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# Proportionality Between Population Descriptors (Covariables) in an Aggregated Acanthocephalan (Helminth) Infection of a Microcrustacean: Crofton Revisited

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Running Title: Proportionality Between Population Descriptors in an Acanthocephalan Helminth Infection

## Abstract

We have previously shown that population parameters of the trematode metacercariae of *Clinostomum marginatum* in black bass (*Micropterus* spp.) were closely correlated with each other with the exception of prevalence (Daly et al. 2007, Daly (2014). Crofton (1971) and Haynes and Nicholas (1963) published raw data on an acanthocephalan, (*Polymorphus minutus*) in an amphipod intermediate host collected on 6 different sites on a stream. That data was used herein to examine similar correlations, as with *Clinostomum*, to see if another helminth infection, in this case from a different phylum, also had similar descriptor relationships. Forty one regression analyses were done on parameter data from the populations of *P. minutus* as described by Crofton and Haynes and Nichols. Descriptors used were mean abundance and standard deviation, maximum number, prevalence, mean intensity and standard deviation, dispersion coefficient, and skewness and kurtosis (shape parameters of the population curves). Results showed that the parameters most usually reported to describe helminth infections were, as with *Clinostomum*, strongly correlated with each other. One difference being prevalence, in which *P. minutus* prevalence was significantly correlated with other parameters.

Dispersion coefficients (standard deviation/mean) were independent (not significant) of an effect by the other parameters. Skewness and kurtosis were very highly correlated within the two population types (mean and mean intensity) but not with other descriptors. Since population parameters are important in the description of parasitic infections, it would seem that more data on more helminths are needed to determine if this proportionality is a universal phenomenon of stochastic and random helminth infections or just coincidentally found in *C. marginatum* and *P. minutus* juvenile infections.

## Introduction

Daly et al. (2007) and Daly (2014) found that population descriptors, with the exception of prevalence, of the metacercariae of the parasite *Clinostomum marginatum* in black bass (*Micropterus* spp.) were highly correlated with each other. However, very little information regarding such correlations can be found in the literature of other parasitic helminth infections. The data of Crofton (1971) offered an opportunity to see if another helminth, this time a member of a different phylum, Acanthocephala, would also show such descriptor relationships (or not). Crofton published the raw data for populations of *Polymorphus minutus* infecting the intermediate amphipod hosts (*Gammarus pulex*) taken from 6 different downstream sites from the source of the definitive infection, a domestic duck farm. Crofton took raw data, originally published by Haynes and Nichols (1963), to develop an understanding of the mathematics involved in the infection and determined that the parasite population conformed to a negative binomial rather than a random distribution of parasites in the hosts. This is due to an overdispersion of the parasites where a few of the hosts contained a relatively large number of worms in contrast to the majority of the hosts. *Clinostomum* metacercariae in black bass also exhibit aggregation or overdispersion (Daly 2014) therefore it was of interest to see if the phenomenon of correlation between descriptors occurs in a similar negative binomial helminth infection. Using data from Crofton regression analyses were done between the descriptors where a correlation coefficient ( $R^2$ ), an intercept, a slope, and a probability factor are obtained. The relative strength of the relationships can be compared with the regression coefficients and probability values. Predictive values can also be obtained for a dependent variable and examined for closeness of fit with the actual dependent values.

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### Methods and Materials

The raw data used for population descriptor determinations is from Crofton (1971). Determination of descriptors was done using Microsoft Excel descriptive statistic package. The descriptor values can be found in Tables 1 and 2. Table 1 contains the descriptors that are found in most population studies. Table 2 contains newer descriptors that are not usually seen in the literature. The data from those tables were used to calculate the correlations between the descriptors using the Microsoft Excel regression analysis package. Definitions of the descriptors followed that of Bush et al. (1997) with the exception of skewness and kurtosis which describes the geometry of the population curves. Skewness (SKEW) is the degree of distance from a hypothetical center of the curve and kurtosis (KURT) measures the height of the curve. Definitions of other descriptors and

abbreviations are as follows: Mean abundance; the average number of parasites per host (MA and its standard deviation MASD), Maximum number; the largest number of parasites in a single host (MAX); Prevalence; Percent of hosts infected (PREV), Mean intensity; The average number of parasites per host but only for infected hosts (MI and its standard deviation, MISD) and dispersion coefficient; the degree of aggregation in the population (MASD/MA and MISD/MI). Also added was a geometric ratio; KURT/SKEW.

