

Chelae asymmetry and trematode parasite load in the crayfish *Orconectes virilis*

Kelly McClure and Stephanie Nguyen

University of Michigan Biological Station
BIO 390 Natural History and Evolution
August 13, 2010
Jordan Price

Fluctuating asymmetry (FA) is random departure from perfect bilateral symmetry of a morphological trait and is hypothesized to be a result of genetics and environmental stress. Parasites play an important role in the development of phenotypic traits, since the full expression of certain traits may reflect the ability of hosts to cope with the debilitating effects of parasites. In our study, we tested the relationship between degree of FA in chelae of North American freshwater crayfish (*Orconectes virilis*) and the magnitude of metacercariae parasite infection (*Microphallus spp.*). Crayfish were collected at two lakes near Pellston, Michigan. We measured chelae length and width and counted the number of metacercariae found in crayfish liver. Our results showed a significant positive correlation between parasite load and chelae width asymmetry, which previous studies have not measured, however, no correlation was found between parasite load and chelae length asymmetry. Our results suggest either that less symmetrical individuals are more susceptible to parasites or that parasitism causes developmental stress in hosts, or both, and that chelae width is more affected by parasite load than chelae length because of its importance in agonistic behaviors.

I grant the Regents of the University of Michigan the non-exclusive right to retain, reproduce, and distribute my paper, titled in electronic formats and at no cost throughout the world.

The University of Michigan may make and keep more than one copy of the Paper for purposes of security, backup, preservation and access, and may migrate the Paper to any medium or format for the purpose of preservation and access in the future.

Signed

INTRODUCTION

Fluctuating asymmetry (FA) is a random deviation from perfect bilateral symmetry of a morphological trait and is hypothesized to be a result of genetics and environmental stress (Li 2002). It may signal in understanding how populations evolve and adapt to an environment, as well as aid in understanding the genetic processes associated with extinction (Leary and Allendorf 1989). Mei-Hui Li (2002) demonstrated that parasitism is associated with an increase in FA in the claws of shore crabs *Grapsus albolineaus*. The relationship between parasitism and FA may be the result of two causes: less symmetrical individuals may be more susceptible to parasites or parasitism may cause physiological imbalances in hosts, making them develop imperfectly (Moller 2005). Some parasites are highly virulent, and this may have important consequences for the effects of parasites on host phenotype by exploiting nutrients from the host when nutrients would go toward host growth and development (Moller 2005). Therefore, the degree of FA allows researchers to evaluate individual capacity to develop properly in the face of environmental stresses (Watson and Thornhill 1994).

Parasites play an important role in the development of sexual ornaments, since the full expression of ornaments may reflect the ability of hosts to cope with the debilitating effects of parasites (Moller 1992). The chelae of crayfish (*Procambarus clarkii*) are both ornamental and functional for both sexes: bigger chelae attract more mates and also help crayfish win territory, burrow, and hunt for food (Aquiloni and Gherardi 2007). Additionally, chelae in males are used in copulation and chelae in both sexes provide a defense mechanism against fish predators (Rutherford 1995; Galeotti 2008). Freshwater crayfish are known for being highly susceptible to parasitism by trematode metacercariae parasites (*Microphallus spp.*) because crayfish are good intermediate hosts (Caveny and Etges 1971). Metacercariae of *Microphallus* species are

encysted in crayfish livers and then consumed by their definitive hosts, birds and fishes, where they develop to sexual maturity in the intestines and become worms (Stunkard 1951). Once trematodes reach the adult stage, they release eggs through their host's feces. Snails become infected when they consume feces bearing parasite eggs and are subsequently consumed by crayfish, repeating the trematode life cycle (Caveny and Etges 1971).

Because less symmetrical individuals are more susceptible to parasites or, alternatively, because parasitism may cause physiological imbalances in hosts, the degree of parasitism by *Microphallus* species in crayfish may correlate with the symmetry of crayfish chelae (Moller 2005). In this study, we tested the relationship between degree of FA in chelae of North American freshwater crayfish (*Orconectes virilis*) and the magnitude of *Microphallus* metacercariae infection. We predicted that crayfish chelae asymmetry would increase with parasite load, and that asymmetry would also be stronger in the male sex, where competition for mates and ability to coerce females increases sexual dimorphism, making them larger and able to bear more parasites due to their sheer size (Alquiloni and Gherardi 2007). We also hypothesized that the average degree of chelae asymmetry would vary between different lakes, due to differences in prevalence of the trematode parasite at each location (Blankespoor and Keller, personal communication).

