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

Janee Kronk
5297 Redding Dr.
Lakeland, MI 48143
jkronk@umich.edu

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Abstract

The sex ratio theory proposed by Fisher (1930) that suggests an equal number of males to females has ignited an interest in studying the sex ratio of various organisms. In particular, this study looked at the tertiary sex ratio of two species of crayfish found in Northern Michigan. Native (*Orconectes virilis)* and invasive species *(Orconectes rusticus)* of crayfish were collected from Burt Lake in Cheboygan County, Michigan during a two-week period in mid-July. Crayfish were sampled at four different sites along the Burt Lake shoreline and were caught by dip nets. The species, sex, carapace length, and total length were determined for each individual. A total of 163 crayfish were caught and by looking at the total length of each crayfish, only 137 of them were of reproductive age. The 26 crayfish not of reproductive age were not included in the data analysis.

The data showed that *O. rusticus* and *O. virilis* were present in nearly equal amounts, 47% and 53% respectively. For *O. rusticus*, 39 females and 26 males were collected for a total of 65 invasive crayfish. For *O. virilis*, 72 crayfish were collected with 52 females and 20 males. The data were analyzed by chi-squared tests. Based on the statistical analysis, the data collected for *O. rusticus* did not show significant bias from a normal sex ratio. However, the data for *O. virilis* was significant. The number of females was substantially higher than the number of males in the population. External factors like differential mortality or differential movement that might be influencing the sex ratio bias of *O. virilis* will be discussed.

Introduction

Sex ratio is defined as the proportion of males in a population. This count of males versus females relates directly to evolutionary fitness, as discussed in Fisher's Theory of Long-term Optimal Sex Ratio (1930). He proposed that natural selection maintains an equilibrium sex ratio in the idea that each gender is equally costly to produce. And if offspring are equally costly to produce, then parental investments and resources should be equally received by each individual offspring. Over time, this should result in a tertiary sex ratio that fluctuates around even male to female numbers. But according to the study done by Trivers and Willard in 1973, a difference in reproductive return between males and females often exists. If one sex happens to reproduce less than the other but is still receiving an equal amount of resources and parental investment, a decrease in future fitness can occur. This tends to result in a departure from the 1:1 sex ratio that natural selection favors when progeny are equally costly to produce.

These ideas have created an interesting field of study that includes all sexual species. In particular, certain crustaceans were studied by Wenner (1972) in order to learn more about sex ratios and possible discrepancies. His results showed that the expected 1:1 sex ratio was unusual for these organisms and that differences in the numbers of males to females were natural. A study was done with freshwater crayfish in Australia that showed an interesting transgression from the expected 1:1 ratio. The proportion of males to females at birth (secondary sex ratio) was found to be 3 males for every 1 female (Austin and Meewan 1999). The fact that this freshwater species had a deviation from the 1:1 sex ratio could mean something for the sex ratios in other similar species of crayfish.

This study in particular focused on the sex ratio of native and invasive species of crayfish at reproductive age (tertiary) in Burt Lake, Michigan. Our hypothesis was influenced by Wenner's 1972 study as well as Fisher's theory of a sex ratio of 1:1 at reproductive age. We proposed that an irregular sex ratio would be seen in both native and invasive species of crayfish.

Materials and Methods

Study Species

At night, the sandy bottom of Burt Lake is a playground for both native and invasive species of crayfish. *Orconectes rusticus*, commonly referred to as the rusty crayfish, stands out from the blue-tinted native species, *Orconectes virilis,* with its robust claws and dark, rusty spots on each side of its carapace. Rusty crayfish were introduced to the north by non-resident anglers who brought them for fishing bait and because they are an aggressive species, the rusty crayfish has been frequently displacing native species ever since (Rubenstein and Hazlett 1974).

