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Proportionality of Population Descriptors of Metacercariae of *Clinostomum marginatum* in the Orobranchial Cavity of Black Bass (*Micropterus* spp.) from Arkansas Ozark and Ouachita Streams

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Running title: Proportionality of Population Descriptors of *Clinostomum marginatum* in Black Bass.

Abstract.

In a previous study of *Clinostomum marginatum* metacercariae in *Micropterus dolomieu*, I reported that the population parameters of mean abundance, standard deviation, maximum abundance, mean intensity and mean intensity standard deviation were proportional between the total population and the orobranchial numbers for 16 locations in Arkansas Ozark and Ouachita streams. This allowed an assessment of the parasite populations by only examining the mouth and gill areas without sacrificing a valued sports fish. The present study examined the same orobranchial parameters utilizing correlation and descriptive statistics to determine if proportionality also existed between the different localities. I have now included an analysis of skewness and kurtosis (drift and shape) of the populations' curves. Proportionality of regression values was highly significant in terms of R^2 and P between all parameters except prevalence, which showed much weaker correlations with the other parameters. The interpretation of these results is that the distribution of infections in the different bass populations are density independent i.e., although the numbers of parasites change from location to location, the pattern of distribution in the host populations remains similar. This may best be explained by a spatiality of distance from the infection source (snails) and nonrandom distribution of hosts (bass) producing aggregation near the snails and a negative binomial distribution throughout the the population.

Introduction

Daly et al. (2007) reported that there was proportionality of the population descriptors between *Clinostomum marginatum* metacercariae in the orobranchial cavity relative to the total population numbers in the entire body of *Micropterus dolomieu*

(smallmouth bass) hosts. The importance of this finding was that by just counting the metacercariae in the gills and mouth of the bass hosts one could get a reasonably close estimate of total population parameters of the parasite without harming a valuable sport fish, which could then be returned to its habitat. It would seem that if such proportionality existed between anatomical sites in the fish hosts then proportionality of population parameters might also exist between the different geographical locales and a single anatomical site (orobranchial). The present study uses similar techniques, correlation and descriptive statistics, from the raw orobranchial data from 17 different locales to test this hypothesis. If such is the case then the parasite distribution in the hosts would appear to follow a pattern, most likely a spatial and stochastic one, independent of the different densities of *Clinostomum* seen in the different populations.

Methods and Materials

Smallmouth and Kentucky bass (KY), *Micropterus dolomieu* and *M. punctulatus* (from 1 locale) hosts were collected from 1 Ozark (Crooked Creek) and 3 Ouachita Mountain (Caddo, Ouachita, and Saline) streams and consisted of 17 different locales on 4 streams in Arkansas with one exception: WR 88 and WR 90 on Crooked Creek, whose values were so greatly different they were treated as separate locales. The total number of hosts collected from May to October 1988-1990 was 579. Details of the collecting locales and necropsy techniques are found in Daly et al. (2002, 2007) and Daly Jr. et al. (2002). Metacercariae (or yellow grubs) were collected from the orobranchial cavity (visible in/on mouth and gill surfaces) of the hosts and counted. The values of population parameters for this site were calculated from these counts using Microsoft Excel (2010). First, descriptive statistics for each locale were obtained and

these results were then used to determine correlation coefficients between the data from the different locales. To test for significance, an F test was applied to determine equal or unequal variances and then an appropriate T Test was used to determine *P* values. Definitions of population parameters followed that of Bush et al. (1997) and, with abbreviations used, are as follows: Mean abundance, MA (average number of parasites per host); Mean abundance standard deviation (MASD); Maximum abundance, Max (largest number of parasites in a single host); Prevalence (percent of infected hosts); Mean intensity, MI (average number of parasites in infected hosts only); Mean intensity standard deviation (MISD); Variance (S); S/MA (Variance to mean ratio, a relative measurement of aggregation – reflected as a negative binomial population distribution where the heaviest infections are in only a few hosts); SD/MA (a simpler measurement of aggregation eliminating a power function ($S = SD^2$) for linear arithmetic comparisons); Skewness, Skew (measure of shift in population curves) and Kurtosis, Kurt (measure of shape of the population curve). One is dealing not only with 17 different populations but also with two different population parameters of MA and MI within those 17 populations. The earliest study in this series of yellow grub and black bass examined length and maximum circumference of the bass hosts and found no correlation with parasite density measurements (Daly et al. 1987) therefore this is not an issue in this study.

