Initial assessment of parasite load in *Clarias batrachus*, *Glossogobius giuris* and *Oreochromis niloticus* in Lake Taal (Philippines)

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Fish parasites are ecologically important because they shape community and ecosystem structure by influencing trophic interactions, host fitness, and food webs. We surveyed fish parasitofauna from Lake Taal and provided initial quantitative and qualitative measures of parasite burden. This was then correlated with fish size, as a preliminary attempt to relate parasitism with fish overall fitness. A total of 60 specimens of *Oreochromis niloticus, Glossogobius giuris* and *Clarias batrachus* were collected from the lake in June, October and November 2010. From these, five taxa of digenetic trematodes (*Opegaster* sp., *Erilepturus* sp., *Euclinostomum* sp., *Orientocreadium* sp., and *Clinostomum* sp.) and one parasitic cyclopoid (*Lernaea* sp.) were collected and identified. Results revealed that the most prevalent parasites, in both occurrence and number, were *Opegaster* sp. in *G. giuris* and *C. batrachus*, and *Lernaea* sp. in *O. niloticus*. *C. batrachus* was the most burdened as it harbored parasites in most of its internal organs. Linear correlations reveal negative trends between parasite burden and fish size for all fish species analyzed, suggesting that smaller fish tend to harbor more parasites than larger fish. However, this relationship was not found to be statistically significant ($p>0.05$). Nevertheless, these results reveal the challenges that many researchers will face in their effort to understand the implications of parasitism to both caged and feral fish, in relation to natural and anthropogenic factors. Moreover, it highlights the need for more ecological studies on parasitism in the Philippines, if we are to improve fish conditions in both open waters and caged areas not only in Lake Taal, but also in all Philippine lakes, especially those that are being utilized for aquaculture.

KEYWORDS

fish parasite, parasitic infection, parasite prevalence, digenetic trematode, *Lernaea* sp.

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INTRODUCTION

Fish are hosts to a variety of parasitic organisms (Yamaguti 1958, Yamaguti 1971) which can influence population dynamics (Minchella and Scott 1991, Roche et al 2010), decrease host fitness and alter host behavior or morphology which increases predation risks (Poulin et al 2005). It is surprising to know that parasitism is more common than traditional predation as a consumer lifestyle (De Meeûs and Renaud 2002) and as parasites can also both function as predator (Raffel et al 2008) and as prey, whether directly (Polis and Hurd 1996) or inadvertently during the consumption of infected hosts (Johnson et al 2010), it suggests that parasites can contribute considerably to energy flow in food webs, despite their small biomass.

Fish parasites, therefore, are ecologically important because they shape community and ecosystem structure through influences in trophic interactions, host fitness, and food webs. Fish parasites are also potential biomarkers for ecology and trophic interactions because they can resolve many things about their host and its environment. For example, the prevalence of some intestinal parasites in a host may reflect long-term trends in feeding patterns since the parasites may stay in the gut for weeks or months (Curtis et al 1995). Parasitism, in such cases, then reflects host diet and foraging behavior because these factors affect the abundance of parasites within the host (Marcogliese and Cone 1997). These interacting factors make parasites good indicators of environmental contaminants and stress (Sures 2004).

Many fish parasite researches in the Philippines had been geared toward effective rearing of cultured fish species, through early detection of infection and treatment of brood stocks, or examination of parasite prevalence in marketable species (Arthur and Lumanlan-Mayo 1997). However, fish parasite studies geared toward better understanding of ecological implications in aquatic environments had not been investigated in great detail. Such studies are of immediate importance because host-parasite relationships at the population level are complex, with many factors related to the host and the host's environment needing to be considered. In farmed fish hosts, culture conditions clearly affect this relationship, as they influence host density and other collateral factors. The spatial distributions of parasites may therefore be the result of physical characteristics of water systems which may reflect differences in the properties of aquatic habitats (Moles and Jensen 1999). This may have different implications in the effects of parasitism on fish overall health because of confounding environmental factors such as pollution and degrading water quality (Marcogliese et al 2005, Martinez-Gomez et al 2006, Morley et al 2006).

