Does Size Matter?
Mating and Predation Pressures for the Amorous *Gammarus*

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Bio 696/496

December 2017

INTRODUCTION

Gammarus is a genus within the Amphipod order. Two hundred species of gammarus have been described, and are found in a wide variety of aquatic conditions. They feed on the decaying plant material in their environment and the associated microflora (Kostalos and Seymour 1976). *Gammarus spp.* have a segmented body. The segment that forms the head houses two compound eyes, antennae, and mouthparts. The segments that form the thorax house accessory mouthparts, swimming legs, and gills. The abdomen contains additional swimming legs and a tail.

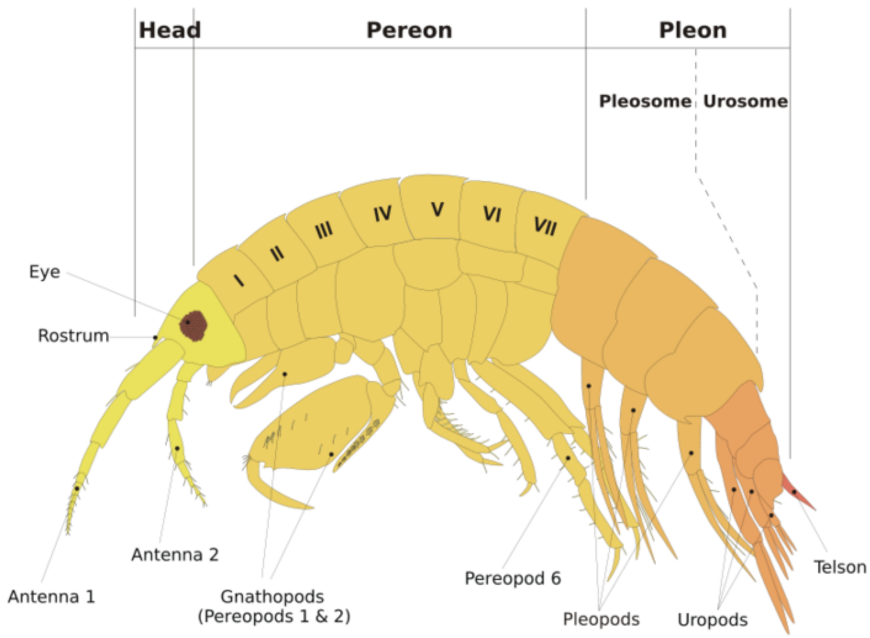

Figure 1: Gammarus anatomy from Hillewaert, 2013.

To reproduce, males attach themselves to females, carrying them for several days until she becomes available to mate with. Mate guarding may be a strategy gives the males an advantage in mating. Guarding one female ensures males of a mating opportunity and excludes other males (Hatcher and Dunn 1996). After eggs are fertilized in the female's oviduct, they are stored in the brood pouch, until the embryos reach maturity and hatch (Sutcliffe 1992).

Gammarus participate in positive assortative mating, meaning that individuals will select a mate of a particular phenotype. This can mean that individuals will seek out others of a similar phenotype, or select mates based directional preference. Hatcher and Dunn observed that larger males are able to attach to larger females (1996). Under assortative mating, high quality pairs will form, while low quality individuals will be left out; it is possible that large females are perceived as higher quality than small females.

Gammarus males will always prefer to mate with high quality females. Since all males are competing, the high-quality males will be more successful in securing the sought after female, and will guard her. High quality males will reproduce with high quality females, and create high quality offspring. Low quality males will only be able to compete with other low-quality males, for lower quality females, and create lower quality offspring. It is possible that not only are large males reproducing with high quality females, but also producing more opportunities. This could be due to greater mating opportunity, but also differences in brood size. Larger females may also have the capacity to produce and carry more eggs. The advantage high quality offspring and a greater number of offspring is that the next generation will survive to perpetuate that genome while low quality offspring have less of a likelihood of passing on their genes, either because they do not survive to reproduce, or are not able to compete with other males (Franceschi, Lemaitre, Cezilly, and Bollache 2010).

While large individuals may have a mating advantage, there are size-specific pressures that may push for smaller body size. Fish that prey on *Gammarus spp.* have been found to prefer larger individuals. One study investigating size-selectivity in sculpin predation on *Gammarus* found that the average size of *Gammarus* found in the stomachs of fish was larger than those in the surrounding environment (Newman and Waters 1984). This difference is evidence to suggest sculpins prefer larger prey. This predation may play an important role in *Gammarus spp.* competition; if large individuals are less likely to survive and reproduce, populations highly preyed upon should drift toward smaller sizes as those that are small survive most often to produce offspring.

Given theoretical foundations and previous research, this study investigated three major hypotheses:

Hypothesis one: Larger male *Gammarus sp.* pair with larger *Gammarus sp.* females.

Hypothesis two: Both male and female *Gammarus sp.* are smaller in springs without fish predation pressure.

Hypothesis three: The largest female *Gammarus sp.* have the greatest number of eggs per brood.

METHODS

All *Gammarus* specimens were collected in May and August 2009 and October 2017 from springs in Greenbrier County, West Virginia. Springs are unique features with water that originates and travels below ground before surfacing. One study site is classified as a karst window, or eroded region once roofed by a cavern. Springs sampled for *Gammarus* throughout Greenbrier County include the Caskey, Whitehouse, Clipps Mill, Cathedral, Agua, Blowing, Taylor, Apple, Davis, and Dickson Springs.

Gammarus specimens were collected at varied locations in each spring. A large, fine mesh net was held against the bottom of the spring while upstream areas of rock, mud, and submerged leaf litter were disturbed with kicking and scraping motions. Rocks were moved and brushed off into the net. The *Gammarus* captured in each netting were housed in plastic bags and kept in a cooler. This was repeated up to three times at each spring. The contents of the bags were analyzed after all springs were sampled.

Gammarus pairs and oviparous females were identified and organized off-site. Bags of samples from each spring were poured into plastic containers in a well-lit environment; pairs were identified and transferred to vials of ethanol for preservation. Oviparous females were removed and preserved similarly. The oviparous females could be identified by dark underbelly coloration that indicates the presence of eggs. Pairs and females from each spring were kept separately.

The head length of each preserved specimen was measured under a dissecting scope. The segmented body makes it difficult to take uniform measurements of each sample, so measurements were taken from only the head segment for comparison consistency. Oviparous females were dissected, eggs were removed and counted.

Using IBM SPSS software, box plots were created with the male and female sizes in each spring and organized by fish presence or absence. Male to female head size was analyzed with Pearson's correlation to determine whether there is a positive linear relationship, as predicted. The closer the correlation coefficient is to 1, the stronger the positive correlation between the variables. The linear relationship decreases the closer the coefficient gets to 0. A strong positive correlation lies in the .5-1 range, a moderate correlation would have a value of .3-.5, and a weak correlation would fall between .1-.3. No correlation would have a value of 0 (Lane). Female head length and brood size were analyzed with a simple linear regression to determine whether variation in brood size can be explained by female size. The higher the R-squared value, the larger proportion of variation in brood size can be explained by female size. A scatter plot comparing all male sizes and all female sizes, and scatter plots comparing male size to female for each spring were created.