Data Collection & Analysis

Native (*Orconectes virilis)* and invasive *(Orconectes rusticus)* species of crayfish were sampled in Burt Lake during a two-week period. The sites included Greenman's Point, Maple Bay, Little Carp River, and Poverty Bay (Figure 1). The time of sampling occurred at night, starting at 11:00pm and ending at 1:00am. Crayfish were caught for one hour at each site, at the same time each night (11:15pm to 12:15am). The sampling was made uniform at each site by catching crayfish in an area 50 meters in length along

the shoreline to 20 meters out into the lake (thigh deep water). Crayfish were located with a flashlight and then scooped up with a dip net. Once captured, they were placed in a plastic bucket halfway filled with water. After one hour of catching samples, all of the captured crayfish were brought to the shore to be measured. Four characteristics were identified: species, sex, carapace length, and the total length. The species were identified by obvious outer characteristics: *O. rusticus* differs from *O. virilis* by dark spots on either sides of its carapace and black bands on its claw tips and the body of *O. virilis* has a bluish tint and no black bands on its claw tips. The sex was determined by shining a flashlight beam on the underside of the crayfish to look at the sex organ. The females have a genital opening on the ventral part of the sixth body segment (between the walking legs) while the males have two appendages facing forward (a sperm duct from the sixth to the eighth body segment) (Figures 2 and 3 respectively). The carapace length was determined by using a caliper to the nearest millimeter (mm). The total length of the crayfish, from the end of the tail to the cephalothorax, was measured in order to determine whether a crayfish was of reproductive age (Figure 4). Total lengths under 25mm were disregarded (Momot et al. 1978). The data were then analyzed using chi-squared tests.

Results

A total of 163 crayfish were collected from the four sampling sites but 26 of them were disregarded because their size (in mm) indicated that they were not of reproductive age. The sample size that was analyzed was 137 crayfish (Table 1).

The data showed that the two species of crayfish, *O. rusticus* and *O. virilis,* were present in nearly equal amounts, 47% and 53% respectively (Figure 5). In terms of sex ratio*, O. rusticus* had 39 females and 26 males (Figure 6) while *O. virilis* had 52 females and 20 males (Figure 7).

After analyzing the data with chi-squared tests*, O.rusticus* did not exhibit a statistically significant deviation from the expected 1:1 sex ratio ($\chi 2=2.6$, df=1, p-value<0.9). Conversely, *O.virilis* did show significant results in regards to the projected 1:1 sex ratio ($\chi 2=14.2$, df=1, p-value<0.001).

Discussion

Fisher's sex ratio theory (1930) has spurred scientists to investigate the sex ratio of various organisms. In particular, this study looked at the tertiary sex ratio of two species of crayfish found in Northern Michigan. Based on our statistical analysis, the data collected for *O. rusticus* does not show significant bias from a normal sex ratio. This illustrates that the population of O. rusticus sampled does not differentiate in the distribution of resources to offspring. It could also mean that differences in the behaviors of males and females that might affect mortality do not exist. Conversely, the data for *O. virilis* was significant. The number of females was substantially higher than the number of males in the population. This indicates that external factors are influencing the sex ratio and causing a female bias for this species.

Multiple theories explaining changes from the expected 1:1 sex ratio in crustaceans were postulated by Wenner (1972). Possible reasons include differential migration, mortality, and growth rate. Males have been shown to be more active than

females in their home range (Rubenstein and Hazlett 1974). This means that there is a greater possibility for males to have more run-ins with predators and less chance that they will reach reproductive age. By risking predation, their mortality rates tend to differ from the females. And in terms of differential growth rate, males spend more time growing than females, meaning that females reach reproductive age quicker (Momot et al. 1978).

The *O. virilis* in Burt Lake appeared in relative even numbers compared to invasive *O. rusticus*. Because this differs from studies showing that the invasive species push out the native species (Olden et al. 2006), it might be possible that an increase in the number of *O. virilis* females ensures enough reproductive success to help them compete with the invading *O. rusticus.*

Several problems associated with the data could have led to the data analysis showing a female bias for *O. virilis*. First of all, the sampling methods were not exhaustive. We did not sample every crayfish in Burt Lake, mainly because we were confined to public access areas. A larger and more geographically diverse sample size would have further benefited this study of sex ratio in crayfish. Secondly, we did not mark the crayfish we caught. Because we did not place markers on the captured crayfish, we are not sure if we measured any of the crayfish more than once. The migration behavior (in terms of long distances) for both the native and invasive species in Burt Lake was unknown for this study.

Possible follow up studies to test additional hypotheses include investigating the sex ratios of *O. virilis* in habitats with and without invasive species present, sampling other crayfish species in similar invasive situations, and studying the sex ratio of species that are unable to compete with invasive species.