Results

Descriptive statistics for *Clinostomum marginatum* metacercariae in smallmouth (*Micropterus dolomieu*) and Kentucky bass (*M. punctulatus*) orobranchial cavities collected from 17 different locales can be found in Table 1 (mean abundance and derivations) and Table 2 (mean intensity and derivations). For mean abundance the average per host ranged from 0.1 to 3.8 giving a low to high spread that provides excellent correlation analysis as opposed to population means being close together. The WR 90 locale is not included in the regression analyses because its values are very high relative to other locales, (the highest ever recorded for yellow grub in fish hosts – Daly et al. (1991) and gives highly skewed but significant results. Although using such a large outlier does give higher R^2 and *P* values the predictive values obtained for the other locales with smaller means, using the applied correlation coefficients of slope and intercept, gives overestimations of mean

abundance values. Excluding WR 90 gives very close predictive values for mean abundance (and other derivations). This skewing of values by WR 90 indicates a break in the linearity required for accurate correlation analysis, therefore the exclusion. However, using the coefficients from the other 16 populations gives a reasonable estimate of the WR90 values, e.g. actual MASD = 97.2 and the predictive value of MASD with MA as the independent variable = 81.3.

Variance to mean ratios.

Discussion of results requires an understanding of the basic population distribution of helminth parasitic infections. First, SDs being larger than the mean (Table 1) indicates a negative binomial distribution (Crofton 1971, Pennycuick 1971) that requires non-parametric statistics for population comparisons as opposed to SDs being smaller than the mean which would assume a normal distribution necessary for parametric statistics. The negative binomial distribution is due to aggregation or dispersion where a few hosts have the majority of the parasites. Mean intensity eliminates zeros and increases the mean and reduces SD. That this reduction of zero values to produce normal distributions does not always work can be seen when the mean intensity SD is still greater than the mean (Table 2). The variance (S or SD^2) to mean ratio reflects the degree of dispersion and is interpreted as less than one = normal distribution, one = Poisson distribution, and greater than one is a negative binomial. For mean abundance (Table 1) the dispersion ratio is 1 or less for only 2 populations and for smaller mean values. For mean intensity dispersion deviates from negative binomials for 6 populations and primarily at the lower mean values. The ratio of SD/MA (or SD/MI) as suggested by Poulin (2007) is preferred here because the variance to mean ratio of S/MA (or SD^2/MI) has a power function, which is not arithmetically linear that is required for parametric statistics. However, the latter is useful for determining relative strength of aggregation. The average for the ratio of mean abundance SD/MA (Mean $\pm$ SD, confidence limit) for 16 populations is 2.1 ± 0.7 , 0.36 and for mean intensity is 0.84 ± 0.5 , 0.23 (WR90 is included since the value is a ratio). A Student T test for unequal variances gave a *p* value of $1.9E-06$ indicating a significantly high probability of the two populations being unequal. This is expected since the MI is a redacted form of MA. A major finding is that the ratios are proportionally the same and a rounded factor of 2 or 0.9 (derived from the averages of both factors) can be used as a rough but

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reasonable predictive estimate of SD/MA, SD/MI.

Proportionality of population descriptors.

Table 3 shows the results of regression analysis of the various descriptors of mean abundances, mean abundance standard deviations (SD), maximum abundances, mean intensities, mean intensity standard deviations (SD), and prevalence.

All regressions showed significant correlations. Log-log transformations were required to obtain more

significant correlations for all prevalence comparisons. Other log-log transformations gave better correlations than arithmetic comparisons, increasing the R^2 and P values slightly. Slope and intercept values can be applied to the independent variables and these give close estimates of the dependent variables. This proportionality can be seen in Figure 1 (A, B, C, D) as examples where the dependent variable was estimated using the correlation coefficients. Maximum abundance (largest number of parasites in a single

Table 1. Population parameters of *Clinostomum marginatum* metacercariae in the orobranchial cavity of *Micropterus dolomieu* and *M. punctulatus* (O KY) from locales in Ozark and Ouachita streams in Arkansas. Locations are identified in Methods. N = host number, Prev. = prevalence, Max = maximum number of parasites in one host, MA = mean abundance, SD = standard deviation, S = variance, Disp. = dispersion, Skew = Skewness, Kurt = kurtosis. Values are for Mean abundance.

