Will biasing sex ratio of invasive signal crayfish populations contribute to controlling its spread and impact on native communities?

Introduction

The North American signal crayfish, *Pacifastacus leniusculus*, is an invasive, non-indigenous crayfish species (NICS) found widely throughout freshwater environments in Europe and Japan. This species poses several major threats to British biodiversity, particularly their native counterpart the endangered white-clawed crayfish, *Austropotamobius pallipes*. Signal crayfish have been shown to drive localised extinctions of white-clawed crayfish by competitive exclusion, as well as through the spread of the oomycete 'crayfish plague,' *Aphanomyces astaci* (Holdich, 1999). They're also known to impact juvenile Atlantic salmon populations via competition for shelter (Griffiths et al., 2004), and have been linked to major reductions in the density of stream invertebrate communities (Crawford et al., 2006). Signal crayfish are also a burrowing species, and in many cases this can lead to river bank collapses and sediment loading which can be a major impact upon the river ecosystem (Guan, 1994).

Despite these significant adverse impacts, there has been very little success in eradicating or controlling signal crayfish populations. Most attempts have failed to significantly reduce the growth and spread of crayfish populations, even when they successfully remove large number of crayfish (Peay, 2001). For example, in 2009 one of the largest scale trapping projects of signal crayfish was undertaken in Loch Ken, a popular tourist and angling loch in South-West Scotland (Cameron, 2010). Signal crayfish were harvested in huge numbers, using 400 traps per day over the course of 56 days to remove over 650,000 *P. leniusculus*. After this heavy trapping session the male component of the population was estimated to have been reduced by up to 60%, which drastically shifted the sex ratio of catch compared to before the removal programme. What's more, the mean size of males in the population also decreased significantly. However, despite these impressive results, many people did not consider it a successful project, simply because they only removed a large proportion of males, rather than the entire population.

There is a short-sightedness in focusing purely on removing the largest possible number of crayfish without accounting for the impact on important population parameters such as reproduction. In order to develop effective management strategies for signal crayfish, we need to better understand their population dynamics and their interaction with management interventions.

it's known that certain trapping methods have significant sexual biases in catch rates. For example, baited traps can show a bias to large (i.e. >18 mm carapace length) male crayfish (Dorn et al. 2005). If successive trapping efforts would impact the sex ratio of the population, understanding how sex ratio affects the reproductive output could prove invaluable in working out how to properly manage signal crayfish.

Some scientists have begun to identify the significance sex ratio could play in managing wild P. Leniusculus populations. In natural populations, signal crayfish usually live at a 1:1 male-to-female ratio in established populations (Celada et al., 2005; Capurro et al., 2007), although some studies have observed a high male ratio in migratory populations (Wutz and Geist, 2013; Rebrina et al., 2015). Experiments in aquaculture environments suggest that a female-biased sex ratio (1 Male: 4 Females or 1M:5F) produces the highest reproductive output (Barki & Karplus, 2000; Celada et al., 2005). However, it is possible that further reducing the ratio would not actually damage the reproduction rates - in one study, for example, a single male noble crayfish fertilized 23 females in less than 40 days (Svensson and Gydemo, 1997). However, all these studies have an antithetical objective to those required for signal crayfish management – namely, they aim to identify the sex ratio with the greatest reproductive output in order to maximise stocking densities (Sheng Yeh & Rouse, 1995), whereas management strategies seek to limit and eradicate signal crayfish populations.

In another study, Stebbing et al (2003) were testing out a new type of trap which was baited with female *P. Leniusculus* pheromones during the breeding season. These traps were almost exclusively attractive to sexually mature males. When discussing their potential use in crayfish management Stebbing said that "the removal of large numbers of mature males during the breeding season could effectively shift the sex ratio, whilst the remaining females would continue to experience inter-individual competition for resources... [this method] could potentially restrict the growth rate and even reduce the size of a *Pacifastacis leniusculus* population."

However, outside of these few studies, or the narrow context of improving stocking densities in aquaculture, there has been little research into how sex ratio impacts reproductive output in wild crayfish populations. Sex ratio has a direct bearing on a populations reproductive rate, and yet it is too often unaccounted for when modelling management strategies for signal crayfish.

With this PTES funded internship, I sought to investigate the importance of sex ratio for signal crayfish reproduction, and for crayfish management. The aim was to understand how control/eradication efforts might influence the sex ratio of populations, and whether any consequent alterations of the sex ratio might impact the reproductive outputs and growth rate of the population in the long term. For this, I designed my own study in which I would analyse the effects of intensive trapping on the male and female components of wild *P. Leniusculus* populations, and another in which I could manipulate the sex ratios of isolated crayfish populations in their natural habitat in order to investigate how it impacts their reproductive output.

Methods

Study Sites

The first study was carried out in two different burns across Scotland: The first site was in the Geddes burn, a lowland stream tributary in the River Nairn; The second site was in Glenshee, in a highland tributary of the River Blackwater. The North American signal crayfish are known to have been established in these rivers for many years. Both sites were roughly 540 metre long, divided into nine 60 metre sections (henceforth referred to as sections A-I). Trapping occurred over 48 capture sessions (24 capture sessions per stream), between the 2nd of June and the 17th of September. During capture sessions 1-8, (2nd to the 26th of June), I used fifteen baited spring-traps per section in Glenshee (135 traps total) and twenty per section used in Geddes Burn (180 traps total). During capture sessions 9-24 (13th of July to the 17th of September), I performed 8 kick samples per section in Glenshee (72 total kick samples) and fifteen per section in Geddes Burn (135 total kick samples).

The sections were separated by small mesh dividers, designed to prevent crayfish movement between sections whilst simultaneously allowing fish and invertebrates to move freely. In doing so, we aimed to halt or reduce migration between sections, thus allowing us to treat each section as an isolated population in the same stream.

For the second study, our site was in a small, man-made loch upstream of the Glenshee site. Trapping in this section occurred between September and October. After trapping was completed, the study itself was carried out in the loch from October to November, during the breeding period of that *P. Leniusculus* population.