The present study is a preliminary survey of the parasitofauna of selected freshwater fish species in Lake Taal, located in Batangas Province, Philippines. A recent review (Papa and Mamaril Sr. 2011) had called for more research effort in Lake Taal, stressing that its unique characteristics, coupled with high biodiversity and endemicity, make it a high priority environment for further study. This is evidently seen in the fact that many of the lake's natural limnological processes, to the best of the authors' knowledge, have yet to be determined and sufficiently monitored. The lack of this needed information, together with reported abuse of fish overstocking, had significantly deteriorated the lake's water quality, contributing to arguably the worst fish stock loss in the history of Lake Taal, reported last May 2011. A number of researches had studied fish parasitism in Lake Taal, but these had focused on incidents of mass mortality due to heavy parasite infection in cultured fish (Lopez 2001, Adorador et al 2006). However, efforts to characterize the parasites of fish from open water and to quantify their effects on fish health have been scarce. The present study intends to provide initial quantitative and qualitative measures of parasite burden in selected caged and open-water fish species in Lake Taal and provide a preliminary assessment on the possible implications of parasitism on fish health.

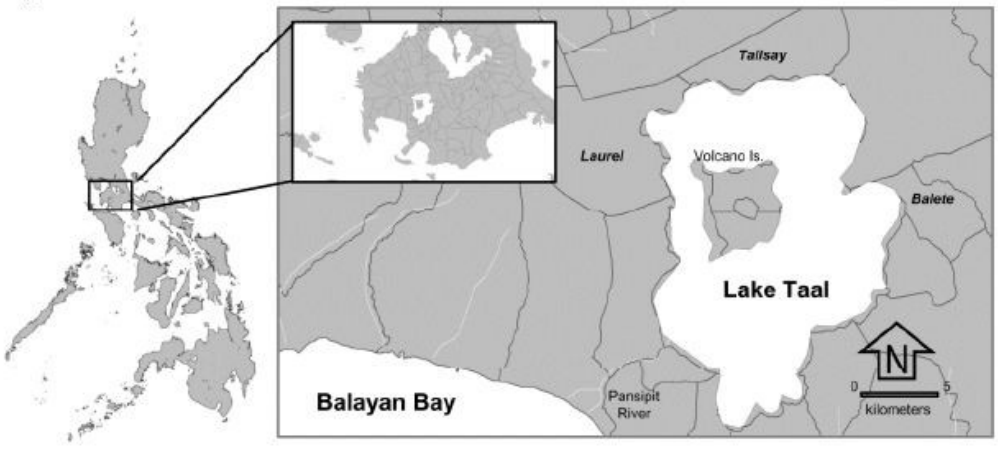

Figure 1. Map of Lake Taal showing its proximal location in Southern Luzon Is., Philippines, and the locality of the three sampling sites in Batangas: Laurel, Talisay and Balete.

MATERIALS AND METHODS

Sampling site and fish collection

Lake Taal is a 236.9 km^2 caldera lake located in southern Luzon in the province of Batangas (Fig. 1). It is nestled within a watershed area of 656.5 km^2 surrounded by 11 municipalities. During the months of June, October and November 2010, we collected a total of 60 fish sampled from landed fish catch (18 *G. giuris* and 18 *C. batrachus*) or harvested from fish cages (24 *O. niloticus*) from the towns of Talisay, Balete and Laurel. For each of the three months, 8 specimens of *O. niloticus* and 6 specimens each of *G. giuris* and *C. batrachus* were analyzed. The collected specimens were frozen and brought to the laboratory for examination. The total fish sample count may be arguably small, but this was because obtaining fish was highly dependent on their availability in the visited sites; this was affected by increased volcanic activity of the Taal Volcano during the sampling period which decreased fishing efforts in the limnetic areas of the lake due to government safety restrictions. Overall, we believe that the present total sample count is adequate enough for an initial assessment of parasitism in the selected fish species from Lake Taal.

