INQUIRY & INVESTIGATION

Using Videography to Study the Effects of Stimulants on *Daphnia magna*

RECOMMENDED FOR *AP Biology*

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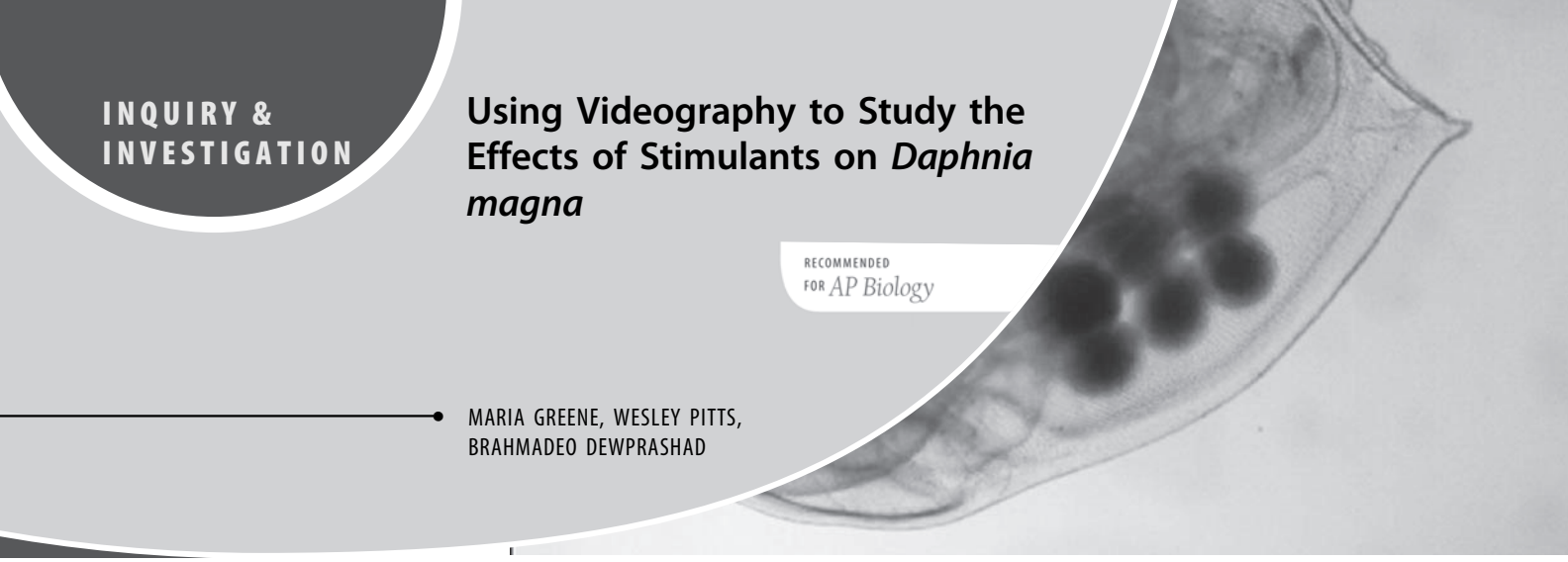

ABSTRACT

Daphnia have been used to demonstrate the physiological effects of stimulants such as caffeine and energy drinks in activities designed for secondary school and college labs. We describe how these activities were enhanced by coupling a microscope to a video recording device and digital data recording system to facilitate more accurate quantification of the rate of heartbeats in daphnia. Also, the technology facilitates measurements of the changes in the size of the heart due to the effect of stimulants. We describe the setup for the video recorder, data acquisition system, and microscope, the results obtained, and how these activities could be replicated in a secondary school laboratory setting to increase student engagement and be used as a primer to enhance learning and understanding of biological systems by students. This work aligns with the Next Generation Science Standards, in that students "use a variety of equipment and software to enter, process, display, and communicate information in different forms using text, tables, and pictures."