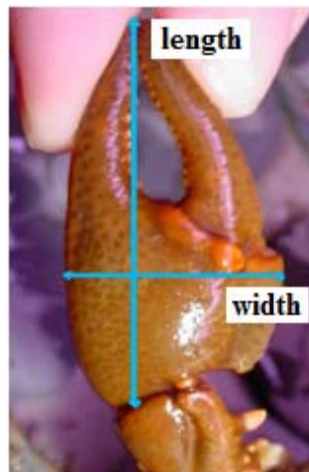
METHODS

We collected crayfish near Pellston, Michigan, in Michigan's upper Lower Peninsula at two separate locations: North Fishtail Bay in Douglas Lake and Maple Bay in Burt Lake.

Douglas Lake is located on University of Michigan Biological Station property and is 1.5 miles

directly north of neighboring Burt Lake. Previous research showed that Burt Lake crayfish were normally highly infected while Douglas Lake crayfish had little to no parasites, due to a recent zebra mussel invasion that eradicated the snail population, which were intermediate hosts for transferring parasites to crayfish (Blankespoor and Keller, personal communication).

We collected a total of 55 crayfish (*O. virilus*) over a period of ten days: 20 from Douglas Lake and 35 from Burt Lake. Collections were carried out using hand nets and buckets during nighttime between 22:00 and 0:00 when crayfish are known to be the most active (Stunkard 1951). After collection, crayfish were directly transferred to five 10-gallon aquaria with shallow lake water, sand, and caves made out of broken pots, with approximately five crayfish per tank. We killed all crayfish within a week of capture by asphyxiation using jars saturated with ethyl acetate. We measured (in millimeters) carapace length, chela length, and chela width for both claws using digital calipers.



Next, crayfish were dissected with forceps and scissors to extract the liver. Each liver was compressed between two glass plates to allow us to count the metacercariae parasites (*Microphallus spp.*).

Asymmetry Measurements -

Asymmetry was measured by calculating the chela proportion of each crayfish. This

was done by dividing smaller chela length over larger chela length, and then repeating the same method for chela width and total area (chela length x chela width). Parasite load was measured as total number of metacercariae cysts present in the liver. Linear regressions were conducted to investigate the relationship between chelae asymmetry and parasite load. Independent samples t-tests were also carried out to test for differences in average parasite load and average chelae asymmetry between sexes and the different lakes.

RESULTS

Chelae symmetry decreased as parasite load increased, revealing a significant positive relationship between chela area asymmetry and parasite load (Figure 1; $R^2=0.201$, $N=55$, $p=0.001$). A further break down of the data in Figures 2 and 3 revealed that chelae length asymmetry was not significantly correlated with parasite load ($R^2=0.001$, $p=0.827$) but chelae width asymmetry did show a significant relationship ($R^2=0.283$, $p<0.001$).

Independent sample t-tests revealed that chelae area asymmetry was not significantly different between males and females (Figure 4; t-test, $p=0.325$) nor was parasite load significantly different between sexes (Figure 5; t-test, $p=0.551$).

Chelae area asymmetry was marginally significantly different between Douglas and Burt Lake (Figure 6; t-test, $p=0.066$), while parasite load between lakes was significantly different (Figure 7; t-test, $p<0.001$).

DISCUSSION

Overall, our hypothesis was supported and chelae asymmetry significantly increased in crayfish that had more parasites. Chela proportions closer to 1.0 indicated greater chela symmetry and were more common among crayfish that had the least amount of liver parasites. Our data were consistent with findings from the Moller (2005) study, which demonstrated

asymmetry is associated with degree of parasite infection. Because less symmetrical individuals are more susceptible to parasites, or because parasitism may cause physiological imbalances in hosts, the degree of parasitism by *Microphallus* species in crayfish was greater in individuals with less symmetrical chelae (Leung 2000; Moller 2005).

Interestingly, after breaking down chela area asymmetry into chela length and chela width asymmetry, we found that asymmetry in chela length alone did not have a significant correlation with parasite load; the trend was only significant in chelae width. This might indicate that chela width was more affected by parasites than chela length. Seebacher (2006) demonstrated that chela width was important in agonistic behaviors including aggression and dominance, and might be related to force power when crayfish close their chelae. Therefore, parasites might be decreasing overall crayfish aggression to make them more easily preyed upon by birds and fish, definitive hosts of the parasite (Stunkhard 1951).

Our hypothesis that asymmetry would significantly differ between sexes was not supported by our results. According to Aquiloni and Gheradi (2007), both male and female crayfish prefer larger mates, resulting in lower sexual dimorphism in overall crayfish size. Additionally, sexual dimorphism is selected against because body and chelae size in both sexes provide important defense mechanisms against predators, help them to burrow, and defend their territories (Rutherford 1995). Lower sexual dimorphism and little difference in their capacity to bear parasites would support neither sex having significantly different asymmetries.