Study 1: Trapping Impacts on Sex Ratio

In order to estimate the impact of *P. leniusculus* control efforts on the sex ratio, we performed crayfish trapping with multiple different intensities of removal in the two streams (Glenshee and Geddes Burn) over the summer harvest season. For both streams, the traps were randomly allocated within their section each day and left overnight, then removed the following day. This, combined with the set number of traps per section, ensured all sections were sampled with a standardised capture effort. All sampling was conducted by a two- or three-man team working upstream, section by section. All sections were sampled in a day, thus each day was a complete capture session.

At the start of the project, each section from both streams was randomly allocated one of three different removal treatments; 100%, 50% or 0% removal. In the 100% removal treatments, all crayfish captured were removed and immediately frozen; in the 0% removal treatments, all captured crayfish were marked and returned to their point of capture unharmed; in the 50% removal treatments, half the catch was returned to the stream and the other half was removed. This set up allowed us to test the direct effects of removing crayfish at different intensities, whilst also controlling for any non-removal effects of trapping.

All crayfish had their capture location and date recorded. I also measured their carapace lengths and identified their sexes on site, as well as other traits such as moult status and missing limbs as part of a larger study. Some of the crayfish were removed and frozen before they had been measured and sexed, as this information could be gathered from future analysis of frozen samples.

I chose to focus the study on capture sessions 1-8, the baited trap sessions, as baited traps are the only standard trapping method known to have a significant effect on sex ratio (Dorn et al., 2005; Cameron, 2010). Previous studies have shown kick sampling to be a non sex selective removal method (Gladman et al., 2009; Houghton, per. comm.), and thus I wouldn't expect any interesting results on sex ratio.

Study 2: Sex Ratio Manipulation

To test the effect of sex ratio manipulation on reproductive output, we constructed twenty $3m^2$ mesh cages to contain small populations of crayfish at different sex ratios over their breeding season. The cages were to be populated with 16 crayfish in different sex ratios as follows: 1 Male: 15 Females; 4 Males: 12 Females; 8 Males: 8 Females; 12 Males: 4 Females; and 15 Males: 1 Female. The crayfish chosen had to be large (carapace length > 45 mm), sexually mature and unmated. In order to check if the females had been previously mated with, we inspected all females for the presence of spermatophores on their abdomens, or eggs on their tails. To check for sexual maturity, we visually inspected under the crayfishes cephalothorax to confirm if they were carrying eggs/spermatophores.

The crayfish were all captured from the same loch, then transferred to the cages which were stored in the loch. These cages functioned as a contained macro-environment within the lake, allowing us to control the exact makeup of individuals in the population (theoretically excluding any escapees or intruders such as predators or other signal crayfish), whilst maintaining them in their natural environment. This experimental design offers the opportunity to gain an insight into the natural reproductive behaviours of the crayfish, whilst offering the advantages of a controlled environment which can be effectively monitored and manipulated in ways the crayfishes natural habitat cannot.

At the end of the breeding season, we retrieved all individuals from the cages and counted the number of successfully bred females, plus their number of fertile eggs, in a lab at the University of Aberdeen. The crayfish were transported live to the lab and placed in tanks. We could thus retrieve the eggs fresh from the live mothers, reducing the likelihood of deterioration or damage which is common when eggs are frozen. The eggs of each female were removed and counted by hand then placed in a petri dish for inspection under microscope in order to determine if they were fertilized and developing. Using the results of previous microscopy studies on signal crayfish embryology as visual and descriptive references (Celada et al., 1985; Celada et al., 1987), as well as help through personal communication with the authors, we were able to identify and distinguish fertilized and unfertilized eggs. After inspecting the eggs fresh under the microscope, we immersed them in a mixture of Tergitol and Bouins solution for 24 hours, then re-examined them. This acted to stain and preserve the eggs, allowing us to dissect the eggs and properly identify certain embryological development stages.

Analysis

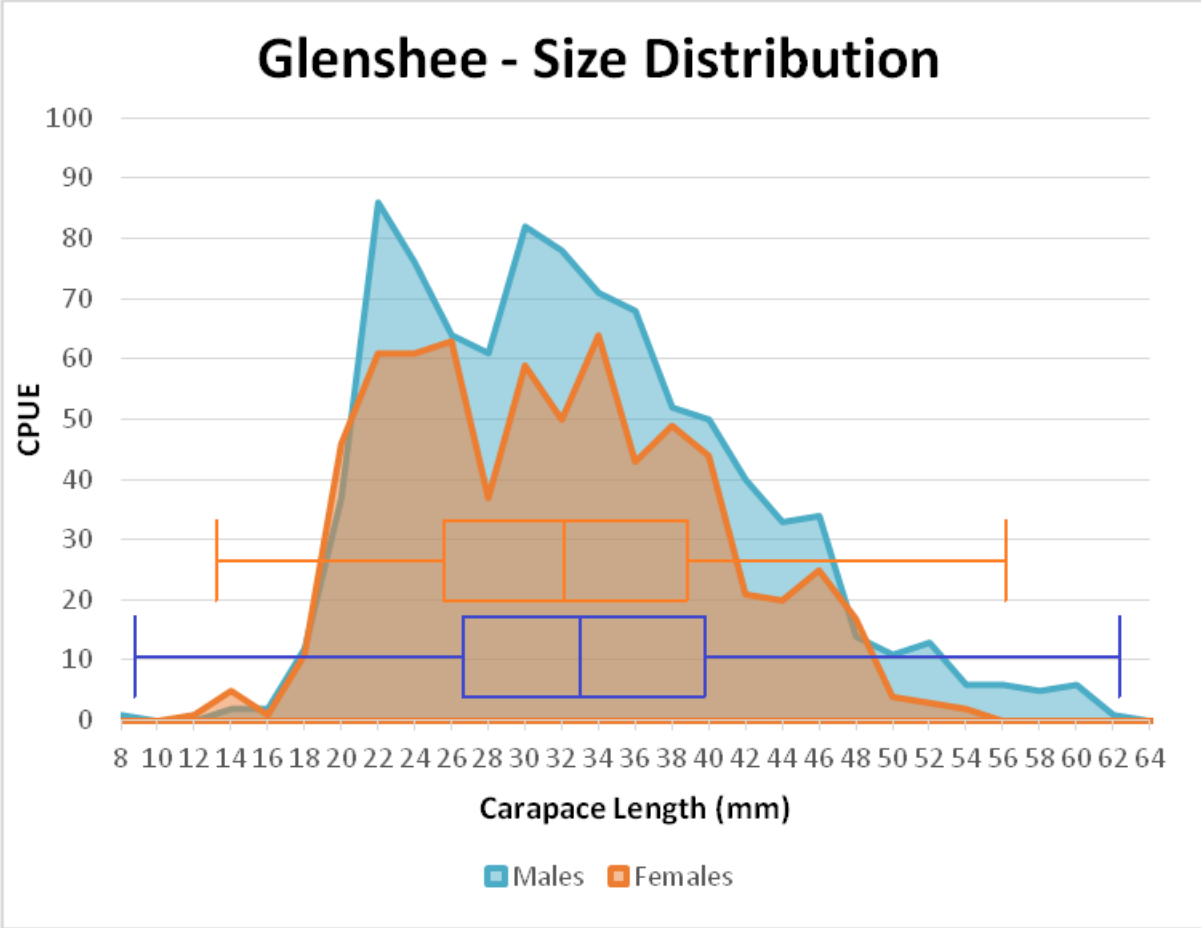
All analyses were conducted using Excel and R. To test the hypothesis that trapping would impact sex ratio over time, I analysed the catch data per treatment using binomial logistic regression, with sex acting as the dichotomous dependent variable. By comparing the 95% confidence intervals of the odds ratios, I could calculate if there were any significant differences in sex ratio between the treatments. This is a similar method used in previous sex-ratio studies (Orton et al., 2006). I also used this method to compare the sex ratios in capture sessions 1-4 versus capture sessions 5-8. By dividing the data into subsets and running individual binomial logistic regressions for each, I could test if the sex ratios had changed within the treatments by the end of the study.