Key Words: *Nikon Basic Research Software; heart rate; Daphnia magna; water fleas.*

○ Introduction

Daphnia magna (daphnia, or water fleas) have been commonly used as models to study the effects of stimulants on the cardiovascular system. Daphnia are transparent and have large hearts, which facilitates direct observation of their organelles under a microscope. In addition, special permission is not required for their use in the laboratory.

Traditionally, the daphnia are immersed in test solutions for specific times and then placed on a slide and viewed under a microscope. Heart rates are observed, directly counted, and recorded. The counting is usually done with the aid of a stopwatch and often includes the use of a pen and paper to tap the beats. When using this method, it can be challenging to count accurately and thus obtain reliable and meaningful quantitative data (Drewes, 1999), and such numerous measurements would be challenging for a typical high school lesson or lab activity. Also, it is difficult to immobilize these organisms, and daphnia have extremely fast heart rates of around 228 ± 2 beats per minute (bpm) (Kaas et al., 2009). The reported effects of caffeine on daphnia heart rate are contradictory. Foster (1997) found that a 0.1% (w/v) caffeine solution increased the heart rate. In another study, Corotto et al. (2010) found that 0.1% (w/v) caffeine solution had no "convincing effect on the heart rate." Accuracy can be improved by coupling a video camera to the microscope, recording the experiment, and using the playback feature for accurate counting. Such a system was used in a kinematic study of the pulsation of the dorsal blood vessel of the blackworm (Halfmann & Crisp, 2011). However, they used a variety of sophisticated software to extract and analyze the data from the videos. Their procedure would be difficult to replicate in a high school setting, as acquisition and setup of the equipment used would be expensive and tedious.

> Daphnia magna *(daphnia, or water fleas) have been commonly used as models to study the effects of stimulants on the cardiovascular system.*

Here, we describe how these activities were enhanced by coupling a microscope to a video recording device and digital data recording system, which is easy to acquire and simple to use. The technology facilitates more accurate quantification of the rate of heartbeats as well as measurements of the changes in the size of the heart due to the effect of stimulants. The traditional methods do not have this ability.

Other features of this software that the students and teacher will find very useful and easy to use include exporting the data to the computer to generate Excel graphs; annotating images; and measuring the length, width, and diameter of an organism and/or its organs (Figure 1). Additionally, the use of this system provides students the opportunity to gain additional technological skills by acquiring and analyzing data.

The use of this procedure lends itself to group activities in which students and the teacher can view the circulatory system of

The American Biology Teacher, Vol. 79, No 1, pages. 35–40, ISSN 0002-7685, electronic ISSN 1938-4211. DOI: https://doi.org/10.1525/abt.2017.79.1.35.