Chela asymmetry was significantly different between crayfish from different lakes (Figure 6); greater asymmetry was found in crayfish from Burt Lake than from Douglas Lake.

We hypothesized that this was because Douglas Lake had fewer *Microphallus* (Figure 7), which lost a crucial intermediate host after the snail population was eradicated by a zebra mussel

invasion in 2001 (Hollandsworth 2006). Zebra mussels attach themselves to snail shells, limiting snail mobility and inhibiting reproduction and predator evasion, potentially leading a snail population to extinction (Van Appledorn 2007). The lack of snails prevented the transfer of *Microphallus* from lake sediment to their next hosts, crayfish, birds, and fish. Therefore, fewer parasites in Douglas Lake might be the main reason for greater chelae symmetry in crayfish. Clearly, environmental influences play an important role in the development of morphological traits.

In conclusion, the presence of parasites (*Microphallus spp.*) at higher densities in crayfish (*O. virilus*) was strongly correlated to the severity of chelae asymmetry. Parasites played an important role in growth and development; the full expression of a phenotype might reflect the ability of hosts to cope with the debilitating effects of infection by parasites (Moller 1992).

Differences between sexes in asymmetry and in parasite load were not significant, suggesting that crayfish were not sexually dimorphic enough as a species to show different levels of parasitic infection. Parasite loads and asymmetry levels were both significantly different between lakes, providing evidence that the environment was a determining factor in parasite prevalence between populations. Lastly, crayfish chelae asymmetry was a significant indicator of level of parasitic infection. However, after taking a closer look at the data, this difference was almost entirely explained by differences in width proportion and not length. This supported that chela width was vital in crayfish agonistic behaviors and was more susceptible to manipulation by parasites. Previous studies have primarily focused on chela length asymmetry (Galeotti et al. 2008; Leary and Allendorf 1989; Leung et al. 2000), but our data showed that chela width was more predominantly affected by parasite load, and is important to be considered in the future.

ACKNOWLEDGEMENTS

We would like to thank Harvey and Curt Blankespoor, professors in ecology and animal parasites at the University of Michigan Biological Station, for assisting in our crayfish dissections, teaching us how to identify parasites, and allowing us to use their lab and dissecting tools for the duration of our experiment. We would also like to acknowledge Troy Keller, professor of the limnology class, for helping us scout locations where *Orconectes virilis* would reside and for advice on how to care for crayfish. Thank you to Brian Scholtens, professor of the insects class, for letting us borrow his ethyl acetate and educating us on the ethics of killing animals. Lastly, we would like to thank our professor Jordan Price and teaching assistant Jennifer Rowe for advice on how to carry out our experiment and guidance in the publication of our research.

LITERATURE CITED

- Aquiloni, L., and F. Gherardi. 2007. Mutual mate choice in crayfish: large body size is selected by both sexes, virginity by males only. *Journal of Zoology* 274: 171-179.
- Caveny, B. A., and F. J. Etges. 1971. Life history studies of *Microphallus opacus* (Trematoda: Microphallidae). *The Journal of Parasitology* 57: 1215-1221.
- Galeotti, P., D. Rubolini, F. Pupin, R. Sacchi, and M. Fasola. 2008. Sperm removal and ejaculae size correlate with chelae asymmetry in a freshwater crayfish specie. *Behavioral Ecology Sociobiology* 62: 1739-1745.
- Hollandsworth, D., Z. Caple, M. Hejna, H. Siersma. 2006. Zebra Mussels (*Dreissena polymorpha*) and unionids in Douglas Lake, Michigan. *Unpublished*.
- Leary, R. F., and F. W. Allendorf. 1989. Fluctuating asymmetry as an indicator of stress: implications for conservation biology. *Trends in Ecology and Evolution* 4:214-217.
- Leung, B., M. R. Forbes, and D. Houle. 2000. Fluctuating asymmetry as a bioindicator of stress: comparing efficacy of analyses involving multiple traits. *The American Naturalist* 155: 101-115.
- Li, M. H. 2002. Fluctuating asymmetry and intersexuality in the shore crab *Grapsus albolineaus* near a coastal landfill in Northern Taiwan. *Bulletin of Marine Science* 70: 75-88.

