects of parasite biology. They are implicated to play vital roles in shaping ecosystems through regulation of their hosts' community structure. Studies have also shown both their potential as agents of disease upon reaching epizootic levels and biomonitors of environmental changes. This research was conducted to describe the distribution pattern of an unidentified acanthogyrid acanthocephalan infecting cultured *Oreochromis niloticus* in Sampaloc Lake, Laguna, Philippines. Results showed that *Acanthogyrus* sp. had a non-random distribution pattern among their fish hosts (P<0.001). Subsequent analysis showed a highly aggregated distribution (D=0.82; Variance/mean=4.403). Larger Nile tilapia appears to harbor more parasites than smaller-sized ones as indicated by positive correlations between intensity and fish length (r=0.40, P<0.001) and weight (r=0.30; P<0.001). This study could serve as baseline information for future investigation on the ecology and infection dynamics of Acanthocephala, especially in the Philippines.

Keywords: Acanthocephala, Acanthogyrus, Aggregation, Correlation, Oreochromis niloticus, Parasitology, Poulin's Index of Discrepancy, Quadrigyridae

INTRODUCTION

The Phylum Acanthocephala is a monophyletic group with all members exclusively parasitic (Kennedy 2006). They are considered highly successful group of parasites for they infect all classes of vertebrates. All species have similar fundamental life history. They all have a free-living egg (acanthor), require an arthropod intermediate host for the larval acanthella and cystacanth stages, and exploit a vertebrate definitive host upon reaching adulthood (Amin 1998). The main underlying feature in studying acanthocephalans is to demonstrate several aspects of parasite biology (Lassiere 1989). This includes understanding more fully the host-parasite relationship in an ecological and evolutionary perspective.

Although acanthocephalans are of little importance as agents of disease relative to other parasites, their role in aquatic ecosystems can be remarkable. They are implicated in host manipulation (Latham and Poulin 2002; Dezfuli et al. 1994). They may impact the food web patterns and community structure of their hosts. Acanthocephalans also respond to changes especially in aquatic habitats, making them good bioindicators of environmental degradation. Several studies have shown the effectivity of acanthocephalans as biomonitors of heavy metal

pollution in aquatic ecosystems (Thielen et al. 2004; Sures 2003; 2002; Sures et al. 1994a; 1994b).

Acanthocephalans may exhibit seasonality in their definitive host. For aquatic species, their distribution and abundance depend primarily on the population dynamics of their intermediate hosts (Ohtaka et al. 2002; Mayama 1989). Some species of acanthocephalans also show some degree of aggregation both in their intermediate and definitive hosts (Outreman et al. 2002; Brattey 1988; 1986; Crofton 1971a). In general, the distribution of parasites fit to the negative binomial form suggesting an aggregated dispersal among their hosts (Outreman et al. 2002; Shaw et al. 1998; Schmid and Robinson 1972; Crofton 1971b). This pattern has been suggested as a significant factor in the dynamics of host-parasite interactions (Wilson et al. 2002; Rosà 2003).

This report describes the distribution pattern of an acanthogyrid acanthocephalan parasite, *Acanthogyrus* sp. in their fish host, *Oreochromis niloticus* from Sampaloc Lake, Philippines. This research could serve as benchmark information for future studies on the ecology of Acanthocephala, particularly for the genus *Acanthogyrus*.

MATERIALS AND METHODS

Description of the Study Site. Sampaloc Lake (14°4'44.77"N; 121°19'47.49"E) is the largest of the seven crater lakes of San Pablo City, Philippines (Fig. 1). The lake is of volcanic origin. It has a circular surface area of 104 ha, a 3.5 km perimeter, an average depth of 20 m with a maximum depth of 27 m. The lake is limnologically classified as warm monomictic and eutrophic (Santiago and Arcilla 1991). Approximately 16 ha of its total area are devoted for cage tilapia aquaculture with 227 active operators as approved by the LLDA in 2002. The lake is inhabited by several species of fish and crustaceans that may serve as potential definitive

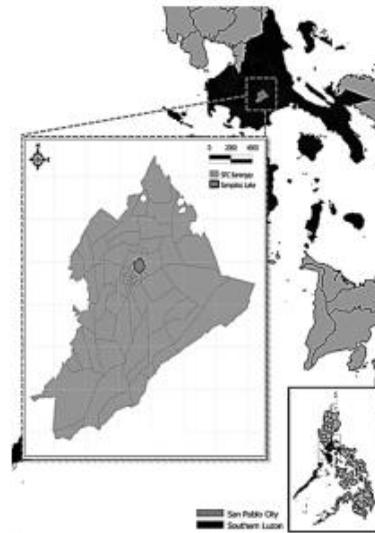and intermediate hosts for acanthocephalan parasites.

