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A Non-Invasive Technique for Assessing the Population Parameters of Metacercariae of *Clinostomum marginatum* in Smallmouth Bass (*Micropterus dolomieu*)

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Abstract.—*Clinostomum marginatum* is a trematode that uses a fish as its final intermediate host. The worms in the fish are in metacercarial cysts and are known as yellow grub. Yellow grubs give the fish's flesh a wormy, unappetizing appearance and are a problem for commercial fish farmers in that heavily infected fish are not suitable for marketing. The parasite is common in smallmouth bass (*Micropterus dolomieu*) living in upland streams of Arkansas where the bass may serve as a wild reservoir for contamination of commercial fish ponds. Because smallmouth bass are a prized game fish, it would be desirable to be able to assess the extent of yellow grub infections by a non-invasive method whereby the fish could be examined and returned to its habitat without destructive necropsy. In this study strong correlations were found between the parasites seen in the orobranchial region and the rest of the host body. These correlations were found for all of the population parameters usually reported. The significance of these findings are mainly three fold: (1) The correlations allowed a reasonable estimate of yellow grub loads in populations of smallmouth bass using only orobranchial counts, (2) *in situ* examination of the mouth and gills alone allows the fish to be returned unharmed to the stream and (3) similar anatomical-site density correlations applied to other parasitic infections might dramatically reduce the amount of necropsy time needed for estimating total parasite numbers. Examples for the latter are given from other studies with *Clinostomum complanatum* and *Proteocephalus ambloplitis* species that show similar anatomical site density relationships in their respective hosts.

Key words:— Clinostomum marginatum, trematode, metacercarial cysts, yellow grub, smallmouth bass, *Micropterus dolomieu*, Arkansas.

Introduction

During a study of yellow grub infection caused by *Clinostomum marginatum* in black bass (*Micropterus* spp.), it was noted that fish which had a large number of metacercariae in the gill and mouth cavities would also most likely have a large number of cysts in the rest of the body. Crooked Creek in northern Arkansas was especially interesting because the smallmouth bass (*Micropterus dolomieu*) in that stream were found to harbor large numbers of the parasite (Daly et al. 1987, Daly et al. 1991). It was hypothesized that if there was a significant correlation between cysts from the gills and mouth to those in the body, it would be useful as a non-invasive approach to determining yellow grub population parameters by visibly counting the grubs seen in those sites. Smallmouth bass are a prized game fish to the extent that the Arkansas Game and Fish Commission has put major restrictions on the number and size of smallmouth that can be taken from Crooked Creek and other smallmouth streams. It would be advantageous for large scale surveys and recapture studies on *C. marginatum* in this stream if the hosts could be returned to the stream relatively unharmed. Smallmouth were collected along the length of Crooked Creek from near its origin to its juncture with the White River, as well as from other Arkansas upland streams. Gill-mouth parameters showed excellent predictive values relative to the total body parameters. Further, re-examination of data from other reports, for similar correlations, showed significant relationships between site densities for yellow grub in South American catfish and for a *Proteocephalus* plerocercoid infection in smallmouth and largemouth bass.

Methods and Materials

The data used in this study were from the surveys of *C. marginatum* from smallmouth bass collected from different sections of Crooked Creek in 1988-90 from the city of Harrison to the White River in Boone and Marion Counties. Collection details and location identifiers can be found in Daly et al. (2002). Locations on the Caddo River were between Black Springs and Glenwood in Montgomery and Clark Counties, respectively (Daly et al. 1999). Locations on the Ouachita River were upstream from Lake Ouachita between Sims and Cherry Hill in Montgomery County with host collections made in 1996. Hosts from the Saline River were collected near Benton (Saline Co.), also in 1996. All three streams are typical of Ozark and Ouachita upland rivers and provide good smallmouth habitat.

This present correlation study included 15 of the 16 locales and 543 smallmouth bass. One location WR90 was not included in the regression analyses because it is a hyperinfection relative to the other populations of yellow grub. Such outliers tend to skew regression analyses and in this case make the correlations seem more significant than they are for prediction purposes. Bass were collected by rod and reel using live or artificial bait.