- Møller, A. P. 1992. Parasites differentially increase the degree of fluctuating asymmetry in secondary sexual characters. *Journal of Evolutionary Biology* 5: 691-699.
- Møller, A. P. 2005. A review of developmental instability, parasitism, and disease. *Infection, Genetics, and Evolution* 6: 133-140.
- Stunkard, H. W. 1951. Observations on the morphology and life-history of *Microphallus limulini* sp. (Trematoda: Microphallidae). *Biological Bulletin* 101: 307-318.
- Rutherford, R. L., Dunham, D.W., Allison, V. 1995. Winning agonistic encounters by male crayfish *Orconectes rusticus* (Girard) (Decapoda, Cambaridae): chela size matters but chela symmetry does not. *Crustaceana* 68: 526-529.
- Seebacher, F., R.S. Wilson. 2006. Fighting fit: thermal plasticity of metabolic function and fighting success in the crayfish *Cherax destructor*. *Functional Ecology* 6:1045-1053.
- Van Appledorn, M., and C. Bach. 2007. Effects of zebra mussels (*Dreissena polymorpha*) on mobility of three native mollusk species. *American Midland Naturalist* 158: 329-337.
- Watson, P. J., and R. Thornhill. 1994. Fluctuating asymmetry and sexual selection. *Trends in Ecology and Evolution* 9: 21-24.

FIGURES

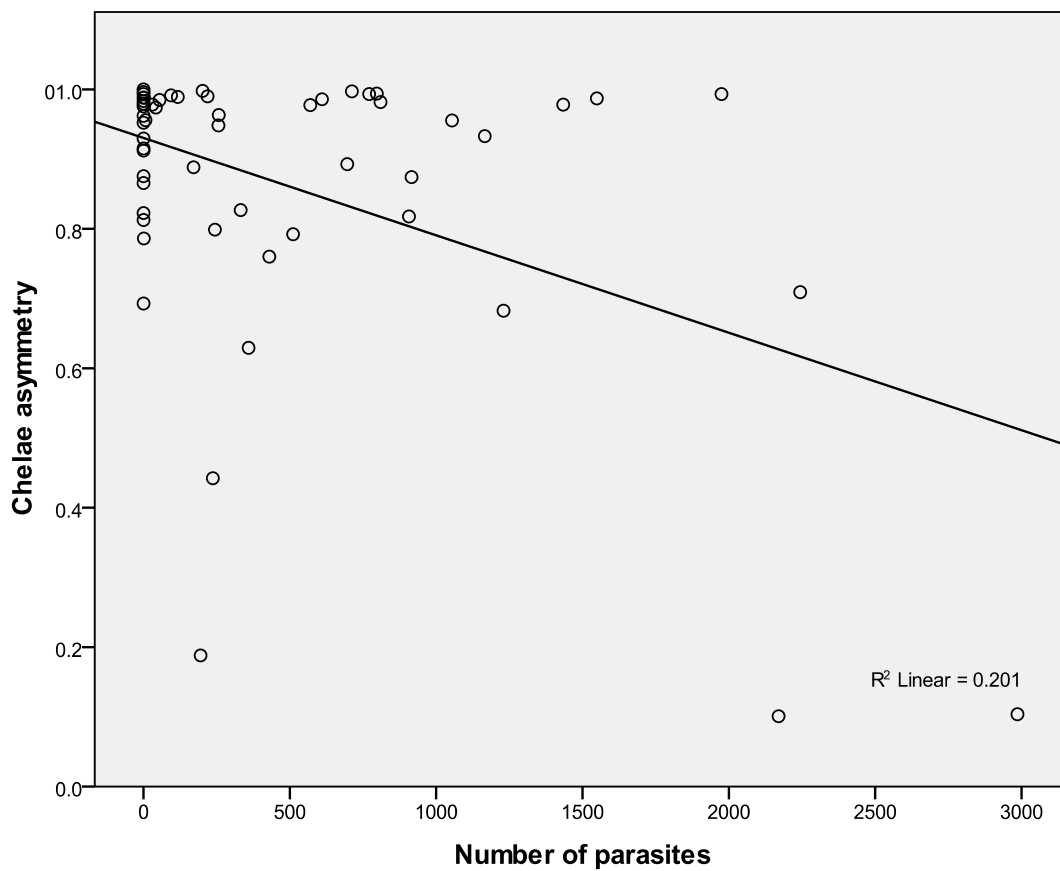


Figure 1: Linear Regression of chelae (area) asymmetry and number of parasites. As chelae asymmetry increased (deviated from 1.00) more parasites were found in crayfish, showing a significant decreasing trend ($R^2=0.201$, $N=55$, $p=0.001$).

