A Study of Sex Ratio in Native (*Orconectes virilis)* and Invasive *(Orconectes rusticus)* Crayfish species in Burt Lake, Michigan

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Abstract

Sex ratio theory has led to numerous studies that both support current ideas and investigate and explain deviations from the expected 1:1 male to female tertiary sex ratio. Crustaceans have been shown to have aberrant sex ratios (Wenner 1972). This study tests the hypothesis that these results apply to crayfish species. In this study native (*O. virilis*) and invasive (*O. rusticus*) species were sampled in Burt Lake in Cheboygan County, Michigan to determine the sex ratios of both populations. Crayfish were captured at 4 sites using dip nets and sex and species were visually determined by examination of the genital pore and color indicators. Carapace length and total length were also measured. The chi-squared tests of the data showed a statistically significant deviation from expected 1:1 sex ratios for *O. virilis*. In this case there was a female bias with 52 female individuals to only 20 male individuals. *O. rusticus* samples did not differ significantly from the expected sex ratio. This sex ratio bias in O. virilis could be the result of differential movement or mortality as well as interactions between native and invasive crayfish species.

Introduction

When discussing evolution and natural selection, examining the role of sexual reproduction and male and female forms is essential. Questions about the evolution of two distinct sexes have expanded to include interest in the total number of individuals of each sex. In his theories about natural selection, Darwin (1871) suggested that equal numbers of individuals of both sexes would be selected for if this ratio proved advantageous. However, he was unsure of the exact reasons or mechanisms that could contribute to such an occurrence and deemed it was "safer to leave its solution for the future" (Darwin 1871).

The idea of equal sex ratio was later undertaken by Fisher who postulated the idea of resource allotment influencing ratio equality. Fisher (1930) hypothesized that if offspring were equally costly to produce that each should receive equal amounts of parental investment and resources. Over evolutionary time, this equality of resources should result in a relatively stable 1:1 tertiary ratio. This is the sex ratio between males and females of reproductive age.

However as Trivers and Willard (1973) brought to light and Frank (1987) supported, there is often a difference in terms of reproductive return between males and females. This means that equal investment in the sex who reproduces less, could decrease potential fitness. Conversely, if resources were selectively distributed fitness could be increased. Unequal investment could then influence a bias that would favor one sex over the other. This would result in deviation from the sex ratio that natural selection favors.

Natural selection and its influence on sex ratio have been widely studied across species. Departures from the hypothetical model have been investigated in numerous organisms including crustaceans. Recent studies in Australia have also shown skewed sex ratios in crayfish. The secondary sex ratio, the proportions of males and females at birth, was recorded at 3 males to 1

female for the freshwater species *Cherax destructor* (Austin and Meewan 1999). A skewed ratio in one species could have implications for other freshwater species who share an evolutionary history with this lineage. These data indicate that freshwater crayfish might have an internal mechanism that can alter sex ratio or that environmental factors are disrupting the expected equilibrium. If sex ratio can be altered at this stage, it could mean that tertiary sex ratio in crayfish could be altered in certain cases.

Wenner (1972) studied numerous crustaceous organisms in attempt to find similarities in sex ratio and learn more about these deviations. His results suggested that in contrast to the proposed 1:1 sex ratio, changes in sex ratio were much more common for these animals. He even went as far to suggest that in marine crustaceans a 1:1 ratio was actually the exception and not the norm (Wenner 1972). This finding is intriguing for those studying marine organisms. If Wenner's ideas hold true then crayfish, a marine crustacean, should show skewed sex ratios in most species.

Previous research and the questions surrounding the origins of changes in sex ratio provide a framework for further investigation. The purpose of this study was to investigate the sex ratios of native (*O. virilis*) and invasive (*O. rusticus*) crayfish in Burt Lake, Michigan. We hypothesize that based on the ideas presented by Wenner (1972); we will see a skewed sex ratio in both species.

