# Biodiversity of Woodlice in Central Park

Magnificent Student and Stupendous Student

**Abstract**

Woodlice will be collected from seven different areas in Central Park to determine the relationship between human activity and species diversity. About 30 woodlice samples will be collected and their DNA will be extracted and sequenced in order to determine their species identification. We hypothesize that there will be greater amounts of species diversity in shady, damp areas with decaying matter and in soil with neutral or alkaline pH, good crumb structure, and high organic matter content, where woodlice can flourish.

**Introduction**

Central Park is an urban park in New York City, containing vast amounts of biodiversity. It is the most visited park in the city, and one of the most well known parks in the world. There are over 42 million visitors annually (Tikkanen, n.d.). Central Park is home to scenic hills, playgrounds, meadows, skating rinks, ballfields, many well-known attractions, and 223 invertebrate species throughout the 842.2 acres of land (Sain-Baird, 2017). Central Park creates a healthier city for New Yorkers, increased opportunities for people to stay active, as well as cleaner air and ecosystems for species to flourish.

Woodlice are included in the 223 invertebrate species that thrive in the park. Woodlice are small terrestrial isopods, a class of Crustacea, of the family Oniscidea. Often they are referred to as bugs, but they are more closely related to lobsters and crabs than they are to insects. They have invaded terrestrial habitats from aquatic environments (Willson, 2020). Most members of the sub-order are small to medium sized organisms, with approximately 5000 species distributed worldwide in temperate to tropical climates. Woodlice can commonly be found in gardens, under piles of leaves, rocks, downed trees, and in soil with neutral or alkaline pH, good crumb structure, high organic matter content, where soil bacteria and other micro-decomposers flourish (Wollney, 2016).

Woodlice are easily identified, but it is not easy to identify the specific species. Woodlice range from 1.2 to 20 mm in length, similar to the size of a pencil tip. They have a gray colored segmented body and seven pairs of legs. In order to protect themselves from predators, they are able to roll themselves into a ball. This ability gives them their common name of "roly-polys" (Wollney, 2016). Woodlice also have a pair of antennas on top of their head, which function as sensory organs that facilitate navigation. To find woodlice, one would commonly look in damp, dark places, which can be under stones, hiding in walls, and in compost heaps. Some species are only found on the coast (Dimond, 2018). There are about 3,500 species of woodlice, but they are often very difficult to tell apart because they look very similar to one another. Identifying different species of woodlice is extremely difficult without the use of DNA sequencing.

Woodlice perform an important role in their ecosystems because they eat decaying matter. This matter includes dead leaves, fallen fruit, detritus, and sometimes includes animal droppings. Woodlice are not harmful due to the fact that they leave living plants and animals alone, so they are not considered pests. The presence of woodlice generally indicates dampness (Wollney, 2016).

Woodlice have four main stages of life consisting of the egg, manca (which includes two sub-stages), juvenile (which includes several sub-stages), and adult. The eggs develop and hatch into mancae. Mancae look like adult woodlice but are signifigantly paler, white or pale yellow, and have fewer legs than adult woodlice. They undergo two molts, the shedding of their skin, during the manca stage, one molt into a more independent mancae, and the next molt into a juvenile woodlice. The juvenile woodlice are smaller versions of adult woodlice. They continue molting periodically until they reach their full size and potential, a year or more later. Finally, in the final stage, adult woodlice can breed (Willson, 2020).

Using DNA barcoding we will discover the DNA sequence of woodlice, uniquely identifying each species of woodlice. We hypothesize that there will be greater amounts of biodiversity in shady, damp areas with decaying matter and in soil with neutral or alkaline pH, good crumb structure, and high organic matter content, where woodlice can flourish.

**Materials and Methods**

First, we will number our test tubes. Then, to collect our samples of insects, we will travel to Central Park. We will collect 30 samples in total; from the Bridle Path in Central Park. While collecting these samples, we will try to the best of our ability to make sure that the samples are from different areas along the Bridle Path, so that we can hopefully have a variety of species that account for the biodiversity of the park. We will transport the samples from the Bridle Path back to the lab, and we will put the samples of leaves and dirt into a tray. Then we will use the forceps to collect insects, and put one insect into each test tube with 95% ethanol. We will clean the forceps and any other equipment we use after using it. We will freeze our samples at -20ºC until we are ready to commence the process of isolating a sample of the DNA.

We will obtain tissues of the specimens, about 10 mg or ⅛- to ¼-inch diameter in size, by removing a piece of the tissue with a razor blade or sterile tweezer. Some organisms or samples will be small enough that we will use the entire specimen. We will be careful not to cross-contaminate specimens, and we will be sure to preserve the remainder of the organism, as well as additional collected specimens, at -20°C, in 95%+ EtOH, or both.

Next, we will gently tap 10% of the Chelex solution tube on a hard surface to ensure the solution is at the bottom and place a tissue into a Chelex tube labeled with a sample identification number. We will twist a clean plastic pestle against the inner surface of the Chelex tube to forcefully grind the tissue for at least 2 minutes, and use a clean pestle for each sample. It is important that we ensure the sample is ground into fine particles and securely close the cap of the tube.

