Heat output by marine microbial and viral communities

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Abstract

The Marine Microbial Food Web (MMFW) includes heterotrophic microbes and their protist and viral predators. These microbes consume dissolved organic matter thereby making the MMFW a major component of global biogeochemical and energy cycles. However, quantification of the MMFW contribution to these cycles is dependent on a handful of techniques, all of which require laboratory-derived conversion factors. Here we describe a differential calorimeter capable of measuring the small amounts of heat produced by marine microbes and viruses at natural populations. Using this ultra-sensitive calorimeter, we show that heat production in the presence of viruses is significantly larger than in their absence. This increased heat output occurs despite a net decrease in the number of microbes. This provides direct evidence for top-down control of microbial populations by viruses and shows that there is increased re-mineralization. A comparative statics model was developed to interpret the calorimeter measurements. The spirit of the model is thermodynamic – it restricts its view to net changes in the populations and net heat produced. The model predicts that approximately 25% of the total heat production during the growth phase of a pelagic microbial community is due directly to viral activities. This result has implications for the energy budget of our planet and for climate prediction.
1 Introduction

Viruses are important marine microbial predators [1–4]. In combination with protist grazing, viral predation is so intense that microbial numbers are maintained at values less than the carrying capacity of the system. This is called top-down control [5,6] and has important implications for cycling rates of nutrients and energy in the world’s oceans.

Dissolved organic matter (DOM) is the largest stock of biologically active carbon on the planet. DOM is essentially only consumed by heterotrophic microbes (both Bacteria and Archaea). Therefore, a major challenge in understanding global biogeochemical cycles, and particularly the carbon/energy budget, is quantifying the use of DOM by heterotrophic marine microbes. This has been a major methodological challenge since the discovery that there are approximately 0.5 million microbes per milliliter of surface seawater. The main approach involves feeding microbes trace amounts of a radio($^3$H)- or chemical(Br-dUTP)-labeled metabolic intermediate (e.g., amino acids or thymidine) and then measuring incorporation into new microbial cells. This yields a measure of new cell production. The energy usage is estimated with conversion factors derived from laboratory experiments [7–12]. These conversion factors are the weak point in this approach and remain major topics of disagreement ever since their introduction. Other methods of measuring cell production include dilution experiments and direct counts. Again, conversion factors must be used to estimate energy expenditure. Changes in dissolved oxygen have also been used to measure microbial production. These methods are limited by sensitivity and the fact that much of the MMFW metabolism may not use typical fixed carbon to oxygen respiration.

The measurement of heat from biochemical processes using a calorimeter offers a direct approach for monitoring microbial activities via their energy usage [13, 14]. Calorimeters have been used to measure biological activity for more than two hundred years, but only in recent years has instrumentation become sufficiently sensitive to potentially perform such measurements on marine microbes and virus communities at natural densities. Due to limited sensitivity of available instrumentation, it has previously been necessary to artificially induce measurable amounts of heat production in these populations by compressing lab samples of pelagic microorganisms far beyond (100 times or more) their naturally occurring densities [15–20]. This causes an observably significant disturbance to the microbial population. For example, one common approach to measuring heat from marine microorganisms has been to concentrate the population
Heat output by marine microbial and viral communities

[19, 20] by filtration and back-washing. This tends to compound the problem; marine microorganisms are typically non-culturable, and any significant disturbance to their natural environment can cause major changes in behavior. Thus it is desirable to conduct measurements without disturbing the natural environment, or at least by simulating that environment as closely as possible. Improved electronics design and computerized control practices have now made feasible calorimetry techniques to measure such natural or simulated communities.

Here we present the design of an isoperibol, temperature-rise, and differential calorimeter capable of measuring open ocean marine microbial heat production at populations of around $10^5$ cells/ml. The calorimeter at temperature of 298 K has a sensitivity of (ca. $10^{-6}$ K). This calorimeter was used to measure the effects of viral predation on heat production. To interpret the measurements, a simple comparative-statics model was formulated. The results show that production-predation cycle in a simulated pelagic community decreases the total microbial cell count (net production) while increasing the total production (number of microbial replications).

