

## Studying *Daphnia* feeding behavior as a black box: a novel electrochemical approach

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**Abstract** We present a novel approach for examining the complex feeding behavior of a filter feeder at a previously unexploited scale. A *Daphnia* lives in a viscous environment and thus creates a feeding current with a distinct laminar inflow and a repetitive pulsed outflow. We propose that by treating the feeding apparatus as a black box, and using the pulsed outflow current as a surrogate to the inside working of the apparatus, we can calculate feeding rate in near real time. The structure of the outflow is interpreted as a direct representation of the organism's response to its environment. Therefore, we examine how the work performed by an organism's feeding apparatus is altered according to environmental factors and metabolic demands. Our approach

is an integration of optical (Schlieren system) and electrochemical (chronoamperometry) techniques that allow for real time visualization and temporal analysis of flow systems, respectively. As electrochemistry requires a tracer chemical, we employed low dopamine concentrations ( $\leq 1\text{mM}$ ), and tested the effect of dopamine on the heart rate and swimming of *Daphnia*. It appears that dopamine free in solution at concentrations below 10 mM has no adverse effects on the organism, and all observed differences in *Daphnia* feeding behavior were due to environmental or metabolic factors. The feeding nature of daphnids in the presence or absence of food, and differences between the sexes is reported. Our results indicate that in the absence of food a *Daphnia* has a strict and repetitive feeding behavior with short delays between pumping actions. However, in the presence of food this behavior becomes complex, with increased delays between pumps, perhaps designed to maximize feeding efficiency. Our observations demonstrate that males have a higher appendage beat frequency than females under identical conditions. We hypothesize that the difference may be dictated by metabolic demand, as a male spends more time actively seeking a mate. The application of electrochemistry to the study of *Daphnia* feeding behavior is an improvement over current methods for its near real time quantification of behavioral response, its versatile application under varying environmental conditions and its extreme sensitivity to changes in the organism's feeding

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behavior. This technique is a valuable addition to the current tools available for studying *Daphnia* feeding behavior and will allow us to learn more about the interactions of an organism with its environment.

**Keywords** *Daphnia* · Feeding rate · Heart rate · Physiology · Electrochemistry

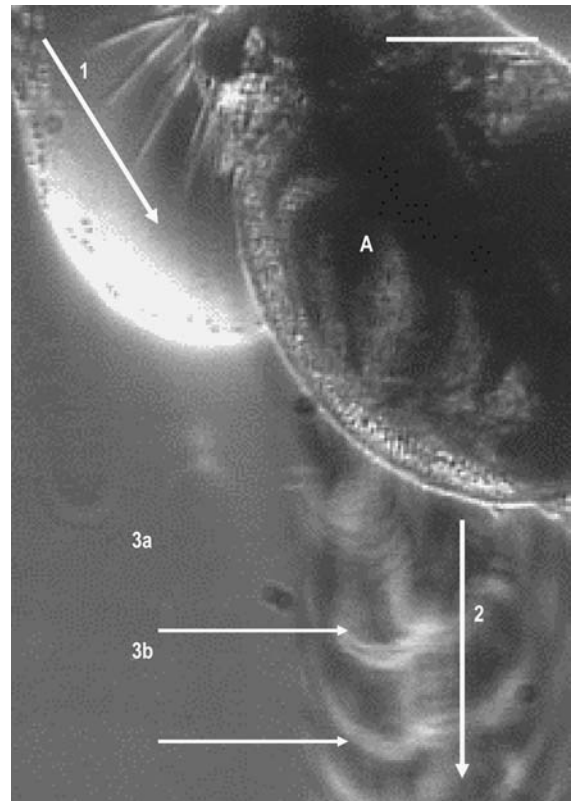
## Introduction

*Daphnia* is a key herbivore species in freshwater systems. Understanding its feeding behavior is important because of its impact on phytoplankton populations and its role in energy conversion up the food web (Lampert, 1987). *Daphnia* populations have the ability to remove a significant portion of the algae from a lake, as a single *Daphnia* can filter all the algae in 4 ml/h (Dodson & Frey, 1991). From the moment it is born *Daphnia* begin endlessly breathing and collecting food particles from the water column by rhythmically beating four morphologically complex thoracic legs that lie within its ventral region enclosed by the carapace. The thoracic legs, also known as feeding appendages, are the main components of the feeding apparatus, and their movement generates a current that brings oxygenated water in and enables *Daphnia* to feed on nanoplanktonic algae and bacteria.