Parasite examination

Parasitological examination followed not later than 24 hours after collection and was done individually under a dissecting microscope. Total body length (in cm) was recorded, after which, sub-samples of scales, fins, and skin were examined for the presence of ectoparasites. Eyes and muscle tissue were extracted and examined for encysted and adult nematodes and trematodes. Gills were removed and, together with the oral cavity, examined for the presence of monogeans, parasitic copepods, and leeches. The body wall was cut parallel to the abdominal midline from the anal aperture upwards. Organs such as the heart, gonads, kidneys, gall bladder, spleen and liver were isolated, immersed in saline solution, teased apart and examined for endoparasites. The gut was extracted and cut along its length, the contents of which were isolated and examined for trematodes, nematodes, cestodes and acanthocephalans. Lastly, smears of the brain and spinal cord was prepared and examined for encysted parasites. All isolated parasites were placed in 75% ethanol for long-term storage, and were mounted in slides and stained with either lactophenol or Eosin-hematoxylin for identification. After identification, parasites were counted and recorded per fish hosts and location of infection. Parasites were identified to genus level using taxonomic keys on tropical fish parasites (Kabata 1985, Velasquez 1986).

Statistical analysis

Measures of parasite burden were described separately for each fish species using various parameters. Percent prevalence (%P) was computed as the total number of infected fish divided by the total number of fish examined and expressed as a percentage. Percent dominance (%D) was calculated as a percentage of N/N-sum (where N is the abundance of a parasite species and N-sum is the sum of the abundance of all parasite species). Abundance, used in the computation of %D, was calculated by dividing the total number of recovered parasites with the total number of fish samples. Mean intensity was determined by dividing the total number of recovered parasites with the number of only infected fish samples. Lastly, range denoted the minimum and maximum parasite counts observed. These descriptive parameters were adapted from a review on quantifying parasites in hosts (Rozsa et al 2000).

Fish total length data and total parasite count data was subjected to normality and homoscedasticity tests using Shapiro-Wilk W and Levene's test, respectively. ANOVA or Kruskall-Wallis test was then used to determine differences in fish size and total parasite count across all three sampling sites. Lastly, linear correlation was used to determine possible fish size-related patterns in total parasite counts. Total parasite counts were used rather than categorizing each parasite according to taxonomic group, since most of the parasites recovered were trematodes. All statistical analyses used an alpha level = 0.05 for significance and were conducted using PAST version 2.04 (http://folk.uio.no/ohammer/past/).

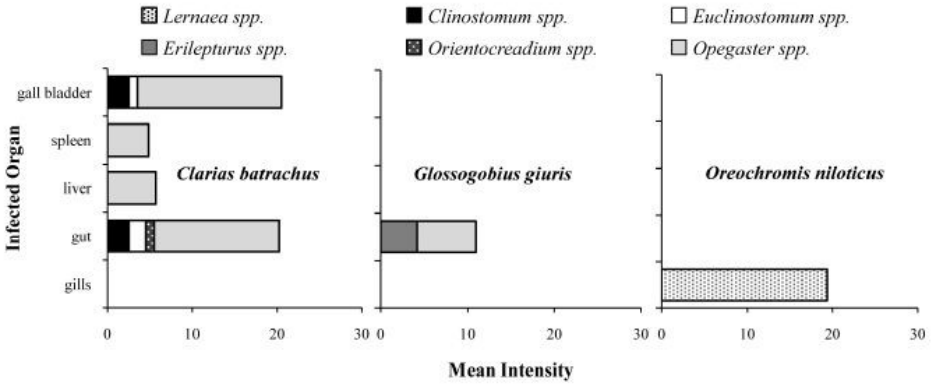

Figure 2. Mean intensity subdivided into location of infection and parasite species for *Clarias batrachus, Glossogobius giuris*, and *Oreochromis niloticus*. Legends represent each parasite species.