2 Materials and methods

2.1 Calorimeter design

Calorimeters are commonly classified into two main types: isothermal, where the system is maintained at a fixed temperature by heat transfer to or from the surroundings, which thus provides a measure of the changes in the system; and adiabatic, where heat transfer between system and surroundings is prevented, so that the temperature change in the system provides the measure of heat. Neither strictly isothermal nor strictly adiabatic calorimeters are suited to measurement of the small heat changes involved in the pelagic systems considered here, because of the intrusive nature of the controls required to maintain the respective conditions. However, approximations to both types have the potential to achieve the desired results.

Currently, the heat conduction calorimeter (pseudo-isothermal) is the most common commercial type, in which heat is allowed to leak to or from the surroundings through a thermopile, which thus provides a direct electrical measure of the heat changes in the system [14]. However, isoperibol (pseudo-adiabatic) calorimeters, by which the system is insulated from isothermal surroundings and corrections are then applied for known heat leakage, have the advantage of simplicity of construction. An isoperibol differential twin calorimeter, in which a reference system is placed inside
the same isothermal enclosure as the measured system so that variations in heat leakage can be compensated continuously, has been constructed in our laboratory and appears to have sufficient sensitivity to monitor heat production from wild marine microbial communities at population densities typically found in pelagic environments ($10^5$ cells/ml).

The calorimeter was constructed as follows. Reaction and reference glass vessels of approximately 25 ml capacity were placed in separate insulated Dewars within a 200-L water bath. The water bath temperature was regulated with a thermostatic controller (Sargent-Welch Co., Model ST; Buffalo, NY) to give a temperature stability of $\pm 0.005$ K. Thermistors (VEECO 0.100 glass probes series, 20 MΩ) were used as the temperature-sensing elements and the reaction and reference vessel thermistors formed two arms of a Wheatstone bridge.

The long stems of the sample vessels are a press fit through the Styrofoam stoppers of the Dewars, which basically act as internal air jackets (25 ml sample vessels in 500 ml Dewars). The Wheatstone bridge is driven by approximately 4 V batteries with the thermistors in series, so the power dissipation in both thermistors combined is on the order of 0.4 $\mu$W. The thermistors were matched as closely as possible, and the difference in temperature change between sample and reference was small, so that self-heating was internally compensated by differential measurement. In 24 h, error due to difference in self-heating is less than 0.1% of the total recorded output. Bridge current was approximately 100 nA. Thermistors were supplied sealed in glass; initially, we tried suspending them in contact with the sample but considerable electrical leakage was found. Placing the thermistors in thin glass tubes with a little oil for thermal contact (NMR tubes worked well) seemed to eliminate the problem. Leads were bundled together and wrapped in Teflon tape to maximize compensation. The Dewars act as additional insulating air/vacuum jackets, and the sample vessels were assumed to be small enough that thermal conduction was adequate without stirring. It was also assumed that initial sample composition distribution was uniform. Temperature sensitivity of the bridge is $10^{-5}$ K. There was some bridge output amplification (normally $10^3$–$10^4$) using a Keithley 155 microvoltmeter.

A personal computer was used for data collection and control via a 16-bit data acquisition board (Keithley, Inc., Model DAS-1802HR; Cleveland, OH). The calorimeter was provided with a calibration heater through which the chemical generation of heat by microbes was simulated by introducing a carefully measured quantity of electrical energy [21]. A simplified diagram of the calorimeter is shown in Figure 1.
An electrical calibration heater (11 Ω) was made of glass-fiber insulated nickel-clad copper wire (number 21 gauge) with a four-wire connection. This insulated electrical calibration heater was inserted into a glass well in the calorimeter through the lid of the vessel. For better thermal contact, the space between the electrical calibration heater and the well was filled with silicone oil. The calorimeter calibration heater is in the feedback of an operational amplifier (op-amp) and a standard resistor of 10.00002 Ω measures the current in the circuit, which is adjustable by the software. This introduces a precisely known amount of electrical energy into the system. Measurement of the voltage across this resistor gives the amount of current. The voltage across the heater is also measured. The software can then continuously calculate the total energy introduced into the calorimetric system until this energy reaches a predetermined value. The circuitry for generating and measuring the electrical energy has been described previously [21].
Electrical leakage to the thermistor was evident, but the bridge recovered in a few minutes and temperature rise appeared nearly vertical on the time scale of the experiment.