A t-test was used to analyse the difference in mean carapace lengths for males and females in each site.

Analysis of Covariance (ANCOVA) was used to compare the compare the regression lines for mean carapace length and CPUE. For example, I plotted 'mean carapace length' by 'capture session,' for males and females in each treatment. Then, by using ANCOVA to compare the slopes of the regression lines, I could test whether the mean carapace length changed over time at different rates for males and females, and whether there was a difference in this rate between treatments.

Results

In Glenshee, a total of 1598 individual *P. leniusculus* were caught in capture sessions 1-8, of which 687 were female and 911 were male. The size of the crayfish ranged from 8.75 mm to 62.03 mm carapace length, with a mean carapace length of 33.21 mm (SE 1.52). A t-test demonstrated that males were significantly larger than females, with a mean carapace length of 33.77 mm for males versus 32.44 for females ($t = -3.04$, $p = 0.002$). (See figure 1.)



<u>Figure 1.</u> Size Distribution of male and female carapace length from the Glenshee site, capture sessions 1-8. For males, min = 8.75, Q1 = 26.41, Median = 32.78, Q3 = 39.53, Max = 62.03. For females, Min = 13.11, Q1 = 25.47, Median = 31.93, Q3 = 38.50, Max = 55.68.

Figure 2. shows the changes in mean carapace length per capture sessions for males and females of each treatment. In all three treatments, the mean carapace length of males and females decrease over subsequent capture sessions. The rate of decrease in mean carapace length is highly similar between males and females in all instances. The 0% removal treatment shows the largest difference between males and females (b = -1.14 for females and -1.62 for males), however a comparison of the two regression lines confirmed there was no statistically significant difference in mean carapace length over time between them (difference in slope = 0.48, SE = 0.42, p = 0.25). The estimated common slope for the 0% removal treatment was -1.43 (p = 0.013). Although they appear closely correlated, there was a nearly significant difference in slope between the males of the 0% and 100% removal treatment (difference in slope = 0.56, SE = 0.32, p = 0.07). There was no significant difference in mean carapace length over time for females. When comparing the 0% and 100% removal treatments, the difference in regression slopes was highly insignificant (difference in slope = 0.17, SE = 0.36, p = 0.64). This suggests there was no sex-specific impact on mean carapace length in any treatment.

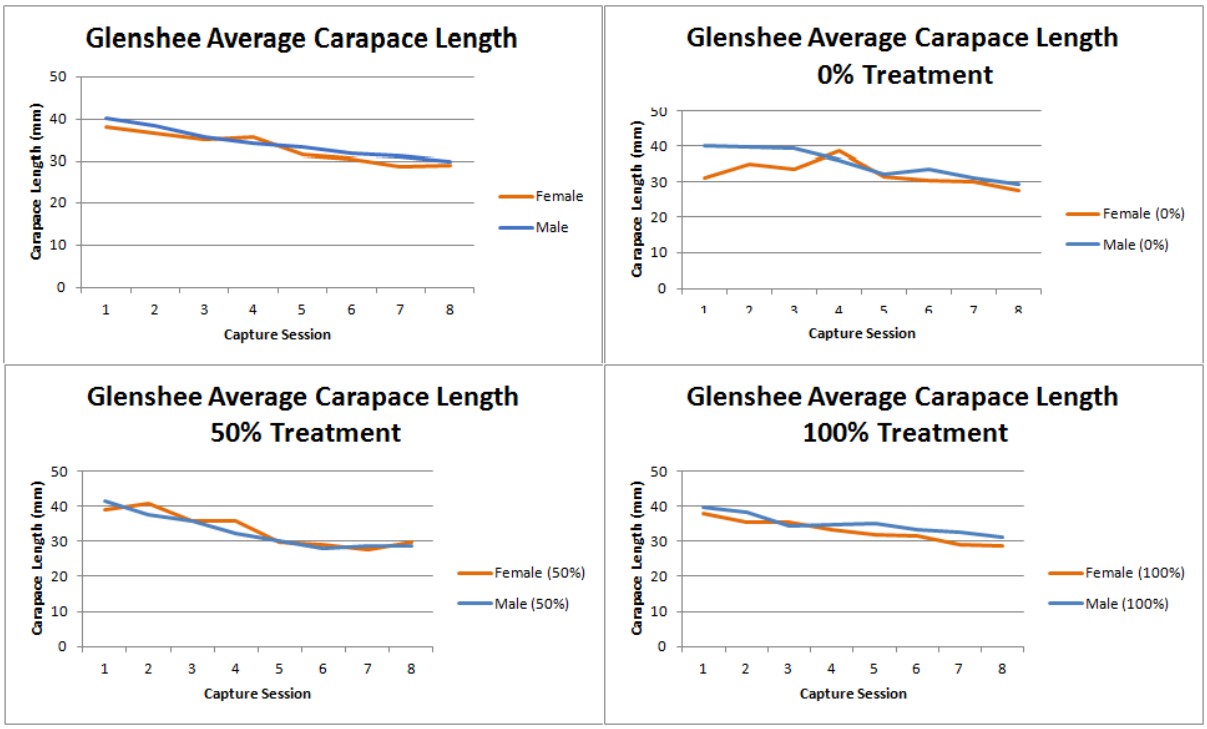


Figure 2. Average carapace length for males and females in Glenshee, capture sessions 1-8.