RESULTS

In this study, a total of 411 parasites were recovered from the fish specimens examined. These were determined to be from five digenean trematode species (*Clinostomum* sp., *Euclinostomum* sp., *Erilepturus* sp., *Orientocreadium* sp., and *Opegaster* sp.) and one parasitic cyclopoid copepod species (*Lernaea* sp.). The percent prevalence, percent dominance, mean intensity and range of the parasites are presented in Table 1.

Fish total length was normally distributed (W: 0.98; 0.91; 0.93 and p-value: 0.95; 0.11; 0.21) and had homogenous variance (Levene's p-value: 0.81; 0.52; 0.26) for *O. niloticus*, *G. giuris* and *C. batrachus*, respectively. Total parasite counts for each fish species also had homogenous variance (Levene's p-value: 0.39; 0.29; 0.46) but *O. niloticus* parasite counts was not normally distributed (W: 0.66 and p-value: 3.3x106) as compared to *G. giuris* and *C. batrachus* (W: 0.97; 0.95 and p-value: 0.86; 0.39), which had normally distributed total parasite counts, respectively.

Fish size represented by fish total length was not significantly different among all sites (F: 1.36; 0.27; 0.07 and p-value: 0.28; 0.77; 0.93) for *O. niloticus*, *G. giuris* and *C. batrachus* respectively. This signifies that variations observed in the total parasite burden of the present samples across sites are not significantly confounded by differences in the fish size of the samples. Also, it was also determined that total parasite counts per fish species did not significantly vary across sites (H: 0.31; F: 1.98; 1.12 and p-values: 0.86; 0.17; 0.90). This suggests that the parameters for parasite burden may be summarized as a whole in this study for each fish species without much variation.

For *O. niloticus*, only *Lernaea* sp. was observed and the parasites were found only in the gills. Of the 24 individuals examined, only 8 (33.3%) were infected showing an average burden of 19.4 parasites in infected samples (Table 1). *O. niloticus* had either no parasites present or was heavily burdened with *Lernaea* sp. with a range of 14 to 24 parasites present per specimen (Fig. 2).

In contrast, all 18 samples of *G. giuris* examined were infected by parasites. However, the range was minimal (~1 to 10 parasites only). Two trematodes, *Erilepturus* sp. and *Opegaster* sp., were observed in the gut (Figure 2) with 33.3% and 100% occurrence, respectively. *Opegaster* sp. (%D – 83) was more dominant than *Erilepturus* sp. (%D – 17), because of a higher parasite burden in infected samples, in addition to the presence of *Opegaster* sp. in all *G. giuris* samples examined (Table 1).

All 18 samples of *C. batrachus* were also infested with parasites, and had the highest parasite species richness as compared to other examined fish hosts, with four species of digenetic trematodes parasitizing various vital organs in the viscera (Fig. 2). *Opegaster* sp. was the most prevalent and dominant parasite with the highest mean intensity, maximum range and was present in most vital organs (gut, liver, spleen and gall bladder) compared to the other parasites.

Linear correlations between total parasite count and fish total length showed negative relationships for all fish species (Fig. 3), but none were significant. This relationship seems to be most recognizable in *C. batrachus*, which has a coefficient of determination value (r^2) of 0.129 as compared to *O. niloticus* and *G. giuris* (r^2: 0.016, 0.088).

