

*Microphallus* spp. presence in *Orconectes propinquus* and their  
affect on asymmetric chela

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University of Michigan Biological Station  
BIO 390 Natural History & Evolution  
13 August 2009

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ABSTRACT

Chela asymmetry and its relatedness to parasite load in Northern Clearwater Crayfish (*Orconectes propinquus*) was tested and we hypothesize individuals with higher parasite loads will also express greater chela asymmetry. 51 *O. propinquus* were sampled in Douglas Lake and the Maple River of northern MI for asymmetry and parasite loads. No statistically significant asymmetry was observed in the individual crayfish and only 3 of 51 individuals were parasitized. This may be explained by an overall absence of asymmetry in chela and/or the absence of the first intermediate host snail host necessary for *Microphallus* to complete their life cycle.

Keywords: *Orconectes propinquus*, *Microphallus* spp., fluctuating asymmetry, chela (Figure 1)

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## INTRODUCTION

*Microphallus spp.* are trematodes that parasitize and form metacercariae in the digestive glands of crayfish hosts, including the Northern Clearwater Crayfish (*Orconectes propinquus*) (Stunkard 1951). Initial hosts for *Microphallus spp.* are mollusks, often a species-specific snail, followed by an intermediate crustacean host where the metacercariae cysts are formed.

*Microphallus spp.* morph into intestinal worms after the intermediate crustacean host is predated by mammals, birds, reptiles, or fish (Caveny 1971). We looked at metacercariae forms of *Microphallus spp.* while in their crustacean intermediate host, *O. propinquus*. *Microphallus* density in grass shrimp has been shown to inhibit physical fitness, which allows us to infer *Microphallus* will have similar effects in the intermediate *O. propinquus* (Kunz and Pung 2004). A reduction in physical fitness makes the individual more susceptible to predators and thus reduces its overall reproductive fitness.

*Microphallus spp.* has previously been observed to heavily parasitize another native crayfish species, *Orconectes virilis*, in Burt Lake, Michigan (unpublished Levick 2008). Burt Lake is located south of Douglas Lake and southeast of the east branch of the Maple River. Noting the myriad parasites found in *O. virilis* of Levick's (2008) study, we *a priori* assumed that *O. propinquus*, likewise, would be heavily parasitized.

A dimorphism exists between male and female chela, which may mean chela size is a sexually selected trait (Anderson 1982). Male chelae sizes are important in agnostic crayfish behavior. Often, individuals with larger chelae win more fights and are more territorial, thus having higher reproductive fitness (Rutherford, D. et al. 1995). However, large chela size does not necessitate increased strength for the individual, making chelae more of a secondary sexually selected trait and reflective of genetic quality of males (Hager 2009). Hamilton and Zuk

hypothesized that sexually selected traits may be exaggerated in individuals with good genes (Hamilton and Zuk 1982). The thought is that it would be too costly for an individual of poorer quality to invest in exaggerated traits and then be unable to cope with environmental stresses like parasitism (Parsons 1990). Large chelas increase the surface area of the individual while decreasing how streamline it is. Reduced swimming effectiveness would be a detriment to their survival because they would no longer be able to evade predators (Wilson, R. et al. 2009). However, if this handicap is representative of good genes, it would be worthwhile for the individual to show-off these good genes (Zahavi 1975). Good genes represented by bilateral symmetry should be sexually selected for by mates, therefore increasing that individual's reproductive fitness (Møller and Pomiankowski 1993).

The purpose of this study was to see if there is any fluctuating asymmetry amongst *O. propinquus* and whether or not there is a correlation between parasite load and chela asymmetry. We hypothesize that any significant asymmetry in chelas observed in *O. propinquus* would be reflected by a heavy parasite load in the digestive gland (Strong and Cable 1972). If an effect on asymmetry is observed, we will draw a conclusion that asymmetry affects sexual selection, ultimately reducing the reproductive fitness of the asymmetric individual because females will be less inclined to mate with asymmetric males.

#### METHODS

*Sample Site.*—*Orconectes propinquus* were collected using hand nets on 21 July 2009 east of Pellston, MI from two sites: the east branch of the Maple River and the south side of Douglas Lake. In the east branch of the Maple River, near a culvert on C-64, *O. propinquus* were abundant and easily collected, especially after agitating the rocky substrate that *O. propinquus* use for shelter. Collection in Douglas Lake was done that night along the south shore at about 1

m depth. Collection was best at dusk with the aid of flashlights when the crayfish were most active. *O. propinquus* were identified upon collection by their characteristic dark band that runs across their carapace and with the help of Troy Keller (Columbus State University).