In conclusion, *O. virilis* and *O. rusticus* were present in equal proportions in Burt Lake. *O. virilis* showed significant deviation from theorized 1:1 tertiary sex ratio, with a female bias. Possible explanations for female bias include differential mortality, movement, and/or growth. Distorted ratios could be significant in the ability of *O. virilis* to compete with invasive *O. rusticus*.

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Figure Legend

Table 1. This table shows the number of individuals caught at each of the four sample sites. It shows the number of O. rusticus and O. virilis sampled, which is then subdivided into the amount of males and females caught for each species.

Figure 1. This map of Burt Lake is labeled with numbers 1 through 4 to show the sampling sites: Greenman's Point (Site 1), Maple Bay (Site 2), Little Carp River (Site 3), and Poverty Bay (Site 4).

Figure 2. This figures shows the sex organ of a female crayfish. The genital pore is showcased by the arrow on the left side of the figure.

Figure 3. This figure shows the sex organ of a male crayfish. The arrow is pointing to the two appendages which are the sperm ducts.

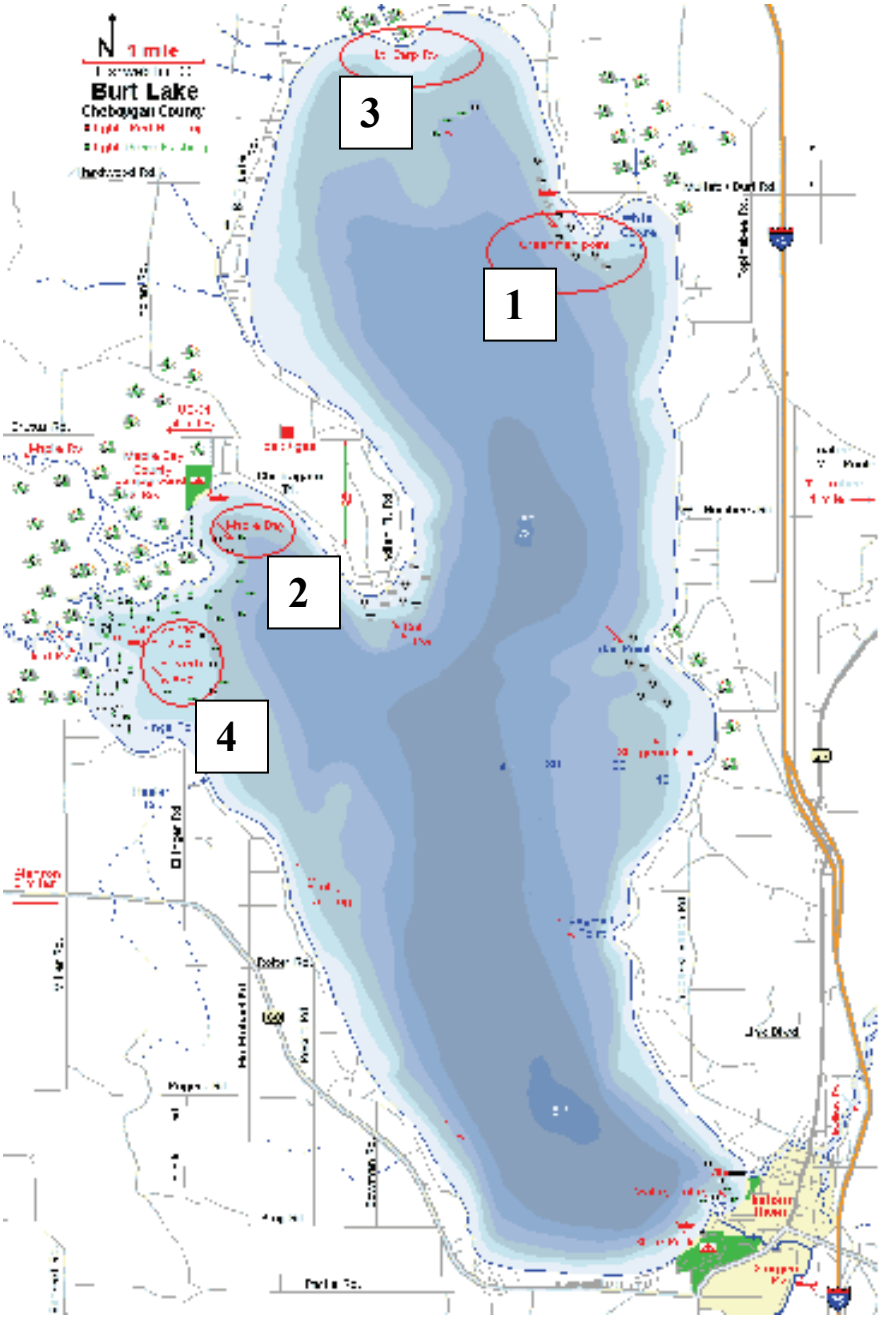
Figure 4. This figure is a diagram of the body parts of a crayfigh. The carapace length and total length (from end of tail to end of the cephalothorax) were measured with a caliper to the nearest millimeter (mm). Total lengths under 25 millimeters were not included in the data analysis.

