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Anatomical Distribution of *Clinostomum* Metacercariae in the Tissues of Pond-Raised Channel Catfish (*Ictalurus punctatus*)

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Running title: *Clinostomum* in Channel Catfish

Previously Daly *et al.* (2007) found that the distribution of *Clinostomum marginatum* (“yellow grub”) metacercariae in the mouth and gills (orobranchial cavity) of smallmouth bass (*Micropterus dolomieu*) was highly proportional to the total body metacercariae. One could use this relationship to estimate the *Clinostomum* larval abundance in a smallmouth population by counting only the number in the mouth and gills without lethal necropsy. Lorio (1989) pointed out that yellow grub in channel catfish could cause a marketing problem for catfish farmers. A simple examination of visible anatomic sites (orobranchial areas) would be helpful for catfish growers as a tool for monitoring yellow grub in their stock. An infection of yellow grub in catfish (*Ictalurus punctatus*) in a pond in Northwest Arkansas offered the opportunity to see if such an approach would be feasible and worthwhile and to see if similar tissue distribution of proportionality existed with another fish host other than smallmouth bass.

Fifty- four catfish of similar age and size (35±3.9 cm SL; range 28-45; weight 326±169 g; range 190-1215) were taken from a pond in Washington Co. in 1995 and necropsied. The recovered yellow grubs (1712 from 54 hosts) into groupings of mouth, muscles, gills, fins and internal sites and counted. Descriptive statistics and regression analysis were done with Microsoft excel 2010.

In Fig. 1. the percentage of cysts in each of the anatomical sites are seen. The majority of the cysts, (59%), are in the orobranchial visible areas of the fish (gills + mouth). The population descriptors for yellow grub in pond-raised catfish are found in Table 1. All sites but one, muscle, have SD/Mean (Index of Dispersion) ratios of much less than one indicating a random infection process. This is unusual since most helminth infections have shown a stochastic and overdispersion of cysts in a few hosts and fewer worms in most of the other hosts. The simplest explanation for this would be that the commercial pond environment

would favor random association with snail-released cercariae because there are few or no areas that the host fish can establish territorial dominance that would otherwise stratify the host-parasite relationship. The muscle SD/Mean data would indicate a different infection route for that particular anatomical site. Mean intensity, i.e. removing zero infections from the calculations, did not show much difference from total population data due to the relatively small number of zero infections. Regression analysis of mean abundance with total population versus other sites were found to be highly correlative (Table 2, Fig. 2). Importantly, total population versus the visible sites (gill-mouth) showed high correlation with $r = 0.89$, $p = 3.7E-19$. This data (gill + mouth = 59% of the cysts) somewhat agreed with that of Vianna *et al.* (2005), with *C. complanatum* in *Rhamdea quellan* (a Brazilian catfish) which showed 42 % of metacercariae in the head region of the host but differed from 16 different Ouachita and Ozark smallmouth infections where less grubs were found in the head region: 14 % with a range of 5-16%, (Daly *et al.* 2014). and 19% (Taber 1972).

Figure 1. Percent distribution of *Clinostomum* sp. metacercarial cysts in different anatomical sites of pond-raised channel catfish (*Ictalurus punctatus*).

Table 1. Population descriptors (Bush *et al.* 1997) of *Clinostomum* sp. metacercarial cysts in different anatomical sites in channel catfish (*Ictalurus punctatus*) from a pond in Northwest Arkansas.

Mean abundance							
	Total	Gill	Mouth	Mouth+Gill	Fin	Muscle	Internal
Mean	31.7	5.7	13.0	18.7	5.8	3.9	3.3
SD	21.4	5.5	8.7	11.8	5.6	5.8	3.6
Max	92	19	34	48	25	28	16
%	100	83	98	100	91	70	67
SD/Mean	0.68	0.78	0.64	0.63	0.85	1.1	0.68

Mean Intensity

	Total	Gill	Mouth	Mouth+Gill	Fin	Muscle	Internal
Mean	31.7	5.7	13.0	18.7	6.5	5.7	5.1
SD	21.4	5.3	8.6	11.8	5.5	6.2	3.4
Count	54	45	53	54	48	37	35
SD/Mean	0.68	0.78	0.5	0.63	0.85	1.1	0.67

Table 2. Regression analyses for key population descriptors of *Clinostomum* sp cysts in *Ictalurus punctatus*.

Independent Variable	Dependent Variable	X	Intercept	r	P
Total	Gill	0.16	0.7	0.60	8.3E-08
Total	Mouth	0.33	2.5	0.82	5.3E-14
Total	Gill + Mouth	1.60	1.5	0.89	3.7E-19
Total	Fins	3.20	13.1	0.83	7.6E-12
Total	Muscle	0.22	1.1	0.78	5.2E-12
Total	Internal Sites	0.09	0.6	0.50	1.0E-04

Figure 2. Regression analysis of total yellow grubs versus gill-mouth yellow grubs. Dashed line is actual total number of grubs and the circles represent the predicted total grubs calculated from regression coefficients.

In conclusion, this study shows that proportionality of *Clinostomum* larval infections exists between anatomical sites in commercial catfish as well as in smallmouth bass and also in an acanthocephalan infection of a microcrustacean. (Daly *et al.* 2014; Daly and Wagner 2016). Furthermore, McAllister *et al.* 2010 used this technique for estimating yellow grub in largemouth bass from a pond that did not require lethal necropsying of a highly valued host. Thus, counting visible grubs in the head region without necropsy gives a good estimate of the total worm burden and can be a useful tool for survey work and for catfish farmers who would not have to sacrifice economically valuable stock in order to monitor for yellow grub infections.

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