*Data Collection.*—Three measurements: length, width, and thickness, were taken on each chela of each individual (Figure 1). Digital calipers, with millimeters as the unit of measure, were used to measure the chela. These measurements were taken for all 109 individuals sampled: 51 individuals were also tested for parasites and 58 individuals only had chela measurements taken. The group of 58 crayfish was sacrificed by freezing them. After freezing, chelas were measured and then the crayfish were frozen again. This group of 58 was put through three cycles of cooling and thawing by the time all the chela measurements had been taken and were ready for dissection. Finally, dissecting the crayfish, parts of the digestive gland were indistinguishable under a dissecting microscope and parasites could not be identified. It was after learning this that we began to sacrifice the crayfish using a probe. For 51 of the crayfish that were sacrificed to check for parasites, a probe was inserted just behind the eyes, through the carapace, and rotated to terminate brain function. Next, an incision was made in the carapace to access the digestive gland where the metacercariae live. The gland's green color contrasted with the rest of the insides making it easy to distinguish and extract. Preparations for viewing required two glass plates that were cut into rectangles, 12.7 cm x 15.2 cm. Each individual's glands were placed on one glass plate and then another glass plate put on top of that, smashing the digestive gland between the glass plates. Observations were then made using a dissecting microscope. Parasites were identified by their roundish morphology and movement. In parasitized individuals, the metacercariae were easy to observe. Carcasses were disposed of in a contained waste receptacle.

*Data Analysis.*—Asymmetry was measured by calculating the magnitude of the difference in chela for length, width, and thickness measurement. Averages of these chela measurements and standard errors for each average were calculated using Microsoft Excel. Using SPSS, three Independent Samples *t*-Tests and one ANOVA test provided statistical analysis on the asymmetry of chela. The number of parasitized individuals were tallied and compared to the number of individuals without parasites.

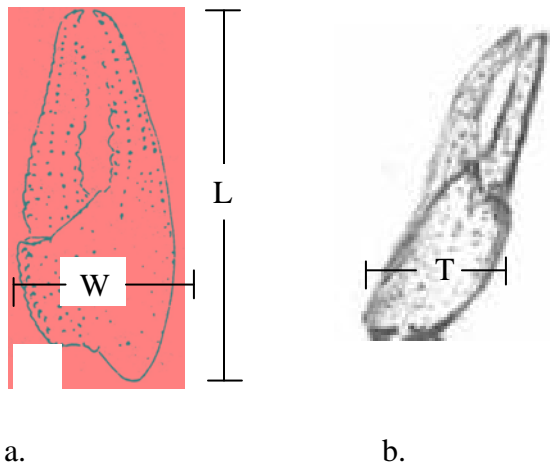


Figure 1: a. Visual representation of chela length and width measurements (Fetzner Jr., J. W. 2006). b. Visual representation of chela thickness measurement (Mortimer, J. 2004).

## RESULTS

Of the sampled females (N=40), chela asymmetry for length, width, and thickness did not differ significantly between the two habitats, river and lake ( $p>0.05$ ). Habitat did not influence chela asymmetry in females (Table 1). Similarly, the sampled males (N=69) chela asymmetry for length, width, and thickness between the two habitats, river and lake, had *p*-values significantly greater than 0.05. Habitat did not influence chela asymmetry in males (Table 2). A One-way ANOVA of all sampled crayfish (N=109) tested for significance in asymmetry within respective habitats and between habitats. For chela asymmetry based on length, width, and thickness, *p*-values $>0.05$  (Table 3).

Table 1. Independent Samples t-Test analyzing differences in chela measurements of female *O. propinquus* (length, width, and thickness) between river and lake habitats (N=40, P length=0.794, P width=0.542, P thickness=0.984). For differences in length, width, and thickness between rivers and lakes,  $p > 0.05$ .

<b>Sex=Female</b>	<b>t-Test Sig. (2-tailed)</b>
<b>Length Asymmetry (equal variances not assumed)</b>	0.794
<b>Width Asymmetry (equal variances not assumed)</b>	0.542
<b>Thickness Asymmetry (equal variances not assumed)</b>	0.984

Table 2. Independent Samples t-Test analyzing differences in chela measurements of male *O. propinquus* (length, width, and thickness) between river and lake habitats (N=69, P length=0.266, P width=0.882, P thickness=0.728). For differences in length, width, and thickness between rivers and lakes,  $p > 0.05$ .