Heater power was 1–100 mW. Platinum thermometers were only used in the stirred outer bath, which was equipped with a control heater with power that was about $10^9$ times larger than the thermometer self-heating. Sample and reference containers and Dewars were switched with no apparent differences. Sample and reference vessels were 80% filled, so there was an air space in the long and relatively narrow stem. The wiring was wrapped with Teflon tape to make a not-quite-airtight seal at the top of the stem to minimize evaporation.

The calorimeter was calibrated electrically. The overall accuracy of the measurements was approximately ±0.2%. From the electrical calibrations, the response time for a short burst of heat is on the order of 100 s; the response time for return to the original temperature is several days.

The experimental procedure for handling and introduction of sample into the calorimeter was revised in repeated experiments. Trial versions of the actual runs using blanks and with sterilized seawater in both sides, in absence of biological activity, produced no observable heat. Setting the initial state took 20 to 30 min.

In practice the A/D readings were output to a spreadsheet file, so essentially the temperature differences were logged in arbitrary temperature units; conversion to a standard temperature scale was done but is not strictly required. Conversion to heat production was done by using the heat capacity calculated from the calibration runs. No significant correction for heat loss to the environment was required: in the absence of heat production in either vessel, the bridge output was essentially constant.

The absolute temperature of the calorimeter was determined by the use of a four-lead standard platinum resistance thermometer (Leeds and Northrup) and a Mueller Temperature Bridge (No. 8069). A platinum resistance element (Degussa Inc.) was calibrated against the platinum standard by mounting both of these elements in a metal block inside the stainless steel container (see Figure 1). Under equilibrium conditions the resistances of these two elements were compared and a calibration curve was constructed. The absolute temperature of the calorimeter vessel was determined by comparing the platinum resistance element with the calibration curve. In the calorimeter, the platinum resistance element was inserted in a metal block inside the Dewar flask (see Figure 1).
2.2 Sample preparation

Seawater was collected off the pier at Scripps Institution of Oceanography (San Diego, CA) and placed in a 200-L aquarium containing an assortment of macrofauna (corals, fish, and live rock), approximately 5 cm of crushed coral on the bottom, a protein skimmer, and carbon filter. The aquarium was outfitted with lighting, so both photosynthesis and respiration continued.

Three types of fractions were prepared from the aquarium samples by filtration using different pore sizes of a 50-mm diameter Sartorius membrane made of polyvinylidene fluoride: a sterile fraction, a viral fraction, and a microbe fraction referred to as the inoculum below. The smallest pore size (0.02 μm) allowed only dissolved organic matter (DOM) to pass. This is the sterile fraction. The medium pore size (0.20 μm) allowed the viral particles as well as some Eukaryotic virus to pass through along with the DOM. Finally, the coarse pore size (0.45 μm) allowed microbes, viruses, and DOM to pass through. The sterile (DOM only) fractions were stored in a dark place and used as the dilution medium in the experiments. This is summarized in Figure 2. Prior to each experiment the sterile (DOM only) fraction was checked for microbial growth and, if any growth was detected, the filtrate was discarded.

Levels of dissolved organic matter varied widely according to the feeding schedules of the macrofauna, so in order to compare biological activity in two samples it was necessary to collect pairs of samples simultaneously. Microbes from aquarium water samples, before and after each experiment, were counted using SYBR Gold (Molecular Probes, Inc.), and direct counting by epifluorescent microscopy. At least 20 fields were counted in each case. Cell counts are reported as the total number of microbial cells in each
vessel. Reaction and reference vessel preparation protocols are summarized in the figures for each experiment. All the experiments were carried out at 23°C. This temperature is close to the aquarium temperature, and near the upper end of the local ocean water temperature range.

3 Results and discussion

The goal of this work was to measure, by direct calorimetry, the effect of viruses on heat production in simulated pelagic communities. Calorimetric measurements are described which show that metabolic activity in the presence of viruses is more intensive than in their absence. Energy flows in viral–host interactions have been studied before by Guosheng et al. [22]. Where comparable, our findings are in line with theirs, although our samples are pelagic communities at natural populations while Guosheng et al. [22] study only the T4/E. coli system. The findings below have broad relevance; assessing the extent of carbon cycling and energy release in pelagic microbial communities is of considerable interest in connection with global warming.