The feeding system of *Daphnia* is a complex mechanism that can be studied at various scales. For one it can be broken down into individual components or it can be observed as a whole. Studying *Daphnia* feeding can be likened to observing the performance of a pianist, where one could observe his hands moving across the keys or by listen to the music. In both cases observing the movement of the appendages or digits can be difficult, both because of their placement and the speed at which they can move. In *Daphnia*, problems with lighting and viewing individual strokes within the carapace arise, which are overcome by relying on high-speed cinematography, computer video analysis, and demanding the animal be restrained in small volumes of water, thus stressing the animal during experiments (Gerritsen et al., 1988; Rudiger et al., 1997). However, we propose that it is easier to know how they are both performing their job by “listening” to the produced effect. In the case of *Daphnia*, this

listening is done by observing and analyzing the flow that the organism is creating, in other words observing the organism’s interpretation of its surroundings.

Aquatic invertebrates, such as *Daphnia*, are known to have a large repertoire of behavioral responses within their aqueous environment. The responses are dictated by the organism’s ability to integrate all sensory input and respond accordingly. Therefore when feeding, each appendage in the feeding system moves in an orchestrated and precise manner to achieve optimal flow through the carapace under the given conditions. A *Daphnia* lives in a viscous environment and thus creates a feeding current with a distinct inflow that brings particles into the feeding apparatus (Fig. 1) and a repetitive laminar outflow (Vogel, 1994; Yen & Strickler, 1996). The outflow is



**Fig. 1** A *Daphnia* tethered to a hair and visualized using the Schlieren optical system. A dextran solution is delivered through a micropipette for visualization of the flow generated by the feeding appendages (A) of *Daphnia*. Arrow 1-point of entry of laminar inflow, 2-direction of parceled outflow, and 3a & b-repetitive waves or fluxes in the solution generated by the feeding mechanism. Bar = 0.2 mm

a direct result of the actions orchestrated by the feeding appendages and we hypothesize that by treating the feeding system as a black box and only investigating the rate of the outflow we can effectively determine the feeding rate of *Daphnia*. This would eliminate the need to observe individual components and would instead use flow rate as surrogate for feeding rate. Past research has equated appendage movement with feeding (Colmorgen & Paul, 1995; Haney et al., 1995; Lampert & Brendelberger, 1996; Ghadouani et al., 2004), yet we believe that in the case of *Daphnia*, because of the dual role the thoracic appendages play, movement of appendages could reflect only respiration and not necessarily feeding. This is an important distinction as it is possible to observe movement of appendages when the carapace is closed and no feeding current is being generated by the organism, thus movement does not expressly mean that feeding is taking place (personal observation), and observing the movement of thoracic appendages alone does not represent feeding behavior. Therefore the need exist for a system that can observe and measure movement of fluid through a *Daphnia*'s feeding apparatus, thus letting the observer know when feeding and breathing are occurring.

Our approach achieves visualization and direct analysis of the outflow by combining Schlieren optical system and electrochemistry. Using Schlieren optics and electrochemistry we can track a tracer fluid's path across the feeding apparatus (for more information on Schlieren techniques see (Strickler & Hwang, 1999)). The combined optical and electrochemical techniques allow for the monitoring of changes in the flow rate created by the feeding system of *Daphnia*.

Microelectrodes have long been used to trace chemicals as they move through flowing systems (Kuhr & Wightman, 1986; Gerhardt, 1987; Wightman et al., 1988). Although developed and widely utilized for monitoring neurotransmitter concentrations within the central nervous system the application of microelectrodes has been successfully transferred to research in aquatic environments (Moore et al., 1989, 1991; Keller et al., 2001). Electrochemistry is an effective tool for studying flow because when a tracer is placed in a current, a sensor can be placed downstream to track the tracer's path. When the tracer comes into contact with the

sensor (i.e., a microelectrode) a chemical reaction occurs that relays a change in current (i). This electrochemical signal is then analyzed to understand the system generating the flow.

Dopamine is a commonly used electrochemical tracer because it has been successfully utilized in aquatic systems, has a diffusion coefficient of  $2 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$  at 20°C in water, and minute concentrations of it can be easily detected by microelectrodes (Schneider et al., 1998). Dopamine is a well-known neurotransmitter in humans, but is also found in many invertebrates (Blenau & Baumann, 2001; Mustard et al., 2003, 2005). There is to date no literature available that investigates the role of dopamine on *Daphnia*. However, dopamine is known to decrease feeding response in honeybees when injected directly into the thorax, but has no effect on the organism when fed dopamine in a sucrose solution (Scheiner et al., 2002). We conducted experiments to observe if the behavior of *Daphnia* was affected by exposing them to various dopamine concentrations free in solution. We hypothesize that in short-term exposures (<5 min) and at low concentrations (<1 mM), dopamine would not adversely affect *Daphnia* feeding behavior.