In Geddes Burn, a total of 321 individuals were caught in capture session 1-8, 161 of which were female and 160 of which were male. The size of the crayfish ranged from 12.04 mm to 60.84 mm carapace length, with a mean carapace length of 32.33 (SE 1.59). Similar to Glenshee, a t-test demonstrated that males were significantly larger than females, with a mean carapace length of 34.42 mm versus 30.23 mm for females ($t = -4.3$, $p = <0.001$). (See Figure 3.)

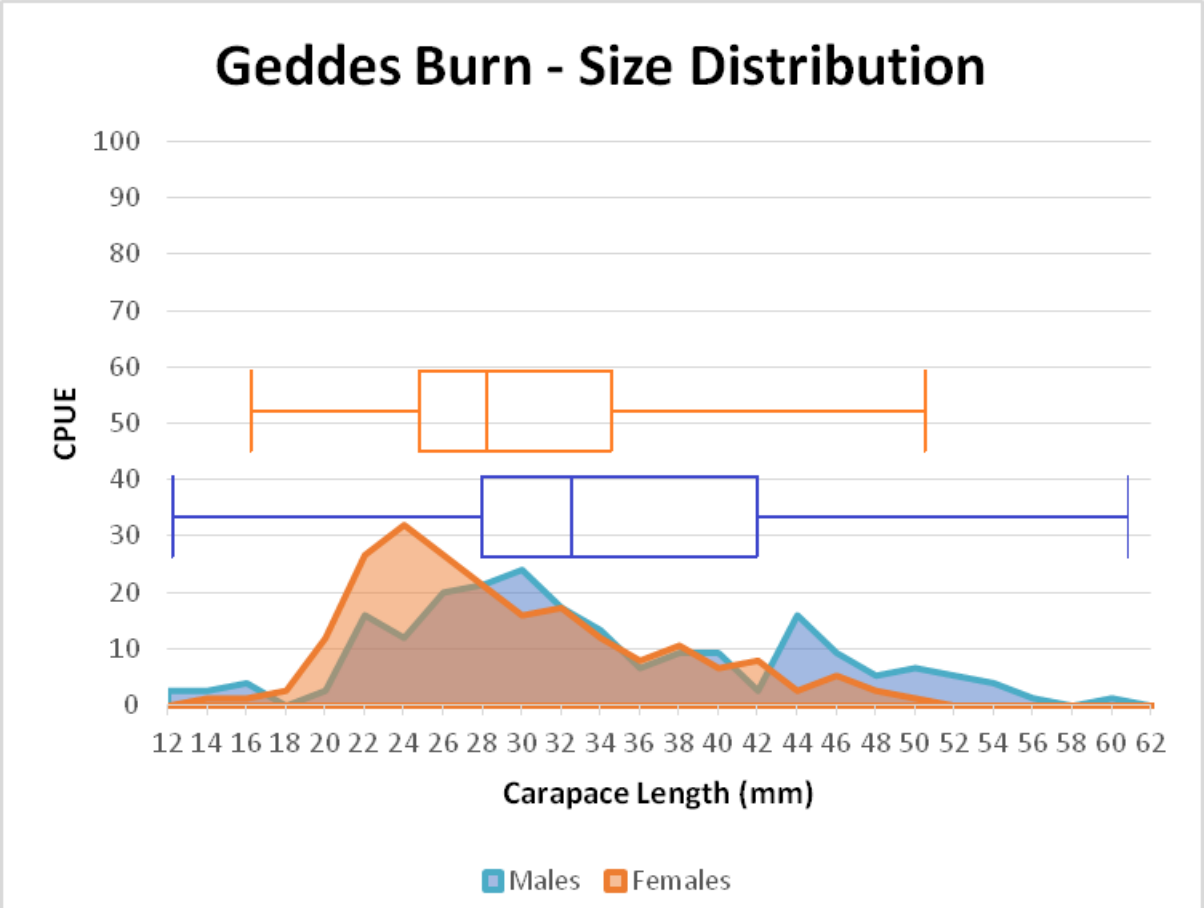


Figure 3. Size Distribution of male and female carapace length from the Geddes Burn site, capture sessions 1-8. For males, Min = 12.04, Q1 = 27.77, Median = 32.32, Q3 = 41.79, Max = 60.84. For females, Min = 15.98, Q1 = 24.57, Median = 28.07, Q3 = 34.38, Max = 50.44.

Figure 4. shows the changes in mean carapace length per capture sessions for males and females of each treatment. All treatments showed an increase in mean carapace length over time. Comparative to Glenshee, there was more stochastic variation in mean carapace length, likely due to the smaller catch numbers overall. For example, in capture session 1 there were no females caught in any of the 0% removal treatments, which can be seen on the graph. However, similar to Glenshee, there were no significant differences between the male and female regression lines in any treatment. The 50% treatment had the largest difference in slope (b = 1.68 for females and 0.67 for males, difference in slope = 1.01) however this difference was not statistically significant (SE = 0.86, p = 0.25). The estimated common slope for the 50% treatment was 1.08 (p = 0.003). This again suggests there was no sex-specific impacts on mean carapace length in any treatment.