3.1 The four experiments

Recall that our goal is to compare the heat production from growing microbial communities with and without viral activity. Since our instrument is a differential calorimeter, i.e., one that measures the difference between the heat productions from two samples, we begin with a description of experiments in which one of the samples was sterile seawater. To facilitate the exposition, we will refer to four experiments, all but the last involving comparison with sterile seawater in one of the vessels. These four experiments are illustrated in the four panels of Figure 3. In reality, variants of each of these four experiments were run many times, from different dilutions and using samples from the aquarium on different days.

As previously reported for near-shore marine environments [5, 6], dilution of the community by 95% inhibits all lytic viral production. This matches our findings even up to 90% dilutions; 2 ml of inoculum added to 18 ml of sterile seawater (DOM) sample produced no measurable viral activity.

Each experiment started with a diluted microbial sample and ended with a stationary phase community producing very small amounts of heat. While the exact cause of this is not certain, the standard explanation of the onset
of this phase is the depletion of nutrients, the buildup of toxins or possible quorum sensing among the microbes. The total heat production varied with the day sampling. This implies that the total heat is probably limited by dissolved nutrient concentration, which varies over time according to the aquarium maintenance schedule, rather than by dissolved oxygen content. It also indicates that meaningful comparison of infected and non-infected populations must be limited to samples collected at the same time. As discussed above, runs from different days were not directly comparable, although the inferred values of the heat associated with reproduction and lysis events were consistent between the runs.

The first three experiments compared reference vessels with sterile seawater to reaction vessels with various dilutions of inoculum: 95%, 90%, and 50%, respectively. The findings for the first two dilutions were consistent with the interpretation that there is no viral activity at these dilutions while the 50% dilution (Experiment 3) resulted in very evident viral activity. The fourth and final experiment compared two samples with the viral fraction added in the ratio of 17:3 bacteria to viruses, one side of the
3.2 Experiment 1: Microbial heat production without viruses

An example of heat production for our first experiment is shown in Figure 4. The calorimeter contained 20 ml of inoculum mixed with seawater (1 ml : 19 ml) in the reaction vessel and an equal volume of sterile seawater, from the same source, in the reference vessel. The microbial cell count changed from $4.6 \times 10^5$ to $9.0 \times 10^6$ during the experiment. The temperature differences were rescaled to energy units using the heat capacities from the calibration runs. Microbial heat is generated in the reaction vessel, raising its temperature relative to the reference vessel, and the heat flow rate decreases as a steady state is reached (see discussion above). The temperature change during the experiment was approximately $0.005 \, \text{K}$. In the present study, the initial population of microbial cells was 2 to 4 orders of magnitude less per ml than the previous study [19]. This resulted in a surprisingly large initial peak power per cell in the $15$–$45 \, \text{pW}$ range in the absence of viruses and presence of excess nutrients. However, the power
output per microbial cell after 2 h decreased to a value of ca. 5 pW, and when population approaches steady state the power was in the range of 30 to 200 fW, consistent with the value previously reported [19]. In a later investigation [20] it was concluded that the average power per microbial cell had an inverse relation with concentration of the microbes present, consistent with our findings. This inverse relationship matches our observations but does not explain their origin. Density-dependent effects are common in ecology and indicate the presence of some feedback similar to quorum sensing. This feedback presumably regulates the release of digestive enzyme stores (ectoenzymes) in such a way that larger drops in concentration trigger more ectoenzyme release. Our initial mixture is a population of bacteria previously in stationary phase at high concentration that suddenly finds itself at low concentration and in the presence of a plentiful food supply. This triggers a change to lag phase and a burst of stored up ectoenzymes. The initial reaction proceeds fastest and most irreversibly releasing a peak initial power from all the extra-cellular digestive reactions. Such bursts may be responsible for the hypersolubilization to which Cho and Azam [23] attribute the deconstruction of particulate organic matter in the oceans. By contrast, the average heat per reproduction event for this experiment is $0.42 \, \text{J} / (9 \times 10^6 - 0.5 \times 10^6) \text{cells} \simeq 50 \, \text{nJ/cell}$, in line with previous measurements.