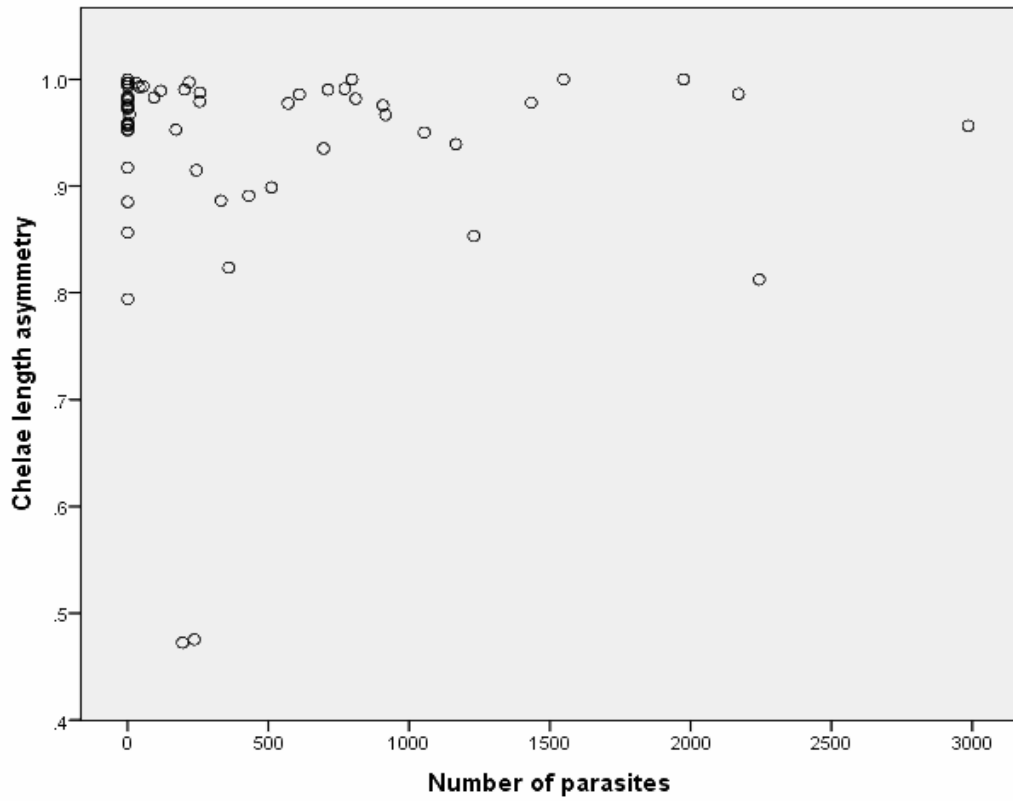


Figure 2: Linear Regression of chelae length asymmetry and number of parasites. No significant correlation was found; chelae length asymmetry was independent of parasite load ($R^2=9.14E^{-5}$, $N=55$, $p=0.827$).