RESULTS

Pair Formations and Size: Male and Female Head Length

Pearson's correlation coefficient for the relationship between male and female *Gammarus sp.* head length was calculated for all springs individually and for all springs combined.

Spring	Location ID	Correlation *P*	Number of Males	Number of Females	Total
Caskey	1	.268	29	29	58
Whitehouse	2	.118	46	53	99
Clipps Mill	3	.058	36	50	86
Cathedral	4	.286	33	46	79
Agua	5	.890	4	4	8
Blowing	6	.324	58	59	117
Taylor	7	.437	76	97	173
Apple	9	.595	5	5	5
Davis	10	.351	59	74	133
Dickson	11	.235	33	97	130
All combined		.516	395	478	873

Table 1: Pearson's correlation coefficient values by spring.

For all springs combined, the correlation coefficient was .516, moderate evidence of a relationship between male head length and female head length (see Figure 2 below). **Graphs for each individual spring are located in the appendix.**

GAMMARUS HEAD LENGTH MALE V. FEMALE (ALL SPRING DATA)

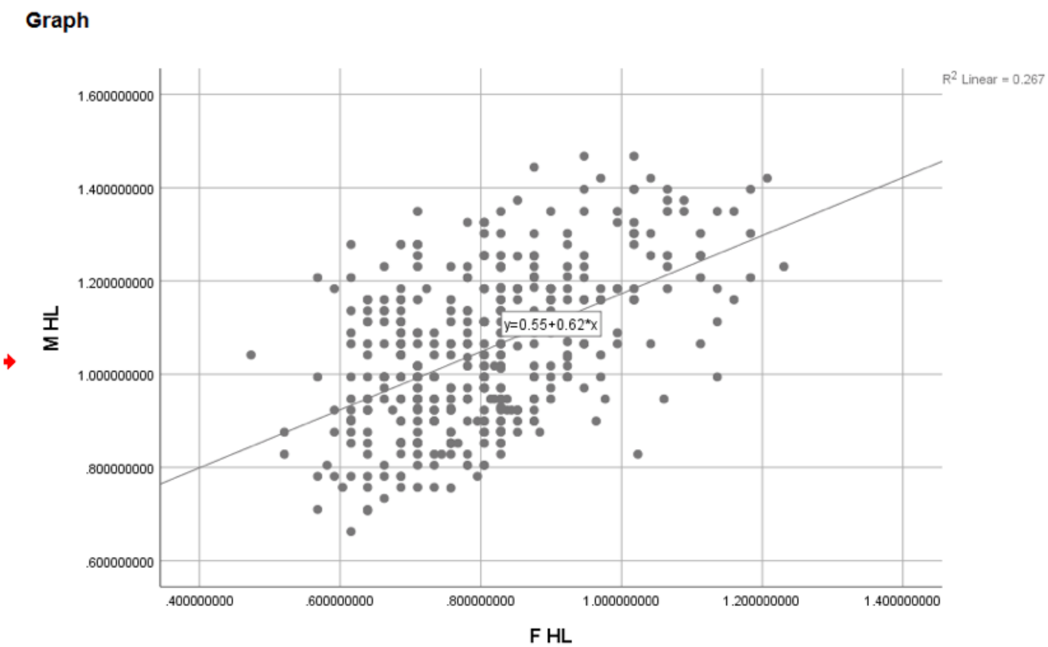

Figure 2: **Gammarus sp.** ***head length for males and females shows a moderate relationship.***
son

Predation Pressures: Head Length by Fish Presence

Of all springs sampled, Whitehouse, Agua, Blowing, Taylor, and Apple did not have fish present (63% of springs). Clipps Mill, Cathedral, Davis, and Dickson did have fish present (36% of springs).

Spring	**Location ID**	**Fish Presence**
Caskey	1	NO
Whitehouse	2	NO
Clipps Mill	3	YES
Cathedral	4	YES
Agua	5	NO
Blowing	6	NO
Taylor	7	NO
Apple	9	NO
Davis	10	YES
Dickson	11	YES

Table 2: Fish presence by spring.

The mean head length for *Gammarus sp.* females for all samples combined in springs with fish was .68, compared to .85 in springs without fish. The mean head length for males in springs with fish was .94, as opposed to 1.1 without fish. While the trend for all samples combined showed mean head lengths were larger in springs without fish, the trend was not ubiquitous. For example, mean head length for males and females in Clipps Mill Spring (ID 3), which has fish, were larger than mean head lengths for Blowing Spring (ID 6), which does not have fish (Table 2).

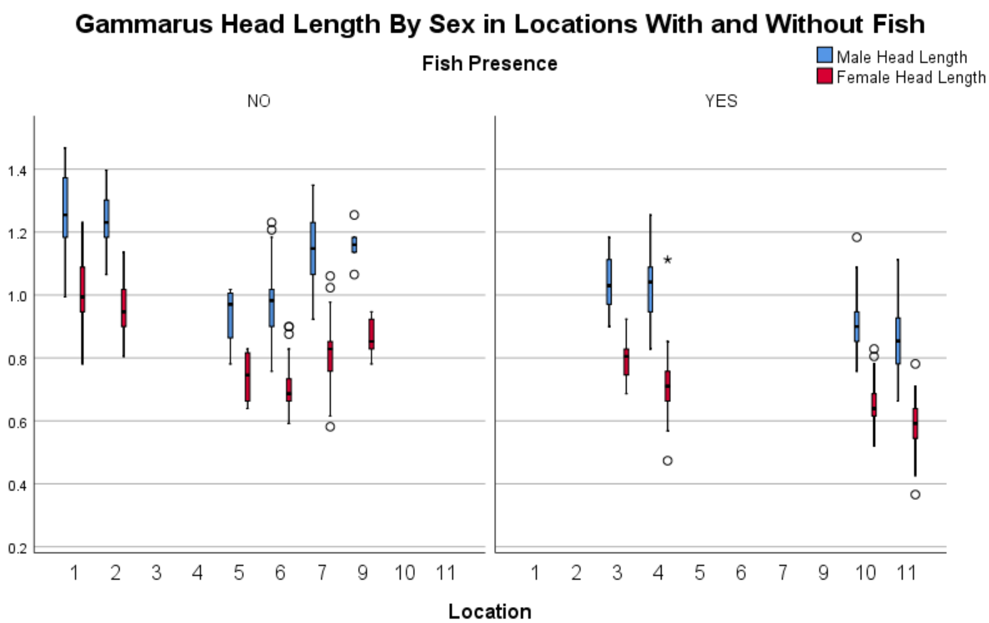

Figure 3: Head length for male and female* Gammarus sp. *by spring.