The objective of this article is to test the hypothesis that electrochemical techniques combined with optical techniques can be used to investigate the feeding behavior of *Daphnia*. In this article the outflow rate and appendage beat frequency (ABF) are used interchangeably as the outflow rate measured is generated by the pulsed beat of the appendages. It is known that *Daphnia* alter their feeding rate according to food availability; therefore, we hypothesized that by monitoring outflow patterns we would observe variations in outflow rates with changes in food levels. We also hypothesized that differences in feeding rates between the sexes must exist to compensate for their distinct swimming tactics. We test the hypothesis that males must have a higher appendage beat frequency (ABF) thus feed more than females to satisfy a higher-metabolic demand as they require more energy to actively search for a mate (Burns, 1968; Porter et al., 1982; Brewer, 1998; Plath, 1998; Kmet & Straskraba, 2004; Darchambeau & Thys, 2005). Success of the application of this system is evaluated by comparing reported appendage beat frequencies with the pulsed outflow rate acquired with our system.

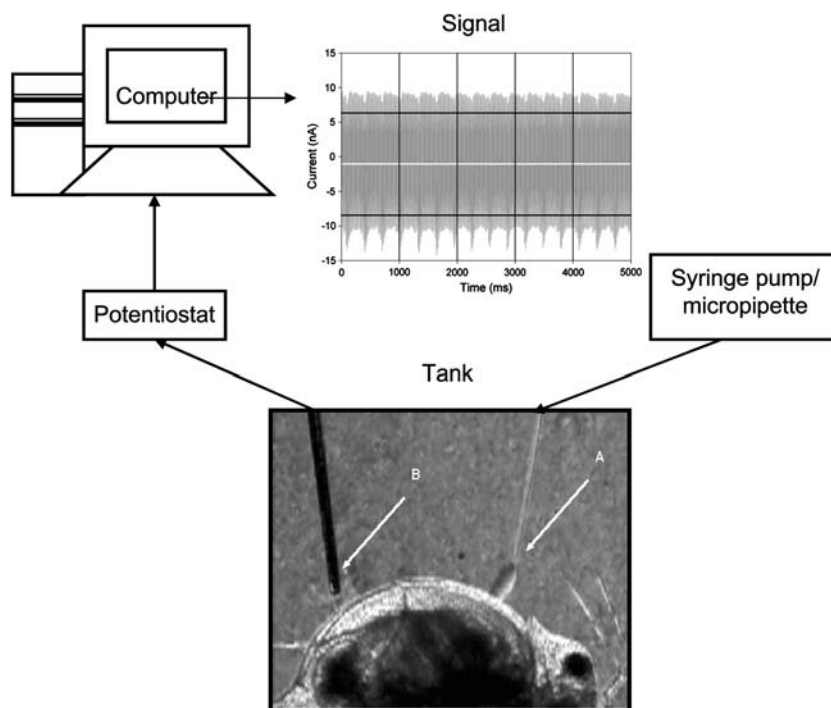
## Materials and methods

### Animal handling and experimental protocol

Experiments were performed at room temperature ( $\sim 22^\circ\text{C}$ ) using adult *D. pulex* and *D. magna* obtained from our lab cultures (clone obtained from S. Dodson, Madison, WI, USA). 10 *D. pulex* females, and 10 male and 10 female *D. magna* were tethered to hair using glue and placed in a 3 l tank containing aerated deionized filtered water or filtered ground water. They were allowed to acclimate to the experimental conditions for 30 min prior to beginning recordings. The tracer solution was delivered into the inflow current at a rate of 5  $\mu\text{l}/\text{min}$  with the use of a microsyringe, a micromanipulator, and a syringe pump. The tracer solution consists of: 1 mM dopamine, dextran ( $8 \times 10^{-5}$  g/ml—for Schlieren visualization), and 0.05 mM ascorbic acid (as an antioxidant). A microelectrode was placed in the

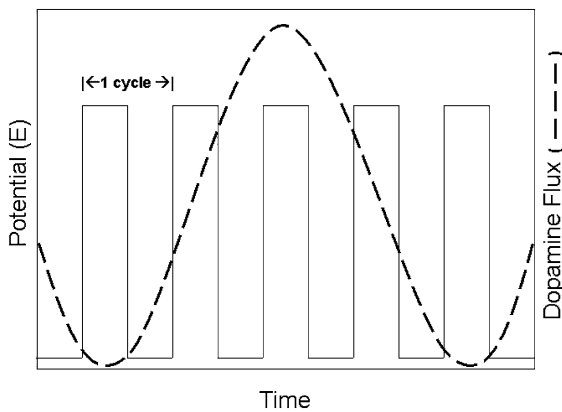
outflow current of the different *Daphnia* to record the fluxes in dopamine generated by the feeding apparatus using a micromanipulator (Fig. 2).