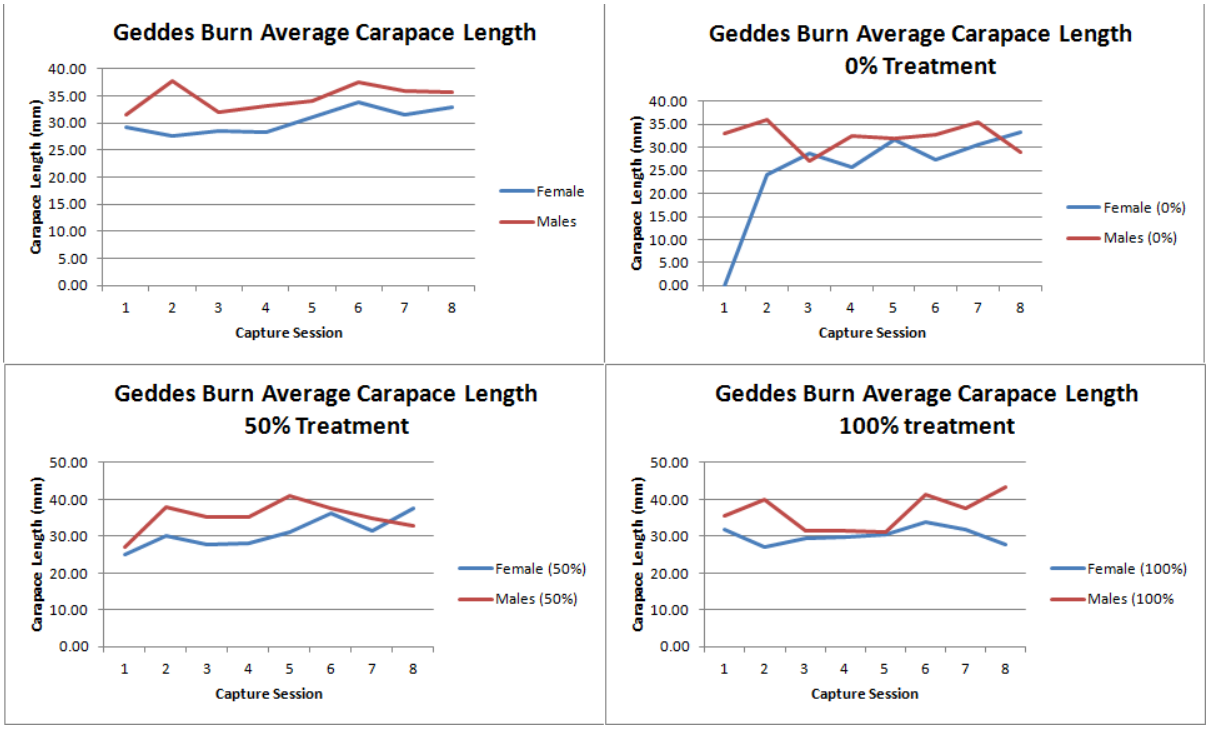


Figure 4. Average carapace length for males and females in Geddes Burn, capture sessions 1-8.

Trapping Impacts on Sex Ratio

For Glenshee, the 100% treatment showed a decrease in the male:female ratio. During capture sessions 1-4, the 100% treatment exhibited a significantly high male:female sex ratio of 1.45M:1F (CI 1.15 – 1.86) (Z value = 3.07, p = 0.002), however during capture sessions 5-8 this ratio had dropped to 1.18M:1F (CI 0.98 – 1.42). This is both significantly lower than the ratio in CS 1-4, and also a reduction from a male-biased to a neutral, 1:1 sex ratio (Z value 1.77, p 0.08). This is in direct contrast to the 0% and 50% treatments, which both started with neutral sex ratios (1.12M:1F, CI 0.81 – 1.55 and 1.24, 0.94 – 1.63, for 0% and 50% respectively) and then increased to significant male biased ratios by capture sessions 5-8 (1.58, CI 1.22 – 2.06 and 1.44, CI 1.14 – 1.84, for 0% and 50% respectively) (Z value = 3.43, p = >0.001 and Z value = 3.03, p = 0.002, for 0% and 50% respectively). However, this does not constitute a significant increase in the sex ratios for the 0% and 50% treatments, as there is overlap in the confidence intervals of the odds ratios from capture sessions 1-4. (See Table 1.)

GLENSHEE									
Capture	**0% Removal Treatment**			**50% Removal Treatment**			**100% Removal Treatment**		
Session	**Males**	**Females**	**Total**	**Males**	**Females**	**Total**	**Males**	**Females**	**Total**
1	19	4	23	27	30	25	61	23	84
2	21	23	44	14	13	25	21	19	40
3	19	25	44	44	27	28	60	48	108

4	20	18	38	28	21	56	20	21	41
5	46	33	79	50	29	35	96	75	171
6	23	19	42	30	21	55	41	40	81
7	41	20	61	30	27	50	53	36	89
8	34	19	53	56	38	0	58	59	117
Total	**223**	**161**	**384**	**279**	**206**	**485**	**410**	**321**	**731**

Table 1. Total catch numbers in Glenshee, capture sessions 1-8

Figure 5. presents the CPUE for Glenshee males and females separately for all three treatments. As you can see from the plots, none of the populations showed a negative slope in CPUE, suggesting that we were not removing enough individuals for a significant depletion effect. The 100% treatment was the only one in which the males CPUE was estimated with a lower slope than the females (b = 1.88 for males, b = 4.37 for females). When comparing the regression lines, there was no statistically significant difference between the slopes of males in 0% and 100% removal treatments (p = 0.80)