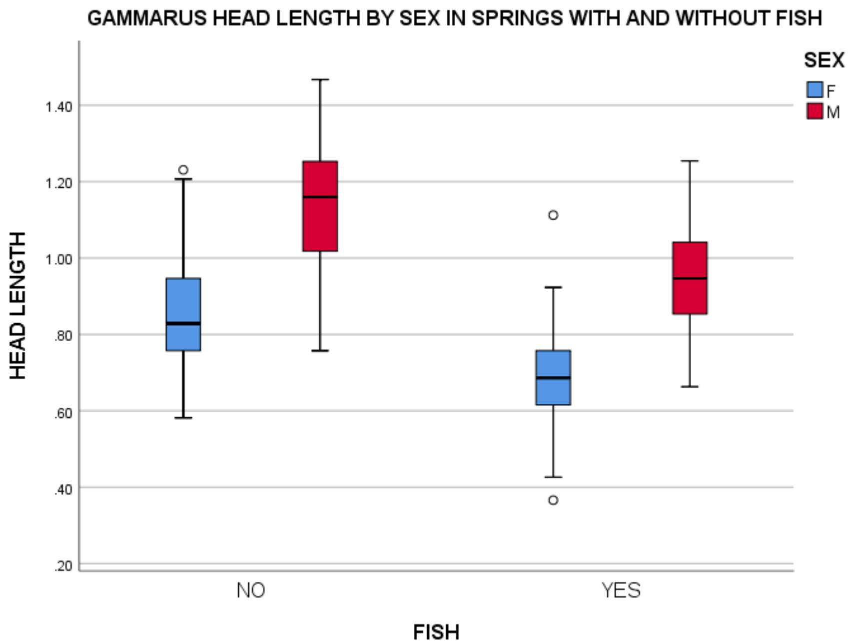

Figure 4: Head length for male and female* Gammarus sp. *for all springs combined.

Female Size and Egg Count

A simple linear regression analysis of female head size and brood size yielded an R-squared value of .394; only 39.4% of variation in brood size could be explained by head length in this model.

Figure 5: Female head length by brood size; R-squared value is .394.

DISCUSSION

The male and female head lengths for all data combined had a moderate correlation of .516; this indicates large males may be pairing with large females. Pairs from Agua Spring (ID 5) and Apple Spring (ID 9) appeared to have the strongest positive correlation, but, small sample size should be taken into account as there are only 4 data points for Agua Spring and 5 data points for Apple Spring. It is possible our small samples aren't representative of the population; these few unusual values may have undue influence on the overall correlation. Taylor Spring (ID 7) had the strongest correlation of all springs at .437. Clipps Mill Spring (ID 3) had the weakest correlation at .058. We conclude that there is some evidence to support our hypothesis that large males are pairing with small females and concur with previous work supporting this conclusion (Hatcher and Dunn 1996); however, there is ample room for further interpretation and additional samples should be collected.

Selective mating pressures that favor large size may be at odds with predation pressures that favor small size. In springs with fish, and presumably high predation pressure, male and female head lengths were smaller than in springs without fish predation when all springs samples were combined. This supports our hypothesis that predation pressures select for smaller size. However, the trend was not ubiquitous. Some springs with fish predation had lower mean head lengths for males and females than springs without fish predation. Future studies could incorporate larger sample sizes in terms of springs surveyed and *Gammarus sp.* individuals measured.

While there is some evidence that large males and large females are pairing, we find only weak evidence that larger females are producing larger brood sizes. Only 39.4% of variation in brood size in our simple linear regression model. While reproductive fitness can be directly measured by mating success and offspring produced, there may be other factors beyond size that are leading to male and female pairing and offspring production. Future studies should explore indicators of female quality beyond brood size. It is possible that our assumption that higher quality females produce a greater number of eggs was unfounded. Number and size of eggs is related to fitness in the specific environment, resulting in different reproductive strategies. In a study of a population of *Gammarus sp.* where adult mortality was high, females were smaller at maturity and produced many small eggs while a population where juvenile mortality was high and females were larger at maturity produced only a few eggs, that were larger (Wilhelm and Schindler 2000). In a population with high adult mortality, it would be more advantageous to produce as many offspring as possible, as soon as possible and release a large amount of eggs as soon as they are able. In a population where juvenile mortality is high but adults do not experience the same pressure, the adults in that population can afford the energy and time investment into larger (but fewer) eggs, that are more hearty to give them a better chance of survival. The springs with fish might experience high predation on adults because sculpin prefer to eat the larger *Gammarus sp.* individuals. Under these conditions, females might be smaller when they reach maturity and carry a larger number of smaller eggs. In springs without fish, *Gammarus sp.* would not experience this type of pressure. To illustrate this, more data should be collected on egg size found in females in these two conditions.

The sampling methods used and data comparison did not account for seasonal variation of *Gammarus sp.* population composition and seasonal variation of predation. A study conducted by Newman and Waters suggests seasonal variation of intensity in predation on *Gammarus spp.,* which could influence body size. Given our results that show *Gammarus sp.* body size varies by predation pressure, comparing seasonal data could show the change in *Gammarus sp.* sizes in relation to fluctuations in predation pressure. Alternatively, data could have all been collected in one season for consistency. Other future research directions should also investigate the relationship between *Gammarus sp.* body size and the level of macroinvertebrate diversity in springs. Varying levels of diversity could be related to *Gammarus sp.* population dynamics.

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APPENDIX

Correlations

[DataSet1]

Correlations

		M HL	F HL
M HL	Pearson Correlation	1	.516**
	Sig. (2-tailed)		.000
	N	395	395
F HL	Pearson Correlation	.516**	1
	Sig. (2-tailed)	.000	
	N	395	478

**. Correlation is significant at the 0.01 level (2-tailed).

Figure 6:* Gammarus sp. *head length for males and females shows a moderate relationship and the correlation is significant.