Subsequent to this, we will fill a beaker nearly to the top with boiling water and cover with aluminum foil. Then, we will punch small guide holes in the foil for the number of samples we are processing. We will prevent the tube from opening in the following step by using a cap lock to secure the cap to the rim of the tube, and we will be sure that both the tube rim and cap are held within the cap lock so that steam can't force the cap open. Next, we will place tubes through foil so that the Chelex and sample mixture is fully submerged, but will not submerge the top of the tube. Then we will incubate tubes for 10 minutes in boiling water. We will remove the tubes from the beaker and allow them to cool for 2-5 minutes. Alternatively, place tubes in a balanced configuration in a microcentrifuge, with cap hinges pointing outward. Centrifuge for 30 seconds at maximum speed to pellet Chelex. We will carefully transfer ~30 µL of supernatant from the Chelex tube, avoiding the Chelex, into a clean 1.5 mL microcentrifuge tube labelled with the sample identification number. This tube will be stored at 4° C temporarily or frozen at -20° C for long-term storage until ready to begin PCR amplification.

Following this, we will obtain a PCR tube containing Ready-To-Go PCR Bead containing dehydrated *Taq* polymerase, nucleotides, and buffer. We will label the tube with your identification number. We will use a micropipette with a fresh tip to add 23 µL of the appropriate primer mix to each tube, and allow the beads to dissolve for 1 minute at ambient temperature. Then we will place the PCR tubes on ice to prevent premature replication of unwanted primer dimers. We will use a micropipette with a fresh tip to add 2 µL of our DNA directly into a PCR tube with a primer and polymerase mixture, ensuring that no DNA remains in the tip after pipetting. We will store our sample on ice until we are ready to begin thermal cycling. Then, we will place our PCR tube in a thermal cycler that has been programmed with the appropriate PCR protocol of the Invertebrate COI primers. For the initial step, the thermal cycler will be programmed to 94°C 1 minute. Then there will be 35 cycles of the following profile: the denaturing step at 95° C for 30 seconds, the annealing step at 50° C for 30 seconds, and the extending step at 72° C for 45 seconds. Our final step will be to preserve the sample at 4° C ad infinitum.

Succeeding the PCR amplification, we will begin Gel electrophoresis. We will seal the ends of the gel-casting tray with masking tape and insert a well-forming comb. We will pour the 2% agarose solution into the tray to a depth that covers about one-third the height of the comb teeth. We will allow the agarose gel to completely solidify which will take approximately 20 minutes. Then we will place the gel into the electrophoresis chamber and add just enough 1× TBE buffer to cover the surface of the gel. Carefully, we will remove the comb and add an additional 1× TBE buffer to fill in the wells and just cover the gel, creating a smooth buffer surface. We will use a micropipette with a fresh tip to transfer 5 µL of each PCR product to a fresh 1.5-mL microcentrifuge tube, and add 2 µL of SYBR Green DNA stain to each tube with 5 µL of PCR product. We will be sure to not add SYBR Green directly to the tubes containing the full 25 µL of PCR product, as SYBR Green interferes with the sequencing reaction. Then we will add 2 µL of SYBR Green DNA stain to 5 µL of 100-bp marker. We will orient the gel so the wells are along the top of the gel. We will use a micropipette with a fresh tip to load 5 µL of 100-bp marker into the far left well, and we will use a micropipette with a fresh tip to load each sample onto our wells. We will store the remaining 20 µL of your PCR product on ice or at -20° C until we are ready to submit your samples for sequencing. Finally, we will send for sequencing using GENEWIZ and analyze the results using BLAST.

**Specific Aims**

In this experiment, our goal is to compare the biodiversity of species of woodlice in different habitats. This will be achieved by collecting woodlice from multiple different areas in Central Park, which will be classified and documented. The samples' DNA will be sequenced and examined to determine the species of each woodlouse. The number of species in each microenvironment will be compared using the Simpson Index to determine the biodiversity of each area.

By comparing the multiple habitats, which may include under rocks, by trees, and in sunny areas, we hope to see how different variables of urban parks affect the biodiversity of different species of woodlice. Some places, such as the Bridle Path in Central Park, may be more exposed to human activity than others, creating more litter and foot traffic, which may affect the different species in the areas. Areas may also have different temperatures due to differing exposures to sunlight. The data we collect will be compared with data collected from other areas to determine the diversity of woodlice in urban habitats. We hope that we will be able to document the effects that human traffic and different habitats will have on biodiversity. Through this documentation, researchers will be able to learn more about the ecosystems in which we live. This will allow us to understand the impact of humans on these ecosystems, and protect organisms such as millipedes.

**Data Analysis**

| **Sample** | **Date Collected** | **Location** | **Observations** | **Species (identified using BLAST)** |
|---|---|---|---|---|
| **Sample 1** | | | | |
| **Sample 2** | | | | |
| **Sample 3** | | | | |
| **Sample 4** | | | | |
| **Sample 5** | | | | |

Once about 30 woodlice samples are collected from Central Park, DNA will be extracted using the chelex methods, amplified using PCR, and identified with BLAST. The composition of woodlice species in each location will be used to analyze the connection between human activity and species diversity. We will also be making graphs to compare woodlice species diversity at the two locations. To measure biodiversity, we will use the Simpson Index.

**References**

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Magnificent Student is a sophomore at Marymount School of New York. She enjoys mathematics and biology. She is on the school math team, and is an Irish dancer at the Aherne Sheehan School of Irish Dance.

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Stupendous Student is a sophomore at Marymount School of New York. She enjoys hands-on activities and learning about advancements in the medical field. She plays with Marymount’s volleyball and lacrosse teams and is part of the Marymount Singers.

**Teacher Statement**
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