<b>Sex=Male</b>	<b>t-Test Sig. (2-tailed)</b>
<b>Length Asymmetry (equal variances not assumed)</b>	0.266
<b>Width Asymmetry (equal variances not assumed)</b>	0.882
<b>Thickness Asymmetry (equal variances not assumed)</b>	0.728

Table 3. One-way ANOVA analyzing variance in chela measurements of *O. propinquus* (length, width, and thickness) within and between river and lake habitats (N=109, P length=0.405, P width=0.578, P thick=0.795). For differences in length, width, and thickness between and within the two habitats, rivers and lakes,  $p > 0.05$ .

Groups=River & Lake Habitats	Sig.
Length Asymmetry (between & within groups)	0.405
Width Asymmetry ( between & within groups)	0.578
Thickness Asymmetry ( between & within groups)	0.795

Slight differences exist between length, width, and thickness asymmetry in males and females between habitats, but in every case there are overlapping error bars. Figure 4 confirms the sexual dimorphism in chela size. Figure 5 illustrates the discrepancy between parasitized and non-parasitized individuals. 48 of 51 sampled crayfish had 0 parasites.

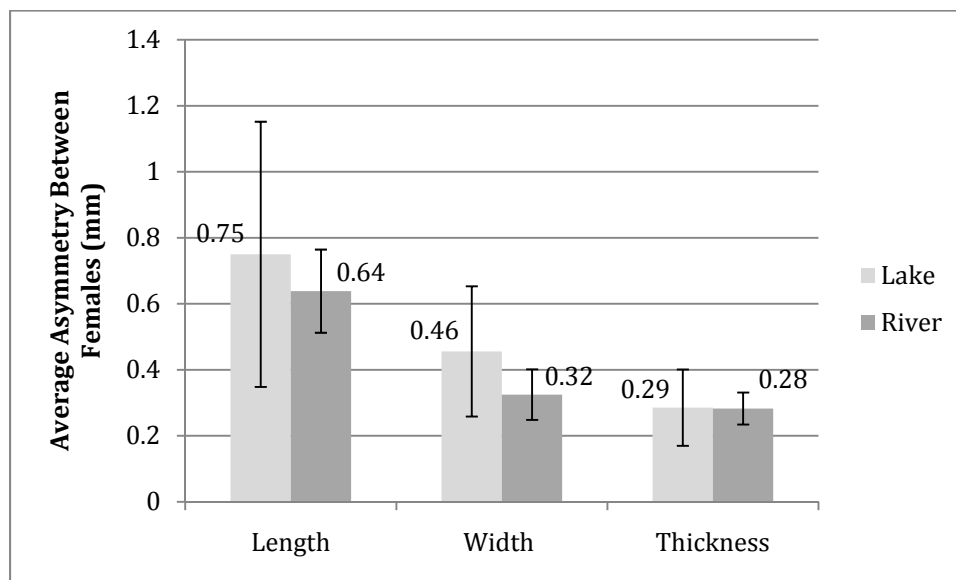


Figure 2. Graphical representation of average asymmetry of female *O. propinquus* between river and lake habitats (N=40). Douglas Lake crayfish are slightly more asymmetric than Maple River crayfish, but error bars overlap (lake length s.e.=0.40, lake width s.e.=0.20, lake thickness s.e.=0.12, river length s.e.=0.12, river width s.e.=0.08, river thickness s.e.=0.05).