Materials and Methods

Orconectes virilis and *Orconectes rusticus* were sampled at four locations around Burt Lake in Cheboygan County, Michigan. Site one (Greenman's Point) and Site three (Little Carp River) were located in northern portion of the lake. Site two (Maple Bay) and site four (Poverty Bay) had western locations on the lake (Figure 1).

Sampling took place on July 21st, July 24th, July 30th, and August 3rd 2007 for sites one, two, three, and four respectively. The sampling area was 100 square meters at each location. Samples were taken along 50 meters of shoreline and then out 20 meters from the shore.

Headlamps were used to illuminate the site and dip nets were used to catch all samples. Sampling took place from 11:15pm to 12:15am due to the nocturnal habits of the crayfish (Davis and Huber 2007).

After crayfish were caught they were placed into a holding bucket and measurements were taken. Sex and species were determined for each individual by examination of the genital pore and color characteristics. A caliper was used to measure the total body length and the carapace or cephalothorax length. Only once data was recorded for all individuals were the crayfish released. This was done to avoid recapture of the same individual.

The data collected was transferred into Excel spreadsheets and sorted based on sex and species at each sample site. Because the study focuses on tertiary sex ratio, the size of the individual was used to determine reproductive maturity. Based on the average sizes for both species at reproductive age as reported by Momot et al (1978), all individuals with a carapace measurement under 25 mm were removed from the data to be analyzed.

The number of crayfish of each sex and species were totaled across all four sample sites because no site had enough individuals to run analysis. Chi-squared analysis was done for each species separately to determine if the populations showed significant deviation from 1:1 sex ratios.

Results

Overall 163 crayfish were captured at all four sample sites. 26 were disregarded before analysis because carapace measurements indicated that they were not of reproductive age. The

total sample size over all four sites that was used for the study was 137 crayfish (Table 1). 65 of the samples were *O. rusticus* while 72 were *O. virilis*. This means that sample was 47% *O. rusticus* and 53% *O. virilis*.

In total there were 39 female and 26 male *O. rusticus* (Figure 2). The *O. virilis* sample was comprised of 52 females and 20 males (Figure 3).

The chi-squared test for each species used one degree of freedom. *O. rusticus* did not show a significant difference between recorded proportions and expected 1:1 ratio with a p value <0.9. On the other hand, the p value for *O. virilis* was <0.001 and showed statistically significant deviation from the expected 1:1 ratio.

Discussion

Based on our statistical analysis, the data collected for *O. rusticus* does not show significant bias from a normal sex ratio. This means that the ratio of males to females that we found for this species was consistent with the 1:1 ratio that Fisher postulated. Because there is no bias in the sex ratio in this study, it appears that O. rusticus populations do not preferentially distribute resources to offspring or engage in behavioral differences between sexes that could skew the sex ratio data.

However, the data collected for *O. virilis* was statistically significant. The number of females in our sample was substantially higher than the number of males in the population. Therefore the sex ratio was shifted to show a female bias. Because there is a change in the ratio from influences other than random chance, we suggest that external factors are influencing the tertiary sex ratio for this species.

In his studies, Wenner (1972) offered several theories about the mechanisms that could alter sex ratio in crustaceous populations which are applicable to the crayfish species we sampled in Burt Lake. Actions such as differential migration and differential mortality could influence the number of females and males present in a population (Wenner 1972). Differential migration refers to the differences in the way that territories are utilized by each sex. Male and female crayfish have been shown to move different distances within similar sized home ranges (Hazlett 1974). This means that although both sexes live in similar sized habitats, that males are more active in their surroundings. In these instances in which males cover more area, they would be more likely to encounter predation or other obstacles before reaching reproductive age. Because females are moving less and encountering fewer obstacles to survival, it is more likely for them to safely reach reproductive age. This differential rate of mortality could impact the number of male crayfish in the population and explain the bias toward females shown in *O. virilis*.