### Results

Values taken from Crofton's raw data were used for both Excel descriptive statistics and regression analyses and can be found in Tables 1 and 2 with new data added. Skewness and kurtosis are new correlations as are mean intensity and its standard deviation.

Table 1. Population descriptors for the acanthocephalan *Polymorphus minutus* in *Gammarus pulex* (data recalculated from Crofton 1971). MA = mean abundance, MASD = MA standard deviation, MAX =maximum number, MI = mean intensity, and MISD = MI standard deviation).

| Site | No. Hosts | Prevalence | MA   | MASD | MAX | MI  | MISD |
|------|-----------|------------|------|------|-----|-----|------|
| 1    | 547       | 70.9       | 2.28 | 2.35 | 10  | 3.2 | 2.2  |
| 2    | 509       | 63.0       | 1.42 | 1.62 | 9   | 2.3 | 1.5  |
| 3    | 633       | 27.7       | 0.60 | 1.2  | 7   | 2.2 | 1.5  |
| 4.   | 486       | 66.0       | 1.31 | 1.37 | 8   | 2.0 | 1.2  |
| 5    | 276       | 48.9       | 0.89 | 1.22 | 6   | 1.8 | 1.2  |
| 6    | 191       | 20.0       | 0.27 | 0.62 | 4   | 1.3 | 0.7  |

Table 2. Population descriptors for the acanthocephalan *Polymorphus minutus* in *Gammarus pulex* (data calculated from descriptors (independent variables) in Table 1 and new data: Dispersion ratios (MASD/MA, MI/MISD); and population curve characteristics of Skewness (SKEW) and Kurtosis (KURT). MA = mean abundance, MASD = MA standard deviation, MAX =maximum number, MI = mean intensity, and MISD = MI standard deviation).

| Site | MASD/MA | MISD/MI | MA SKEW | MA KURT | MI SKEW | MI KURT |
|------|---------|---------|---------|---------|---------|---------|
| 1    | 1.03    | 0.69    | 1.08    | 0.61    | 1.00    | 0.49    |
| 2    | 1.14    | 0.65    | 1.47    | 2.73    | 1.61    | 3.17    |
| 3    | 2.00    | 0.68    | 2.59    | 7.07    | 1.37    | 1.36    |
| 4    | 1.04    | 0.60    | 1.22    | 1.56    | 1.43    | 2.20    |
| 5    | 1.37    | 0.66    | 1.70    | 2.90    | 1.56    | 1.96    |
| 6    | 2.22    | 0.54    | 2.94    | 10.32   | 2.28    | 5.15    |

Dispersion coefficients for both mean abundance and mean intensity are also new and calculated with mean abundance standard deviation/mean abundance (MASD/MA) and mean intensity standard deviation/mean intensity (MISD/MI). These coefficients can determine the degree of dispersion of the population. Greater than 1 is overdispersion and less than 1 is tending toward random distribution. It can be seen that the MASD/MA of the unredacted data shows overdispersion (Table 1). The average and standard deviation of the 6 sites is  $1.66 \pm 0.85$ . However, removing all zeroes (uninfected) from the host population (MI) increases the population mean to an average of  $2.13 \pm .66$  producing a random distribution of parasites in the host population.