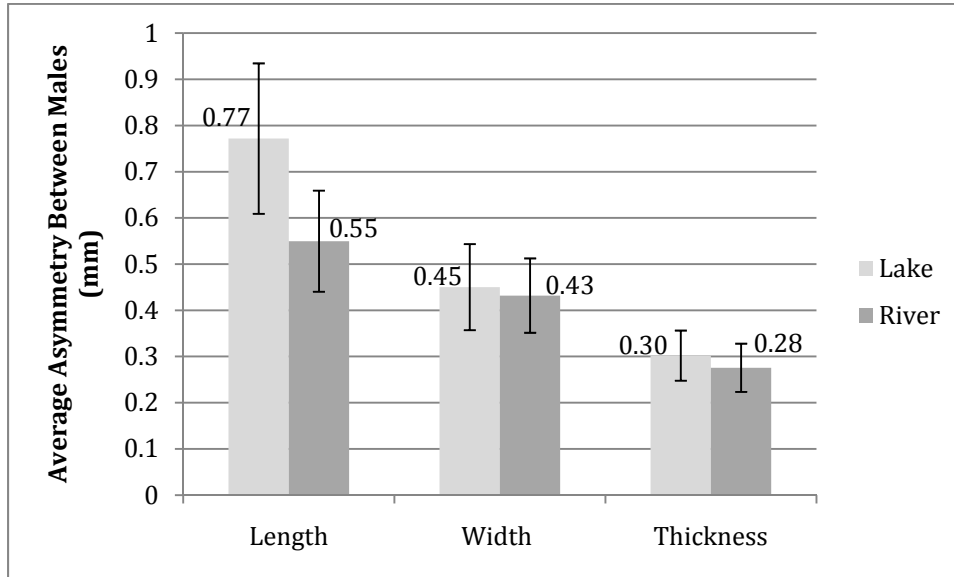


Figure 3. Graphical representation of average asymmetry of male *O. propinquus* between river and lake habitats (N=69). Douglas Lake crayfish are slightly more asymmetric than Maple River crayfish, but error bars overlap. (lake length s.e.=0.16, lake width s.e.=0.09, lake thickness s.e.=0.05, river length s.e.=0.11, river width s.e.=0.08, river thickness s.e.=0.05).

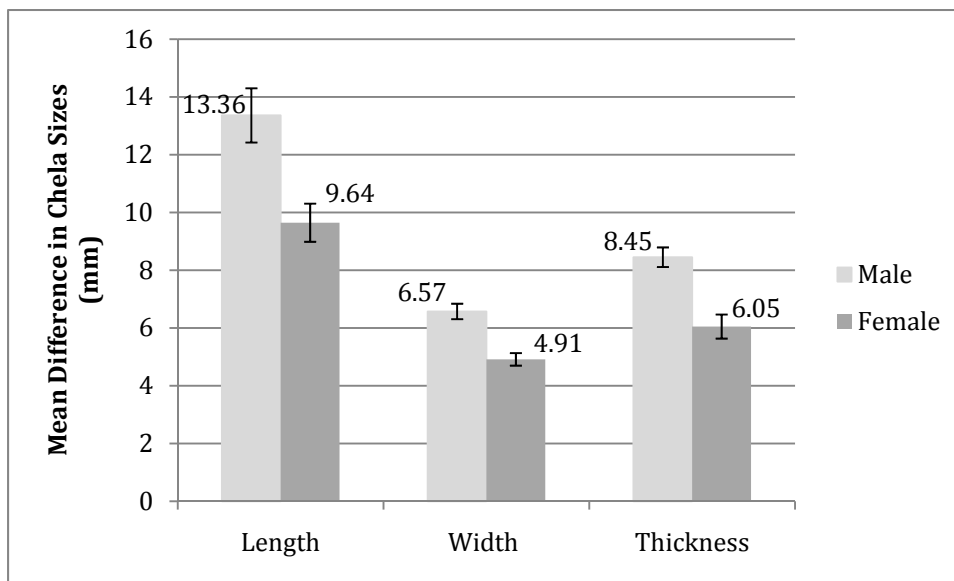


Figure 4. Graphical representation of average chela size between *O. propinquus* sexes (Male: N=69, Female: N=40). Male crayfish have larger chelas than female crayfish and error bars do



not overlap. (male length s.e.=0.94, male width s.e.=0.27, male thickness s.e.=0.34, female length s.e.=0.66, female width s.e.=0.22, river thickness s.e.=0.42).

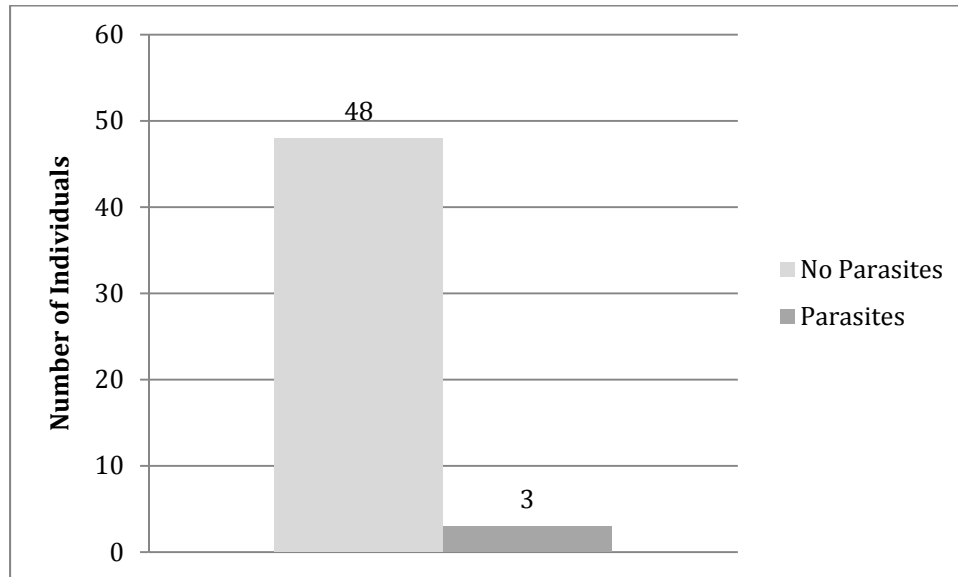


Figure 5. Graphical representation of number of parasitized individuals compared to number of non-parasitized individuals (N=51). Of the 51 *O. propinquus* sampled, 48 had 0 parasites and only 3 had any parasites at all.