DISCUSSION

Results of the study are consistent with previous parasite

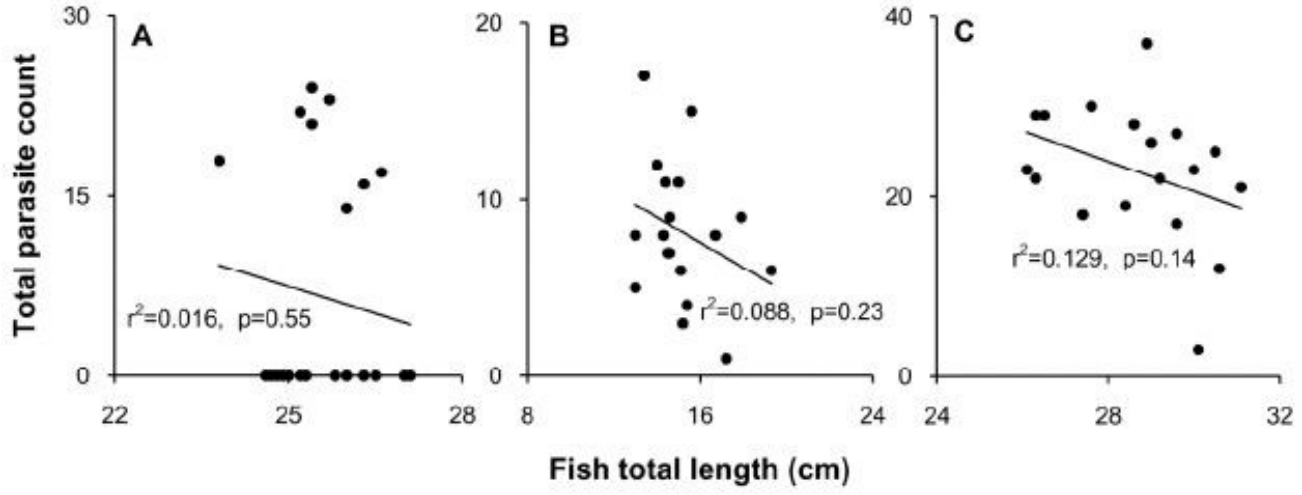

Figure 3. Linear correlation between total parasite count and fish length for a) *Oreochromis niloticus*, b) *Glossogobius giuris*, and c) *Clarias batrachus*. Values for coefficient of determination (r^2) and their corresponding p-values have been provided.

studies of the same fish species in different localities in the Philippines and also in other countries. Lernaeosis, caused by the parasitic copepod *Lernaea* sp., has been reported locally and internationally to cause increased mortality in cultured fish (including *O. niloticus*) in cases of heavy infection (Kabata 1985) which may be caused by the parasite penetrating its anchors in the fish host's vital organs (Khalifa and Post 1976). In some cases, the penetrating attachments of *Lerneae* sp. open routes for secondary infection (Hoffman 1973, Dempster et al 1988). Infections of *Opegaster* sp. in the viscera of *G. giuris* and *C. batrachus* have also been reported in various parts of the Philippines (Tubangui and Masilungan 1944, Lopez 1979), and the parasite has been also documented to infect a wide range of other fish species (Velasquez 1986). The omnivorous feeding behavior and benthic nature of *C. batrachus* (Robins et al 1991) may have made it susceptible to the high species diversity and heavy burden of parasites observed in the study, as both traits most likely increase its chances of ingesting prey that serve as intermediate hosts. This is supported by the fact that parasites from the genera *Opegaster*, its most infective parasite, have been observed in a wide array of intermediate hosts and take advantage of ingestion by infected prey as a route for infection (Tubangui 1933). These various modes of infection hint how changes in the environment, both by anthropogenic and natural factors, may affect the variability of parasite burden by possibly promoting or hindering certain stages in the life cycle of each parasite species.

Many environmental factors may have affected the variability found in the parasite composition of both the free-swimming and caged fish species in the present study. Environmental changes have been documented to affect parasitism, regardless of whether the fish species are caged or feral, because they are both always exposed to these intrinsic factors. For example, meteorological events such as rainfall coupled with wind patterns had been noted to induce a time-lagged increase in parasite burden in many fish species because of its tendency to mix the water column, thereby increasing the possibility of ingesting prey that are intermediate hosts (Steinauer and Font 2003, Pech et al 2010). Even certain limnological characteristics such as differences in water temperature (Poulin 2006a, Hirazawa et al 2010) may potentially affect host susceptibility and parasite growth, because it may trigger the increased activities of the free-living larval stages of certain parasite species. Even more, anthropogenic disturbances such as pollution (Valtonen et al 1997, Dusek et al 1998, Galli et al 2001) have been well documented to cause a myriad of oftentimes determinable effects on fish parasite communities. This has been the reason many researchers are tapping into this deterministic potential of fish parasites to use as environmental bio-indicators (Sures 2001, Sures 2004). However, it is possible for certain parasite species to be unaffected by local environmental conditions, due to their inherent biological traits, and still influence even the dynamics of the local parasite community (Poulin 2006b). For this reason, precaution should be taken when interpreting the results of the present study in relation to many of these possibilities, since necessary data are currently unavailable. Nevertheless, these are all serious issues that need to be considered if researchers intend to provide information that will help improve both feral and caged fish health, and prevent fish mortalities due to parasite infections. In this light, the possible role of the presence of aquaculture in