The outflow is comprised of a series of waves or beats. With this system, when the outflow consists of variations in dopamine flux, the observed current ( $i$ ) is the highest at the strongest point of the appendage beat followed by a brief rest period, seen as a trough (Fig. 3). The pulsed outflow rate of *D. pulex* was determined using a custom-designed recording system built around the IVEC-5 (Medical Systems Corp.). The electrochemical recordings were made at a 25 Hz frequency: analog to digital conversion of input occurred at 1 kHz. Data were acquired in ASCII format and saved for analysis. The frequency measurements involved the recording of square wave pulses (0- +0.55 V vs. an Ag/AgCl reference electrode) with 2200 mA sensitivity. For analyzing the ABFs of *D. magna* of different sexes we used a series of 20 s chronoamperometric recordings made at



**Fig. 2** A basic schematic of the outflow recording system. The micropipette (A) is used to deliver the tracer, which is picked up by the laminar inflow current of the *Daphnia* and travels through the feeding mechanism. The microelectrode (B) is placed in the outflow current. The flux, the moles of dopamine flowing over the electrode per second per unit area of the

electrode surface, is detected by the potentiostat and recorded by the computer during the multiple chronoamperometric experiments (see methods). This permits an analysis of the dynamic movements of the feeding appendages by calculating the number of spikes over time. Bar = 0.2 mm



**Fig. 3** The standing square wave chronoamperometry approach. The dotted line represents the flux in dopamine expected as the waves created by the feeding mechanism of the *Daphnia* pass over the microelectrode. The square waves are the potential imposed on the microelectrode over time (input waveform) and the current ( $i$ ) of the output due to dopamine oxidation is measured. A rate of 50 Hz was used to map out the appendage beat frequency of *Daphnia*, which in turn provide a total of 125 current ( $i$ ) measurements to detect each *Daphnia* generated flux (which occur at a rate of 3–6 Hz)

50 Hz, employing a new square wave pattern built around a CS-1200 Computer Control Potentiostat (Cypress Systems). The chronoamperometric recordings were made at a frequency of 50 Hz (analog to digital conversion of input occurred at 100 kHz), a 450 mV positive potential step starting from 300 mV versus Ag/AgCl reference electrode, 20 nA sensitivity, and a 0.10 s sample interval. When running the experiments at 50 Hz and a 0.10 s sample interval, there are about 12.5 potential steps (or chronoamperometric runs) per wave generated by the organism, assuming an approximate outflow frequency of about 4 Hz (i.e.,  $50/4 = 12.5$ ). This in turn means that a total of 125 current ( $i$ ) measurements are taken per beat; allowing us to trace each individual peak and trough with increased clarity. When current response output is graphed over 20 s, we see a series of “peaks,” from which the feeding rate or ABF is calculated. We designed the electrochemical wave pattern to reduce the high degree of variation observed using the IVEC system.

#### Optical and video system

A Schlieren optical system was used to visualize the organism and the tracer solution as it traveled through the feeding apparatus of each *Daphnia*. It also

assisted in the correct placement of the micropipette for tracer delivery, positioning the microelectrode and aided in assessing overall animal condition. The image was displayed using a BW-camera and a BW monitor (Sony).

#### Microelectrode fabrication

Working microelectrodes were constructed from 10- $\mu$ m radius carbon fiber (Thornel P-55) sealed in glass capillaries (for detail description see (Baur et al. 1988). Under our experimental conditions recording frequencies of 10–200 Hz can be achieved with these microelectrodes. All microelectrodes were tested and calibrated in the tracer solution using cyclic voltammetry prior to beginning an experiment, pH 7.4 (Skoog et al. 1998).

#### Dopamine exposure experiments

In order to determine if dopamine alters *Daphnia* behavior, we conducted control experiments. Free swimming *Daphnia* were exposed to a range of dopamine concentrations (0–100 mM), and observed over a 6 h period for differences in swimming behavior or lethal effects in 1 l tanks. Using high-speed cinematography we also monitored the heart rate of 10 tethered *Daphnia* exposed to the same range of dopamine concentrations. An inverted microscope (Zeiss Axioskop) was placed on its side and fitted with a high-speed camera (Fastcam, Photron) set to record at 125 frames/s. Micromanipulators were utilized for placement of the tethered *Daphnia* in the field of view within a 20 ml vessel. Each *Daphnia* was continuously exposed to the given concentration for 1 h, and the heart rate recorded every 5 min. The microscope’s light source was kept at its minimum setting to reduce possible changes in temperature during each experiment.