Correlations

Correlations

		M HL	F HL
M HL	Pearson Correlation	1	.268
	Sig. (2-tailed)		.072
	N	46	46
F HL	Pearson Correlation	.268	1
	Sig. (2-tailed)	.072	
	N	46	53

Figure 7: **Gammarus sp.** *head length for males and females in Caskey shows a weak relationship and the correlation is not significant*

Graph

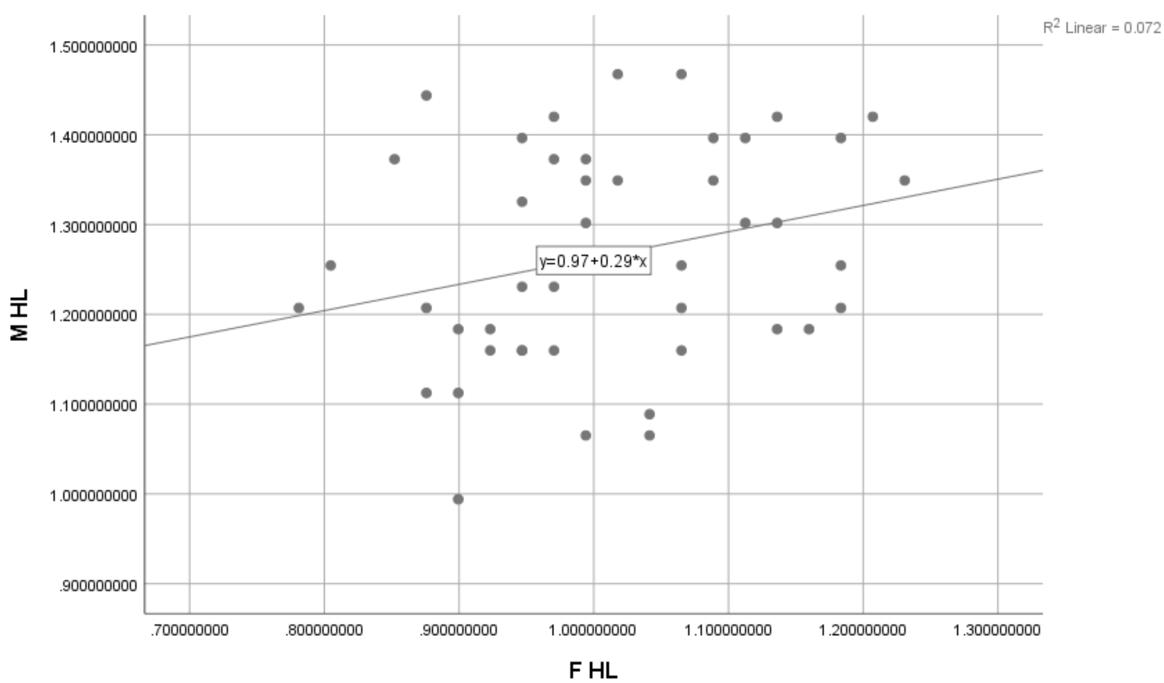

Figure 8: **Gammarus sp.** *head length for males and females in Caskey Spring shows a weak relationship.*

Correlations

Correlations

		M HL	F HL
M HL	Pearson Correlation	1	.118
	Sig. (2-tailed)		.542
	N	29	29
F HL	Pearson Correlation	.118	1
	Sig. (2-tailed)	.542	
	N	29	29

Figure 9: **Gammarus sp.** *head length for males and females in Whitehouse shows a weak relationship, and the correlation is not significant*

Graph

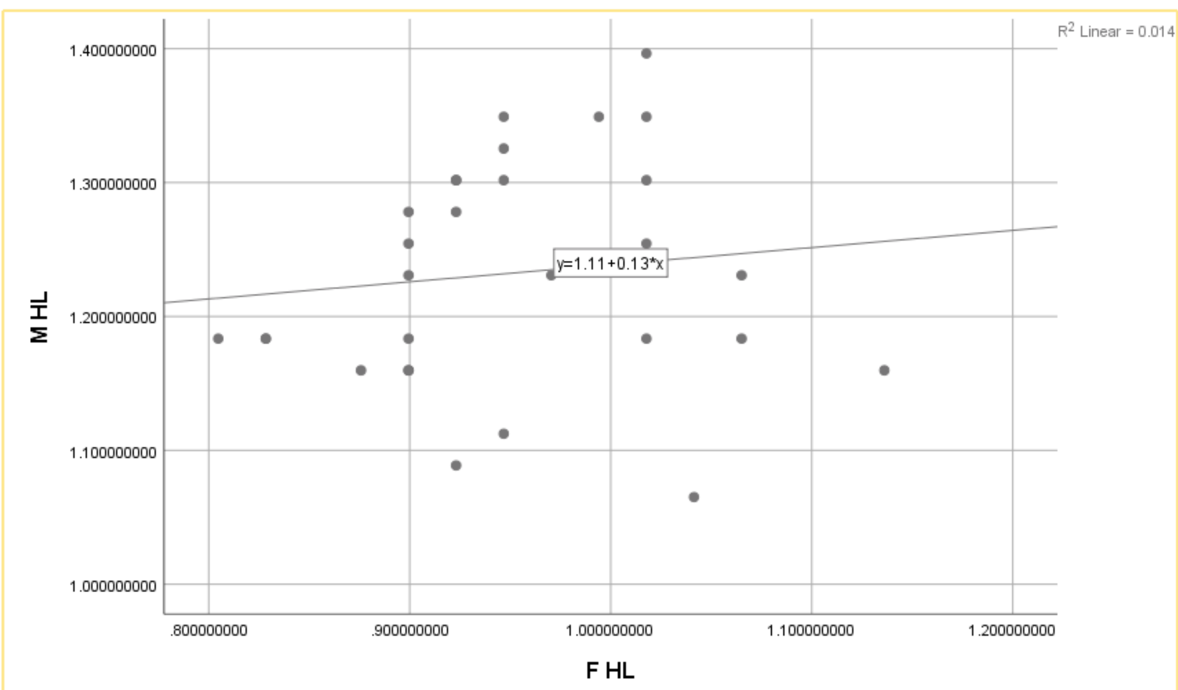

Figure 10: **Gammarus sp.** *head length for males and females in Whitehouse Spring shows a weak relationship.*

Correlations

Correlations

		M HL	F HL
M HL	Pearson Correlation	1	.058
	Sig. (2-tailed)		.737
	N	36	36
F HL	Pearson Correlation	.058	1
	Sig. (2-tailed)	.737	
	N	36	50

Figure 11: **Gammarus sp.** ***head length for males and females in Clipps Mill Spring shows a weak relationship, and the correlation is not significant***