Table 1. List of parasite species indicating taxonomic classification and values of parasite burden. Namely, percent prevalence, percent dominance, mean intensity and range across all examined hosts separated by fish species. Dash represents no value because of absence of infection.

Parasite Species	*Oreochromis niloticus* (N=24)				*Glossogobius giuris* (N=18)				*Clarias batrachus* (N=18)			
	%P	%D	Mean intensity ±SD	Range	%P	%D	Mean intensity ±SD	Range	%P	%D	Mean intensity ±SD	Range
Arthropoda												
Cyclopoida												
***Lernaea* sp.**[1]	33.3	100	19.4 ±3.6	14-24	---	---	---	---	---	---	---	---
Platyhelminthes												
Trematoda												
***Clinostomum* sp.**[2]	---	---	---	---	---	---	---	---	22.2	13.4	3.8 ±1.9	2-7
***Euclinostomum* sp.**[2]	---	---	---	---	---	---	---	---	16.7	5.9	1.7 ±0.7	1-3
***Erileptus* sp.**[3]	---	---	---	---	33.3	17.0	4.7 ±3.4	1-10	---	---	---	---
***Orientocreadium* sp.**[4]	---	---	---	---	---	---	---	---	5.6	3.6	1	1
***Opegaster* sp.**[5]	---	---	---	---	100	83.0	6.8 ±2.4	1-11	100	77.1	21.7 ±6.9	3-35

Superscripts represent family classification 1: Lernaeidae, 2: Clinosomatidae, 3: Hemiuridae, 4: Allocreadiidae and 5: Opecoelidae, respectively.
%P: Percent Prevalence. %D: Percent Dominance. SD: Standard Deviation.

contributing to changes in parasitism must also be considered, since this serves as a major anthropogenic input source.