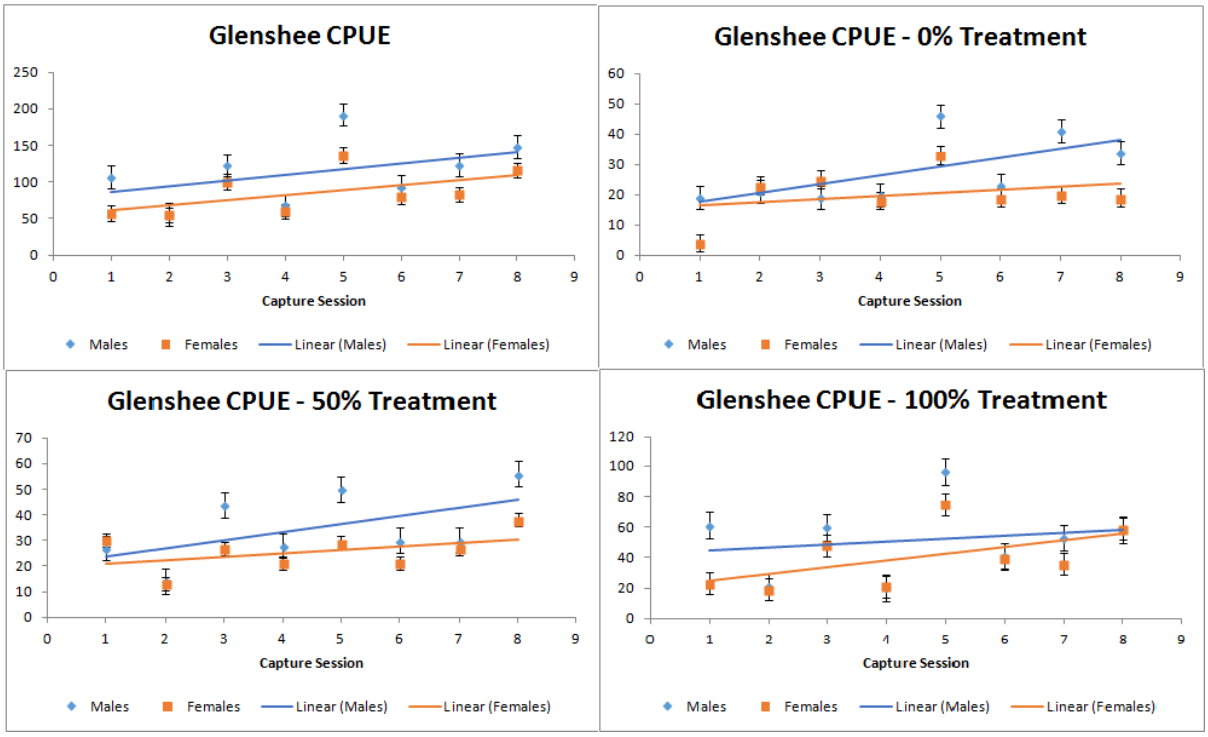


Figure 5. Catch Per Unit Effort (CPUE) of males and females in the Glenshee site, capture sessions 1-8.

In Geddes Burn, all three treatments show insignificant changes in sex ratio between CS 1-4 and CS5-8. However, although the results were insignificant, there was some appearance of a pattern. The 0% treatment was the only one in which the sex ratio remained above the 1:1 threshold in both capture sessions 1-4 and 5-8 (1.35 and 1.21, in CS 1-4 and 5-8 respectively). The 50% treatment showed a nearly significant reduction in sex ratio, dropping from 1.39 (CI 0.86 – 2.28) in CS 1-4 to 0.80 (CI 0.44 – 1.43) in CS 5-8. This was the largest reduction in sex ratio for any treatment, although there was marginal overlap in the 95% confidence intervals. The 100% treatment had the lowest

ratio of males in both capture session 1-4 and 5-8, although it still fell within the neutral 1M:1F in both cases. Therefore, there was no evidence of any sex ratio impact in Geddes Burn. (See Table 2.)

Geddes Burn									
Capture Session	**0% Removal Treatment**			**50% Removal Treatment**			**100% Removal Treatment**		
	Males	**Females**	**Total**	**Males**	**Females**	**Total**	**Males**	**Females**	**Total**
1	4	0	4	7	3	10	6	5	11
2	4	2	6	9	5	14	4	6	10
3	7	6	13	13	9	22	10	9	19
4	7	9	16	10	11	21	12	19	31
5	6	9	15	5	10	15	7	10	17
6	6	4	10	9	10	19	7	8	15
7	9	3	12	3	3	6	5	11	16
8	2	3	5	4	3	7	3	3	6
Total	**45**	**36**	**81**	**60**	**54**	**114**	**54**	**71**	**125**

Figure 6. presents the CPUE for Geddes Burn males and females separately for all three treatments. As you can see from the plots, both the 50% and 100% removal treatment show declining CPUE over time for males (b = -0.80 for 50%, b = -0.35 for 100%), whereas the 0% treatment shows a marginal increase (b = 0.08). What's more, the regression line for female CPUE remains flat for the 50% and 100% removal treatments, yet increases for the 0% treatment (b = 0.24).

However, analysis of the regressions did not detect any significant difference between the slopes for either males or females. When comparing the male CPUE between 0% and 50% there was no statistically significant difference between their slopes (difference in slope = 0.89, p = 0.15). Thus, there was no statistically significant differences in CPUE between treatments.