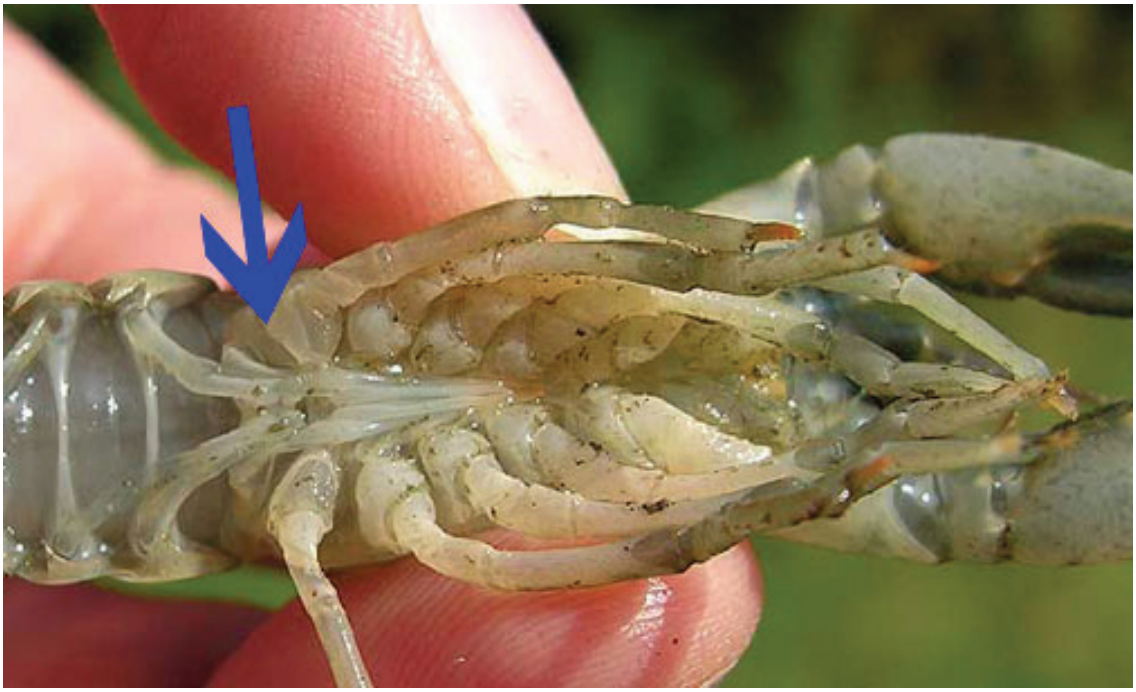
Figure 5. This chart shows the % of the two crayfish species, *O.rusticus* and *O. virilis,* that were collected in Burt Lake.