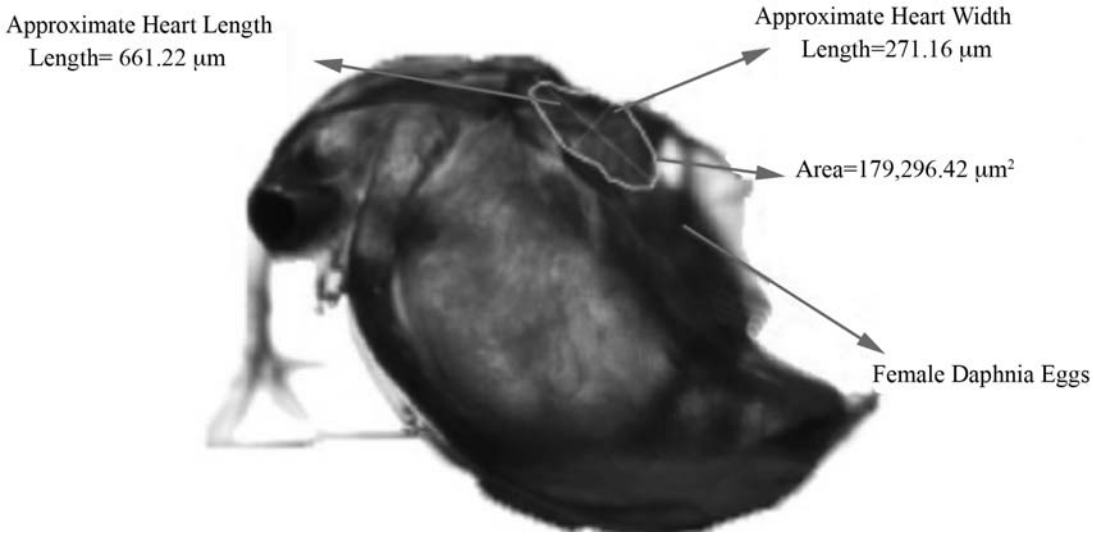

Figure 1. *Daphnia magna*.

the heart of the daphnia in real time. The group can record the organism's response to stimulants and use the playback features to analyze the data. A replay of the video can generate more opportunity for discussions between students and teacher. This can also be extended to a whole-class discussion in which each group can compare its findings to those of the other groups. Since "modal preferences influence individuals' behaviours, including learning" (Fleming & Baume, 2006), teachers and students can access data for multiple modes of representation, thereby accommodating students with auditory, visual, and kinesthetic learning styles. Visual learners can benefit from the text, images, and graphs generated by the software. The hands-on activity and video replay cater to kinesthetic learners. Auditory or aural learners will benefit greatly from the group and class discussions. Students are also expected to transcribe their observations in real time. These activities provide them with such experiences.

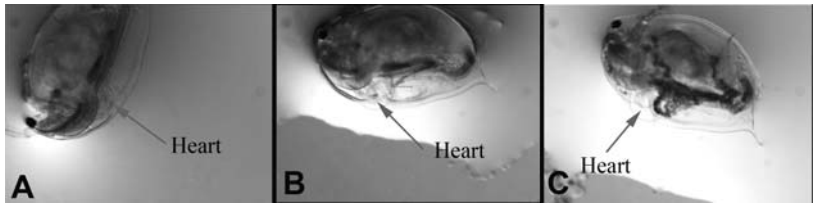

Figure 2. *Daphnia magna* in water (A) and in 1% caffeine at (B) 5 minutes and (C) 15 minutes immersion time.

○ Experimental Procedure

A Nikon SMZ 800 special imaging system (NIS) consisting of a camera and the Nikon Basic Research (BR) and Analysis Research (AR) software (cost: $3,300) was coupled to a microscope and used to visualize and record 1-minute video clips of daphnia after they were placed in test solutions for specific times. NIS documentation software with similar functions can be obtained for $500 less; the software is compatible with other Nikon or third-party microscopes such as the Nikon SMZ 800N, CMO stereomicroscope, or any other type of stereo microscope.

Alternative but more expensive software to be coupled with the stereomicroscope, such as Metamorph and Image Suite, can be used in place of the BR software. Also, ImageJ, which was written by the National Institutes of Health (NIH), is a free, Java-based applet unsupported program. This free program is not very user friendly, in the sense that the user will need to install and calibrate it before use. Also, it is a very basic program and does not include some of the features of the region of interest (ROI) of the software we used for our research.

Heart rates of daphnia were determined from a replay of the recorded video. In addition, heart widths of daphnia (Figure 2) were determined using the ROI in the basic research software. Also, the software was used to generate histograms representing the change in reflection of light due to contractions of the heart.

○ Preparation

The procedure for preparing the daphnia for this experiment is the same as described in a study of the effects of melatonin and ethanol on the heart rate of *D. magna* (Kaas et al., 2009). Installing the NIS software is quick and simple. First, connect the digital microscope's USB cable to the computer. The software package includes an installation disk and a Hardware Against Software Piracy (HASP) key. Insert the installation disk into the computer; an icon will appear on the computer screen. Select the research package (advanced research or basic research) to be installed (i.e., the one you purchased and obtained the license for). Follow the directions for installation and run the NIS-Elements software. When the software is installed, an icon will appear on the computer screen, indicating that the software was successfully installed and is ready to use (Figure 3). After installing the software, plug the HASP key into the USB port of the computer and click on the icon to open the program.