Location	N	Prev.	Max	MA	MASD	S	Disp.	MA/SD	Skew	Kurt	Kurt/Skew
HU	10	10	1	0.10	0.30	0.09	0.9	3.0	3.2	NA	NA
H1	38	11	2	0.13	0.41	0.17	1.3	3.2	3.4	11.8	3.5
H3	37	32	3	0.41	0.69	0.48	1.2	1.7	2.5	6.2	2.5
CG	29	11	2	0.18	0.55	0.30	1.7	3.1	3.0	8.2	2.7
GL	23	20	2	0.29	0.63	0.40	1.4	2.2	2.3	4.7	2.0
H2	45	16	2	0.18	0.43	0.18	1.0	2.4	2.0	4.7	2.0
S	20	20	8	0.64	1.73	2.99	4.7	2.7	4.8	4.8	1.0
BS	20	47	5	1.00	1.49	2.22	2.2	1.5	1.5	2.2	1.5
O	37	44	10	1.07	2.07	4.28	4.0	1.9	1.0	1.8	1.8
Y	44	49	11	1.14	1.90	3.61	3.2	1.7	3.5	16.2	4.6
CC	42	49	17	1.84	3.25	10.56	5.7	1.8	2.9	10.4	3.6
P	27	59	10	1.85	2.24	5.02	2.7	1.2	2.4	6.1	2.6
G	30	67	13	2.70	3.40	11.56	4.3	1.3	2.3	2.9	1.2
O KY	19	37	26	3.60	7.40	54.76	15.2	2.1	1.9	8.3	4.4
T	105	64	25	3.70	4.90	24.01	6.5	1.3	1.9	3.5	1.9
WR88	36	53	67	3.80	11.04	122	32.1	2.9	3.3	11.3	3.5
WR90	17	65	400	42.50	97.20	9447	222.3	2.3	5.4	31.0	5.7

Table 2. Population parameters of *Clinostomum marginatum* metacercariae in the orobranchial cavity of *Micropterus dolomieu* and *M. punctulatus* (O KY) from locales in Ozark and Ouachita streams in Arkansas. Locations are identified in Methods. MI= mean intensity, SD = standard deviation, S = variance, Disp. = dispersion, Skew = Skewness, Kurt = kurtosis. Values are for Mean intensity (all infected hosts only – prevalence = 100 %).

Locale	MI	MISD	MI S	Disp	SD/MI	Skew	Kurt	Kurt/Skew
HU	1	NA	NA	NA	NA	NA	NA	----
H1	1.25	0.43	0.18	0.10	0.34	2.4	6.0	2.4
H2	1.14	0.35	0.12	0.11	0.31	2.6	6.2	2.4
H3	1.25	0.50	0.25	0.20	0.40	2.0	4.0	2.0
CG	1.67	1.58	2.50	1.50	0.95	-1.7	NA	-----
GL	1.25	0.52	0.27	0.22	0.42	2.0	4.0	2.0
S	3.20	3.77	14.21	4.44	1.18	0.5	NA	NA
BS	2.29	1.38	1.90	0.83	0.60	1.3	1.5	1.2
O	2.40	2.57	6.60	2.75	1.07	3.2	6.5	2.0
Y	1.80	1.05	1.10	0.61	0.58	3.2	12.1	3.8
CC	3.86	3.80	14.44	3.74	0.98	2.2	6.0	2.7
P	3.13	2.40	5.80	1.84	0.77	1.9	3.6	1.9
G	4.05	3.50	12.25	3.02	0.86	1.6	1.5	0.9
T	5.70	5.10	26.01	5.60	0.89	1.6	2.2	1.4
O KY	9.70	9.70	94.09	9.68	1.00	1.7	2.4	1.4
WR88	7.26	14.40	240	30.26	1.95	2.9	15.6	5.4
WR90	65.6	120	14634	213	1.83	2.6	7.0	2.7

host), is related to the degree of aggregation since its value produces a right shift of population distribution. Maximum abundance also shows excellent correlation with other variables.

All of the correlations show proportionality between the parameters of both MA and MI populations.

Skewness and Kurtosis.

Regression analyses were performed on the skewness and kurtosis values from 14 populations

(including WR90) that had available values for analysis as seen in Table 4. Correlations were significant for skewness and kurtosis within the parameters of MA and MI but not between the two parameters with $P = <0.05$. The descriptor values change but the ratios and the shape of the curves do not. A major difference is the effect of the elimination of zero values from MI which is clearly shown by the shift of the mean or mode (skewness) of the population distribution from the MA values (Tables 1 and 2).

In summary, these results overall show highly

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significant proportionality amongst the population descriptors. Population curves also have similar shapes proportionally but differ in skewness when the population values are changed (redacted zeros).

SD/MA and SD/MI ratios have a common factor for each of the population sets of about 2.2 and 0.9 respectively and are also significantly different between the two sets of populations.