Graph

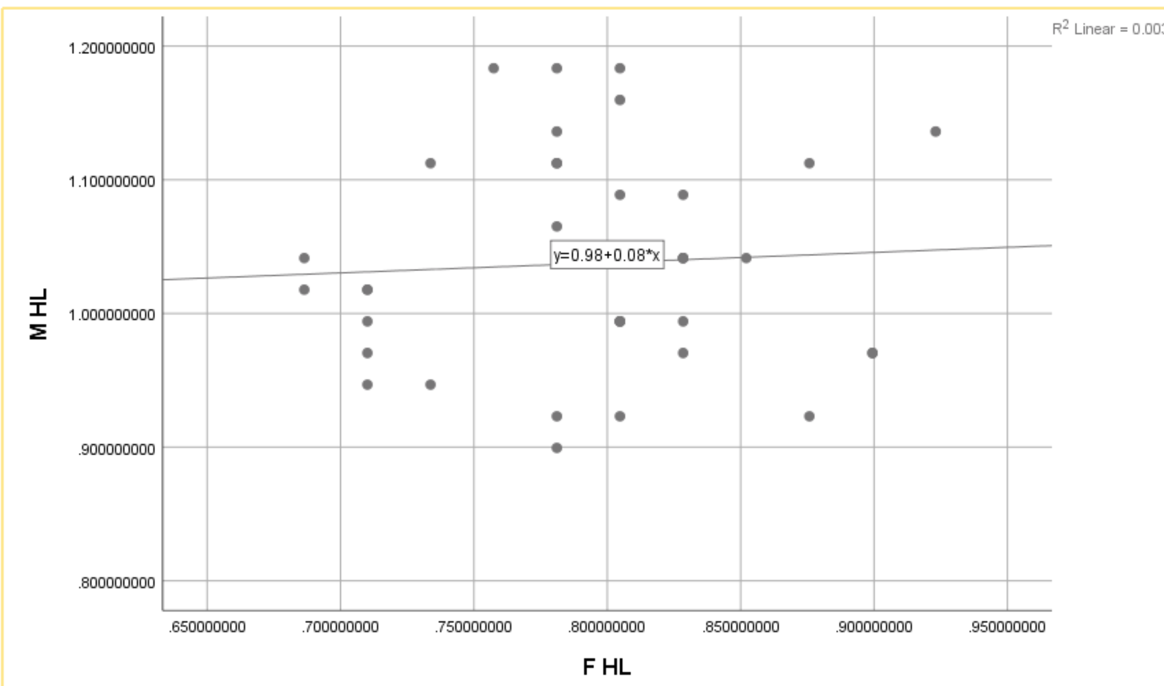

Figure 12: **Gammarus sp.** ***head length for males and females in Clipps Mill Spring shows a weak relationship.***

Correlations

Correlations

		M HL	F HL
M HL	Pearson Correlation	1	.286
	Sig. (2-tailed)		.106
	N	33	33
F HL	Pearson Correlation	.286	1
	Sig. (2-tailed)	.106	
	N	33	46

Figure 13: **Gammarus sp.** *head length for males and females in Cathedral Spring shows a weak relationship, and the correlation is not significant*

Graph

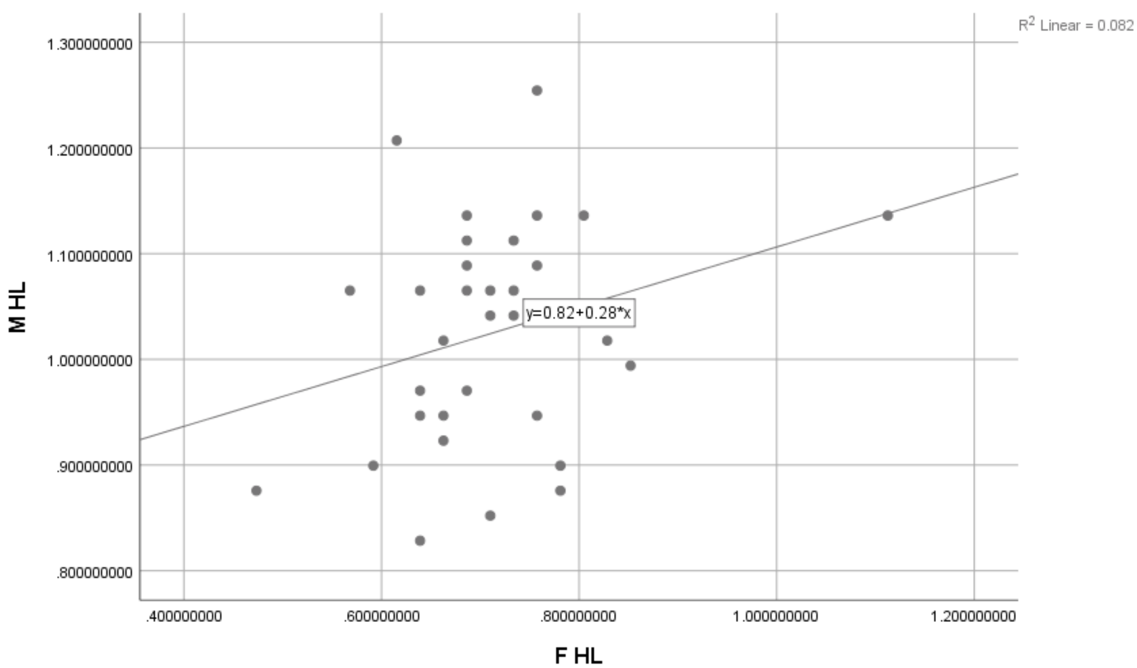

Figure 14: **Gammarus sp.** *head length for males and females in Cathedral Spring shows a weak relationship.*

Correlations

Correlations

		M HL	F HL
M HL	Pearson Correlation	1	.890
	Sig. (2-tailed)		.110
	N	4	4
F HL	Pearson Correlation	.890	1
	Sig. (2-tailed)	.110	
	N	4	4

Figure 15: **Gammarus sp.** ***head length for males and females in Agua Spring shows a strong relationship, and the correlation is not significant.***