### 3.3 Experiment 2: The effect of autoclaved DOM

To further validate the assumption that the viruses were sufficiently dilute in Experiment 1 to suppress any viral activity, experiments were performed on aquarium water samples at various dilutions and using different DOM solution sterilization methods. In the second experiment, shown in Figure 5, the inoculum was only diluted to 90% (2 ml added to 18 ml). No viral activity was detected in either of the two variants: one in which the DOM solution is autoclaved to ensure sterility, while in the other sterility arises only as a result of the filtration. While the total heat produced was very nearly the same (0.63 vs. 0.62 J), the autoclaved sample gave much less power initially, possibly due to structural changes in the nutrients causing slower metabolism without changing the total available energy.

The average heat per reproduction event for this experiment was

- $0.62 \, \text{J} / 14.7 \times 10^6 \, \text{cells} = 42 \, \text{nJ/cell}$ (Figure 5, red) and
- $0.63 \, \text{J} / 12.6 \times 10^6 \, \text{cells} \simeq 50 \, \text{nJ/cell}$ (Figure 5, black), respectively.
3.4 Experiment 3: Heat production with combined microbial and viral communities

At sufficiently high dilutions, viruses are not able to infect their target microbes before they degrade. The first two experiments, shown in Figures 4 and 5, constitute a calibration where the heat production is essentially all due to microbial growth. In the third experiment, to assure that viral activity would be present, the dilution was decreased to 50%, again using a sterile reference sample. The measured heat is shown in Figure 6. The average heat per reproduction event for this experiment is masked by the viral activity, but if one repeats the naïve calculation carried out above, the mean heat per unit increase in microbial count is $0.48 \text{ J} / 1.3 \times 10^6 \text{ cells} \approx 370 \text{ nJ/cell}$. This additional heat is accounted for more carefully after the discussion of a comparative-statics model presented in Section 4.
3.5 Experiment 4: Heat production with intact and autoclaved viruses

For our final experiment shown in Figure 7, the reference and the reaction vessels both contained mixtures of all three fractions, the difference being that the viral fraction was sterilized in the autoclave for the reference samples but not for the reaction samples. The three runs shown differ in the initial microbial populations and show, in agreement with Mukhanov et al. [20], that the more dilute the solution, the higher the difference in the amount of heat produced. The side with live viruses always produced significantly more heat and resulted in lower final microbial counts than the side with the autoclaved viruses. We postpone a quantitative discussion of these three runs until after a presentation of the comparative statics model in the next section.
Figure 7. Differential heat output vs. time comparing microbial heat production in the presence of concentrated live and autoclaved viruses. Measurements from three different initial counts of inoculum are shown: $0.1 \times 10^6$ (red), $0.4 \times 10^6$ (blue) and $0.9 \times 10^6$ (black) microbes. This time heat is produced in both the reference and in the reaction vessel. The analysis is presented in Section 4.2.3. The results of the calculations are shown in Table 1.

4 Accounting for heat production by microbes in the presence of viruses

4.1 Background

Two biological sources of heat were considered: (i) microbial growth and replication, and (ii) viral replication and subsequent lysis of the host. The average heat from growth and cell division leading to the formation of one new microbial cell (a replication event) is approximately constant for the pelagic communities measured. This heat was directly available in the first two experiments. As discussed above, the samples varied greatly with the extent of dilution, times of cleaning the tank, and the addition of nutrients. There was no consistency in the levels of microbes before and after the runs or in the total amount of heat produced. Nonetheless, the total heat produced divided by the increase in the number of microbes in all the runs with negligible viral activity was constant and approximately 50 nJ/cell.
The heat produced during the destruction of a single microbial cell by viral replication and enzymatic lysis is more elusive. Given our findings that the average heat of a microbial reproduction event is constant, it is reasonable to assume that such lysis events also have a constant associated average heat. The model below enables one to count the number of lytic events based only on the initial and final microbial counts and the net difference in heat produced. The central variable in this model is the ratio \( r \) of the heat in one lytic event to the heat in one reproduction event. We strive to keep all the modeling at the simplest level and thus stick to the net changes observed in each experiment. In particular, we make no attempt to model the dynamics of the complex communities in our samples, as we believe such modeling to lie well beyond the scope of this paper. Rather we focus on the net changes in the experiments that present food to a diluted population of an initially stationary phase microbial population and follow the heat evolved and microbes produced in this population until it reaches stationary phase once again. Our thesis below is that such net changes can be simply and consistently interpreted.