Captured fish were placed on ice and transported for necropsy and length measurements to the University of Arkansas for Medical Sciences, Little Rock, AR. Metacercariae were removed for counting from all parts of the fish and placed into two Petri dishes containing saline; one dish for those parasites from the orobranchial cavity and the other for those from the rest of the body. Nomenclature of population parameters (descriptors) of prevalence, maximum abundance, mean abundance, and mean intensity follows that of Bush et al. (1997). The term "site" used herein refers to anatomical region of the host with "location" defined as the geographic host collection place. An important caveat is that the gill-mouth metacercariae are combined with the count in the rest of the body as a Total Body count and separately as a Gill count. Although both mouth and gill cavity parasites were counted, they will be referred mainly to as gill in this paper. Regression analyses were done between the gill and total body parameters using the parameters from each of the locales as the dependant and independent variables. Data from Table 4 in Vianna et al. (2005) was used for the calculated values in Table 4. Data from Gillilland and Muzzall (2004) used in Table 4 were from their Tables 2 and 3 and were recalculated. Total numbers for the parenteric sites used in Table 4 were calculated by multiplying the mean intensity of each site by the sample number corrected for prevalence. The total count was determined by adding the values from the 4 sites. The sample size N for each regression was 4 representing the gender classes as different populations. Population statistics, graphs and regression analysis were done with Microsoft Excel (2003).

Results

Tables 1 and 2 show the population parameters obtained from necropsy counts of the gill/mouth area and the total body metacercariae in the bass hosts. Data on 15 populations (excluding WR90) from those two tables were used to determine the coefficients of the regression analyses of the paired parameters seen in Table 3. Gill data were always the independent variables and the total body data the dependent variables since the purpose was to determine if the gill counts could reasonably predict the total counts. All of the correlations of gill parameters versus other parameters had high r values and were significant with most being at p = <0.001, set at 95% confidence limit. Gill prevalence did not correlate as well with total body prevalence as did the other parameters. The reason for this is that total body prevalence quickly reaches a plateau and becomes asymptotic even at relatively low mean abundances. Gill prevalence more closely correlates with total mean abundance ($r = 0.90$, p = < 0.0001) than with total prevalence ($r = 0.77$, p = <0.001) which means that gill prevalence is a more sensitive and better predictor of parasite load than total body prevalence itself. Interestingly, prevalence values from the 16 infections ranged from the 60s to 90s percent, but never reached 100 %, which is true for some heavy parasitic infections, such as with *Proteocephalus ambloplitis* (Gillilland and Muzzall, 2004).

Table 4 shows data taken from two studies: *Clinostomum complanatum* in South American catfish (Vianna et al. 2005) and the cestode *Proteocephalus ambloplitis* in smallmouth and largemouth bass in Michigan (Gillilland and Muzzall 2004). In the first paper, catfish were examined and the number of metacercariae was divided into different anatomical sites, with one being the head. Seven class sizes of fish were used for the counts. This data was re-analyzed in this present study to examine the relationship between head and total counts by regressing the data as seen in Table 4. The regression coefficients were then used to determine a predicted total parasite load based on the head counts. The correlation was highly significant. The predictions gave a reasonable estimate of parasite load except at very low densities. In the second paper, the *P. ambloplitis* plerocercoid distribution was studied between largemouth and smallmouth bass with the emphasis on gender differences and differences between infected sites. Parenteric sites in the hosts included the mesentery, gonads, liver and spleen which we have used for regression analysis. Using the total number of *P. ambloplitis* larvae found in each anatomical site, regression was done between the parenteric organs and the total for all parenteric sites. The results can be seen in Table 4. The r and p values were significant for three of the four organs even with the low number of degrees of freedom. The number of *P. ambloplitis* plerocercoids in the gonads, mesentery, and liver should be predictive of the total parasite load in the host.

Figure 1 (A-F) shows the relationships of the actual total body counts in Tables 1 and 2 and the predicted values obtained from the regression coefficients in Table 3. It can been seen that using gill values gives a reasonable estimate of yellow grub infections, most especially for distinguishing light, moderate, heavy and super heavy infections.

The smallmouth bass collected from the 16 locations ranged in length (standard) from 9.5 to 36.2 cm. There was no readily apparent relationship seen between fish length and parasite loads. Regression between bass length and total body number of metacercarie for each of the 16 locales gave r values ranging from 0.03 to 0.35 and p values from 0.06 to 0.96. Regression of all 560 lengths and all metacercariae numbers gave values of $r = 0.03$ and $p = 0.48$. There is no clear evidence indicating that size of the bass would affect the regressions obtained between the gill and total body parameters.