#### Data analysis

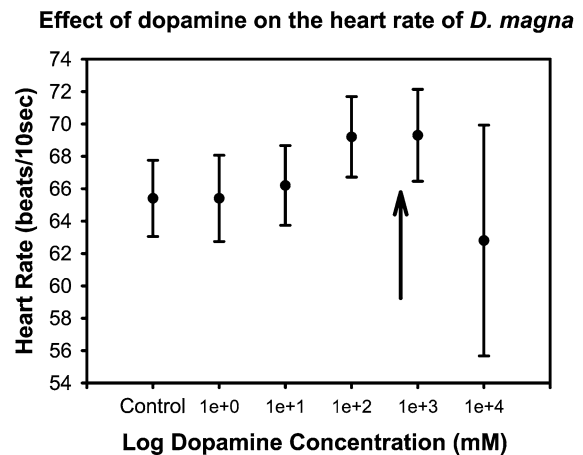
Due to shifts in the tethered *Daphnia*’s position, not all electrochemical recordings collected could be used for analysis. Only those which contained a full uninterrupted electrochemical signal were utilized. The feeding frequency data of *D. pulex*, recorded in the presence and absence of food using the IVEC system were examined using various time series analysis

techniques. However, none were capable of capturing the periodicity observed in the outflow signal. Therefore the signal was examined using a dynamic system theory approach. An associated nonlinear time series analysis is capable of extracting the dynamic characteristics of the feeding mechanism based on the electrochemical data. By using 3D phase space diagrams (PSD) we were able to graphically represent the structure of the responses, and compare them quantitatively (Guckenheimer & Holmes, 1983; Wang & Wang, 2000). PSDs are a way of autocorrelating data that is non-linear. It graphs the data against itself by shifting the data points, similar to an autocorrelation, until a strong correlation is achieved. PSDs allow for a dynamic and apparent chaotic system to be analyzed, and if the signal is not random, the strongest correlation is found when the shift results in all points lining up along a plane. This plane is known as an attractor. This graphical representation allows you to visualize the near-linear periodicity of the system, and compare each treatment's lag to approximate the time it takes for the signal to repeat itself. The 3D phase space plots were produced using MatLab (Mathworks Inc.). All graphs contained >5,000 points and were divided for examination by *Daphnia* and food treatment. Assessment of the effect of different dopamine concentrations on the heart rate of *Daphnia* was done with a Friedman's Repeated Measures ANOVA test (SigmaStat). Analysis of covariance (ANCOVA) was used for comparing the difference in ABF (dependent variable) according to sex and body size (JMP statistical software).

## Results

### Short-term effects of dopamine exposure on *Daphnia*

Our results on the heart rate of *Daphnia* are in agreement with previous reported rates of approximately  $65.4 \pm 2.36$  beats/10 s (Spicer and Morrill 1996; Baumer et al. 2002). This indicates that dopamine does not significantly alter *Daphnia* heart rate at concentrations below 10 mM after 1 h. Most variation in heart rate (Fig. 4, SEM = 7.13) is seen when the *Daphnia* are exposed to concentrations  $\geq 10$  mM of dopamine, yet it was not statistically significant. A qualitative observation on the swim-



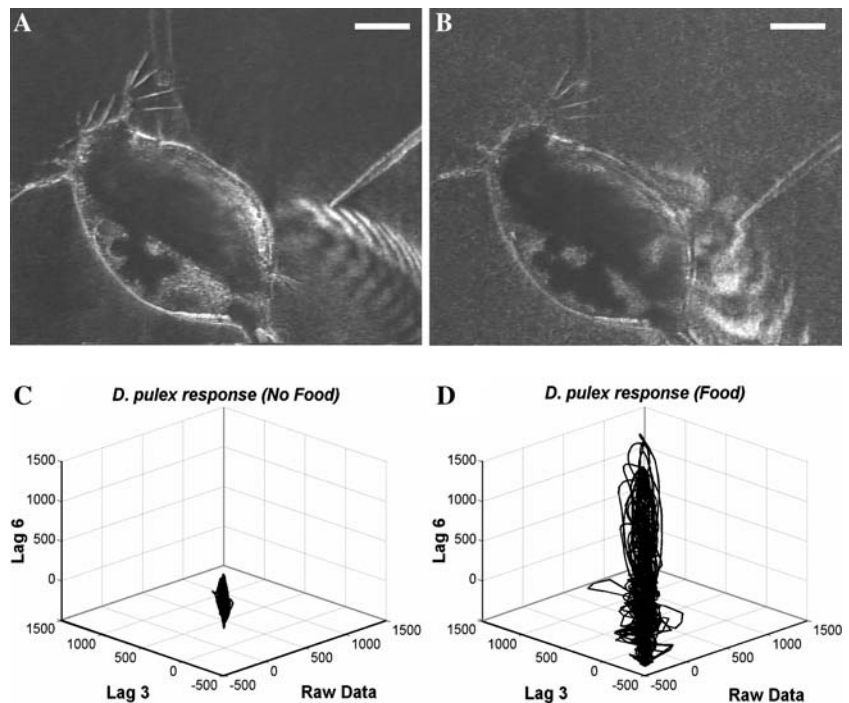
**Fig. 4** Effect of increasing dopamine concentration on the heart Rate of *D. magna*. The heart rates of 10 different *D. magna* were monitored as dopamine concentrations were increased from 0 to 10 mM by a factor of 10 each time. Each *Daphnia* was monitored for a period of 1 h and heart rate was recorded every 5 min for 10 s at 125 frames/s, using a high-speed camera. Standard error is reported for each concentration after averaging the response of all 10 animals. Treatment means were not statistically distinguishable, but the 10 mM treatment was the most variable ( $F_{5,54} = 0.448$ ,  $P > 0.80$  One-way ANOVA). Arrow indicates our working concentration of  $\leq 1$  mM