Table 3. Regression analysis of population parameters of *Clinostomum marginatum* metacercariae in the orobranchial area of *Micropterus dolomieu* and *M. punctulatus* (O KY) hosts from 16 stream locales in the Arkansas Ozark and Ouachita highland areas. Variables used are from Tables 1 and 2. SD = Standard deviation.

Independent Variable	Dependent variable	R ²	Intercept	Slope	P
Mean abundance	Mean abundance SD	0.81	-0.12	2.0	2.0E-06
Log ₁₀ mean abundance	Log ₁₀ maximum abundance	0.91	0.93	0.9	1.2E-08
Mean abundance	Maximum number	0.67	-1.37	10.0	1.1E-04
Mean abundance	Mean intensity	0.83	0.81	1.7	7.7E-07
Mean abundance	Mean intensity SD	0.70	-0.19	2.4	4.9E-05
Mean abundance	Mean abundance variance	0.56	-9.60	17.5	9.0E-04
Log ₁₀ mean abundance	Log ₁₀ MA Variance	0.94	0.57	1.7	4.1E-10
Mean abundance SD	Mean intensity	0.82	1.16	0.8	1.4E-06
Mean abundance SD	Mean intensity SD	0.96	-0.20	1.3	8.6E-11
Mean abundance SD	Maximum abundance	0.94	-1.62	5.4	9.8E-10
Mean abundance SD	Mean abundance variance	0.91	-12.10	10.3	1.0E-08
Mean intensity	Mean intensity SD	0.81	-1.30	1.4	1.0E-06
Mean intensity SD	Maximum abundance	0.90	-0.40	4.1	6.9E-08
Log ₁₀ mean intensity	Log ₁₀ MI variance	0.96	-0.69	2.8	3.7E-10
Prevalence	Mean abundance	0.58	-0.49	0.05	5.8E-04
Log ₁₀ Prevalence	Log ₁₀ mean abundance	0.85	2.70	1.7	4.4E-07
Prevalence	Mean abundance SD	0.29	-0.29	0.08	0.03
Log ₁₀ Prevalence	Log ₁₀ mean abundance SD	0.67	-1.70	1.30	1.0E-04
Prevalence	Mean intensity	0.25	0.90	0.06	0.05
Log ₁₀ prevalence	Log ₁₀ mean intensity SD	0.45	0.60	0.70	0.004
Prevalence	Maximum abundance	0.26	-2.73	0.42	0.05
Log ₁₀ Prevalence	Log ₁₀ maximum abundance	0.67	-1.30	1.43	1.2E-04

Figure 1. Examples of regressions of descriptive parameters from 16 populations of *Clinostomum marginatum* from the orobranchial cavity of stream black bass (*Micropterus dolomieu* and *M. punctulatus*) from Ozark and Ouachita streams in Arkansas. A, B, C regressions are with mean abundance as the independent variable with the graphs showing the dependent variable and the predicted values of the dependent values using the regression coefficients from Table 3. A. Standard deviation of mean abundance, B. Mean intensity, C. Maximum abundance and D. Prevalence as the independent variable and mean abundance as the dependent variable showing the predicted mean abundance from the prevalence values.

Table 4. Regression analysis of skewness and kurtosis of mean abundance and mean intensity of population curves of *Clinostomum marginatum* in black bass orobranchial cavities.

Independent variable	Dependent variable	R ²	Intercept	Slope	P
Mean abundance skewness	Mean abundance kurtosis	0.89	-8.8	6.7	3.4E-07
Mean intensity skewness	Mean intensity kurtosis	0.70	-6.6	5.5	2.1E-04
Mean abundance skewness	Mean intensity skewness	0.12	1.7	0.2	0.23*
Mean abundance kurtosis	Mean intensity kurtosis	0.21	3.6	0.2	0.10*
Mean abundance skewness	Mean intensity kurtosis	0.23	1.1	1.7	0.08*
Mean intensity skewness	Mean abundance kurtosis	0.15	1.0	3.2	0.17*

* Not significant with $P = > 0.05$

Discussion

Population parameters of helminth parasites have traditionally been used to compare differences and/or

similarities between parasite populations (Dobson and Beveridge 1987, Poulin 2007). At least 62 reports in just the last 10 years of the Journal of Parasitology have utilized these parameters for comparisons and

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predictive power. This is the first study to show close correlations between all the commonly used population parameters of prevalence, mean abundance, mean abundance standard deviation, mean intensity, mean intensity, mean intensity standard deviation, and of variance to mean ratios. The 17 populations examined herein are useful for these parameter analyses because they are from geographically different locales spread along a single stream, or even from different streams, and therefore represent a range of parasite densities in the hosts that can give a reasonable spread for representative correlations. The correlation coefficients of R^2 and P are so significant that the relationships do not require more advanced statistics to demonstrate their existence. The positive correlations between MA and MI parameters were not unexpected, since one population (MA) was derived from the other (MI) and they are different only due to the redacted zeros.