Place the specimen on the microscope stage to view. The organism can be viewed directly from the computer screen (Figure 4). Also, the computer can be connected to an overhead projector for whole-class observations. To capture a video recording of an image, set the duration time (in the case of our experiment, 30 seconds) and

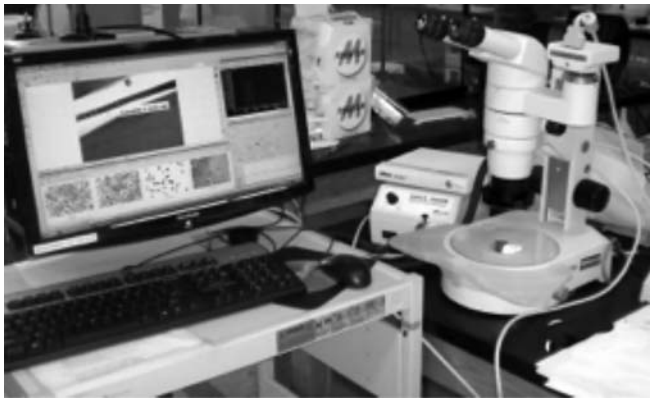

Figure 3. Computer and digital camera.

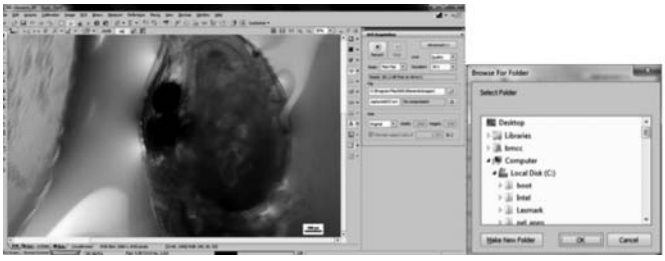

Figure 4. *Daphnia magna* on slide mounted on computer stage. Duration time of 30 seconds. Image (footage) file of slide mount can be saved to a designated project folder.

click "record." The image will be automatically saved in AVI format to a folder created by the NIS-Elements. Also, the recordings can be saved in other file formats to the NIS-Elements designated folder, a USB, or another designated folder. For our experiment, we recorded videos of daphnia to analyze. We also took still images of the specimens by clicking on "acquire" and selecting "image." The images will also be saved to the NIS-Elements designated folder and can likewise be saved to a USB or another designated folder.

Histograms are generated from the live capture of the organisms. To view and generate histograms, click on the "customize" button (Figure 5), scroll down to "visualization," and click on "histogram." The histograms can be exported to Excel. "A histogram is a graphical display of tabulated frequencies. In NIS-Elements, it displays frequencies of pixels with a certain intensity value" (NIS-Elements user guide).

○ Analyzing & Annotating Recordings of Organisms

Analysis and counts of the organism's response (i.e., heart rate of daphnia) to the stimulants was done by a slow replay of the videos. Annotations were done using the software's ROI tools. Click on the ROI tool and select the ROI form (in our case, we chose the "bezier" ROI; Figure 6). Draw around the desired area, then right click the mouse (Figure 7). Additional annotations and measurements can be added to the image (Figure 8).

A similar method was used to measure the change in the area of the heart of the daphnia (Figure 2B, 2C). Measurements were taken

Figure 5. Selecting the ROI histogram tool.

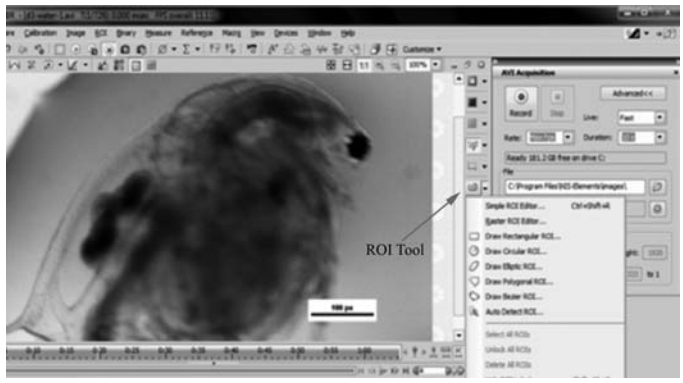

Figure 6. Selecting the ROI tool.