Let \( q^+ \) represent the average heat associated with one replication event and \( q^- \) represent the average heat associated with one lytic event. The crucial unknown is the ratio \( r = q^-/q^+ \) of the heat per lytic event divided by the heat per reproduction event. For the T4/E. coli system studied by Guosheng et al. [22], the reported values lead to this ratio equaling 0.78 (see [22, p. 141]).

Quite generally, this ratio is expected to be on the order of one, probably between one half and one. Evolutionary forces select for viruses that optimally use up their hosts and thus recycle most of the material within the microbial cell. Micrographs of microbes crowding with viral particles add credence to this expectation. On thermodynamic grounds, one would expect a comparable amount of irreversible heat production from the salvage operations carried out by virus on a microbe to the heat produced during the construction of that microbe. Most of the biosynthesis required for microbial and viral growth does not require complete chemical dismantling of nutrient monomers. For example, it is possible in principle to construct the DNA of one species simply by rearranging the nucleotides from the DNA of another species. The heat of reaction for this rearrangement is about zero although the biosynthesis still requires ATP. While the net heat of the reaction is zero, the spontaneity of the reactions requires that some free energy be degraded to heat. The oxidation of nutrients to generate ATP is exothermic and usually much larger than the average heat of formation of one biopolymer from another. Since the virus is actually using
the metabolic system of the microbe, biochemical strategies and associated heat production are likely to be similar for microbial and viral growth.

4.2 The calculations

Let $N^+$ represent the number of microbial replication events during the experiment and $N^-$ the number of lysis events. The total heat produced is

$$Q^{\text{tot}} = q^+ N^+ + q^- N^-$$

(1)

with an associated net change in microbial population of

$$\Delta N = N^+ - N^-.$$

(2)

Note that this net change in the number of microbes is given as all “births” minus all “deaths.” Given the measured values of $Q^{\text{tot}}$ and $\Delta N$ for a vessel and an assumed value of $r$, Eqs. (1) and (2) determine the values of the number of reproduction events, $N^+$, and the number of lytic events, $N^-$, in that vessel.

4.2.1 Experiments 1 and 2

In the first two experiments (Figures 4 and 5), the reference vessel contained sterile seawater and so produced no heat and no microbes. The reaction vessel was inoculated with aquarium water at a dilution level sufficient to render any virus harmless. Consequently, in these experiments, the production of heat can be attributed exclusively to microbial growth. That is, $N^-$ is equal to zero in Eqs. (1) and (2), while $\Delta N$ and $Q^{\text{tot}}$ (equal to measured heat, $Q^{\text{meas}}$) are measured directly as the change in cell count and the total differential heat produced. Thus these experiments can give calculated values of the average heat of one reproduction event

$$q^+ = Q^{\text{tot}} / N^+ = Q^{\text{meas}} / \Delta N.$$

(3)

The values of $q^+$ calculated from the data shown in Figures 4 and 5 are $q^+ = 50, 42$, and $50 \text{ nJ/cell}$, respectively. In Experiment 2 (Figure 5, red), a decrease in heat flow rate is evident between 11 and 16 h. The decrease is most likely a result of minor contamination of the reference vessel. Consequently, the observed heat per bacteria cell, $q$, decreased from $50 \text{ nJ}$ to $42 \text{ nJ}$. For the subsequent analysis of Experiments 3 and 4 (Figures 6 and 7) the value of $q^+$ is taken as $50 \text{ nJ/cell}$. 
4.2.2 Experiment 3

In the third experiment (Figure 6), the dilution of the inoculum was 50%, resulting in considerable viral activity in the reaction vessel. Since the reference vessel had no microbes present, we may again identify $Q_{\text{meas}} = Q_{\text{tot}} = 0.48 \text{ J}$. This total heat can then be used to determine $N^+ + rN^- = Q_{\text{tot}}/q^+ = 9.6 \times 10^6$ cells using Eq. (2) while the measured change in cell count, $\Delta N = 1.3 \times 10^6$ cells, determines $N^+ - N^-$. The values of $N^+$ and $N^-$ can then be determined by solving these two linear equations in two unknowns. For $r = 1$, we find $N^+ = 5.45 \times 10^6$ cells and $N^- = 4.15 \times 10^6$ cells. For $r = 0.5$, we find $N^+ = 6.8 \times 10^6$ cells and $N^- = 5.5 \times 10^6$ cells. As discussed above, the true values are expected to be somewhere in between. Note that to one significant figure the number of replication events $N^+$ is in either case about five times the net observed increase of $\Delta N = 1.3 \times 10^6$ cells.