The relationship between prevalence and mean abundance have been most popularly examined as a predictor of population density and/or intensity, but prevalence has not always been shown to be a reliable predictor of abundance or intensity of infection. Janovy et al. (1997) and recently Shostak (2014) resorted to log transformations to show such a relationship and in the latter case the curve obtained was asymptotic at the top end. In this present study, prevalence without log conversions was shown to have the weakest correlations of all the combinations of parameters between mean abundance or mean intensity. Prevalence can be a problem for estimations of population numbers at higher values because at 100% or values near 100% they cannot recognize a further increase in the number of parasites. Conversely, Shostak (2014) found that abundance actually leveled off at 90% prevalence, but this is not necessarily the case, as with the total population values for *Clinostomum* in smallmouth bass (Daly et al. 2007) which increased. There is an interesting relationship that can be detected between prevalence and the two density measurements. If one knows the prevalence and the value of one of the two means, then simply by multiplying a factor determined by dividing 100/prevalence or prevalence/100, the other mean can be closely estimated (e.g., Prevalence = 50 %, then MA multiplied by 2 = MI or MI multiplied by 0.5 = MA).

This is the first report that reveals the close relationships that exist between population curves for skewness and kurtosis for the same population parameter (mean abundance or mean intensity). The correlations between skewness and kurtosis showed

that the population curves are similar within MA and MI parameters, but are statistically dissimilar between the curves of the two groups as shown by a T test which did not show a difference between the average skewness' within MA and MI but did with correlations which paired the values between MA and MI. This is to be expected since the two population curves are obviously different because of redacted zeros.

The negative binomial distribution of most helminth parasite populations has been a problem for comparisons of populations requiring transformations such as log values (or nonparametric techniques) to obtain a normal distribution required for parametric statistics. Mean intensity removes zeros which raises the mean value and lowers the standard deviation, but even this does not necessarily produce normal distributions for parametric comparisons. This redaction of zeros (only) is somewhat subjective and questionable methodology since removing zero values defies the logic of population sampling. Despite this, in the current study, close correlations were found between the two populations' MA and MI parameters. This indicates that removal of zeros did not affect the basic relationships except for geometry of the curves.

One caveat is the exceptionally large parasite density in WR 90 that shifts the R^2 and P values to greater significance, but lowers the predictability for smaller density populations. This suggests that the relationships between the variables may not be totally linear but are probably polynomial when including very high values (outliers?). This does not diminish or exclude the use of correlations since the predictability of the independent variable is still high for most parameters (Table 3; Figure 1).

Degree of aggregation has traditionally been the use of S/mean or a calculated K value (Esch et al. 1977, Poulin 2007). The use of SD/MA removes a power function (SD^2) that can make linear correlations questionable. A strong similarity between means and standard deviations can be seen in the ratios obtained for the different populations, which can be rendered to a factor of approximately 2. Standard deviation/mean can also be a comparative measure of degree of aggregation, although values such as assigned to S/mean (>1 = aggregation) have not yet been generally accepted. Aggregation is not dramatically different amongst the populations, as seen herein with the ratios, but has a relativity; i.e., the SD/MA , that unites the populations' distributions. Maximum abundance is also a useful tool to indicate aggregation and it is also proportional although the high value distorts the population curve from a random, normal distribution.

In conclusion, the mean and standard deviation relationships show populations with a strong stochastic characteristic with the interaction of black bass and yellow grub in an aquatic environment. This is evidence for the presence of a relatively stationary snail host producing cercariae into a setting where the bass hosts are not randomly distributed (Etnier and Starnes 1993). The proportionality of parameters is further evidence for the non-randomness in that it also implies very structured populations. A simple way to look at the proportionalities of the population parameters is to consider the different populations similar to a nesting set of Russian dolls that are alike in appearance but different in geometric proportions and size. Empirical evidence is necessary to further substantiate the theory that these relationships are due, at least in this case, to the spreading cercariae encountering a non-random host distribution of smallmouth bass producing a stochastic aggregated population, but where the parameters remain mathematically proportional.

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