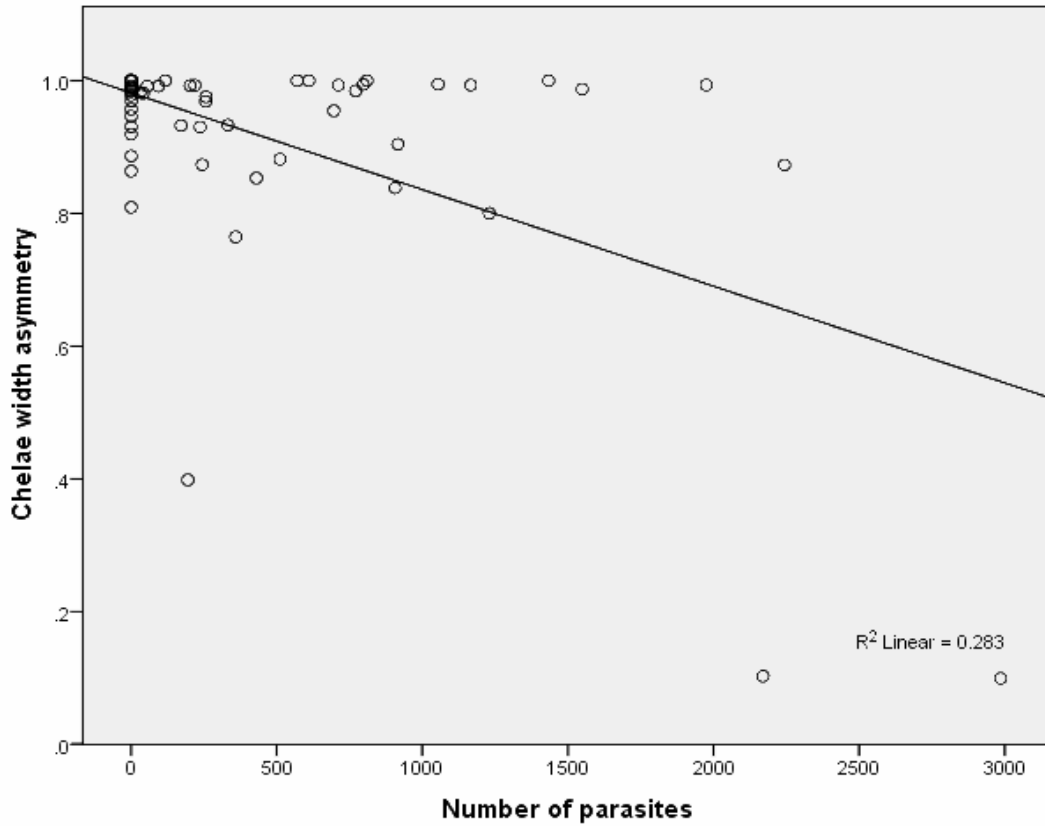


Figure 3: Linear Regression of chelae width asymmetry and number of parasites. As chelae width asymmetry increased (deviated from 1.00) more parasites were found in crayfish, showing a significant decreasing trend ($R^2=0.283$, $N=55$, $p<0.001$).

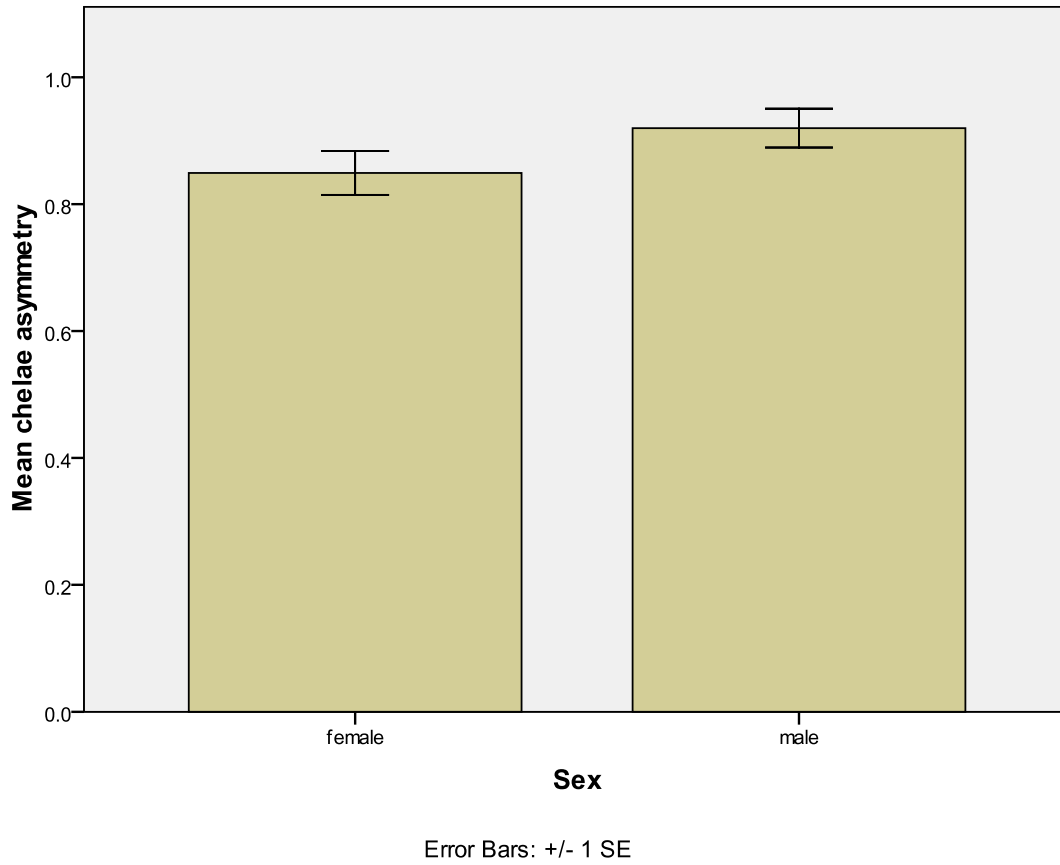


Figure 4: Comparison between crayfish sex and mean chelae asymmetry. There was no significant difference in chelae asymmetry between males and females (t-test; $N=55$, $p=0.325$).