Figure 7. Use of the ROI tool to draw around selected area (red line) of the heart of *D. magna*.

at the same time frame when the heart was dilated. Click on the annotations and measurements button (Figure 8) and select the ROI measuring tool under the "length" section. Right click the mouse, and the measurement will appear on the image. After completion of annotations and measurements, the image can be exported to Excel. Click on the "export" button (Figure 8) to export to Excel.

○ Investigation: Effects of Caffeine on Heart Rate of *Daphnia*

A baseline control (166 ± 9 bpm) was determined by measuring the heart rates for each of three daphnia in spring water. The effect of caffeine was next determined by immersing three daphnia in 1 mL of a 0.05M (1% w/v) aqueous caffeine solution for 2, 5, and 15 minutes and then determining the heart rates for 30 seconds. We used the

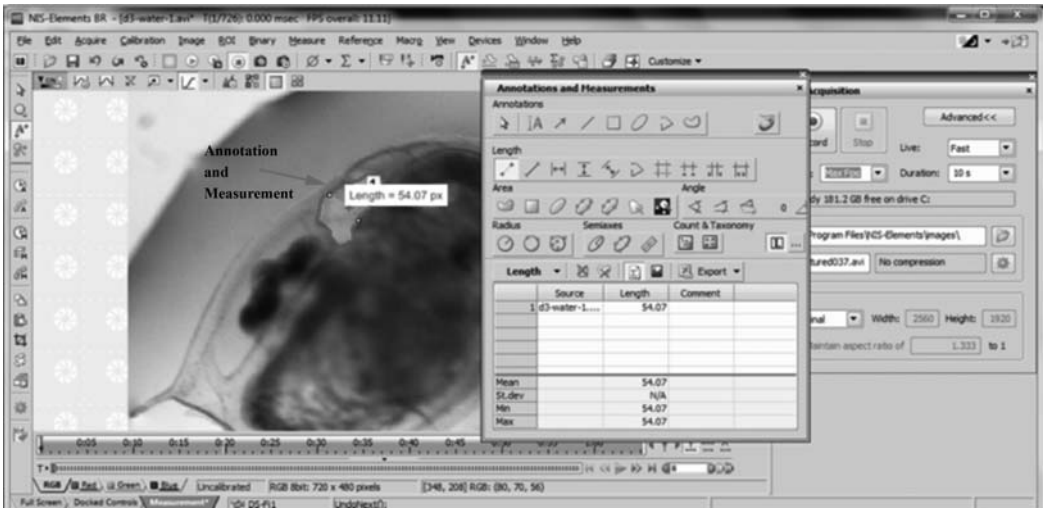

Figure 8. Annotation and measurement of the area of the heart of *D. magna*.

Table 1. Effects of 0.05 M caffeine on the heart rates of *Daphnia magna* at 2, 5, and 15 minutes immersion times.

Daphnia no.	Immersion Time (2 minutes)			
	Before Treatment	After Treatment	Increase	Percent Increase
1	174	184		
2	166	186		
3	172	190		
Mean rate	171 ± 4	187 ± 3	16	9
Daphnia no.	**Immersion Time (5 minutes)**			
	Before Treatment	**After Treatment**		
1	166	198		
2	172	184		
3	164	194		
Mean rate	167 ± 4	192 ± 7.2	25	15
Daphnia no.	**Immersion Time (15 minutes)**			
	Before Treatment	**After Treatment**		
1	170	128		
2	146	144		
3	166	134		
Mean rate	161 ± 13	135 ± 8	–26	16 (decrease)

procedure described by Kaas et al. (2009). The results are shown in Table 1 and Figure 9. Comparative histograms (Figure 10) were generated using the export feature in the NIS software.