4.2.3 Experiment 4

The fourth experiment (Figure 7) compared microbial activity with and without viruses. Microbes were delivered to both reaction and reference vessels in the form of a 5% inoculum, by itself dilute enough to suppress viral activity. Viruses were introduced separately into the reaction vessel in a concentrated form to guarantee the presence of viral particles in excess. In this case, since the reference vessel contains growing microbes, the determination of $N^+$ and $N^-$ is slightly more involved.

The heat produced in the reference vessel (with no viral activity) is calculated as

$$Q_{\text{ref}}^{\text{tot}} = q^+ \Delta N_{\text{ref}}$$

and the heat produced in the reaction vessel as

$$Q_{\text{rxn}}^{\text{tot}} = Q_{\text{meas}} + Q_{\text{ref}}^{\text{tot}}.$$  

Combining this with the observed $\Delta N_{\text{rxn}}$, in the reaction (rxn) vessel, enables us to determine $N^+_{\text{rxn}}$ and $N^-_{\text{rxn}}$ using Eqs. (1) and (2).

As an example, consider the red curve in Figure 7. The change in the cell counts in the reference vessel is $22.7 \times 10^6$ cells, giving $Q_{\text{ref}}^{\text{tot}} (50 \text{ nJ/cell} \times 22.7 \times 10^6 \text{ cell})$ equal to $1.13 \text{ J}$. This then determines the total heat from the reaction side, $Q_{\text{rxn}}^{\text{tot}}$ (Eq. (5)), as $2.5 \text{ J}$. From Eq. (1),

$$\frac{Q_{\text{rxn}}^{\text{tot}}}{q^+} = N^+_{\text{rxn}} + rN^-_{\text{rxn}}.$$  

Combining this with the net change in cell counts for the reaction vessel $N_{rxn}^+ - N_{rxn}^-$ gives

$$N^+ = \frac{5 \cdot 10^7 + 1.66 \cdot 10^7 r}{1 + r},$$

$$N^- = \frac{3.3 \cdot 10^7}{1 + r}. \tag{7}$$

For $r = 1$ these become $N_{rxn}^+ = 33.3 \times 10^6$ cells and $N_{rxn}^- = 16.7 \times 10^6$ cells. For $r = 0.5$ these become $N_{rxn}^+ = 38.8 \times 10^6$ cells and $N_{rxn}^- = 22.2 \times 10^6$ cells. The calculations for the other initial concentrations proceed in the same way; the results are summarized in Table 1.

### 4.3 Fraction of heat attributable to viral activity

In this section we decompose the total heat released in one vessel into three parts. The first part is the heat directly attributable to viral activity, $Q_{phage}^{dir} = q^- N^-$. The second part is the heat indirectly attributable to viral activity $Q_{phage}^{indir} = q^+ N^-$. The latter is the heat generated by the microbes in effectively producing food for the viruses. The final part $Q_{prod} = q^+ \Delta N$ is the heat associated with net microbe production. Note that these three terms add up to the total heat $Q_{tot} = q^+ N^+ + q^- N^- = q^+ \Delta N + q^+ N^- + q^- N^-$. The fraction of heat directly or indirectly attributable to viruses

$$\alpha = \frac{Q_{phage}^{dir} + Q_{phage}^{indir}}{Q_{tot}} = \frac{Q_{tot}^{dir}}{Q_{tot}} = \frac{q^+ N^- + q^- N^-}{q^+ N^+ + q^- N^-} = 1 - \frac{q^+ \Delta N}{Q_{tot}}, \tag{9}$$