Forty two regression analyses were done. The independent variable of each set was always what was

considered to be the major descriptor of the relationship: mean abundance, and standard deviation, maximum number, mean intensity and mean intensity standard deviation. The results of the regressions can be found in Tables 3 and 4. Table 3 regressions are those that are considered to be the usually reported descriptors found in most publications with the addition of dispersion coefficients of MASD/MA, MISD/MI. Table 3 regressions are those involving the descriptors found in Table 2 and the population distribution curves geometric analyses of skewness and kurtosis are in Table 4. The correlations in the Tables were ranked based on the strength of the probability coefficient for significance and for Table 3 ranged from  $8E-05$  to 0.35. Using 0.05 as the standard for statistical significance the strongest correlations were those variables that are considered to be somewhat

Table 3. Regression values for population descriptors for *Polymorphus minutus* infection in *Gammarus pulex*. Calculations were made using the data in Table 1. Abbreviations can be found in Tables 1 and 2.

| Independent variable | Dependent variable   | R <sup>2</sup> | Intercept | Slope | p      |
|----------------------|----------------------|----------------|-----------|-------|--------|
| Mean Intensity       | Mean Intensity SD    | 0.99           | -0.28     | 0.78  | 8E-05  |
| Mean Abundance       | Mean abundance SD    | 0.95           | 0.53      | 0.76  | 0.001  |
| Mean Abundance SD    | Mean Intensity       | 0.94           | 0.64      | 1.10  | 0.0013 |
| Mean Abundance SD    | Mean Intensity SD    | 0.91           | 0.23      | 0.83  | 0.003  |
| Mean Abundance SD    | Maximum Number       | 0.88           | 2.30      | 3.57  | 0.006  |
| Mean Intensity       | Maximum Number       | 0.84           | 0.66      | 3.10  | 0.01   |
| Mean Abundance       | Maximum Number       | 0.83           | 4.30      | 2.70  | 0.01   |
| Mean Abundance       | Prevalence           | 0.82           | 19.80     | 26.00 | 0.014  |
| Mean Abundance       | Mean Intensity       | 0.82           | 1.20      | 0.78  | 0.014  |
| Mean Abundance       | Mean Intensity SD    | 0.75           | 0.72      | 0.58  | 0.03   |
| Mean Abundance       | SD/Mean Abundance    | 0.74           | 2.20      | -0.63 | 0.03   |
| Maximum Number       | SD/Mean Abundance    | 0.69           | 3.00      | -0.21 | 0.040  |
| Mean Abundance SD    | Prevalence           | 0.69           | 6.40      | 30.80 | 0.042  |
| Mean Intensity SD    | SD/Mean Intensity/MI | 0.66           | 0.51      | 0.09  | 0.048  |
| Mean Abundance SD    | SD/Mean Abundance    | 0.64           | 2.50      | -0.76 | 0.057* |
| Mean Intensity       | SD/Mean Intensity    | 0.56           | 0.49      | 0.06  | 0.18*  |
| Mean Intensity       | Prevalence           | 0.47           | -0.04     | 23.20 | 0.13*  |
| Mean Abundance SD/MA | Mean Intensity SD/MI | 0.22           | 0.71      | -0.05 | 0.35*  |

\*Not significant with  $p = 0.05$

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required for any description of a parasitic helminth population (mean abundance, mean abundance standard deviation, maximum number, prevalence and in many publications, mean intensity values). Only four of these regressions were not significant. Of these, three had as one of the variables a coefficient of dispersion. The other non-significant correlation, which did not include a dispersion coefficient, was mean intensity versus prevalence. Prevalence in this latter correlation uses the non-redacted data and does not mean intensity prevalence which is 100% for all sites. Since prevalence does not change for the MI populations it can be assumed beforehand that this relationship should not be significantly correlated. This relationship (PREV vs. MI) deals with an artificially modified population (mean intensity) of the original nonredacted population and acts as a control on the efficacy of the regression analyses. Surprisingly dispersion coefficients are somewhat independent of the mean and standard deviation (and MISD and MI) which are both used to calculate the dispersion coefficients. Table 4 included the regressions involving skewness and kurtosis as the dependent variables. These ranged from 7.9E-05 to 0.46. The strongest relationships were between skewness and kurtosis (both mean abundance and mean intensity populations). Eight of the correlations were not significant based on  $p = 0.05$ . Prevalence and skewness or kurtosis showed surprisingly strong correlations with Mean abundance (MA). Other descriptors paired with skewness and kurtosis were either slightly significant,  $p = 0.041$  to 0.048, or not significant. Therefore skewness and kurtosis are somewhat independent of the other descriptors except for each other. A caveat for all these regressions is that only 6 samples ( $n = \text{sites}$ ) are used for each variable. Such a small sample size emphasizes the significance of some of the comparisons.