ming behavior of *Daphnia* in the presence of dopamine was performed. Only concentrations  $\geq 100$  mM elicited erratic swimming behavior in free swimming *Daphnia* and were lethal after 10 min of exposure.

### Monitoring *Daphnia* feeding behavior

The IVEC/Schlieren system allowed us to visualize, analyze, and compare the feeding behavior and generated outflow of *D. pulex* under two conditions: when no food was present ( $n = 6$ ) and when *Chlamydomonas reinhardtii* ( $n = 4$ ) was added free in solution to the tank above the organism at unmeasured concentrations and allowed to be suctioned into the feeding current (Fig. 5A & B). The structures of the PSDs were analyzed using various time lags in order to find the most fitting for our organism's appendage beat rate. All of the time lags examined had a similar structure and none occupied the entire 3D space, as expected if the data are completely random and have no attractor. The data were best represented by a lag of three for all treatments and individuals, concentrating all points along a tight

**Fig. 5** Schlieren system images under the two different conditions with their respective 3D Phase Space Diagrams. The difference can be seen visually between: **(A)** The more regular beat pattern of *Daphnia* when no food present; and **(B)** the more dynamic beat pattern when food is present. Dopamine concentration distribution as visualized using phase space diagrams over 60-s ( $n = 5,000$ ) when no food present **(C)**, and when food present **(D)**. Bar = 0.25 mm



plane (i.e., an attractor). The PSD of the feeding behavior of *D. pulex* when no food is present is more concentrated and predictable, indicating that the organism is pumping water at a near constant rate. When food is present the feeding behavior changes to a more complex array of responses from the organism. The more complex plane indicates that there is a larger delay between beats when algae is present, yet the system remains predictable as it falls along a given plane. Therefore, PSDs help to evaluate the IVEC signal, and demonstrate that a *Daphnia's* feeding behavior differs with the availability of food. When food is present the organism slows down its feeding frequency (longer delay between beats). Any deviations from the plane represent different behaviors exhibited by the organism, in Fig. 5D, the line away from the plane represents a rejection event, as the abdominal claw was raised to remove a bolus of food. At this point the signal experienced a delay between peaks and received a pulse of dopamine at a time outside the normal cycle (Fig. 5C & D).

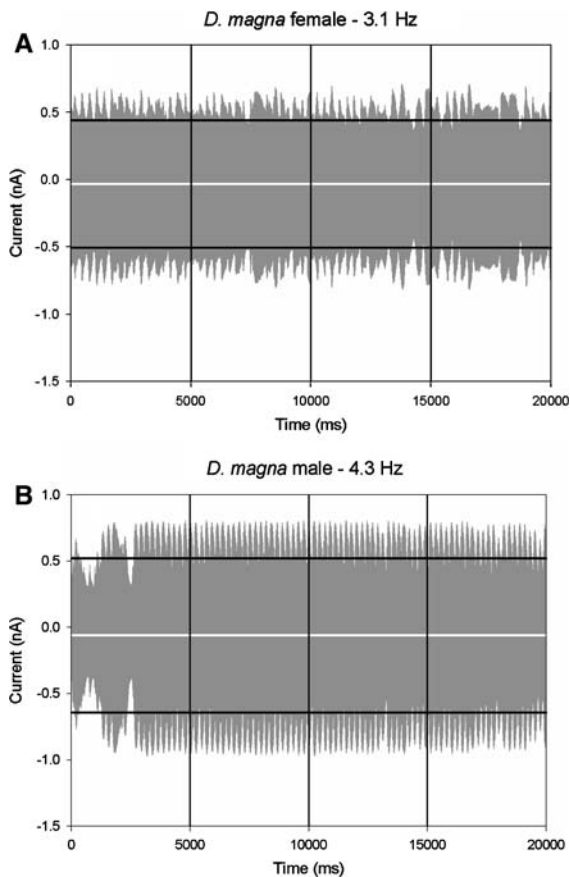
Appendage beat frequency differences according to sex