Graph

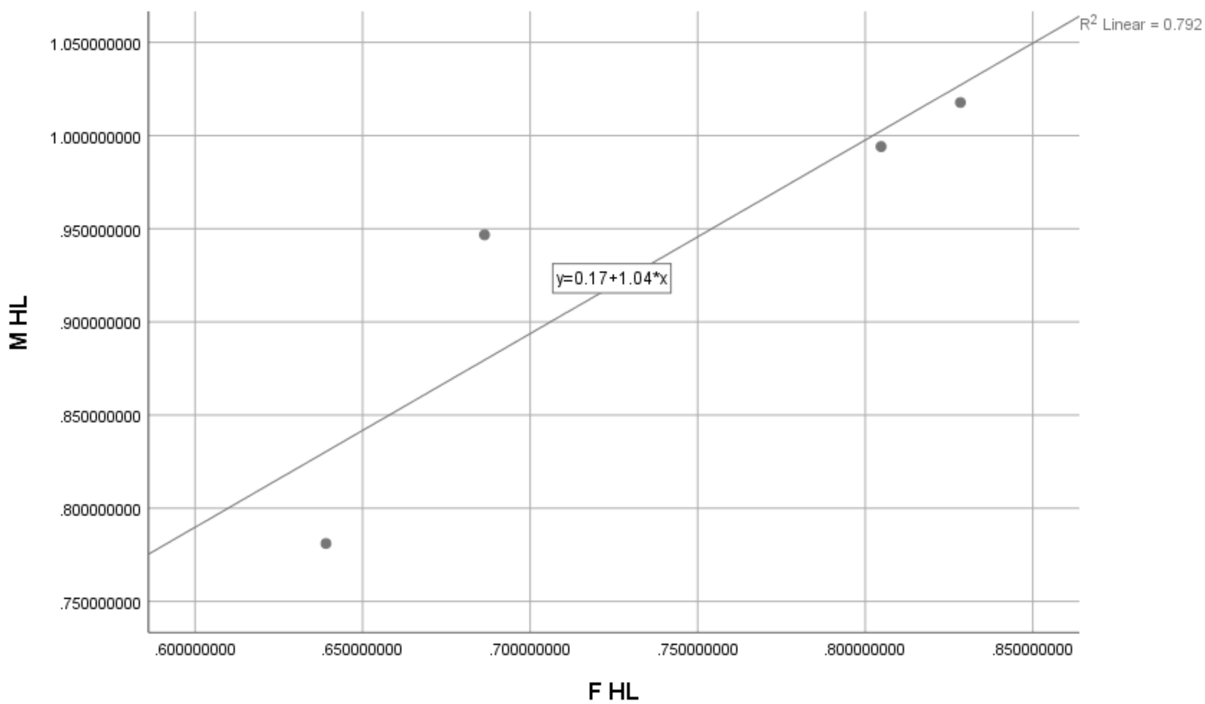

Figure 16: **Gammarus sp.** ***head length for males and females in Agua Spring shows a strong relationship.***

Correlations

Correlations

		M HL	F HL
M HL	Pearson Correlation	1	.324*
	Sig. (2-tailed)		.013
	N	58	58
F HL	Pearson Correlation	.324*	1
	Sig. (2-tailed)	.013	
	N	58	59

*. Correlation is significant at the 0.05 level (2-tailed).

Figure 17: **Gammarus sp.** ***head length for males and females in Blowing Spring shows a moderate relationship, and the correlation is significant.***

Graph

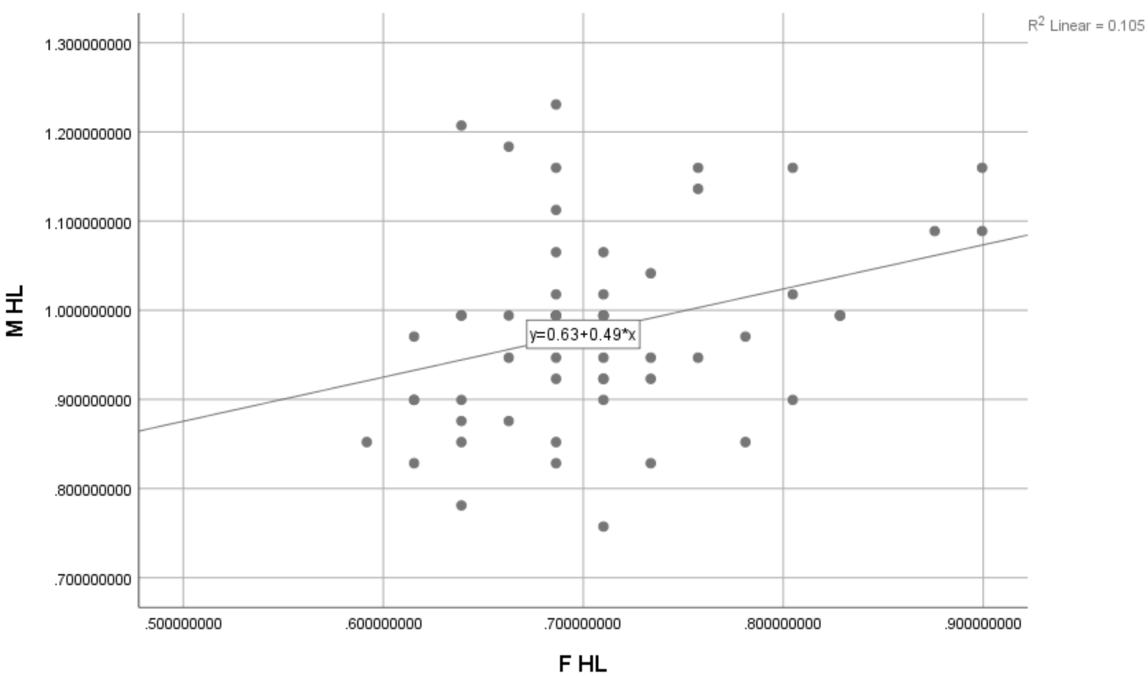

Figure 18: **Gammarus sp.** ***head length for males and females in Blowing Spring shows a moderate relationship.***

Correlations

Correlations

		M HL	F HL
M HL	Pearson Correlation	1	.437**
	Sig. (2-tailed)		.000
	N	76	76
F HL	Pearson Correlation	.437**	1
	Sig. (2-tailed)	.000	
	N	76	97

**. Correlation is significant at the 0.01 level (2-tailed).

Figure 19: **Gammarus sp.** *head length for males and females in Taylor Spring shows a moderate relationship, and the correlation is significant.*

Graph

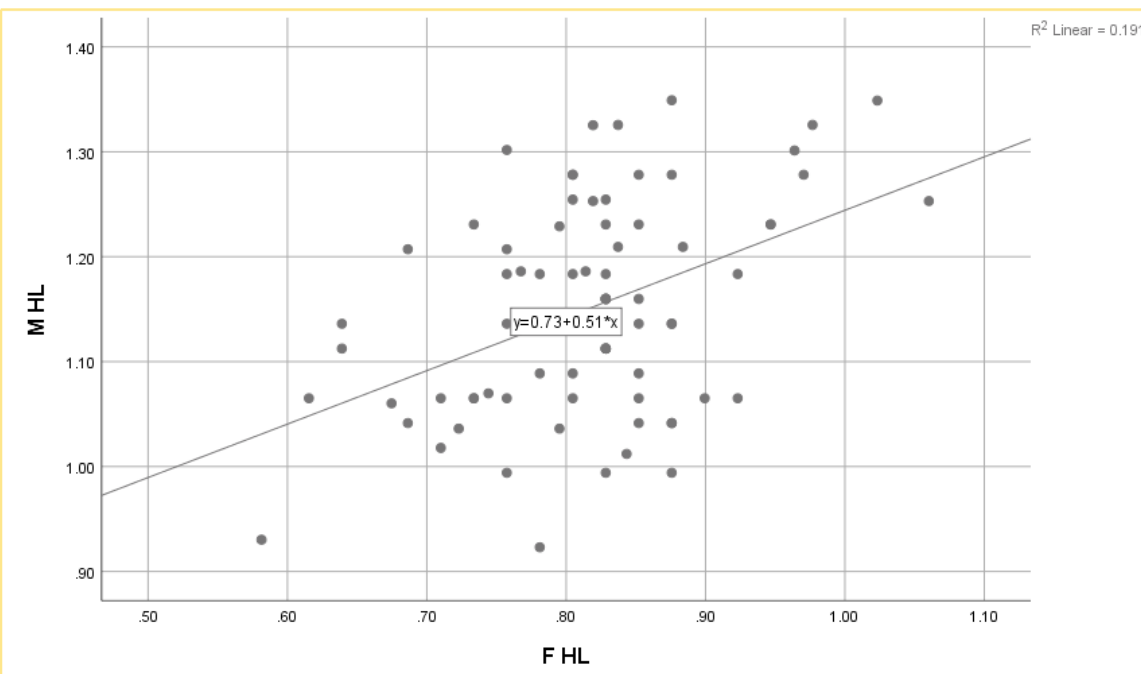

Figure 20: **Gammarus sp.** *head length for males and females in Taylor Spring shows a moderate relationship.*