Figure 1. Map showing the location of Sampaloc Lake in the Philippine archipelago.

Fish Collection and Processing. A total of 550 fish (*Oreochromis niloticus*) were randomly sampled using scoop nets from different cages in 2010 to 2012. The samples were transported on ice to the laboratory and were processed immediately within the day. The standard length (cm) and weight (g) of each fish were measured using a caliper (Mitutuyo, Japan) and a digital weighing scale (Tamika, Japan), respectively. Each fish was dissected and the intestine was isolated into a Petri dish containing physiological saline solution. These were examined for the presence of adult *Acanthogyrus* sp. under a dissecting stereomicroscope (Leica EZ4, China), counted, and identified using the key to species provided by Amin (2005). The parasites were photographed under scanning view (x20) using a digital camera (Canon A2300, China).

Distribution Pattern of *Acanthogyrus* sp. (Acanthocephala: Quadrigyridae) in Nile Tilapia (*Oreochromis Niloticus* L.) From Sampaloc Lake, Philippines

Statistical Analyses. The distribution of the acanthocephalan in their fish hosts was characterized using goodness-of-fits including Kolmogorov-Smirnov test, index of discrepancy, and negative binomial distribution (NBD) models. Where appropriate, the k parameter of the negative binomial and/ or the discrepancy value (D) was interpreted to estimate the degree of aggregation. Pearson's product moment correlation was used to determine the relationship between infection intensity and size in terms of length and weight (two-tailed) of infected fish. The tests were performed at 95% confidence level. Statistical computations were done using the software Quantitative Parasitology (QP) version 3.0 (Rózsa et al. 2000) and Predictive Analytics SoftWare (PASW) version 18.0 (IBM Corporation, CO, USA).

RESULTS AND DISCUSSION

The over-all dataset for the frequency distribution of *Acanthogyrus* sp. (Fig. 2) in fish host, *O. niloticus*, was examined to determine the type of dispersion pattern. There was a very strong evidence (P<0.001) that the distribution pattern of *Acanthogyrus* sp. in their final host followed a non-random pattern of dispersal, not the usual normal distribution. According to Kennedy (2006), the skewed to the left frequency distribution shown by *Acanthogyrus* sp. (Fig. 3) is characteristic of fish acanthocephalans. Consequently, the dataset was examined using Poulin's index of discrepancy, D, as an appropriate measure to estimate the degree of aggregation in their fish hosts (Poulin 1993).
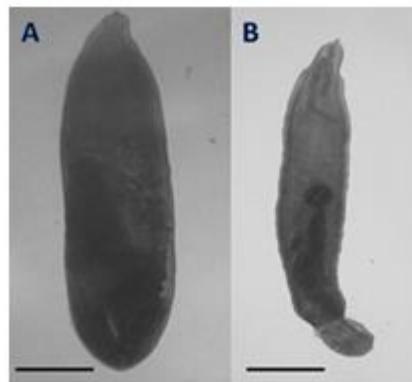

Figure 2. Photomicrographs of (A) female and (B) male *Acanthogyrus* sp. infecting Nile tilapia in Sampaloc Lake, Philippines. (x20, bar = 1 mm).

The discrepancy index is a mathematical expression that quantifies parasite aggregation as the discrepancy between the observed parasite distribution and the hypothetical distribution whereby all hosts are used equally and the infrapopulation in or on the hosts are of the same quantity (Wilson et al. 2002). It is a measure of the relative departure of the observed frequencies from a uniform distribution. According to Poulin (1993), parasite is said to be aggregated when a large proportion of the host population comprises lightly-infected or uninfected individuals and a large proportion of the parasite population occupies the few heavily infected hosts. The index, D, has a given range of zero to one. Zero (0) suggests the theoretical value of no aggregation so that all hosts in the sample are equally infected with the same number of parasite. One (1) is the theoretical limit where all parasites in the sample are concentrated in one host (Wilson et al. 2002). The computed D value of 0.819 in this study indicates that *Acanthogyrus* sp. was highly aggregated among their fish hosts.
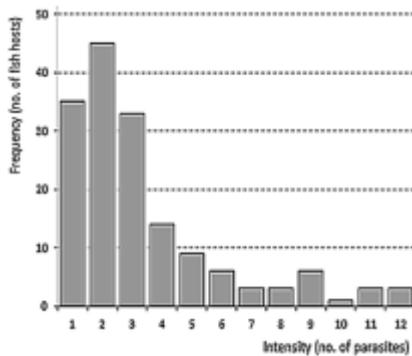
Figure 3. Frequency distribution of *Acanthogyrus* sp. (N=539; mean intensity and standard deviation=3.35±0.37) among infected cultured Nile tilapia (N=161).