ranges between 0.44 and 0.88 in the experiments presented. Note that its value is independent of the value of $r$ as can be seen from the last expression in Eq. (9). The fraction of heat directly attributable to viral activity,

$$\beta = \frac{Q_{phage}^{dir}}{Q_{tot}} = \frac{q^- N^-}{q^+ N^+ + q^- N^-}, \tag{10}$$

does show an $r$ dependence. The dependence of $\beta$ on $r$ is fairly simple; however,

$$\beta(r) = \left(\frac{2r}{1 + r}\right) \cdot \beta(1), \tag{11}$$

where $\beta(1) = \alpha/2$ is the fraction of heat directly attributable to viruses assuming $r = 1$. As seen in Table 1, assuming $r = 0.5$, results in $\beta$ values that range from 0.15 to 0.29, while assuming $r = 1.0$, results in $\beta$ values that range from 0.22 to 0.44.
Table 1. Calculated quantities for analyzing microbial energy use and viral activity indices in the four experiments. The last row lists ranges of $\beta$ values corresponding to the assumptions of $r = 0.5$ and $r = 1.0$.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Without phage activity</th>
<th>With phage activity</th>
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<tbody>
<tr>
<td>Figure</td>
<td>4 (red)</td>
<td>5 (black)</td>
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<tr>
<td></td>
<td>6 (red)</td>
<td>7 (blue)</td>
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<td>$Q_{\text{meas}}$ (J)</td>
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<td>$10^6 \Delta N_{\text{rxn}}$</td>
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<td>$10^6 \Delta N_{\text{ref}}$</td>
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</tr>
<tr>
<td>$q$ (nJ)</td>
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<tr>
<td>$Q_{\text{tot}}^{\text{rxn}}$ (J)</td>
<td>0.43</td>
<td>0.62</td>
</tr>
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<tr>
<td>$Q_{\text{tot}}^{\text{ref}}$ (J)</td>
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<tr>
<td>$10^6 N_{\text{rxn}}^+$</td>
<td>8.54</td>
<td>14.67</td>
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<tr>
<td></td>
<td>12.61</td>
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<tr>
<td>$10^6 N_{\text{rxn}}^-$</td>
<td>0</td>
<td>0</td>
</tr>
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<tr>
<td>$\alpha$</td>
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<tr>
<td></td>
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<tr>
<td>$\beta, r =$</td>
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<td>0.22–</td>
</tr>
<tr>
<td>0.5–1</td>
<td>0.44</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>0.22</td>
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5 Conclusions

The present paper reports calorimetric measurements of marine microbes smaller than 0.45 $\mu$m at pelagic population, albeit from an aquarium. The goal was to demonstrate a calorimetric method for assessing what fraction of the heat produced in a microbial community is due to viral activity. The measurements showed that in the presence of viruses, the total heat production is increased, while the final microbial cell count is decreased. Microbes apparently reproduce more in the presence of viruses. This has been observed by other investigators for the T4/E. coli system [22]. The likely explanation is that viral lysis creates DOM accessible to other microbes [24]. The final microbial count being decreased in the presence of live viral particles is evidence for top-down control of the microbial population.

The apparatus described here makes it possible to perform calorimetric measurements on intact pelagic microbial communities. Furthermore, this design could be adapted to a portable “nano-calorimeter” which could be
deployed in the field to measure microbial heat production in natural environments.

The heat production in all our experiments reaches a steady state. Our measurements for the heat produced in such steady states are in close agreement with Mukhanov et al. [19]. The heat production per microbe increased with dilution contrary to what was naively expected but in line with the results of Mukhanov et al. [20] who reported an inverse proportionality between heat production and microbial concentration. Such density-dependent effects are common in ecology and indicate the presence of a feedback mechanism like quorum sensing. The large surge of initial power observed suggests a possible connection to hypersolubilization [23] and points to several important future experiments.

A simple comparative-statics model was introduced to account for the heat production in the presence of virus activity. The model infers the number of lytic events from the excess heat production. To within a factor of 2, the fraction of heat directly attributable to chemistry carried out by viruses is about 0.3, while the fraction of heat directly or indirectly attributable to viral activity is about 0.7. This makes viruses major players in the energy budget for our planet and has important implications for carbon cycling.

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Heat output by marine microbial and viral communities

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