Using the chronoamperometric technique, we observed that male and female *D. magna* have different

appendage beat frequencies, regardless of size of the individual organism in the absence of food ( $P < 0.001$  effect of sex and  $P = 0.17$  effect of size) (Fig. 6). Male *D. magna* have a higher appendage beat frequency, averaging 5 Hz. Female ABF remained close to 3.3 Hz (Fig. 7). No statistical significance was observed within the sexes with regard to size (Table 1—ANOVA, females  $P = 0.1305$ , males  $P = 0.2731$ ).

## Discussion

Monitoring the feeding behavior of *Daphnia* with the IVEC system proved successful as our calculated outflow rates and changes in feeding behavior are in agreement with other research conducted on *Daphnia* appendage beat frequencies and copepod feeding behavior under changing food conditions (Burns, 1968; Yule & Crisp, 1983; Fryer, 1987). Exposure to low levels of dopamine for short durations does not appear to adversely affect *Daphnia*. This is not surprising as noted previously that feeding dopamine alone does not affect feeding behavior in other invertebrates. Both IVEC and chronoamperometric signals demonstrate a periodicity in the generated outflow. The acquired signals showed comparable

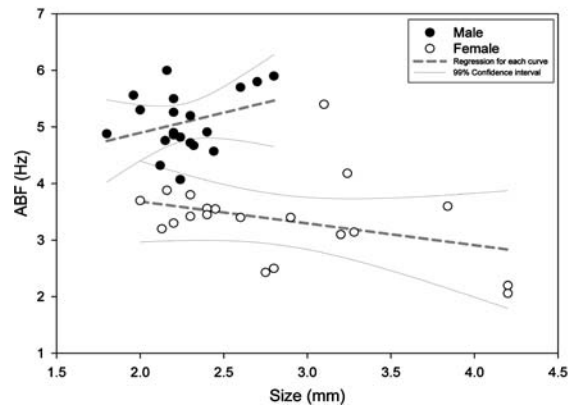


**Fig. 6** Chronoamperometric signal. As the outflow carrying the tracer crosses the microelectrode surface we record the appendage beat frequency (ABF) of the organism. Example of electrochemical signal, where the number of peaks in 20 s intervals were counted over time to assess ABF. (A) A single female signal—appendage beat frequency of 3.1 Hz; average ABF for all females 3.3 Hz. (B) A single male signal—appendage beat frequency of 4.3 Hz, average ABF for all males 5 Hz. This individual example of frequency differences corresponds to approximately 72 more beats per minute for the male

results across individuals. Changes in dopamine flux can be observed whenever the outflow is altered by the organism; as is the case when the *Daphnia* raised its abdominal claw to cleanse, rejected particles or simply changed the rate of appendage beating to satisfy environmental conditions.

No apparent adverse effects of dopamine on heart rate were observed on the organism during our control experiments while exposing them to concentrations from 0 to 10 mM (Table 1). Heart rates of exposed *Daphnia* were well within previously reported rates, and treatment means were not

Appendage Beat Frequency of Male vs. Female *D. magna* according to size



**Fig. 7** Relationship between body size and appendage beat frequency (ABF) for males and females (○—females, ●—males). Males have a significant higher ABF than females, ANCOVA ( $P < 0.0001$ ). Dotted lines represent a regression fit for each sex and a 99% confidence interval of each. Most data points fall within their corresponding confidence interval demonstrating the strong correlation of the ABF according to sex

**Table 1** Analysis of covariance for sex and body size of *D. magna*

Source	df	Sum of squares	F ratio	Prob > F
Size	1	0.7654	1.8907	0.1774
Sex	1	18.0969	44.6986	<0.0001

The appendage beat frequency of 20 male and 20 female *D. magna* were analyzed using one-way ANCOVA, with ABF as the dependent variable. The appendage beat frequency was determined for each daphnid from 20 s chronoamperometric experiments, for a total of 10 min measuring time. Statistically significance was seen only between ABF and sex ( $P < 0.0001$ ) but not for ABF and size ( $P = 0.1774$ , One-way ANCOVA, 39 total degrees of freedom)

statistically significant even at concentrations above 1 mM. However, at 10 mM we saw the highest level of variation in heart rates. To date it is unknown whether *Daphnia* possess the necessary receptors for dopamine uptake free in solution, and whether it has an effect on its feeding rate. However from our observations we conclude that changes in outflow rates between *Daphnia* and the sexes are not in response to the low levels of the tracer chemical.