The results indicate that there is a distinct measurable change in heart rate when daphnia is immersed in a 0.05 M (1% w/v) caffeine solution. The heart rate increased for the 2-minute (187 ± 3 bpm) and 5-minute (192 ± 7.2 bpm) but decreased for the 15-minute immersion time (135 ± 8 bpm). Our results indicate that daphnia can be used to demonstrate to students that caffeine is a stimulant and that it speeds up heart rates. The effects at 0.1%, 0.5%, and 2% (w/v) caffeine solution were previously studied, and no "convincing effect on heart rate" of *D. magna* was found (Corotto et al., 2010). That study's results contradict those obtained from an experiment in which stroboscopic methods were used to measure heart rates of daphnia immersed in 0.1% (w/v) and lower doses of caffeine solution (Foster, 1997). Significant increases in heart rates that were concentration dependent were found (Foster, 1997). Also, Drewes (1999) found similar increases in the heart rate of daphnia using

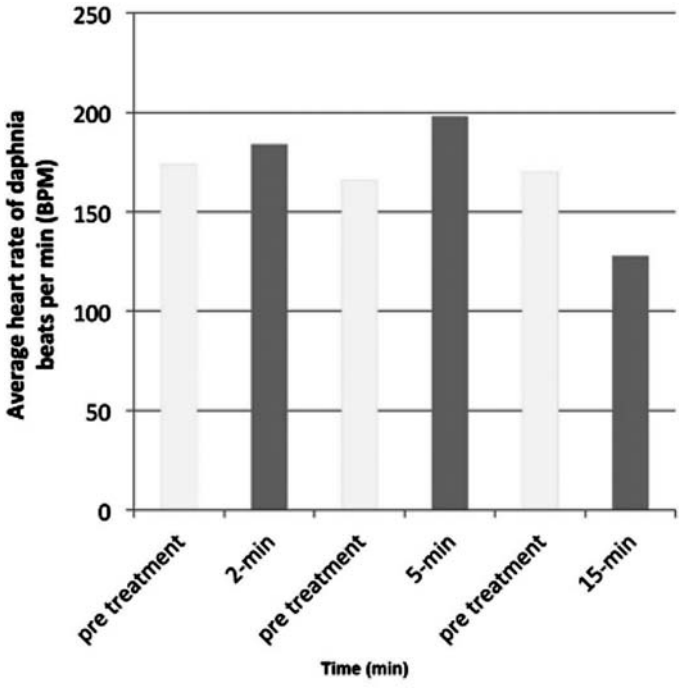

Figure 9. Effects of 0.05 M caffeine on the heart rates of *D. magna* at 2, 5, and 15 minutes.

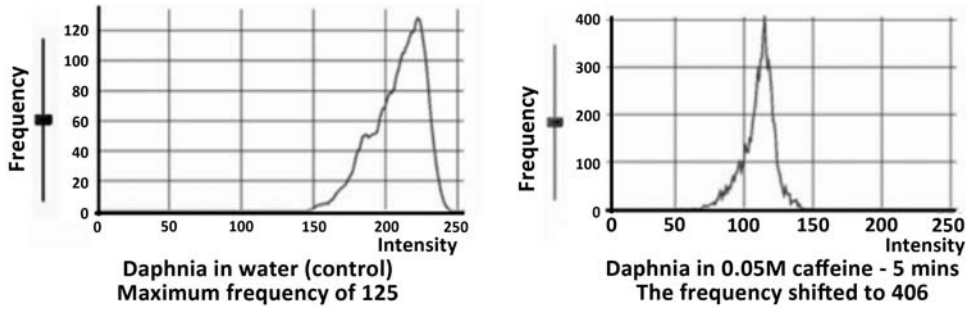

Figure 10. Effects of caffeine on light reflected by *D. magna*.

5, 3, 1, 0.1, and 0.05 mM concentrations of caffeine. Our work, and that of Foster (1997) and Drewes (1999), suggests that appropriate technology can be used to more accurately count the heartbeat of daphnia and use these organisms to demonstrate the stimulatory effect of caffeine on heartbeats.