Discussion

The population parameters of *Clinostomum marginatum* metacercariae in the gill cavity and mouth can serve as a reasonable estimate for total parasite parameters in smallmouth bass. It can be postulated that it was possible to observe this because of the relatively large number of locales (16) on 4 different streams with a spectrum of mean abundance that ranged from 2.3 to 279.3 metacercariae per fish, combined

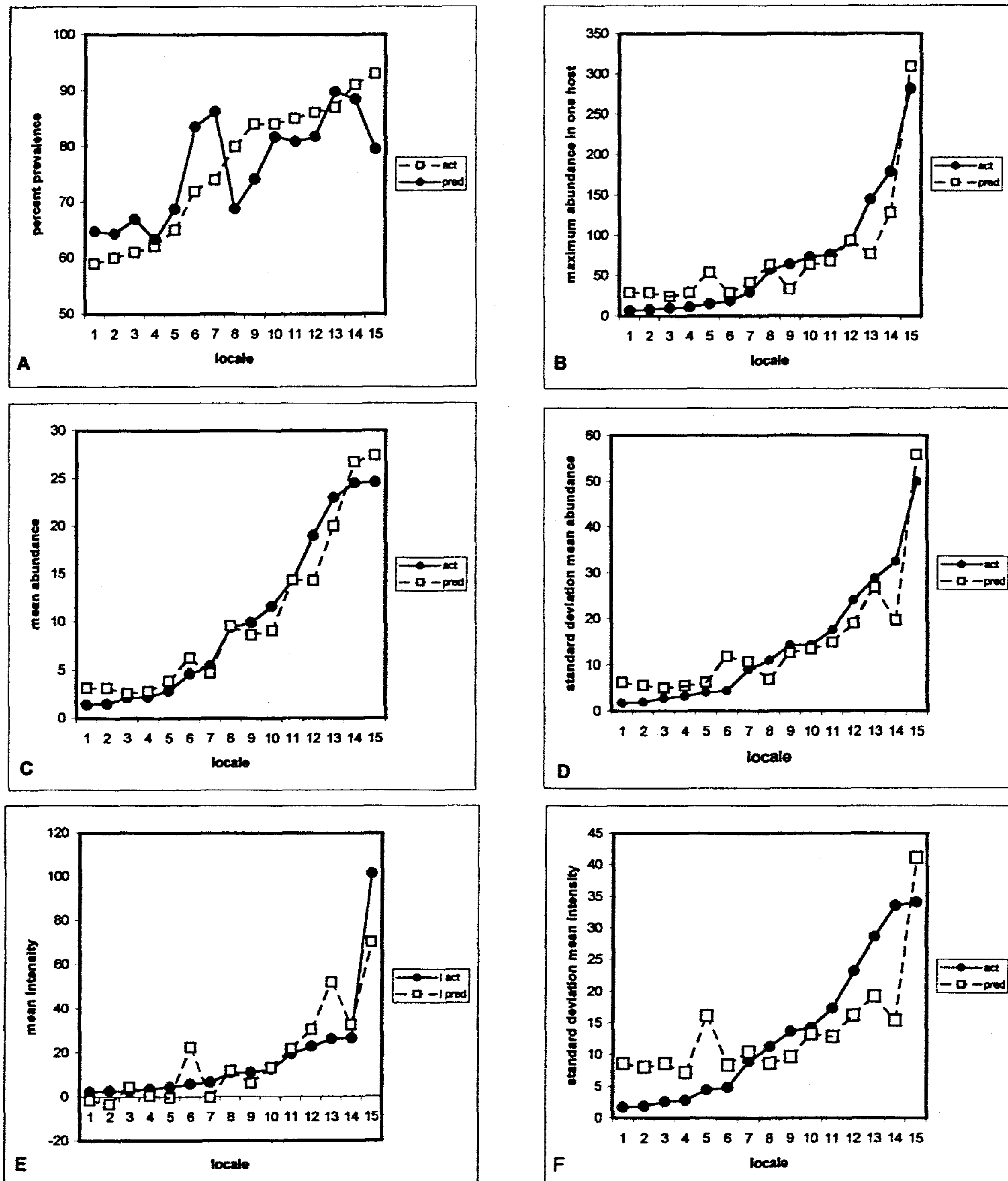

Fig. 1. Comparisons of total body parameters from 15 locations with predicted total body values calculated from gill values using the regression coefficients in Table 3. (A) Prevalence, (B) Maximum Abundance, (C) Mean Abundance, (D) Mean Abundance Standard Deviation, (E) Mean Intensity, and (F) Mean Intensity Standard Deviation. Dark closed circles are the total body values and open squares are predicted values from the gill counts. Actual total values are from Table 2 and predicted values are from residual values calculated by computer regression analysis. The graphs are standardized by plotting the lowest to the highest total body values for each parameter.