The possible contributory role of aquaculture in the variation of parasitism observed from the selected fish species in the present study cannot be ignored, in light of decadal information showing that the presence of aquaculture has indeed induced changes in the measure of parasitism in both caged and wild fish populations in environments with the presence of aquaculture (Scholz 1999, Costello 2006, Krkošek et al 2007, Frazer 2008, Heuch et al 2011, Saksida et al 2011). Changes in the aquatic environment are indeed a main driving force in the population dynamics of parasites of both caged and open-water fish species, but we speculate that the intensity of its effects may either be significantly pronounced or mitigated in aquaculture fish due to a variety of possibilities. We understand that in the natural environment, parasite populations usually show an aggregated distribution in host populations (present in very few individuals with heavy infection) because of different factors, such as host size, host density, and other ecological determinants (Arneberg et al 1998, Poulin 2000). As an example, the size of wild fish shoals has a direct relation to the possibility of parasite transmission (Sasal 2003). Similarly in aquaculture, fish cohorts are usually confined within relatively small areas with variable stocking densities, and it has been established that large fish densities in cages can induce significant stress to the caged fish (Ashley 2007). This can be correlated with parasitism since an increase in stress has an impact on fish parasite infection (Lafferty and Kuris 1999). This aspect may have contributed to the Percent Prevalence (%P) of *Lernaea* sp. in *O. niloticus* in the present study since stocking density preferences for *O. niloticus* in Lake Taal have been variable, ranging between 65 and 100 fish for a 10 x 10 x 6 m cage (Vista et al 2006), and since the samples were not taken from a single cohort but from different months. Also, another aspect that must be considered is that if the species of caged fish has been introduced to a non-native range, its resistance to parasites may either be stronger or weaker than other local fish species, since it had not co-evolved with the local parasitofaunal community (Roche et al 2010). *O. niloticus* (tilapia) is native to Africa and was introduced in the Philippines for aquaculture because of its fast growth, rapid breeding and tolerance to a variety of water conditions (De Silva et al 2004). This must explain why we did not find any other parasites (%D – 100%) in *O. niloticus* during the duration of the study other than *Lernaea* sp., which is a parasite that has a cosmopolitan distribution. However, it can be argued that this is because the cages may act as a "barrier" to the open waters, limiting the contact of this caged fish species with potential intermediate hosts and parasites. This does not seem to be the case since it has been reported that *Corallana grandiventra*, an ectoparasite that has been known to infect a wide variety of local fish species in Taal, was also found in certain cases to be in high numbers among caged *O. niloticus*, even causing high mortality among this cage-cultured fish (Adorador et al 2006). These events suggest that in spite of the fitness advantage among introduced species based on the "enemy release" hypothesis (Williams 1954, Elton 1958) and the seeming advantage of minimizing the interaction with potential intermediate hosts, aquaculture conditions may still make caged fish as favorable hosts and reservoir for parasites. To make it even more difficult to assess, different fish species have been documented to have varied immunological responses in mitigating the effects of parasitism in cases of heavy infection, or even possibly total resistance from certain types of parasites (Secombes and Chappell 1996, Jones 2001, Sitja-Bobadilla 2008). These complexities make it hard to determine in separate case-to-case basis what fish morphological characteristics should be assessed in order to determine if parasitism has had a significant effect on the overall fitness of individual fish and of the fish population.

In the present study, we initially evaluated if fish size can be a good indication of the effect of parasitism to overall fish health. We discovered potential but non-significant ($p>0.05$) negative relationships between total parasite count and total fish length, suggesting that smaller fish tend to have higher number of parasite counts as compared to larger fish, in all the fish species analyzed. However, due to the limited fish sample size, and the small representative fish size range of the present data, the correlation may be masked by variability. There may be a true negative correlation, but it cannot be presently validated with the available data. A resulting possibility if the above holds true maybe that parasitism may delay growth by significantly competing nutrition with the host. This can be interpreted as parasitism affecting the degree of the host's immunological response, decreasing the need to allocate energy to other physiological responses when certain environmental factors change making it unfavorable for fish host growth (Rohlenová et al 2011). However, this explanation will require that fish samples to be examined be of the same age and same population in the wild or same cohort in caged systems, undergoing similar environmental stimulus, to validate that the difference in growth is indeed a result of parasitism. This will require in-depth monitoring of many ecological determinants, as well as age determinants in fish morphology such as otolith examination. As a counter-point, it might very well be possible that smaller fish of younger age are more prone to parasitism because of a less developed immune system as compared to larger and much older fish, as the latter have been observed to have better chances of survivability against natural mortality in both the wild and in caged systems (Lorenzen 1996). This difficulty in explaining the possible reasons and implications of the variability of parasitism in aquatic environments that have both open-water and caged ecosystems is one of the hurdles to be overcome by researchers who intend to determine ecological implications of fish parasites in these aquatic ecosystems.

In summary, the present study analyzed parasites of some fish species in Lake Taal and had revealed variable measures of parasite burden among different species, explaining possible routes of infection, and showing possible effects on fish overall health. We recommend that when further studies on fish parasitology are performed, it must be with the intention of also

understanding the ecological implications on both the host and the host's environment. We encourage researchers to look into improved measures in evaluating parasitism that will better empirically explain overall fish health and apply those measures in fish parasitology studies.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

GC and RDP wrote parts of manuscript, supervised the analyses and facilitated the sampling. JCB performed the statistical analyses and wrote the manuscript for publication. EDL, JAG, EOP, MCIP and MAP conceptualized the initial study design, collected and identified the samples and wrote the preliminary version of this manuscript.

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