Correlations

Correlations

		M HL	F HL
M HL	Pearson Correlation	1	.595
	Sig. (2-tailed)		.290
	N	5	5
F HL	Pearson Correlation	.595	1
	Sig. (2-tailed)	.290	
	N	5	5

Figure 21: **Gammarus sp.** ***head length for males and females in Apple Spring shows a strong relationship, and the correlation is not significant.***

Graph

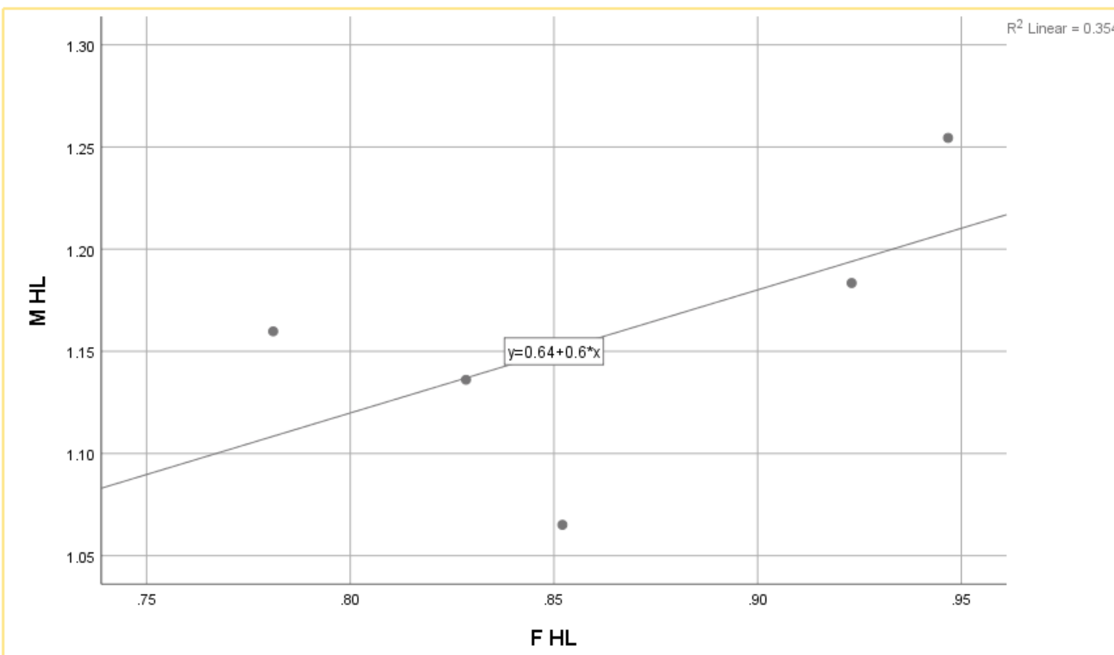

Figure 22: **Gammarus sp.** ***head length for males and females in Apple Spring shows a strong relationship.***

Correlations

Correlations

		M HL	F HL
M HL	Pearson Correlation	1	.351**
	Sig. (2-tailed)		.006
	N	59	59
F HL	Pearson Correlation	.351**	1
	Sig. (2-tailed)	.006	
	N	59	74

**. Correlation is significant at the 0.01 level (2-tailed).

Figure 23: **Gammarus sp.** ***head length for males and females in Davis Spring shows a weak relationship.***

Graph

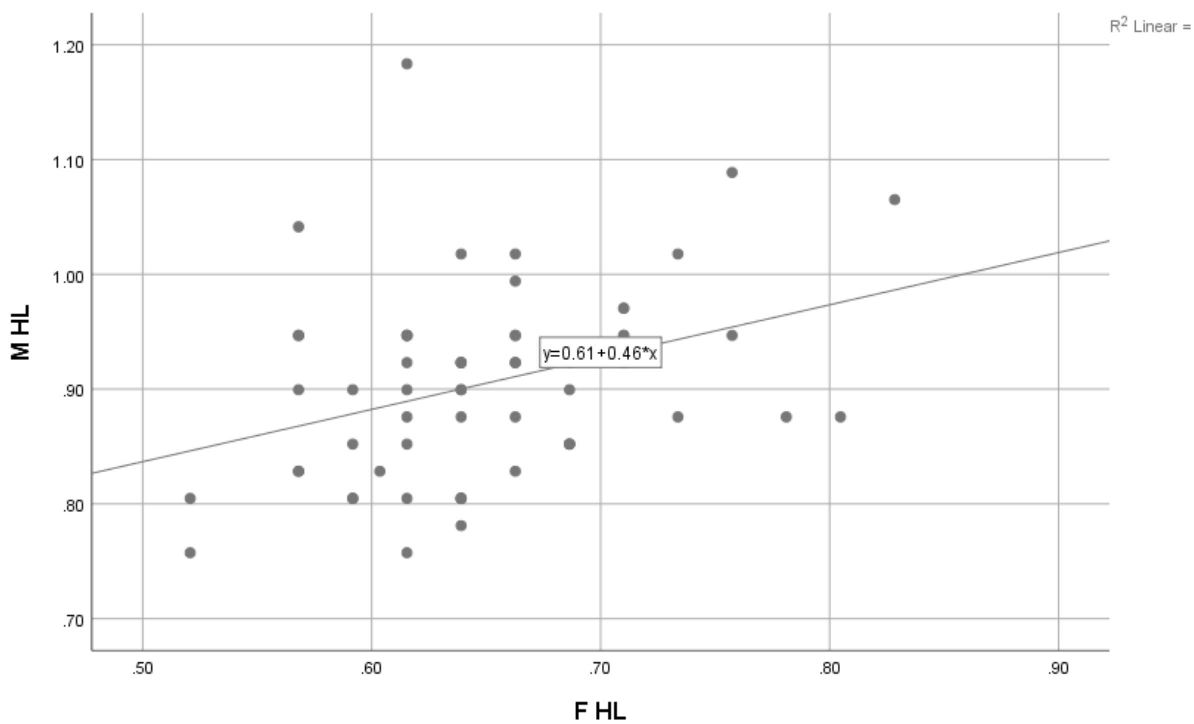

Figure 24: **Gammarus sp.** ***head length for males and females in Davis Spring shows a weak relationship.***