with the large overall number of hosts sampled (560). This allowed enough data for in-depth regression analyses to be done on the possible gill:total site relationships. In order for the parameters examined to be so well correlated, the highly over-dispersed or aggregated populations, which are usually associated with negative binomial distributions, must have some unknown biophysical factors at work that function to regulate these relationships in the environment in an equivalent manner. The geographic locale or biological factors that produce the separate parasite burdens at the different collection locales are probably relatively consistent throughout the stream. Janovy et al. (1997) showed that major fluctuations in a river could perturb parasite populations in fish, but evidently any changes in stream conditions on Crooked Creek did not seem to upset the relationships seen between the population parameters of yellow grubs and the smallmouth populations in this study. Ecologically, smallmouth bass are known to have a limited migration range, as a rule, of about several hundred yards in a stream although there are exceptions (Etnier and Starnes 1993). Added to that are the environmental obstacles such as sections of "dead water" where there are no smallmouth because of poor habitat conditions. These factors would increase the possibility of isolation of the different host populations sampled giving rise to the different loads of yellow grub. Also, there appears to be little, if any competition, with other similar parasites that could affect the *C. marginatum* infrapopulation numbers because of the effect of parasite interactions. Such parasites, as *C. marginatum* have been referred to by Janovy (2002) as "isolationists". Blackspot (*Diplostomum spp.*), a potential competitor for host sites, from superficial observation, did not seem to be a major problem, nor were there any other trematode infections that would penetrate and utilize the same tissues of the bass host as does *C. marginatum*.

The feasibility of using a specific site in the host as being representative of the whole parasite population can also be shown by applying the regression coefficients to the WR90 hosts collected in 1990 (Tables 1, 2). This hyperinfection, or superinfection (Daly et al. 1991) was not included in the regression analyses because such outliers tend to distort regression values, which in this case would give even more significant r and p values. This population of hosts had a mean abundance of 279.3, with a maximum abundance of 2500 for the total body. If the regression coefficients in Table 3 are applied to the hyperinfection's gill values (42.5 and 400, respectively) for predicting total body values they produce close estimates of 287 and 2740 (mean and maximum abundance respectively). Taber (1972), examined smallmouth from southwest Missouri streams for yellow grub parasite densities from various anatomical sites. Taber did not analyze the relationships between numbers of yellow grub and the different body sites, but we have found that his data supports a similar relationship seen herein for gill:total ratios.

Further, the gill:total methodology may have a more broadly important aspect by using this anatomical site density approach for short-cut estimations for other parasite survey work (or the relative significance of parasite site distribution in the host). Data from *Clinostomum complanatum* in South American catfish (Vianna et al. 2005) and the cestode *Proteocephalus ambloplitis* in smallmouth and largemouth bass in Michigan (Gilliland and Muzzall 2004) indicates that this approach maybe feasible in other genera and species. Using a noninvasive method for yellow grub infections would also be very useful in tagging experiments to follow population changes as was done with the less precise estimations done by Fischthal (1949) with yellow grub in *Perca flavescens* and sunfish centrarchids. Finally, another use is that of quickly screening hosts for heavy infections of yellow grub that can be harvested for laboratory studies. Although the probability was good that more grubs in the gills meant more in the body, this was not always as reliable an individual predictor as the relationships seen in the overall populations. In some cases smallmouth had few grubs in the gills and many in the body and the converse might also be true; large numbers of grubs in the gills and much fewer in the body. Overall, however, the probability of the gill:total body relationship held up for selecting heavily infected bass to collect yellow grubs for experimental purposes.

A limitation in this study is the range of host sizes examined which did not include the smallest and largest sizes of the bass population. Small bass could not be collected with bait and there are much fewer of the larger bass available relative to other class sizes. Lastly, this study was done primarily from June to August and may not represent the status of the yellow grub population during the other months of the year.

The Crooked Creek data and other data included herein points the way to a more serious consideration for using this technique for short-cut estimates of parasite populations as well as studying the distribution and relationships of parasites in the site locations in the hosts.

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Table 1. Population parameters of *Clinostomum marginatum* metacercariae in the orobranchial cavity of *Micropterus dolomieu* from locales in Ozark and Ouachita mountain streams in Arkansas. Locations are identified in Materials and Methods. N = host number, Prev. = Prevalence, Abund. = Mean Abundance, Abundance SD = Abundance standard deviation, Int. = Mean Intensity, Int. SD = Mean Intensity standard deviation.