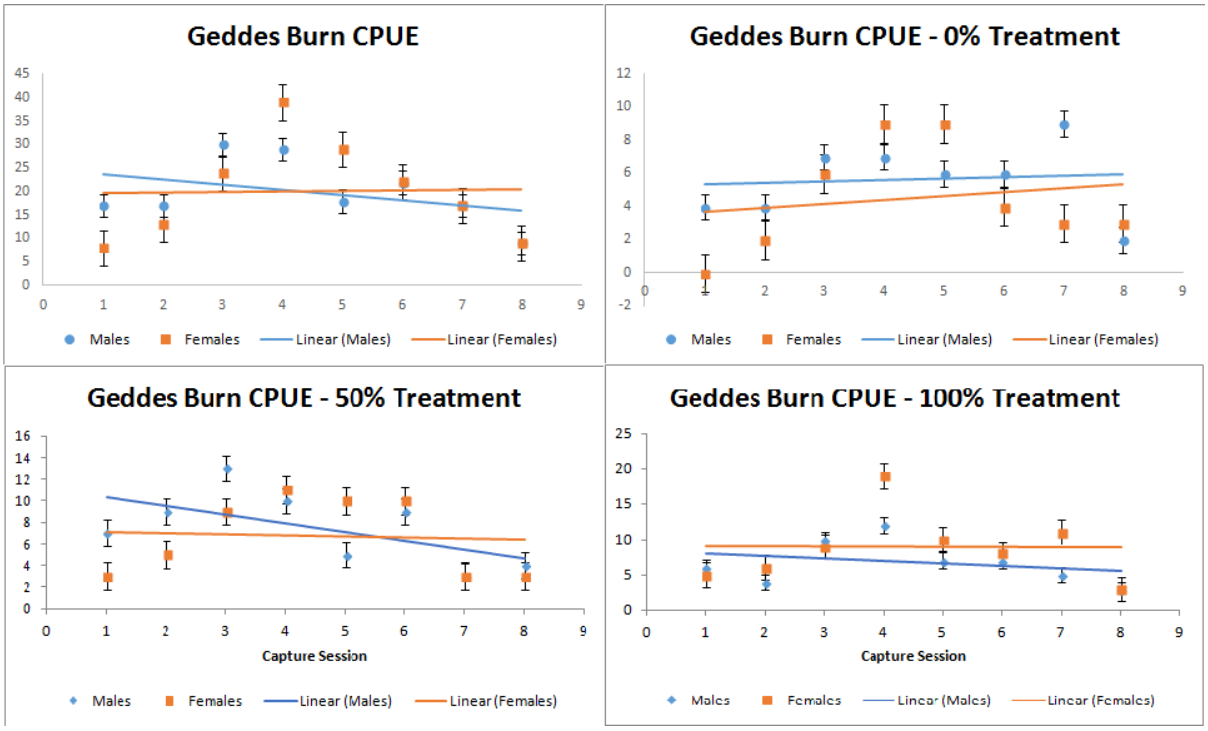


Figure 6. Catch Per Unit Effort (CPUE) of males and females in the Geddes Burn site, capture sessions 1-8.

Sex Ratio Manipulation

Unfortunately, the cage experiment could not be completed in its entirety. Due to an unfortunate design overlook in the early stages of the cage study, a large number of our captured, virgin mature females were mated with by external non treatment crayfish before the experiment begun. As such, we did not have a sufficient number of females to fill all the trials we required for the complete experiment, and the numbers we had to use were insufficient for any meaningful statistical analysis. In the end, the experiment consisted of six cages; three cages with a ratio of 1M:7F, and three cages with a ratio of 7M:1F.

However, although we could not gain any statistically significant results, we were able to gleam some interesting details. Of the 48 crayfish in total, there were only 4 mortalities (1 male, 3 females), which appears to be within the natural mortality level. What's more, the majority of females (19 out of 24) had successfully mated after the breeding season, with an average clutch size of 159.8 eggs (+-46.01). This seems to indicate that the cages provided adequate conditions for crayfish survival and reproduction, which could support our idea that the cages function as close-to-natural microcosm of the lake environment.

In all three of the majority-female cages, most or all of the females were carrying eggs. In one, all 7 females were successfully mated with large clutches (213.4 +-61.25); in the second, 1 female had died and the other 6 were all mated, however 2 of these females had noticeably fewer eggs per clutch; in the last, only 5 crayfish were mated with, and 3 of which had less than 12 eggs each. This seems to suggest that, although the males are capable of mating with at least 7 females per season, reproduction may become less successful after consecutive mates, most likely due to smaller ejaculates of spermatophore.

Microscopic analysis of the crayfish eggs proved to be somewhat useful for distinguishing fertilized and non-fertilized eggs. I had been told that unfertilized eggs generally do not stay attached to the female for any length of time as the female usually grooms them off (days or a few weeks), and would turn a orange or brown colour (Reynolds 2015, per comm.). Using this information, as well as the detailed descriptions and photographs of the developing embryological stages, I was able to differentiate unfertilized and dying eggs from the developing ones. This process was easier the further developed the eggs were, as their embryological features became more pronounced and easier to identify. Figure 7. shows a visual comparison between fertilized and unfertilized eggs. The staining process aided in identifying later stage embryological features, and allowed for the eggs to be dissected. Figure 8. shows a comparison of a tergitol-stained egg from my study with one from Celada et al (1987), where they used an electron microscope to identify the developmental stages of signal crayfish eggs. The photos show the *P. Leniusculus* embryo with the appearance of masticatory appendages and abdomen. Using this paper as a reference, I estimated the eggs in my study to be between 30 and 37 days old.

Figure 7. Visual comparison between fertilised and unfertilised P. Leniusculus eggs.

Figure 8. Left: photograph of a tergitol-stained P. leniusculus egg under a binocular microscope. Right: Detail of the embryo in a 30-day old P. leniusculus egg (Taken from Celada et al., 1987).

Conclusions

For the Geddes Burn site, there was no evidence to suggest any significant impact on sex ratio. The binomial regression and the ANCOVA analysis generated no significant results, suggesting there was no difference in CPUE, mean carapace length or sex ratio for males and females in this site. As the catch numbers were so low compared to Glenshee, it's possible the population was too small to effectively impact the male and female components separately.

For the Glenshee site, however, I found some evidence to suggest that intense trapping had impacted the sex ratio of *P. leniusculus* populations. After 4 capture sessions of intense trapping, the 100% removal treatment showed a significant reduction in the proportion of males captured, whereas both the 50% and 0% treatments showed an increase. This is a similar pattern observed in the Loch Ken study, and consistent with known male bias of baited traps. From this, I can conclude that intensive trapping in the Glenshee site altered the populations sex ratio by significantly reducing the proportion of males. Likewise, there was a significant decrease in the mean carapace length over time in Glenshee as there was in Loch Ken, although there was no difference in impact between males and females. This suggests that the baited traps initially removed both large males and females from the population. It's also interesting to note that the CPUE in Glenshee showed no indication of decline, despite the large numbers of crayfish removed and the already noted impact on sex ratio. This suggests to me that we had not yet removed a significant proportion of the population, and yet we were already detecting impacts on sex ratio.

What this project has shown is that, at least in large crayfish populations, its relatively easy to induce a change in the sex ratio of wild *P. leniusculus* populations by using baited traps at a high intensity.

Although the cage experiment was not executed in its entirety, it proved to be an extremely insightful pilot study. Once completed, the cages functioned perfectly for their design – there were no escapees, mortalities were within natural ranges, and the crayfish did in fact reproduce. This experimental design offers a huge range of possibilities for crayfish behavioral analysis. As I mentioned before, there has been a lot of study in aquaculture focusing on crayfish reproduction at a narrow range of sex ratios – almost all the studies I could find fell between 1 male: 2 females and 1 male: 5 females. My study has already demonstrated that a male signal crayfish is at least capable of mating with up to 7 females in natural conditions. If this methodology was repeated with greater

numbers and a wider range of sex-ratio treatments, we could begin to paint a complete picture of the role sex ratio plays in their reproduction. The importance of sex ratio on long term population dynamics is still unknown for signal crayfish, however this study has demonstrated a powerful means by which to investigate it.