Because the *O. virilis* in Burt Lake appeared in relative even numbers compared to invasive *O. rusticus* it is important to look at the differences between the species to help explain the bias. Both species are members of the genus *Orconectes* and the mating habits of this genus characteristically show inter-male competition for females (Berrill and Arsenault 1984). This would imply that females are the limiting resource for which males must compete in both of these species. Since our results indicate that females are not a limited resource in the *O. virilis* population as they might be in the *O. rusticus* are data provides a challenge to this idea. It is possible that while one species conforms to this mating pattern that a population with a larger number of females might have other mating systems. *O. virilis* females might engage in competition for the limited male mates or copulation with multiple males may occur. This could discount previous ideas of *O. rusticus* life history; however other factors are also at work.

The fact that one species is native and one is invasive could also have an impact on the way that individuals interact and the habitats of both the *O. rusticus* and *O. virilis*. The impact of the invasive *O. rusticus* on native populations of *O. virilis* have been widely studied and recorded. Literature attests that the invasive species is able to displace native species after establishing themselves in the habitat. In fact, *O. rusticus* has now replaced *O. virilis* as the most abundant crayfish species (Olden et al 2006). This is known because the increase in population size for *O. rusticus* is directly related to the decline in populations of *O. virilis* (Lodge et al 1986). If this displacement is occurring then direct male-male competition might be having an impact on the number of males surviving to reproductive maturity.

However, despite these predictions both species were present in our sample in relatively equal numbers. *O. virilis* in Burt Lake have been able to compete with the invasive species and remain part of the Burt Lake ecosystem. However, even though the ratio remains around 50-50, this does not mean that the crayfish are evenly spaced throughout the habitat. *O. virilis* might have resisted total displacement, but males may have been displaced from areas near the shore where sampling took place. A local displacement by the invasive species could have resulted in less *O. virilis* being present in our sample areas.

If our sampling was flawed this could have led to the appearance of a bias sex ratio when one does not really exist. Errors in sampling could have resulted from the previously discussed habitat differences and changes in locomotion through the habitat. If *O. virilis* males have been displaced outside our sample site or if males move more often and farther in the habitat, this could have made them more difficult to sample or caused them to be outside of our chosen sampling range. Because of the possibility of sampling error, our study should be repeated with larger sample sizes, greater diversity of sites, and increased sampling time to confirm our results.

Overall our study shows an indication that external factors are influencing the tertiary sex ratio of O. virilis crayfish in Burt Lake. Statistical analysis shows that the proportion is significantly different from the expected ratio of one female for every one male. We also noted that this bias favored females for this species. In contrast, the competing invasive species, O. rusticus does not differ from the sex ratio as predicted by Fisher (1930) under the influence of natural selection. A combination of factors such as movement and differential mortality could result in the bias that was observed. Also the influence of native and invasive species as they compete for resources across and within species could have a role in altering sex ratio. Further study is needed to confirm these hypotheses and to solidify the results presented here.