Graphs (Figure 10) of the frequency versus intensity of light reflected by the organism were constructed for daphnia immersed in 0.05 M caffeine solution for 5 minutes and for a control. The graphs show that the control exhibited a maximum frequency of 125 frames per second (FPS); this corresponds to a mean heart rate of 167 ± 4 bpm as shown in Table 1. On the other hand, for daphnia immersed in 0.05 M caffeine for 5 minutes, there was a shift in the frequency peak to a maximum of 406 FPS; this corresponds to a mean heart rate of 192 ± 7.2 bpm as shown in Table 1. This change in frequencies results from a change in the reflection of light due to changes in the size and shape of the beating heart. The changes in frequency observed are not directly proportional but are correlated to the changes in heartbeat and size. These are visually compelling data.

Heart widths of daphnia in water and in 0.05 M (1% m/v) caffeine with 2 minutes immersion time were determined using the ROI in the basic research software to measure the width once every second. The results, shown in Figure 11, indicate that caffeine induced a significant increase in the width of the heart (and thus its size). Such an increase would lead to more forceful contractions of the heart. Caffeine is known to cause more forceful contractions in the daphnia heart, resulting in increased volume of blood ejected and, thereby, cardiac output (Nuffield Foundation, 2012). Measurement of the change of heart areas (as described) can be used by teachers to make concrete the concept that stimulants increase not only heartbeats but also cardiac output.

○ Conclusions

The use of daphnia to demonstrate and/or study the pharmacological effects of stimulants can be enhanced by videography such as described in this paper. The integrated imaging system is user friendly and can be easily installed and used in a high school setting. Its use can lead to more accurate and less tedious counting of heartbeats in daphnia. The system can also be used to provide complementary data such as the effect of pharmacological agents on the size of specific organs such as the heart. In addition, activities such as described in this paper can be used to introduce students to medical imaging.

The activities described in this paper are in line with many of the "eight practices of science and engineering" of the *Next Generation Science Standards*. For example, students undertaking the activities described will "use a variety of equipment and software to enter, process, display, and communicate information in different forms using text, tables, and pictures" (NGSS Lead States, 2013). These activities will likely lead students to ask questions about the physiological effects of stimulants, such as the following: How do stimulants work? What are the long-term effects of stimulants on the heart rate? What's the connection between Starling's law of the heart and this activity? Through guided inquiry, students will be able to plan and undertake investigations of the physiological effects of stimulants. In addition, students will be able to enter, analyze, and interpret data patterns using the technology described in this paper. Since many (including students) consume stimulants (such as caffeine) in their beverages on a daily basis, the activities lend to "engaging in argument (debate) from evidence."

○ Acknowledgments

We thank Lisa R. White and Vinton A. Melbourne of the media center at the Borough of Manhattan Community College for the tech support that they have provided us. A special thank you to Aijo DeCastro of Hunter College for his assistance with the live culture.

We also thank Abdulrahman Bah of the Learning Resource Center at the Borough of Manhattan Community College for his assistance and expertise in digital file formatting.

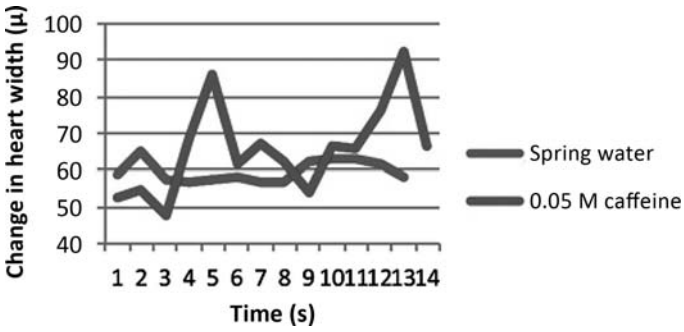

Figure 11. Effect of caffeine on width of the heart of *D. magna*.

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