Effects of vegetation on freshwater shrimp

The aim of this experiment was to discover whether the presence of vegetation affected the abundance of the species of freshwater shrimp Gammarus pulex. To do this an experiment was carried out involving samples taken from a variety of vegetated and non-vegetated areas. The abundance of the species in each environment was measured and compared and found to be statistically significant. Freshwater shrimp do prefer vegetated areas over non-vegetated areas due to either; increased availability of detritus, increased oxygen content or additional protection from predators.

Introduction:

Gammarus pulex: (freshwater shrimp) are Crustacean's that are members of the Amphipoda order. Their bodies are curved into a 'C' shape and they average approximately 20mm in length. As seen in figure 1, Gammarus pulex have 7 pairs of legs, two of which perform a grasping function similar to human's hands whilst the other 5 are adapted to swimming. The species gills are provided with oxygen by the vibrations of their legs and the 3 pairs of structures seen on the abdomen, the gills are located on the inside of the back five legs. Gammarus pulex have a high oxygen requirements and as a result are typically found in faster flowing, oxygen rich water. They are hatched from eggs and emerge fully developed and ready to reproduce. This results in a fast rate of reproduction. The offspring's sex is determined by the temperature of the water. These shrimps mate whilst the male carries the female on its back, this will occur several times per year. Gammarus pulex Have no natural defence mechanism other than their camouflage and as a result they are primarily active during low sunlight periods and under shelter.

As a result of Gammarus pulex's lack of a defence mechanism they are typically found under and within the aquatic fauna and rocks within rivers.

Gammarus pulex are primary consumers that typically consume detritus, i. e. they consume dead and decaying matter in order to provide energy for themselves. Gammarus pulex main predators are fish which that rely on them as one of their primary sources of food, fish commonly found to consume these shrimp include: Trout, Bullheads and Salmon.

Rationale:

Gammarus pulex can be used as an indicator species due to their affinity for highly oxygenated water. 'They (Gammarus Pulex) are intolerant of polluted waters or reduced dissolved oxygen concentrations, and can therefore be used as an indicator species for water quality.' A decrease in the oxygen content of an ecosystem can have serious adverse effects on the other species within the ecosystem and is one of the most common causes of 'Fish Kills' as a result it is a variable regularly monitored by fishing agencies Due to increasing awareness of the impact humans are having on their environment and the ecosystems that we interact with, biological indicator species will become increasingly important in the identification of suitable habitats for species to be introduced to.

In addition to Gammarus pulex acting as an indicator species, they also require highly oxygenated water to survive and reproduce. A lack of oxygen within the water could potentially lead to anaerobic conditions within the water. Due the fact that they require oxygen for respiration (Aerobic) this would reduce the population of the shrimp within an area. This would have a knock on effect on the secondary and tertiary consumers within the food chain that also rely on Gammarus pulex as their primary food source such as trout and salmon. As a result, the abundance of the species is of key ecological importance within these ecosystems and steps should be taken to ensure high oxygen content of the water and adequate foliage and vegetation for the species to thrive within its niche. These goals can be achieved relatively easily through the planting of vegetation for cover. This would also provide raised oxygen levels due to the respiration of the plant life to produce oxygen as a by-product.

In a study by H. H Costa on investigating the affect of low concentrations of oxygen on the number of reactions per minute in Gammarus pulex, strong correlations were made between the concentration of oxygen within the water and the number of reactions taking place per minute. Low levels of oxygen would lead to decreased metabolic activity, resulting in slower growth and reproduction. As a primary consumer this would have a detrimental effect on the rest of the ecosystem as a lower population of the species would result in food being a limiting factor for fish that relied on the species as a source of food. This could also lead to the gradual decline of other primary and secondary consumers as they are used as a source of energy by the tertiary consumers to sustain themselves.

Aims:

The aim of the experiment was to determine whether the presence of vegetation within the water at Nettlecombe church pool would have a significant impact on the abundance of Gammarus pulex measured within an area and whether any changes in abundance could be attributed to the independent variable (presence of vegetation) by controlling or measuring the other abiotic factors within the ecosystem.