Acknowledgements

Working on this PTES internship has been a highly rewarding experience. Having the responsibility for planning and managing a long-scale project was a fantastic opportunity that I can transfer to my future career in academia and science. This project has offered me personal and professional challenges which, not too long ago, I wouldn't have thought myself capable of handling.

Firstly, the sheer amount of work my workmate and I took on was remarkable! Each day we were waking up at the early hours of morning (early for me, at least!), faced with 8, 10, sometimes 12 hours of trekking up and down a river bank, hauling out traps and counting crayfish. It was physically exhausting work for a couple of scrawny guys like us! What's more, half the time we were sleeping in tents overnight, from which I learned perhaps the most important lesson of all – if you're going to be camping in the Highlands, spend more than £30 on your tent! This work taught me perseverance, far beyond what I'd ever shown before, and for that I am proud and grateful.

Without a doubt, though, the most demanding work was constructing the cages. This was a mammoth undertaking, considering neither of us were experienced in design and construction. We set ourselves the ridiculous task of hand building 20 giant bamboo-net cages and somehow, with a little help from some lovely volunteers, we managed to pull it off. It was a trial-by-fire for design and construction, and it proved to be a valuable, skill-building experience; the ability to conceive of a design intended for my own specific project, select the appropriate materials, construct it from scratch and put them to use.

This project also helped me develop my ability to work as a team. Every aspect of the project required highly co-ordinated team work, as there was always some task to be done and we had to know who was doing what at all times. We each had our roles, and we were constantly communicating and co-ordinating in order to increase our work efficiency. On multiple occasions, we had a third person volunteering to help us for the day. This offered me the chance to work as a team leader, explaining tasks to someone who did not usually work with us and managing them to work effectively in the group. I thoroughly enjoyed working in such a close-knit team dynamic.

Finally, through this project I was offered the opportunity to produce and present a poster at the Findhorn, Nairn & Lossie River Festival. The festival itself was a delightful and entertaining day, and it gave me the chance to communicate complex scientific ideas to the public in a digestible manner. This is an invaluable skill in the scientific community.

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References

Guan, R. Z. (1994) *Burrowing behaviour of signal crayfish, Pacifastacus leniusculus (Dana), in the River Great Ouse, England*, Freshwater Forum, 4.

Capurro, M., Galli, L., Mori, M., Salvidio, S. & Arillo, A. (2007) *The signal crayfish, Pacifastacus leniusculus (Dana, 1852) [Crustacea:*
Decapoda: Astacidae], in the Brugneto Lake (Liguria, NW Italy).
*The beginning of the invasion of the River Po watershed?*Aquatic Invasions, 2(1), pp. 17-24

Celada, J. D., Antolin, J. I., Carral, J. M., Saez-Royuela, M. & Rodriguez, R. (2005) *Successful sex ratio of 1M:4F in the astacid crayfish Pacifastacus leniusculus Dana under captive breeding conditions*, Aquaculture, 244(1-4), pp. 89-95

Cameron, S. (2010) *Loch Ken Crayfish Trapping Project Report*, Planning, Housing and Environment Committee, Marine Scotland

Barki, A. & Karplus, I. (2000) *Crowding female red claw crayfish, Cherax quadricarinatus, under small-tanks hatchery conditions: what is the limit?* Aquaculture, 181(3-4), pp. 235-240

Sheng Yeh, H. & Rouse, D. B. (1995) *Effects of Water Temperature, Density, and Sex Ratio on the Spawning Rate of Red Claw Crayfish Cherax quadricarinatus (von Martens)*, Journal of the World Aquaculture Society, 26(2), pp. 160-164

Gladman *et al*. (2009) Signal crayfish in Scotland. Crayfish Conservation in the British Isles, *Proceedings of a conference held on 25th March 2009 at the British Waterways, Leeds*, pp. 43-48

Crawford, L., Yeomans, W.E. & Adams, C.E. (2006) *The impact of introduced signal crayfish Pacifastacus leniusculus on stream invertebrate communities*, Aquatic Conservation: Marine and Freshwater Ecosystems, 16(6), pp. 611–621.

Griffiths, S.W., Collen, P. & Armstrong, J.D. (2004) *Competition for shelter among over-wintering signal crayfish and juvenile Atlantic salmon,* Journal of Fish Biology, 65, pp. 436-477.

Peay, S. (2001) *Eradication of Alien Crayfish Populations*. Scottish Natural Heritage, R&D Technical Report W1-037/TR1. Available at: http://www.snh.gov.uk/docs/C209050.pdf (Accessed: 4 April 2016).

Wutz, S. & Geist, J. (2013) *Sex- and size-specific migration patterns and habitat preferences of invasive signal crayfish (Pacifastacus leniusculus Dana),* Limnologica - Ecology and Management of Inland Waters, 43(2), pp. 59-66.

Svensson, M., Gydemo, R. (1997) *Mating capacity in male noble crayfish, Astacus*

astacus, under laboratory conditions, Freshwater Crayfish, 11, pp. 311-318.

Rebrina, F., Skejo, J., Lucić, A. & Hudina, S., (2015) *Trait variability of the signal crayfish (Pacifastacus leniusculus) in a recently invaded region reflects potential benefits and trade-offs during dispersal,* Aquatic Invasions, 10(1), pp. 41-50.

Dorn, N. J., Urgelles, R. & Trexler, J. C. (2005) *Evaluating active and passive sampling methods to quantify crayfish density in a freshwater wetland*, Journal of the N. A. Benthological Society 24, pp. 346-356.

Orton, S. M., Herrera, B. M., Yee, I. M., Valdar, W., Ramagopalan, S. V., Sadovnick, D. A. and Ebers, G.C. (2006) *Sex ratio of multiple sclerosis in Canada: A longitudinal study*, The Lancet Neurology, 5(11), pp. 932–936.

Celada, J. D., Fernandez, R., Gaudioso V. R. & de Paz, P. (1985) *Identification et chronologie des phases de developpement des oeufs de l'ecrevisse* (*Pacifastacus leniusculus Dana) par l'observation directe*, Pisciculture Francaise, 82, pp. 5-8.

Celada, J. D., de Paz, P., Gaudioso V. R. & Fernandez, R. (1987) *Embryonic Development of the Freshwater Crayfish (Pacifastacus leniusculus Dana): A Scanning Electron Microscopic study,* The Anatomical Record, 219, pp. 304-310.