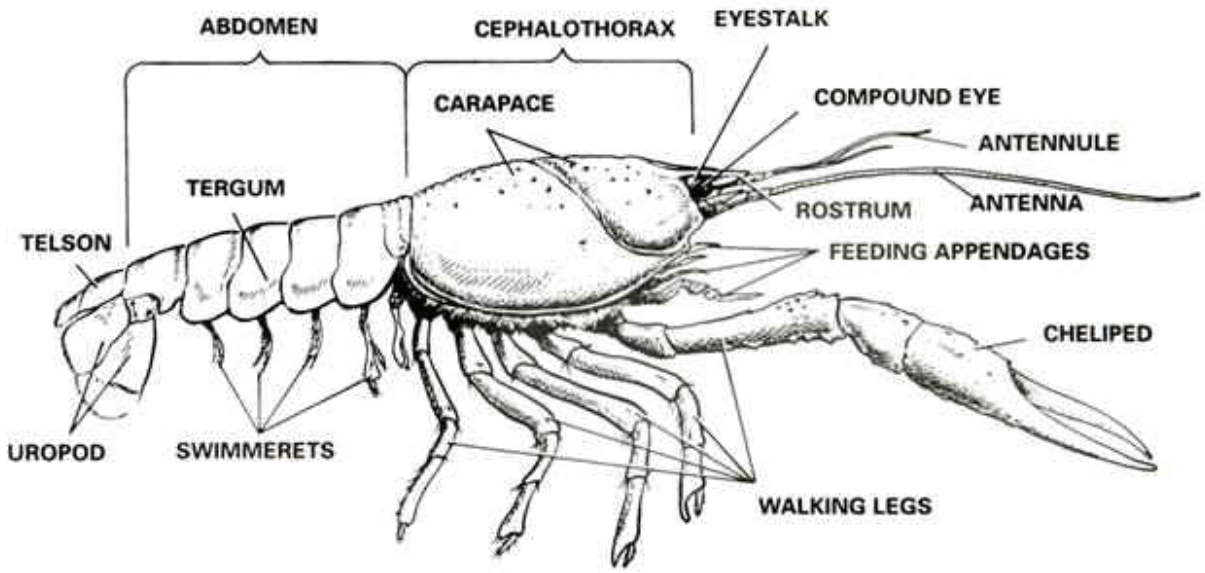
Figure 6. This chart shows the number of *O. rusticus* males and females collected in Burt Lake.

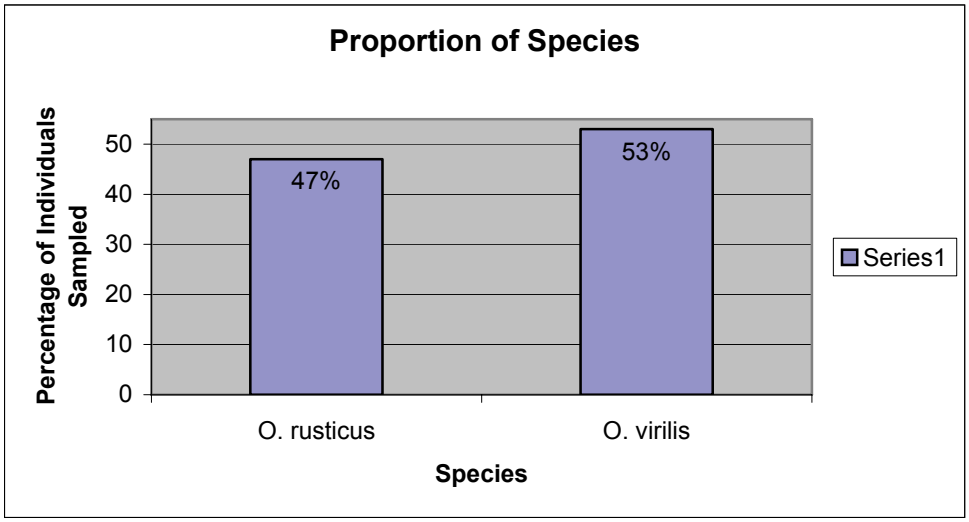
Figure 7. This chart shows the number of *O. virilis* males and females collected in Burt Lake.