Poulin's aggregation index is one of the several indices used to estimate parasite aggregation. One method examines the variance (s^2) and the mean (m) of parasite distribution whereby random pattern corresponds to a statistical result indicating an approximate equality of variance and the mean (i.e. s^2=m); whereas, an aggregated distribution is indicated when the variance is significantly greater than the mean (i.e. s^2>m). Consequently, the degree of aggregation can be quantified by the ratio of the variance to the mean (i.e. s^2/m) (Wilson et al. 2002). In this study, the resulting variance of 4.315 was greater than the mean 0.98, and the corresponding variance-to-mean ratio of 4.403 showed a significant departure from uniform distribution.

Another method of predicting the distribution pattern of parasites is by fitting the observed dataset to the negative binomial distribution (NBD) model. This is the widely used index of parasite crowding among epidemiologists since majority of parasite datasets are usually described by the NBD (Crofton 1971a, 1971b; Shaw and Dobson 1995; Grenfell et al. 1995; Shaw et al. 1998). Moreover, the exponent k of the expression's expansion [i.e. $(q - p)^{-k}$, where

q=1 + p and k is positive (Crofton 1971a)] can be easily interpreted to capture parasite over-dispersion (Wilson et al. 2002). The exponent k of NBD is an inverse measure of aggregation. That is, as the k value decreases, the degree of aggregation increases (Elliot, 1977). In this study, the dataset did not fit the NBD (P<0.05); hence the computed k value of 0.204 cannot be interpreted to quantify aggregation. This inconsistent result may be due to statistical artifacts or the unsuitability of NBD to characterize parasite distribution patterns (Poulin 1993). In fact, the use of NBD fitting has been criticized, as the k value is not sensitive to the tail of the distribution that corresponds to superinfection (Scott 1987). The study results suggest that NBD is not universal for parasite datasets and the use of index of discrepancy is relatively robust in profiling parasite distributions.

The overdispersed pattern of parasite distribution especially in the case of parasitic helminthes and arthropods is a usual encounter (Crofton 1971a; Shaw and Dobson 1995). A meta-analysis on patterns of macroparasite aggregation in wildlife host populations was conducted, whereby 90% of the datasets have corresponded to an aggregated form (Shaw et al. 1998). In this type of distribution pattern, it appears that among the susceptible hosts available for infection, only few are actually infected by a certain species of parasite (Poulin 1993). For instance, Newey et al. (2005) showed that the helminth *Graphidium strigosum* had low prevalence and was highly aggregated among its hare hosts *Lepus timidus* from the Central Highlands of Scotland. Aggregation of a trematode parasite *Diplostomum spathaceum* in their fish hosts was also reported (Pennycuick 1971). According to Kennedy (2006), acanthocephalan populations are over dispersed under most conditions. In this study, only 161 or 29% (95% CI: 26-33%) of the fish samples were infected with *Acanthogyrus* sp. Similarly, by using the dataset of Hynes and Nicholas (1963), Crofton (1971a) showed in his proposed model that the acanthocephalan parasite *Polymorphus minutus*

was over dispersed among their hosts. Moreover, among the infected hosts sub-population, most harbors few parasites while the remaining succumbs to superinfection (Shaw and Dobson 1995). This was also evident in the present study wherein majority of infected fish harbors one to three adult acanthocephalans whereas relatively few (less than 50 samples) had up to 12 acanthocephalans per fish.

Wilson et al. (2002) expressed that such heterogeneities are generated when individual hosts vary in their exposure to infective stages of the parasite and/or differences on the susceptibility of each potential host once they encounter the infectious agent. Even small differences in susceptibility between hosts can rapidly produce non-random distributions of parasites (Anderson and May 1978). The sampled Nile tilapia from Sampaloc Lake apparently has varying level of exposure to cystacanth-infected intermediate hosts. Variability in exposure to infective stages can be attributed to external heterogeneity, which is not considered attributes of the host or the parasite. Such heterogeneity can be manifested by difference in spatial distribution of infective external stages (Wilson et al. 2002). Heterogeneities in parasite intensities across the host population can occur when there is spatial variation in the density of infective stages in the environment and when different hosts exhibit certain preference of location in their habitat. Keymer and Anderson (1979) showed in their experiment that the mean intensity of the tapeworm *Hymenolepis diminuta* in flour beetle (*Tribolium confusum*) increased as the density of the egg increased per unit area. Clearly, the rate of infection acquisition increases with the frequency of contact between the host and the infective stages. Moreover, as the eggs in the external environment became increasingly aggregated, so did the parasite distribution in the host population (Keymer and Anderson, 1979).