Correlations

Correlations

		M HL	F HL
M HL	Pearson Correlation	1	.235
	Sig. (2-tailed)		.139
	N	41	41
F HL	Pearson Correlation	.235	1
	Sig. (2-tailed)	.139	
	N	41	50

Figure 25: **Gammarus sp.** ***head length for males and females in Dickson Spring shows a weak relationship, and the correlation is not significant.***

Graph

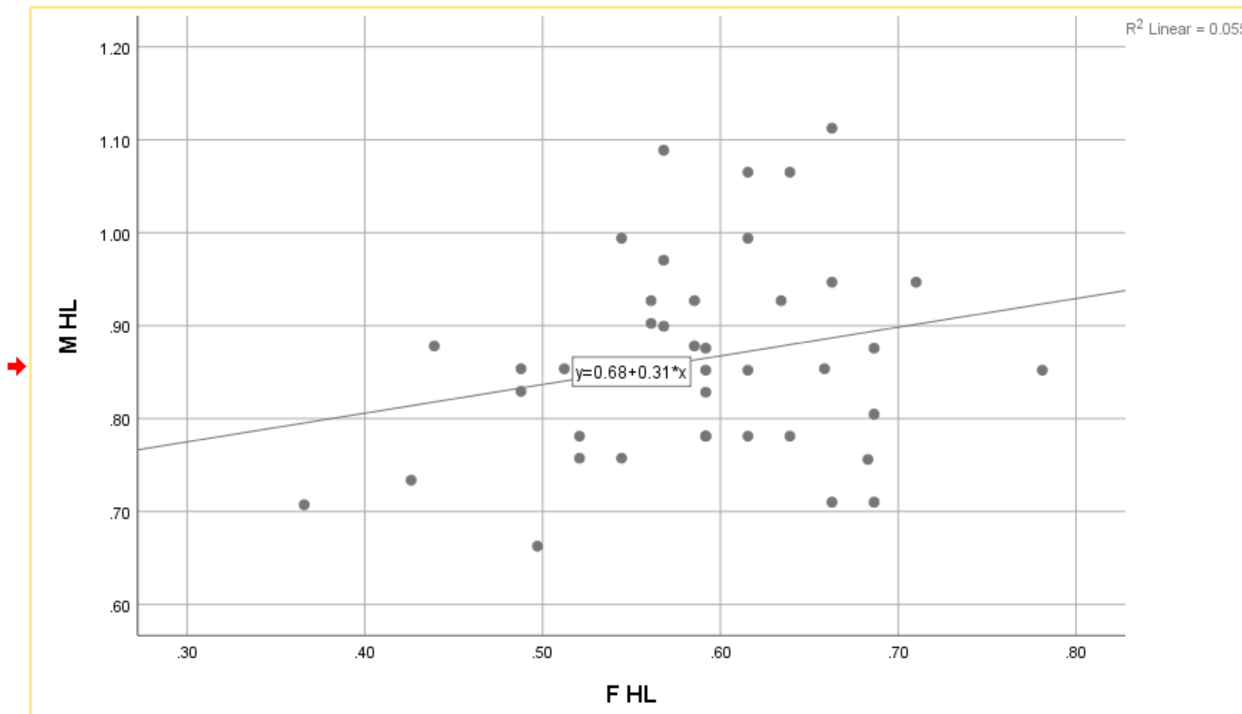

Figure 23: **Gammarus sp.** ***head length for males and females in Davis Spring shows a weak relationship.***

Correlations

Correlations

		Brood size	F HL
Brood size	Pearson Correlation	1	.578**
	Sig. (2-tailed)		.000
	N	33	33
F HL	Pearson Correlation	.578**	1
	Sig. (2-tailed)	.000	
	N	33	97

**. Correlation is significant at the 0.01 level (2-tailed).

Figure 24: **Gammarus sp.** ***female head length and brood size shows a strong relationship, and the correlation is significant.***

Graph

[DataSet7]

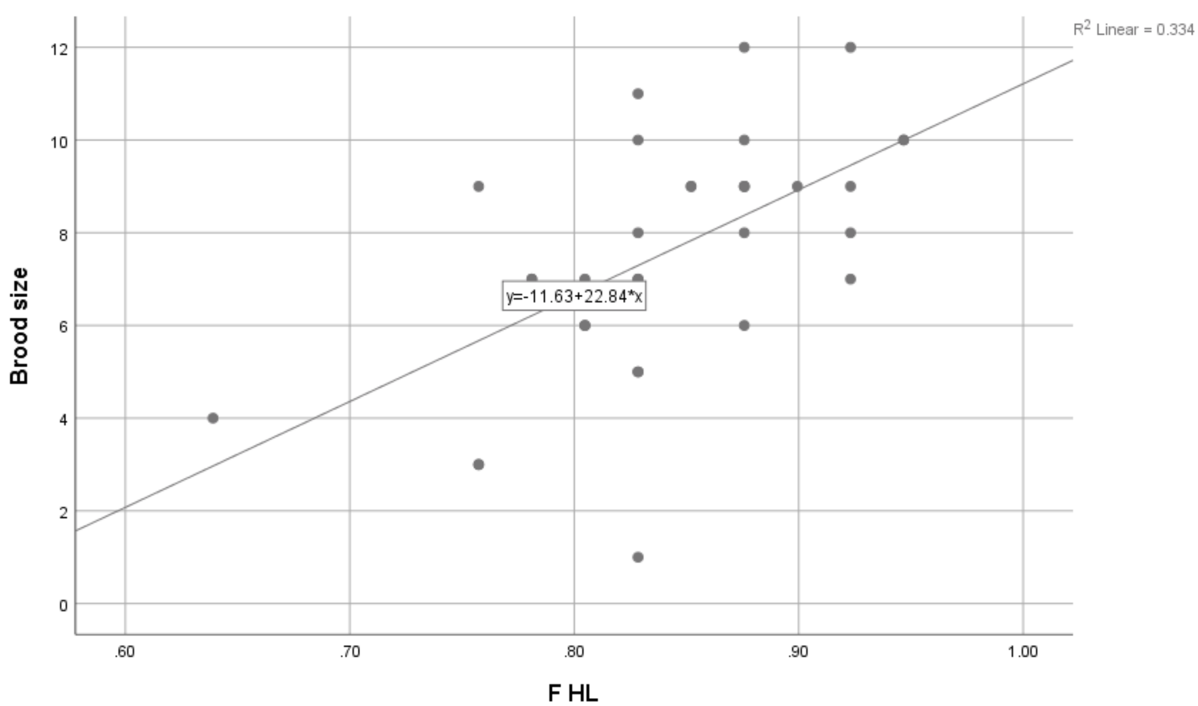

Figure 25: **Gammarus sp.** ***female head length and brood size show a strong relationship***