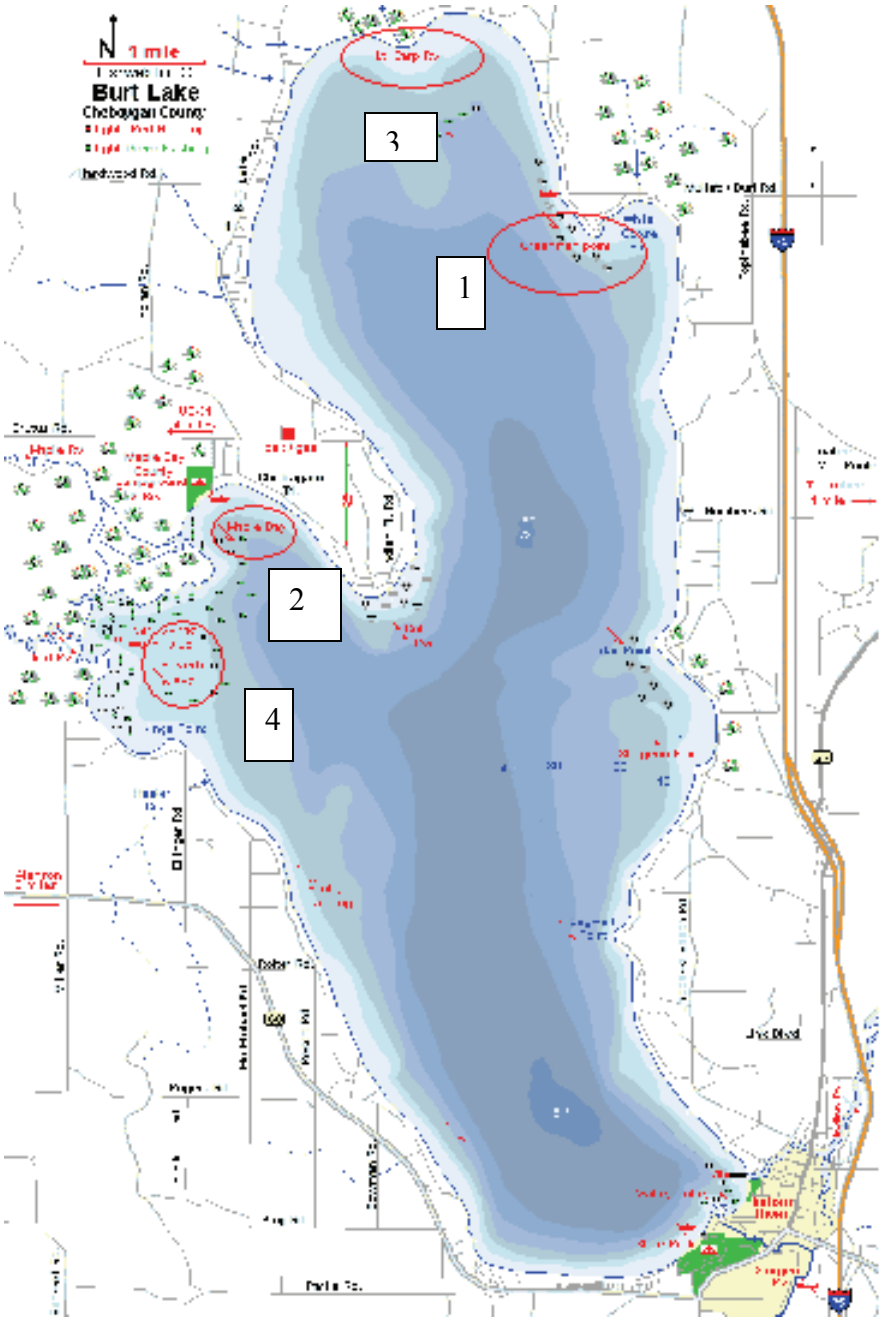
Tables and Figures

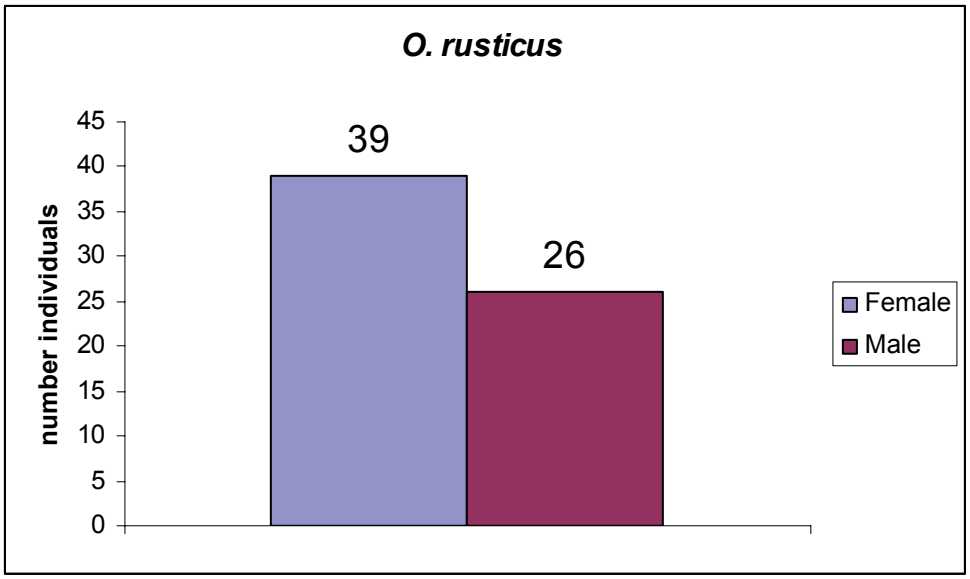
Figure 1. This map of Burt Lake shows the four sites where crayfish were captured. Site one is Greenman's Point, site 2 is Maple Bay, site 3 is Little Carp River, and site 4 is Poverty Bay.