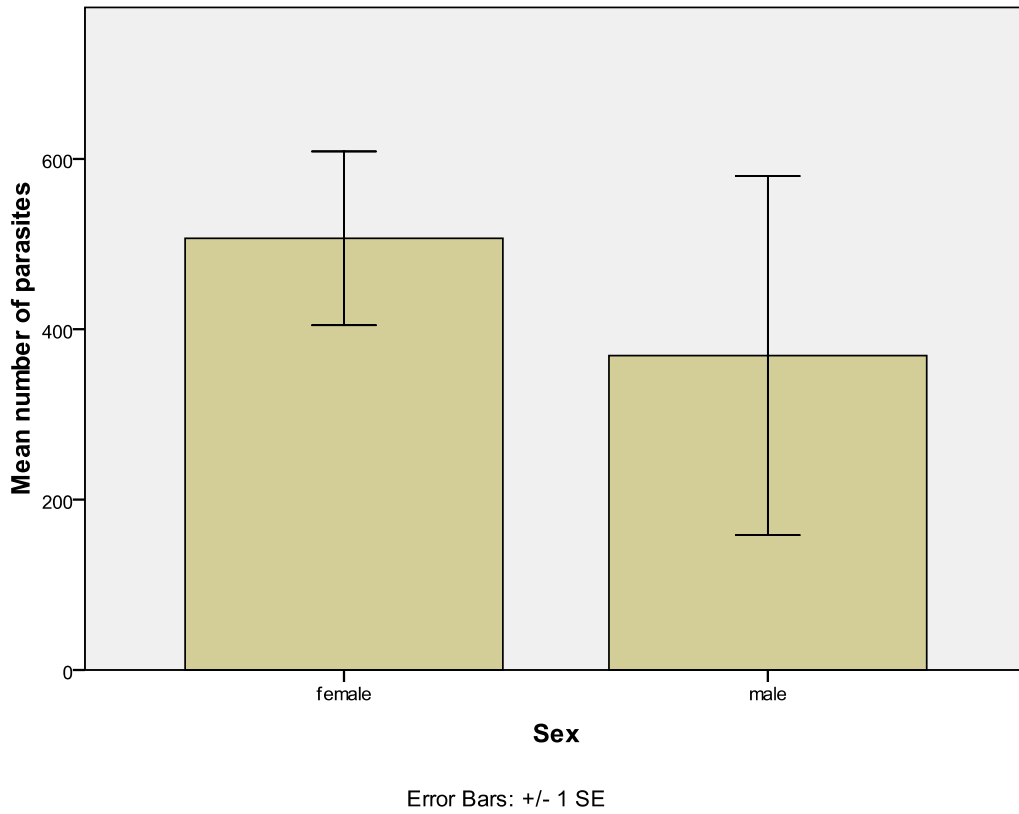


Figure 5: Comparison between crayfish sex and number of parasites. Differences were not significant (t-test; $N=55$, $p=0.551$).