All p-values testing significance of chela asymmetry in *O. propinquus* were greater than 0.05 and thus not statistically significant (Tables 1, 2, 3). Moreover, Figures 2 and 3 represent graphically chela asymmetry differences in both males and females in the Maple Rivers and Douglas Lake and although lake crayfish of both sexes show more asymmetry than river crayfish, the error bars overlap and nothing conclusive can be drawn from this. This supports all calculated p-values being  $>0.05$ . Furthermore, because only 3 of the 51 *O. propinquus* tested for parasites had any parasites, we cannot test any correlation between asymmetry and parasite load.

#### DISCUSSION

As a result of all *O. propinquus* chelas not having statistically significant asymmetry and only 3 of 51 sampled for parasites had any, our hypothesis that an individual's asymmetry would be reflected in its parasite load must be rejected, or remain unanswered until further research is done. However, we can confidently conclude that, as expected and observed, a dimorphism does

exist in average chela sizes between sexes (Figure 4). Unfortunately, nothing further can be concluded from the data collected.

With no significant data to support our hypothesis, we are left to explain why this might have been the case. Procedurally, three actions could have been taken which might have led to more significant data. First, preliminary testing for asymmetry would have shown if asymmetry exists in *O. propinquus*. Having known this, we could have, with more confidence, asked evolutionary questions like if fluctuating asymmetry in chela is reflected by an individual's parasite load. Also, before collecting *O. propinquus*, sampling the east branch of the Maple River and Douglas Lake for the first molluscan host of *Microphallus spp.*, a species-specific snail would have ensured whether numbers were high enough to support the parasitization of *O. propinquus* by *Microphallus spp.* Without the presence of viable snail hosts *Microphallus* cannot be present in the system because they would have no way to complete their life cycle. Moreover, knowing that *Microphallus spp.* are in the river and lake systems, collecting and sampling only older *O. propinquus*, identifiable by their larger size, could lead to more significant parasite densities for statistical analysis. Collecting older crayfish means that these individuals have had more time to acquire parasites. By taking these steps, a more significant data set could be under analysis.

In addition to more in depth preliminary studies, one procedural error could have given us much more data to work with, especially if the *O. propinquus* had been found to be parasitized. This error was sacrificing 59 *O. propinquus* by freezing them. After freezing them, individuals were then removed from the freezer to have chela measurements taken and then stored in the freezer before we looked at their parasite loads. We suspect that this freezing and

thawing damaged cell membranes and was responsible for making the digestive gland homogenous to the point of nothing being distinguishable (Lovelock 1957).

*O. propinquus*, for the most part, were not being parasitized by *Microphallus* in the east branch of the Maple River and on the south side of Douglas Lake. The absence of exaggerated chela asymmetry is consistent with this observation. Because of support for Hamilton and Zuk's and Zahavi's hypotheses in other organisms like barn swallows, we believe it would still be worthwhile to test for a correlation between fluctuating asymmetry in chela and parasite load (Møller 1994). However, sample sites should be limited to ones that have been sampled first for the prevalence of *Microphallus spp.*, like in Burt Lake, MI (unpublished Levick 2008). Until a study is conducted with heavily parasitized individuals, a conclusion cannot be made on whether fluctuating asymmetry exists in individuals that have high parasite loads.

#### ACKNOWLEDGEMENTS

Thanks to Troy Keller for his help in *Orconectes propinquus* identification

Thanks to Harvey Blankespoor for his help with *Orconectes propinquus* dissection and *Microphallus* identification

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IMAGES CITED

Figure 1a:

Fetzner Jr., J. W. 2006. 13 August 2009

<http://iz.carnegiemnh.org/crayfish/keys/IMAGES/Fig79.jpg>

Figure 1b:

Mortimer, J. 2004. 13 August 2009

[https://kb.osu.edu/dspace/bitstream/1811/3345/1/V43N05\\_201.pdf](https://kb.osu.edu/dspace/bitstream/1811/3345/1/V43N05_201.pdf)