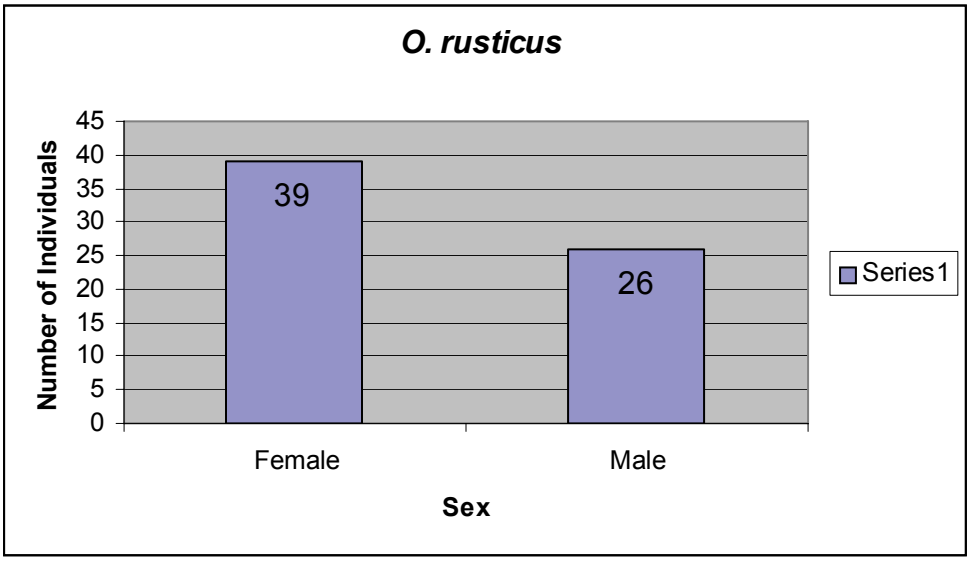
Sample Location	**Species Total**			**Species Total**		
	O. rusticus	Female	Male	*O. virilis*	Female	Male
Greenman's Point (Site 1)	21	11	10	6	6	0
Maple Bay (Site 2)	5	2	3	35	21	14
Little Carp River (Site 3)	25	18	7	12	8	4
Poverty Bay (Site 4)	14	8	6	19	17	2
Total	65	39	26	72	52	20

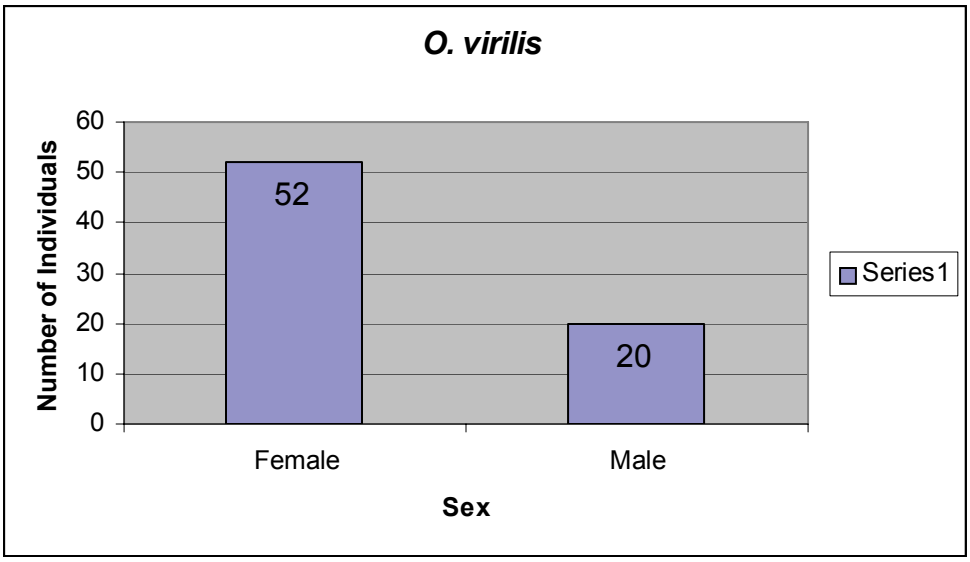

N
1 mile
Burt Lake
Cheboygan County
3
1
2
4




ABDOMEN
CEPHALOTHORAX
EYESTALK
CARAPACE
COMPOUND EYE
ANTENNULE
TERGUM
ROSTRUM
ANTENNA
TELSON
FEEDING APPENDAGES
CHELIPED
UROPOD
SWIMMERETS
WALKING LEGS


Proportion of Species
Percentage of Individuals Sampled
50
40
30
20
10
0
47%
53%
O. rusticus
O. virilis
Species
Series1


O. rusticus
Number of Individuals
45
40
35
30
25
20
15
10
5
0
39
26
Female
Male
Sex
Series1


O. virilis
Number of Individuals
60
50
40
30
20
10
0
52
20
Female
Male
Sex
Series1