Assuming that all individuals of Nile tilapia in Sampaloc Lake have equal chances of ingesting an infected intermediate host, it was possible that some Nile tilapia resisted the establishment of infection by the acanthocephalan parasite. Infected fish samples were not able to do so, as manifested by the presence of adult worms. Infection physiology, by operational definition, is a series of events required prior to development and/ or reproduction (Brown et al. 2005). Evidently, the mature forms of *Acanthogyrus* sp. in the gut of Nile tilapia had demonstrated efficiency in establishing infection physiology. Direct reproduction of parasites within the hosts may also cause overdispersion of (Anderson and Gordon 1982). However, as in the case of *N. rutili* from Loch Maragan, Scotland (Lassiere 1989) the cultured Nile tilapia could not have autoinfection of *Acanthogyrus* sp. because the latter was an obligate endoparasite that required an intermediate host to complete its life cycle.

The distribution of parasites can also be a function of host age and is termed as age-intensity profile (Duerr et al. 2003). This may change throughout the lifespan of a host with changes in demographic, physiological, and ecological factors (Kennedy 2006). The analysis of infection intensity as influenced by age of hosts may reveal demographic related causes of aggregation. However, age-intensity profiles are difficult to interpret because several processes underlying the parasite metapopulation can affect them. These processes may include age-dependent exposure to parasites, parasite-induced host mortality, and density-dependent parasite mortality and establishment (Duerr et al. 2003).
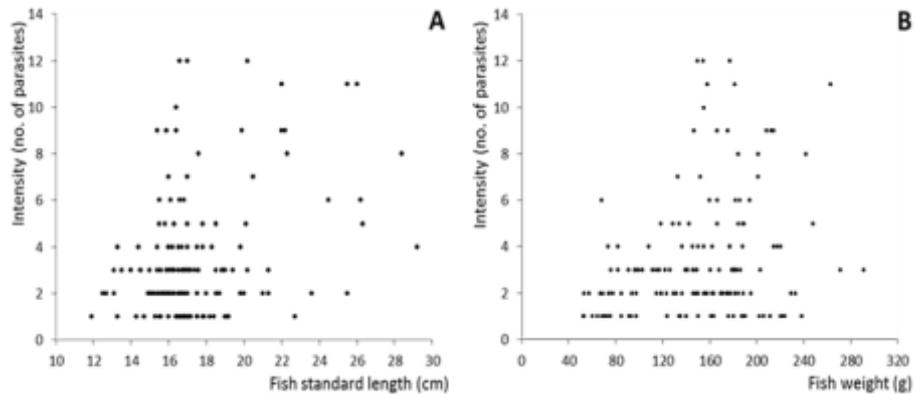
Figure 4. Correlation between parasite infection intensity and fish (A) length and (B) weight.

In this study, size-intensity profile was used to find association between host's size and degree of infection with *Acanthogyrus* sp. Correlation analyses (Fig. 4) showed a significant but weak positive relationship between intensity and length (r=0.40; P<0.001) as well as the weight (r=0.30; P<0.001). This suggests that the parasite load increased with increasing fish size, and indicates that *Acanthogyrus* sp. appeared to aggregate in larger Nile tilapia.

Some studies have been conducted to describe size-intensity profiles for natural populations of acanthocephalans in fish. The studies of Muzzall and Bullock (1978) and Walkey (1967) on *N. saginatus* in fallfish and for *N. rutili* in sticklebacks showed that the number of worms was directly proportional to the fish length. Diamant (1989) also found similar results and attributed such size-based distribution of acanthocephalans to host habitat, feeding habits, extended exposure to infections, age-related segregation, larger microhabitats, and physiological conditions to maturity. In this study, the relative heavy infection encountered in larger Nile tilapia might be due to its higher feeding behavior in response to metabolic demands for growth. Cultured Nile tilapias in Sampaloc Lake