Null hypothesis:

Statistically, there will be no significant correlation between the abundance of freshwater shrimp and the presence of vegetation within the stream.

Alternate hypothesis:

Statistically, there will be significant correlation between the abundance of freshwater shrimp and the presence of vegetation within the stream.

Prediction

Gammarus pulex will likely show a significantly higher presence in the vegetated areas of the stream due to several key biological factors:

Defense: Due to the lack of any inherent defense mechanisms, the species relies on its camouflage as its sole mechanism for defense. Naturally, high amounts of vegetation present in an area provide an aid to the protection of the freshwater shrimp from its predators and allow a greater chance of the survival of the species.

Oxygen content: The oxygen within water is derived primarily from submerged plants present within the water. As these plants photosynthesize, Oxygen is released as a byproduct and dissolves into the surrounding water. This increased oxygen content would attract freshwater shrimp to an area, as they are reliant on the surrounding water for the oxygen that is necessary for them to respire aerobically.

Detritus: Organic material such as decaying plant material is the primary food source of Gammarus pulex as detritivores. If vegetation is present in an area an increased amount of detritus will also be present due to the death and decay of the submerged plants. This provides an ideal food source for the Gammurus pulex and increases the chances of survival and reproduction. As a result you would expect to see an increase in the abundance of the species in areas with vegetation.

Independent variable

The independent variable within the experiment was the presence of a significant (+10cm) amount of vegetation submerged within the water and the lack of +10cm of vegetation present. This was varied by locating two areas, one with +10cm of submerged vegetation and another without.

Dependent variable

The dependent variable within the experiment was the relative abundance of the species within the two separate areas per sample. This was measured through obtaining a sample and counting the number of Gammarus pulex present per sample.

Control variables

Several abiotic variables were controlled within the experiment to ensure that any changes in the abundance of the freshwater shrimps could be assigned to the changing in the independent variable. In the cases in which an extraneous variable could not be controlled without compromising the integrity of the experiment, the variable was measured and any anomalous readings recorded could possibly be assigned to that abiotic factor.

- PH: The acidity (concentration of hydrogen ions) within the water was measured by obtaining a sample in the two areas and measuring the sample through the addition of universal indicator. The PH of the water was measured as Gammarus pulex have an aversion to water with more extreme Ph's. In 'Responses of Gammarus pulex to modified environment II. Reactions to abnormal Hydrogen ion concentrations ' H. H Costa states that at the PH values of <6 the species showed negative reactions to the PH and avoided water of that PH. He also states that they avoided water at a PH of > 9. 6. These negative reactions can be explained through the inability of the shrimp's enzymes to function at these extreme PH's.
- Temperature: Temperature was measured in degrees centigrade through the use of a digital thermometer that was placed in each sample, allowed to stabilize and a reading was taken. Temperature was controlled due to the reaction of Gammarus pulex's offspring to temperature, at lower temperatures, offspring within eggs will hatch as male, whilst above this they will hatch as female. This would lead to one sex becoming dominant at extremely hot or cold temperatures, which would result in a reduced abundance as the difficulty of locating a mate increased.
- Light: The amount of light in each sample was recorded through the use of a lux meter as close to the water as possible. This variable was measured in lux and was measured as it is likely the shrimp will be more abundant in darker areas due to the increased effectiveness of their camouflage in these areas and increased protection this offers.

- Depth: The depth of each location was measured through the insertion of a ruler to measure the depth of each location. The ruler was placed on the surface of the sediment to stop it being pushed too far into the soft sediment and producing an inaccurate reading. Areas were chosen as close to 10cm deep as possible to rule out the depth of the water having any significant impact on the results produced.