Location	N	Prev.	Max.	Abund.	Abund. SD	Int.	Int. ISD
HU	10	10	1	0.10	0.30	1.00	0.00
H1	38	11	2	0.13	0.41	1.25	0.43
H2	45	16	2	0.18	0.43	1.14	0.35
CG	29	11	2	0.18	0.55	1.67	1.58
GL	23	20	2	0.29	0.63	1.25	0.52
H3	37	32	3	0.41	0.69	1.25	0.62
S	20	20	8	0.64	1.73	3.20	3.77
BS	20	47	5	1.00	1.49	2.29	1.38
O	37	44	10	1.07	2.07	2.40	2.57
Y	44	49	11	1.14	1.90	1.80	1.05
CC	42	49	17	1.84	3.25	3.86	3.80
P	27	59	10	1.85	2.24	3.13	2.40
G	30	67	13	2.70	3.40	4.05	3.50
T	105	64	25	3.70	4.90	5.70	5.10
WR2	36	53	67	3.80	11.04	7.26	14.40
WR90	17	65	400	42.50	97.2	65.63	120.00

Table 2. Population parameters of *Clinostomum marginatum* metacercariae in the total body of *Micropterus dolomieu* from locales in Ozark and Ouachita Mountain streams in Arkansas. Locations are identified in Materials and Methods. N = host number, Prev. = Prevalence, Abund. = Mean Abundance, Abundance SD = Abundance standard deviation, Int. = Mean Intensity, Int. SD = Mean Intensity standard deviation.

Location	N	Prev.	Max.	Abund.	Abund. SD	Int.	Int. ISD
HU	10	60	10	2.1	2.8	2.6	2.8
H1	38	62	11	2.2	3.2	3.5	2.6
H2	45	61	8	1.4	1.9	2.3	1.9
CG	29	59	7	4.5	1.7	2.7	1.7
GL	23	65	19	2.8	4.1	4.5	4.8
H3	37	84	64	5.4	10.9	6.4	11.3
S	20	80	15	4.6	4.4	5.8	4.4
BS	20	85	30	9.9	9.0	10.7	8.9
O	37	93	73	11.6	14.3	12.4	14.3
Y	44	86	76	9.4	14.2	10.9	13.7
CC	42	84	92	19.0	24.0	22.6	23.2
P	27	74	57	14.3	17.5	19.3	17.3
G	30	87	144	23.0	32.4	26.4	33.6
T	105	91	179	24.5	28.8	26.0	28.7
WR2	36	72	282	24.6	50.0	101.6	34.1
WR90	17	76	2500	279.3	604.0	363.9	695.5

Table 3. Regression analysis of gill and total body parameters of populations of *Clinostomum marginatum* from smallmouth bass (*Micropterus dolomieu*) collected from Ozark and Ouachita streams in Arkansas. N = 15 populations (excluding WR90). X variables are the gill values and the Y variables are the total body values taken from Tables 1 and 2. All p values are < 0.001.

Variable	Intercept	Slope	R
Prevalence	60.0	0.45	0.77
Maximum Abundance	19.1	4.3	0.92
Mean Abundance	1.9	6.7	0.97
Mean Abundance SD	3.6	4.7	0.93
Mean Intensity	-15.0	11.7	0.86
Mean Intensity SD	5.4	2.2	0.76

Table 4. Regression relationships between the number of parasites in a specific site in the host and the parasite load in the entire host. Regressions were calculated from data taken from the references cited below. *Clinostomum complanatum* data are total numbers harvested from each size class. *Proteocephalus ambloplitis* data are total numbers harvested from each organ and gender/bass species.

Vianna et al. (2005)

Clinostomum complanatum ($r = 0.91, p = < 0.001$, x = 2.92, $b = -146$)

Class size	Head	Total Body	Predicted Body
1	2	4	-139
2	24	49	-70.4
3	259	557	606
4	1605	5573	4506
5	2344	6639	6652
6	1208	2212	3354
7	1001	2159	2559

Gillilland and Muzzall (2004)

Proteocephalus ambloplitis

Regressions are done with each parenteric organ column as the independent variable and the total column as the dependent variable. Regression coefficients for each comparison are found under the organ column

Bass Type	Gonad	Mesentery	Liver	Spleen	Total
Largemouth Female	99	270	312	32	713
Smallmouth Female	1054	688	477	149	2367
Smallmouth Male	341	416	334	178	1269
Largemouth Male	60	362	261	42	725
r	0.99	0.98	0.96	0.71	
p	0.003	0.02	0.03	0.29*	

*not significant