### Discussion

Almost any study of a parasitic helminth population includes descriptive parameters. These are used to monitor changes in the population and/or compare populations to see if they are different. Any additive information regarding these parameters or the introduction of newer available parameters (skewness and kurtosis) would assist investigators of helminth populations in achieving the above goals. Poulin (2007) performed a review of these descriptors and the possible reasons to explain the variety of results that parasite populations have shown. Such factors as

parasite density dependent, density independent, immune response of hosts, fecundity, mortality, host size, recruitment and environmental impact are all considered. Daly (2014) found with the yellow grub (*Clinostomum*) that metacercarial infections in smallmouth bass (*Micropterus dolomeiu*) showed strong proportionality between descriptors, so much so that the regression coefficients were strongly predictive within the populations. Prevalence was not well correlated with other descriptors but this is due to the high parasite density in some of the populations where parasites can reach 100% prevalence but parasite abundance can still keep increasing (Daly and Wagner, *in prep.*). With the *Polymorphus minutus* regressions the parameters were almost all significantly correlated with the exception of standard deviation/mean and some geometric values (kurtosis and skewness). This makes *Polymorphus* the second helminth to have these population proportionality characteristics. Although in two different phyla both *P. minutus* and *C. marginatum* are similar and dissimilar in important ways. Both studies used juvenile worms in intermediate hosts. Neither worm reproduces in that host. Both studies used different sites on a stream, the difference being that *P. minutus* decreases overall infection density as it progresses downstream and *C. marginatum* increases overall abundance downstream. However, both parasites exhibit overdispersion or a negative binomial dispersion. Redacting zero infected hosts (MI) in both infections produces a normal distribution but still does not alter basic correlations, except for skewness and kurtosis and the contrived standard deviation/mean ratio. Why parasitic helminth populations favor a negative binomial distribution over random distribution is not clear (Poulin 2007) but Daly (2007) has proposed that in many cases that the most heavily infected hosts are closer to the source of the infection and the less infected hosts are further away. Parasites and hosts are not randomly or evenly distributed in the environment. In the case of *C. marginatum* the important factors would be the presence of infected snails with a nearby population of definitive hosts (great blue herons). The infecting zone would then be determined by stream hydraulics and the path of the migrating cercariae. With *P. minutus* stream hydraulics would be the most important factor since eggs of *P. minutus* are inert and dependent for distribution on a current. It is known that stream flow is different from area to area in a stream and the position and numbers of the microcrustacean hosts in those zones would be the major determinate for infection and an aggregate distribution in the hosts.

Table 4. Regression values for population descriptors using data for SKEW and KURT, for *Polymorphus minutus* infection in *Gammarus pulex*. Calculations were made using the data in Table 1 and 2. Abbreviations can be found in Tables 1 and 2.