Pilot study:

Introduction:

Before carrying out the main study, a pilot study was used to determine the final procedure and method of the study. This pilot study was used to identify the best sampling technique to use to collect a sufficient number of shrimps per sample. This technique had to be efficient enough to collect enough data for a statistical significance to be shown and collect the maximum number of shrimp within an area to ensure that the true abundance of the species was actually being measured. In addition, the method used to collect the shrimp had to be standardized, so that the identical technique could be carried out in different areas to ensure the reliability of the technique. This meant the same technique could be carried out again to produce as similar results as possible.

In addition, the 'best' local spot to produce enough shrimp to state a significant difference had to be located. This pilot study was used to locate the area's that would be sampled in the main experiment by locating the areas with enough vegetation to have an impact on the dependent variable. This meant looking for area's with high levels of submerged vegetation within several different rivers until enough within as Single River was found.

This was to make the control of extraneous variables easier as the variation in the variables would be far larger in separate bodies of water.

Within the pilot study, I also had to assess the extraneous variables that would have to be measured in my final study to prevent them interfering with the data produced. During the pilot study, I identified that the amount of light in an area seemed to be having an effect on the abundance of the species within that area. This was potentially reducing the validity of the experiment. This was because any results produced were no longer necessarily down to just the independent (presence of vegetation) variable. As a result, I included the use of a light meter in the final experiment to control and limit the impact on the data produced. Along with deciding the variables to be measured, I also had to finalize the equipment used to measure the variable in a standardized manner.

The pilot study was also used to identify any potential risks or hazards within the area. Once these were identified, appropriate controls could be taken to reduce or nullify the risks from these sources.

Equipment list:

- A 15cmx10cm 'Scoop net'
- A 1 meter long ruler
- A digital thermometer
- A square 10x25 cm tray that was not filled with pond water
- A digital stopwatch
- Freshwater identification sheet
- Pipette

Method:

1. Whilst 3 rivers were found in close proximity, only one, the church stream, contained an appropriate number of vegetated areas to non-vegetated area's close enough to carry out the experiment expediently. In addition the proximity of the locations reduced any extraneous variables that were acting on the study. Thus the church stream was used for the final experiment.
2. 2 methods of sampling with the net were carried out, 'the figure of eight sample ' (A)and the 'Kick sample'(B)
    1. A) This involved locating an area to sample and standing still whilst moving the net in a figure of eight through the water. This was timed for 10 seconds and 20 seconds . This method did not disturb the sediment of the area.
    2. B) The kick sample involved placing the net downstream of the area to be sampled and kicking up the sediment into the net without moving the net. This was timed for both 10 seconds and 20 seconds using the stopwatch.
3. After collecting each sample the contents of the net was upturned and placed into the empty tray. The tray was then filled with water from the stream.
4. Using the identification sheet, I timed for 1 minute on the stopwatch and counted all of the Gammarus pulex present in the tray within the time. Using a pipette to move them to a separate bucket once counted
5. A variety of sampling techniques for the control variables were then carried out in the area.

6. The ruler was used to measure depth at a variety of locations. This provided practice at accurately measuring the depth of the water without disturbing the sediment on the bed of the stream.
7. The digital thermometer was then placed in the water, however not placed at the bed of the stream.

Risk assessment:

Before carrying out the pilot and main study, a risk assessment of the risks that could pose a threat to me or those in the surrounding areas was carried out. Through this I identified the hazard (negative effect 1-10) and the risk factor (chance of the hazard actually occurring 1-10) to control this I also decided on sensible controls to minimize the chance of the hazard occurring or minimize the severity of the hazard. The risk rating was calculated by multiplying the chance of the hazard occurring with the severity of the hazard (1-100).

Hazard Severity of the Hazard Chance of occurring Risk rating Control

Slipping and falling. 2 6 12 Ensure the muddy parts of the bank are noted and avoided. Wear appropriate footwear to avoid tripping/slipping.

Drowning in unexpected deep water. 10 1 10 Ensure all personal can swim. Identify any deep areas before beginning.