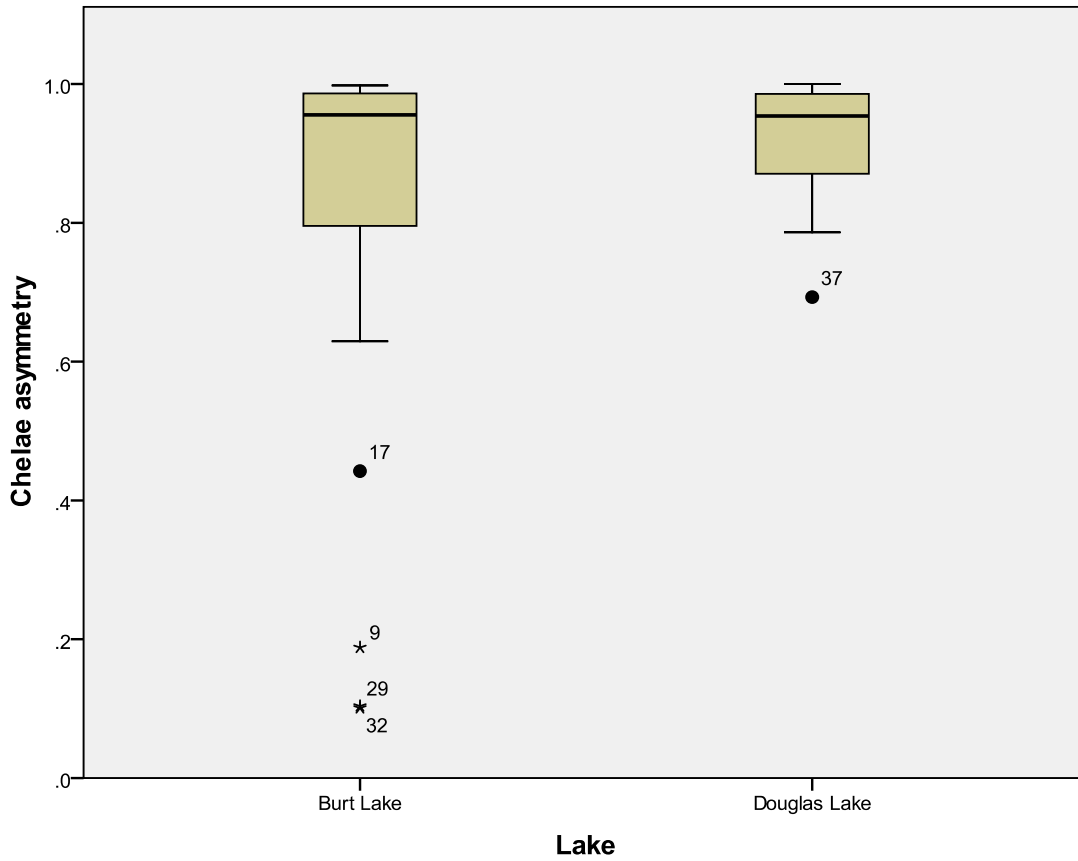
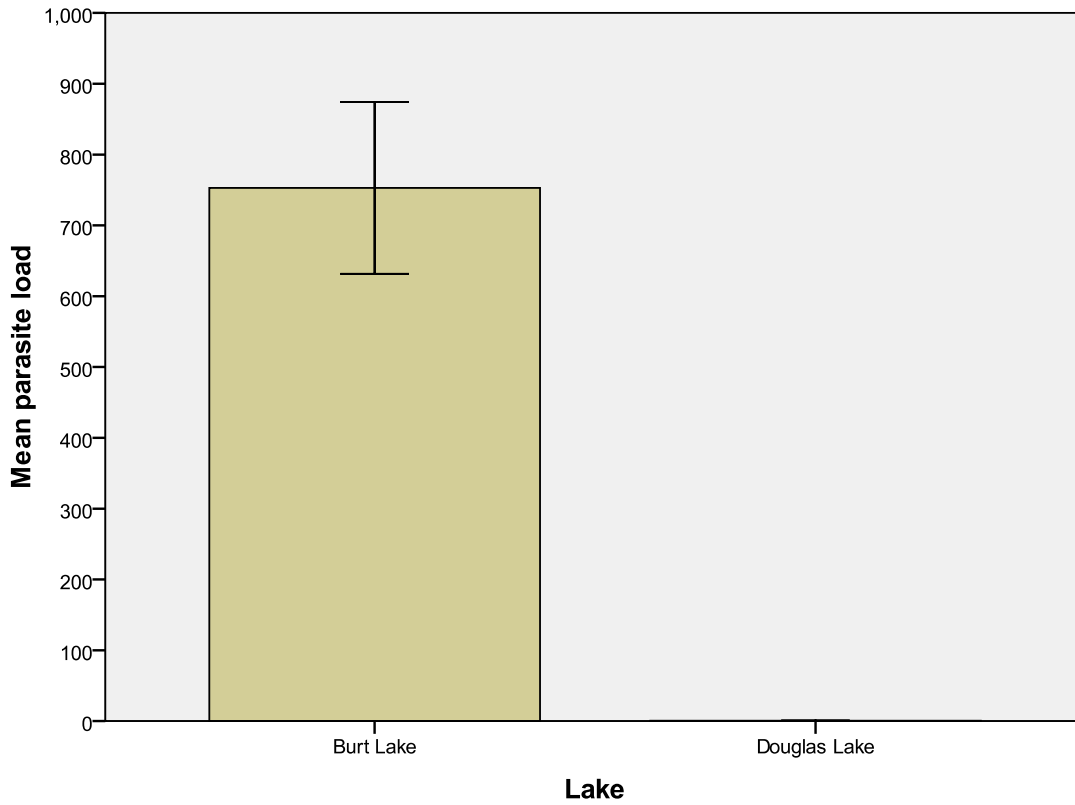


Figure 6: Comparison between chelae asymmetry and different lakes. Chelae asymmetry varied more in Burt Lake crayfish than in Douglas Lake, illustrating that these two populations were significantly different (t-test; $N=55$, $p=0.066$).



Error Bars: +/- 1 SE

Figure 7: Comparison between number of parasites and different lakes. Mean number of parasites was significantly greater in Burt Lake than in Douglas Lake (t-test; $p < 0.001$).