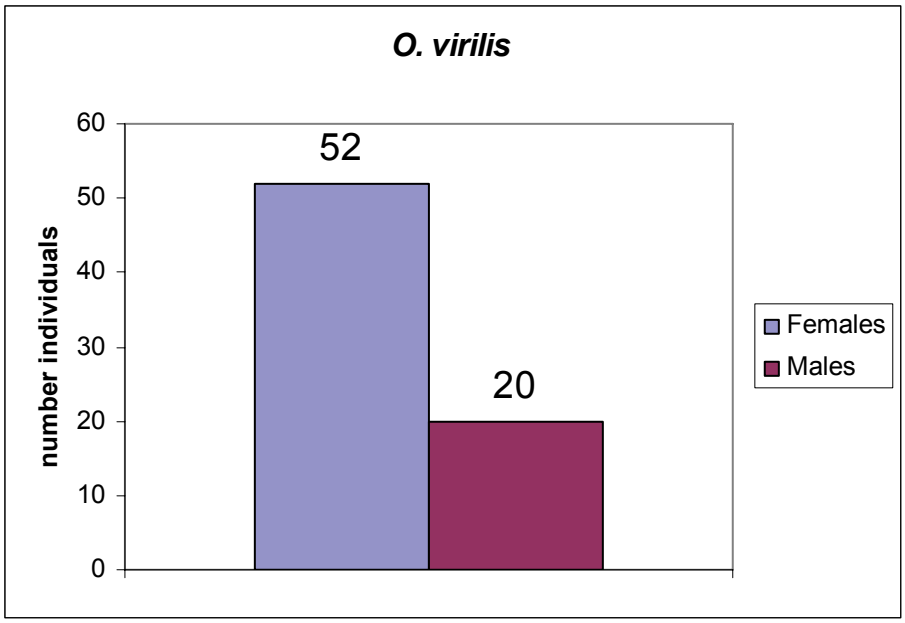
Figure 2. This figure shows the total number of female and male *O. rusticus* collected over all four sample sites. Of the 65 individuals recorded, 39 were females and 26 were males.

Figure 3. This figure shows the total number of female and male *O. virilis* collected over all four sample sites. Of the 72 individuals recorded, 52 were females and 20 were males.

Table 1. This table shows the number of female and male *O. rusticus* and *O. virilis* caught at each of the four sample sites. The total number of individuals of each species is in the "species total" column and the breakdown between sexes is in the next two columns. Totals for each species and sex for all four sites are also shown


1 mile
Burt Lake
Cheboygan County
3
1
2
4


O. rusticus
number individuals
45
40
35
30
25
20
15
10
5
0
39
26
Female
Male


O. virilis
number individuals
60
50
40
30
20
10
0
52
20
Females
Males

Sample Location	species total			species total		
	O. rusticus	female	male	*O. virilis*	female	male
Site 1 Greenman's Pt	21	11	10	6	6	0
Site 2 Little Carp River	25	18	7	12	8	4
Site 3 Maple Bay	5	2	3	35	23	17
Site 4 Poverty Bay	14	8	6	19	17	2
total	65	39	26	72	54	23

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