Contamination from any water/aquatic life. 8 3 24 Wash hands before and after touching the water. Do not handle the aquatic life, use a pipette instead

Tripping with net/ruler. 4 4 16 Wear appropriate footwear, I. e. hiking boots

Discussion of results:

The number of shrimp produced by using method B for 20 seconds produced the largest total number of shrimp in both vegetated areas and non-vegetated areas. In comparison, method A produced the fewest number of shrimp in both vegetated areas and non-vegetated areas. The temperatures produced seemed to be pretty constant throughout the pilot study however the two 9. 8 readings were likely caused by the thermometer not being pressed to the sediment properly, reducing the reliability of those readings. The preliminary findings reflected that more shrimp were indeed found in the vegetated areas in comparison to the non-vegetated.

Evaluation of pilot study:

Through the use of the pilot study I was able to identify the main problems with the study and remedy them for the final study:

- The measuring of the temperature using the thermometer was standardized by pressing the thermometer to the bed of the stream on each measurement.
- The sample I chose to use was the 20 seconds 'kick sample' This method of sampling was far superior to the figure of eight sample. This was likely to the freshwater shrimp inhabiting the upper layer of the sediment; the kick sample dislodged them into the net whereas the figure of eight did not disrupt the sediment at all resulting in only the swimming shrimp being picked up. As a result, only the number of shrimp swimming in a sampled area was being measured not the total number of shrimp in the area. I chose to use the 20 second sample as, although it was less expedient than the 10 second sample, it did

provide more species and reduced the chance of anomalies cropping up through not all of the shrimp being collected.

- Whilst measuring the depth in step 8, I noticed that the vegetated areas where normally darker than the non- vegetated areas. To counteract this variable, I decided to control it in the main experiment by measuring the light using a lux meter. This was to ensure that the results were down to the vegetation present.
- During step 1 and the selection of the location of the samples, I realized that I hadn't set any specification on what classed as 'enough' vegetation to qualify as a vegetated area. After discussing this with my tutor I decided to use 10cm measurements of submerged vegetation qualify an area as vegetated. In addition, I used areas without any vegetation for the final experiment.
- Whilst collecting the samples in step 4 I realized that I was disturbing the ecology further downstream on further kick samples. This was an unfortunate side effect of using the kick sample from the top of the stream down. As a result, I decided to reverse the direction for the final experiment and take the values from downstream before taking those upstream. This stopped earlier experiments influencing later ones.

Main Experiment:

My final experiment took into account the findings from the pilot study and used these to modify the final experiment to produce the best results I could. The changes listed above were introduced to tailor the results of the experiment into a form I could use to compare the effect of the independent variable.

Intro:

My final experiment consisted of 10 samples of each vegetated and non-vegetated areas being taken, with the variables light, depth, temperature, PH and number of shrimp present being measured. The revised aim of the final experiment was to measure whether the presence of vegetation affected the abundance of Gammarus pulex present and whether this difference was statistically significant. To do this I carried out a stratified sampling technique to locate areas to work in, I, e areas with +10 cm of submerged vegetation. I then used a systematic sampling technique to collect the data as I timed how many of the species I could count from a sample in 1 minute.

Equipment list:

- A 15cmx10cm 'Scoop net'
- A 1 meter long ruler
- A digital thermometer
- A square 10x25 cm tray that was pre-filled with pond water
- A digital stopwatch
- Freshwater identification sheet
- Pipette
- 1 Lux meter
- 1 bottle of universal indicator, with a beaker to collect a sample of water
- A small bucket to place counted shrimp in
- Writing equipment and paper to record results
- Pipette to collect the shrimp with

Method:

1. 10 vegetated areas were first located. This involved measuring the total submerged vegetation present and ensuring it was over 10cm long.
2. The non-vegetated areas were then located, these were areas within as close proximity to the vegetated areas as possible but without any vegetation present.
3. Starting from downstream and moving upstream, the kick sample was performed in the first vegetated area. This involved placing the net downstream from the sampling area and kicking the sediment for 20 seconds into the net. This was timed with the stopwatch.
4. The sample in the net was upturned into the tray.
5. The Gammarus pulex were identified using the species identification sheet and removed from the tray and placed in the bucket using a pipette. The total number of shrimp counted in 1 minute was recorded.
6. The contents of the tray and bucket were emptied back into the water.
7. The depth of the sampling area was then measured using the ruler and placing it on the stream bed. The lowest value of the water rising up the ruler was measured as the depth.
8. The thermometer was then placed lightly on the stream bed, this was left to stabilize for 10 seconds and the value was recorded
9. The lux meter was then placed as close to the water as possible and held still. After 10 seconds the value was taken.
10. This method was repeated for the non-vegetated areas.
11. This procedure was carried out at all of the 10 areas.