The new chronoamperometric wave pattern designed by our team proved to be a more precise method for monitoring the dynamic outflow generated by the feeding appendages of *Daphnia*. Of great

significance is to note that with either electrochemical technique it is apparent that *Daphnia* has a dynamic feeding mechanism. During feeding a *Daphnia*'s feeding apparatus repeats its movements constantly, yet they do not repeat at exact intervals, thus making it a dynamic system. This was observed in copepods by Cannon as well when studying their filter feeding (Cannon, 1928). This new understanding supports the idea that *Daphnia* are constantly adjusting their feeding rates to current conditions. It is possible to see in the phase space diagrams that when no food is present *Daphnia* have a higher ABF, which can be deduced from the shorter delay between beats. However, with the introduction of food the outflow becomes a complex interaction of behaviors and the beats are delayed, thus having a lower ABF than when scanning for food. In the presence of food the daphnids exhibited a large array of feeding movements, from slowed pumping to increase rejection events. It will be important to investigate whether the behavior is a response to chemical and/or mechanical cues. However, our results makes it possible to support the concept that in the presence of food a *Daphnia* may be a selective feeder by controlling how food is collected and the amount of energy spent collecting particles.

Appendage beat frequencies acquired by measuring pulsed flow rate are overall lower than rates previously reported using individual appendage movement alone (e.g., 6–9 Hz for males in Porter et al. (1982) vs. 4–6 Hz for males in this study). Our results are the only study to date that utilizes the outflow pattern as a surrogate for appendage beat frequency and directly compares the appendage beat frequencies of male and female *D. magna*. Porter (1982), while exploring the effect of food concentration of *Daphnia*, reported the ABF of females and included male data in a graph, but there is no mention of the males in the text, and no conclusions are drawn from the observed differences.

Males had a significantly higher appendage beat frequency than females (5 vs. 3.3 Hz, ANCOVA  $P < 0.001$ ). Male daphnids are produced under times of stress and their priority is to mate. Unlike females that hop and sink in the water column, males demonstrate a swimming pattern known as scanning, swimming horizontally across the water in search of mates. This swimming behavior could in turn place a higher metabolic demand on the male, as more energy

would be required for maintaining consistently high swimming speeds. Allometric effects do not appear to play a role when comparing individuals of the same species (Plath, 1998), and appear to not to play a role between the sexes either (ANCOVA  $P > 0.177$ ).

These methods efficiently monitored the feeding behavior of *Daphnia* and has the capability of real time monitoring. Currently a basic application of time series analysis cannot be applied to the data due to the signal's peak structure and dynamic non-linear nature. Therefore the next step will be to develop a program that will evaluate the frequency of the outflow based on peak separation, thus obtaining an average ("true value") of frequencies. This will stream the data and immediately determine any changes in the animal's feeding response. Our potentiostat is capable of acquiring data at frequencies of up to 200 Hz, which may help to provide us with an increasingly detailed frequency analysis to determine minute changes in behavior. It is also our goal to integrate the electroanalytical signal output (see Figs. 3 & 5) with high-speed cinematographic recordings, so that behavior and physiology (e.g., heart rate) can be jointly assessed in real time, thus also creating a library of visible behavioral responses.

It is important to note that the electrochemical system can be utilized without the need of the Schlieren optics. Once the organism and tracer solution are tethered in place, simply moving across the *Daphnia*'s ventral region with the microelectrode can allow you to detect and record the outflow with the potentiostat. The system does not require the use of deionized water, as we have tested filtered lake water and have had similar results. This system is accessible and relative inexpensive as the potentiostat is the most valuable piece of equipment, and the microelectrodes can be easily made in the laboratory. Thus, this system can become widely used to explore feeding and breathing behavior of *Daphnia*.

A debate still ensues on the existence of chemoreceptors on *Daphnia* and whether food particle quality can be sensed by it (Demott & Schoenberg, 1988; Kerfoot & Kirk, 1991). We hope to apply this method to the examination of how feeding is altered in the presence of different chemicals and food particles to assess changes in feeding behavior. This method is a new tool for analyzing the larger picture. It is able to record the frequency at which the organism is functioning, giving us a sense of its

perception of the surrounding waters, and can also illustrate the control and refined intricacies of the feeding apparatus and all its components. By designing a new approach that examines a behavioral response at a different scale than previously done, we are able to improve our overall understanding of the organism's response to its environment. We believe this method holds the key to appreciating the plethora of feeding responses this organism can have in its surroundings and help to examine differences among species and taxa.

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