| Independent variable   | Dependent variable | R <sup>2</sup> | Intercept | Slope | <i>p</i> |
|------------------------|--------------------|----------------|-----------|-------|----------|
| Skewness (MA)          | Prevalence (MA)    | 0.99           | 100.0     | -28.0 | 7.9E-05  |
| Skewness (MA)          | Kurtosis (MA)      | 0.97           | -4.7      | 4.9   | 0.0003   |
| Skewness (MI)          | Kurtosis (MI)      | 0.94           | -3.3      | 3.7   | 0.001    |
| Kurtosis (MA)          | Prevalence (MA)    | 0.93           | 72.6      | -5.5  | 0.002    |
| Kurtosis/Skewness (MA) | Prevalence (MA)    | 0.89           | 86.6      | -19.4 | 0.004    |
| Kurtosis/Skewness (MA) | Abundance (MA)     | 0.85           | 2.3       | -1.63 | 0.01     |
| Mean Intensity SD      | Skewness (MI)      | 0.82           | 2.6       | -0.77 | 0.014    |
| Mean Intensity         | Skewness (MI)      | 0.79           | 2.8       | -0.59 | 0.02     |
| Abundance (MA)         | Skewness (MA)      | 0.78           | 2.9       | -0.91 | 0.03     |
| Abundance (MA)         | Kurtosis (MA)      | 0.74           | 9.2       | -4.4  | 0.03     |
| Mean Intensity SD      | Kurtosis (MI)      | 0.70           | 6.2       | -2.7  | 0.04     |
| Maximum Number         | Kurtosis (MA)      | 0.69           | 14.7      | -1.43 | 0.041    |
| Maximum Number         | Skewness (MA)      | 0.69           | 4.0       | -0.29 | 0.043    |
| Mean Abundance SD      | Kurtosis (MA)      | 0.68           | 11.7      | -5.4  | 0.041    |
| Mean Abundance SD      | Skewness (MA)      | 0.66           | 3.3       | -1.1  | 0.049    |
| Mean Intensity         | Kurtosis (MI)      | 0.64           | 6.8       | 2.1   | 0.051*   |
| Kurtosis/Skewness (MI) | Abundance (MI)     | 0.61           | 3.1       | -0.8  | 0.07*    |
| Kurtosis (MA)          | Skewness (MI)      | 0.61           | 1.2       | 0.06  | 0.07 *   |
| Skewness (MA)          | Skewness (MI)      | 0.50           | -0.15     | 1.28  | 0.11*    |
| Kurtosis (MA)          | Kurtosis (MI)      | 0.47           | 1.1       | 0.3   | 0.13*    |
| Skewness (MI)          | Prevalence (MA)    | 0.47           | 102.6     | -34.5 | 0.13*    |
| Skewness (MA)          | Kurtosis (MI)      | 0.34           | 0.11      | 1.2   | 0.22*    |
| Kurtosis (MI)          | Prevalence (MA)    | 0.29           | 66.2      | -7.0  | 0.27*    |
| Kurtosis/Skewness (MI) | Prevalence (MA)    | 0.14           | 67.7      | -12.4 | 0.46*    |

\*Not significant with  $p = 0.05$

Possibly the faster flow would contain more eggs and a slower flow more *Gammarus*.

Nevertheless, regardless of the parasite distribution in the host population, it appears that because of the proportionality that the same factors are at work at different sites, in this case on streams. Daly (2014) has compared the proportionality to that of nesting Russian

dolls. They look similar except for size but proportionally they look the same. It is interesting that even the geometry (kurtosis and skewness) of the population curves change proportionally to abundance changes. It would seem that more studies of this proportionality phenomenon are needed and would add important data to metazoan parasite infections. The

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most important impediment factor for such studies is to have enough different sites to regress with markedly different population intensities.

### Conclusion

To summarize: Significant correlations are seen between the major population descriptors of MA, MASD, MAX, MI, MISD, and PREV. Dispersion coefficients correlate poorly or are not significant with other descriptors. Skewness and kurtosis (both MA and MI) correlate well with prevalence, MA, MI, MISD; However they do not correlate significantly with other descriptors including most of the standard deviations but are closely correlated with each other within the same population (MA SKEW vs MA KURT, MI SKEW VS MI KURT).

This is only the second parasitic helminth to exhibit so many such significant correlations. It implies that the factors producing this uniformity are the same, for the most part, at different population sites with different parasite densities. The aggregated populations may be a result of a similar spaciality between host and source of infection at each of the different sites.

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