Table of results for main experiment

Calculations of the means for Vegetated data:

Mean PH: (7. 5 x 10)/10 = 7. 5

Mean temperature ' C: (9. 8 x 2 + 9. 9 x 8)/10 = 9. 88

Mean Lux reading (Lux) :

(240+234+194+30+13+109+603+719+560+335)/10 = 303. 7

Average depth (cm) : (10. 0+9. 5+5. 0+4. 0+4. 0+5. 0+5. 2+8. 2+12. 8+13. 2) /10 = 7. 69

Mean Shrimp (Arbitary units) : 341/10 = 34. 1

Calculations of the means for Non-Vegetated data :

Mean PH: (7. 5 x 10)/10 = 7. 5

Mean temperature ' C: (9. 8 x 1 + 9. 9 x 9)/10 = 9. 89

Mean Lux reading (Lux) :

(846+645+300+71+24+123+441+739+506+389)/10 = 408. 4

Average depth (cm) : (12+11. 5+10. 5+12+8+10. 5+12. 4+14. 5+12. 4+13. 3) /10 = 11. 71

Mean Shrimp (Arbitary units) : 56/10 = 5. 6

Anomalies:

Both of the values highlighted in red, are examples of possible anomalous data that could have been caused by procedural errors. 6 is far below the average shrimp reading of 34. 1, this could have been caused by the high light reading on this particular reading, reducing the shrimps camouflage and therefore their safety in this area. The value of 17 was far ahead of the mean value of 5. 6 and could have been caused through the procedural error of the pipette not sucking the shrimp up and them being counted twice. These samples also could have had the random error of large amounts of sediment resulting in some shrimps not being counted or others being counted twice.

The clear variation in the number of Gammarus pulex sampled in the two areas would indicate that the vegetation did have an effect on the abundance of the population. Likely due to the increased defense offered by these areas and also how much easier it would be to obtain decomposing plant matter (Detritus) as a food source. The largest value for a vegetated shrimp was 79, with the lowest being 6. This is in comparison to the highest value for non-vegetated areas being 17, with the lowest being 2. The similarities between the abiotic factors, primarily temperature and P. H also increase the validity of the results produced. We can be sure that the results are actually as a result of the presence of the vegetation. It should be noted that none of the samples contained no freshwater shrimp, leading to the assumption that the water likely has a high oxygen content and has few pollutants. This is backed up by the location of Nettlecombe, a relatively rural area with few large roads running nearby.

Statistics:

Within this study I decided to use the Mann Whitney U test to ascertain whether the difference between the two values was enough to reject the null hypothesis and convey a significant difference between the two sets of data.

Null Hypothesis: There will be no significant statistical difference between the data drawn from vegetated samples and non-vegetated samples in the Nettlecombe church stream on the 14/10/12

The lowest U value calculated was 6, The critical value at 0. 1 is 16 meaning that the Null Hypothesis can be rejected at the 0. 1 significance level This allows us to accept the alternate hypothesis, and although there were small overlaps in the data, the difference between the two sets of data values is significant.

Evaluation:

Throughout the experiment, every effort was made to control or measure extraneous variables that could have an effect on the results of the experiment. As a result, the validity of the experiment is quite high; the values for all the abiotic variables were measured and have similar values for both the Vegetated areas and the non-vegetated areas. As a result we can make the assumption that any changes in the dependent variable, were in fact down to the independent variable.