were dependent on organic feeds provided by the farmers. However, larger fish may encounter intermediate hosts such as shrimps or copepods and may feed on these unselectively as part of their age-related diet preference. Lassiere (1989) showed that larger trout from Lock Maragan were more heavily infected relative to smaller trout; and reasoned the adoption of piscivorous feeding habit on minnows by larger trout (indicating the importance of post-cyclic transmission) as basis for this observation. On the other hand, parasite accumulation may also be a factor for the observed high frequency of acanthocephalans in larger fish. This can be due to the life history of Neoechinorhynchid acanthocephalans in having 60 to 90 days of cystacanth development to adulthood (Harms 1965). It could be inferred that parasite recruitment occurred during the juvenile stage of the fish (when they were smaller in size) and greater number of adult parasites were observed as the fish became older and larger. This result could only be interpreted for certain size-range of Nile tilapia. It was observed in this study that very large and older fish samples did not harbor *Acanthogyrus* sp., signifying the termination of the parasite generation.

The characteristic aggregated distribution of macroparasites in their hosts is a key feature of host-parasite population biology (Grenfell et al.

1995). The over dispersion of *Acanthogyrus* sp. among Nile tilapia in Sampaloc Lake has allowed the regulation and stability of the host-parasite system. The observed superinfection in a few of the sampled hosts demonstrated that acanthocephalans have ensured both the overproduction of eggs while maintaining minimum rates of host mortality. Over dispersion promotes the mating probability of parasites since the chance of a parasite meeting the opposite sex increases when they are numerous in a single host (Kennedy 2006). Over dispersion, as such, can be interpreted as an adaptive mechanism by macroparasites. In fact, Crofton (1971a) suggested that over dispersion should form part of parasite definition. Another feature of parasite aggregation pertains to host survival and mortality. It is empirical that parasites increase mortality of hosts in instances of superinfection (Kennedy 2006). Death of host would also mean death of parasites. Hence, aggregation, in effect, ensures that only few members of potential hosts are superinfected and may succumb to death as a consequence.

The importance of predicting the distribution pattern of parasites also underscores which appropriate statistical methods should be used in quantifying infection patterns. Considering an over dispersed parasite distribution, there is an increasing argument on whether central tendencies (e.g. mean intensity, mean abundance, etc.) would still be the best measure to quantify the degree of parasitism in samples of hosts (Rózsa et al. 2000). When the distribution of the parasite is highly aggregated (i.e. non-random), comparing central tendencies between two or more samples of hosts may be inappropriate as it violates the normality assumption of parametric tests such as the Student's t-test and Analysis of Variance (McDonald 2009). Techniques involving log transformation or omission of some data, which appear as outliers to achieve Gaussian datasets, are conventional. However, parasite distributions are generally considered highly aggregated as shown in loads of empirical datasets, such that even log transform cannot fix the skewness nature (Rózsa et al. 2000). This was demonstrated in the present study whereby a non-log normal dataset was obtained. Moreover, intensity values indicating superinfection (also observed in this study) should not be considered as outliers because it is one of the prominent features of the host-parasite population dynamics (Crofton 1971a). Non-parametrics, bootstrapping, and randomization tests are recommended as appropriate statistical analyses in comparing highly aggregated infrapopulations of parasites in samples of hosts (Rózsa et al. 2000).

CONCLUSION

The frequency distribution of the adult worms was significantly different from the theoretical normal distribution suggesting a non-random pattern of dispersal. The adult worms were over dispersed among their fish hosts. There was also a positive correlation between infection intensity and size of fish in terms of length and weight. It was observed that larger Nile tilapia harbored more parasites relative to smaller sized ones. However, this could not be a general pattern because of the weak relationship between fish size and infection intensity. The characteristically aggregated distribution of *Acanthogyrus* sp. in their fish hosts was attributed to heterogeneities in host susceptibility to infection. These include the hosts' chance to encounter infected intermediate hosts and/ or differential reaction towards the cystacanth as a function of varying immunity levels. However, these factors were difficult to confirm in the field. Results herein would remain open for assumptive interpretations until further field and laboratory-based investigations. The over dispersed distribution of *Acanthogyrus* sp. was consistent with most empirical datasets of macroparasites. This type of distribution has been proposed as a mechanism to maintain the equilibrium of the host-parasite relationship.

STATEMENT OF AUTHORSHIP

All authors contributed equally on the study design, gathering of empirical data and related literature, and writing of the manuscript. Results presented herein are portions of the thesis of the first author for the degree of Master of Science in Zoology at the University of the Philippines Los Baños. The first three authors conducted the actual fieldwork and laboratory analyses. The last author served as the research adviser.

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