The pilot study was carried out to aid in the identification of any confounding variables, risks or extraneous variables that could potentially impact on the validity of the results produced and as a result of the pilot study, controls were put in place to counteract these variables.

In addition, due to the standardized methodology carried out within both the pilot experiment and the final experiment, the method could be repeated under similar conditions to produce similar results. Resulting in a high reliability of the data.

As the Mann Whitney U test allowed us to reject the null hypothesis at the 0. 01% significance level, we must assume that the results produced show a significant change between the two environments.

Although the results proved to be statistically significant, several procedural errors should be considered. Even with a 20 second kick sample, not all of the shrimp in a certain area could be collected as the shrimp likely inhabit different layers of the sediment. The speed at which the shrimp were counted at could also vary considerably based on the amount of sediment present in a sample, in a sample with high amounts of sediment, it would take longer to count and remove all of the shrimp within a minute than it would be in a clear sample. To counteract this, a stratified sample could be put in place; all of the shrimp from a sample could be counted rather than just counting for one minute.

Systematic errors within parts of the equipment could result in repeatedly wrong measurements, e. g the Lux meter may not be calibrated properly and give readings that are continually 50 higher than they should be.

Percentage error can also become a problem and whilst small, can be noticeable when accounted for in multiple datasets e. g. 0. 01/500 x100 = 0. 002 % percentage error

There are also several ethical considerations to take into account when using live organisms within experiments. For instance the ecosystem is being massively disrupted through the use of a kick sample. All of the layers of sediment are being removed and many organisms are being collected in a sample that didn't have to be. A more specific and less environmentally invasive procedure could instead be carried out using the figure of eight sampling method so as not to disturb the stream bed ecosystem as much.

Conclusion

From the data and evidence provided above, a reliable argument can be put forward that the species Gammarus pulex does favor vegetated conditions over non-vegetated conditions. This could be down to a variety of reasons:

- Gammarus pulex is an aerobic organism and thus favors oxygenated conditions. During photosynthesis in submerged plants, oxygen is produced as a byproduct which diffuses into the surrounding environment (the water) this results in increased oxygen content. The oxygen content at this point is not acting as a limiting factor in the population of Gammarus pulux and thus an increase in the activity within the reproductive cycle of the species. This is demonstrated in the image to the right.
- Gammarus pulex favors the vegetated environment due to the increased source of detritus present through the decomposition of the plants present in the environment. This increased source of food and energy means the species expends more energy within its reproductive cycle and fewer of the species die due to starvation. Thus the population of the species in these conditions increases.
- Gammarus pulex could also potentially favor the vegetated environment due to the increased camouflage and protection it provides it. This could be part of a behavioral adaptation the organism has developed to protect itself from the fish that hunt it. This would result in large numbers gathering in the areas that they were safe and

fewer of them being consumed by secondary and tertiary consumers resulting in larger numbers of them in the vegetated areas.

Source evaluation

Within my experiment, I extensively referenced and used H. H Costa's work into the differing effects of modified environments on Gammarus pulex. This work was published in the scientific, peer reviewed journal Crustaceana. This journal is 'is a leading journal in the world on crustacean research.' As a result I feel the peer reviewed work is both reliable and accurate and although published in 1967, the more recent research (Field guide to the water life of Britain 1984) also agrees with the core principles described in H. H Costa's work. In addition to several peer reviewed journals, one of my sources was an Information pack produced by the University of Florida, a well respected academic institute unlikely to publish inaccurate or unreliable information. As well as the 'paper' sources listed, I used a variety of websites to gather data on various aquatic processes and mechanisms. Whilst I trust the majority of the websites, certain websites such as source 2, don't back up many of the claims with any real evidence which could be a sign of unreliable or inaccurate information.

Further research

Further research could be carried out into the preferences of Gammarus pulex through an experiment designed to ascertain the type of vegetation that they prefer and also the nutritional value of the varying types of detritus to the organism. In addition, research could be done into pinpointing the exact reason that the species prefers to reside within vegetative conditions rather than non-vegetative.