

TECHNICAL NOTE

Tools for Physiology Labs: An Inexpensive Means of Temperature Control

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We describe a simple means of modulating preparation temperature, which may be useful in undergraduate physiology laboratories. The device was developed in an effort to make teaching exercises that involve temperature modulation accessible at low cost. Although we were interested in using the device specifically with the larval fruit fly preparation, it is applicable to many preparations and temperature sensitive phenomena. Feedback driven thermoregulators offer superior precision in experiments

requiring temperature control, but can be prohibitively expensive, require power supplies and circuitry, and often generate large switching transients (artifacts) during physiological recording. Moreover, many interesting exercises involving temperature control can be carried out with a slightly reduced level of temperature precision.

Key words: temperature control, cooling, heating, physiology exercises, Q_{10}

An ongoing interest in our laboratory is the development of inexpensive and useful equipment for undergraduate neurophysiology exercises. During development of physiology exercises using the larval fruit fly preparation (Krans et al., 2005), it became clear that some means of temperature control was necessary. In general, temperature control can be useful for physiological recording. Cooling extends the lifespan of numerous preparations, and an abundance of biological phenomena are temperature sensitive (Somero, 1997).

Any laboratory exercise that utilizes a preparation stand – an elevated platform upon which a dish rests (Figure 1B) – can be fitted with the temperature control system. Applications of the system are thus broad reaching and include many illustrations of Q_{10} in biological processes. Some possible preparations and experiments that involve temperature dependence are: locomotion and grazing in protozoa (*Paramecium*), photosynthesis in pond weeds such as *Elodea*, conduction velocity of giant fibers in cockroach (*Periplaneta*), distribution of compound action potential waveforms in earthworm giant fibers (*Lumbricus*), larval locomotion in fruit fly larvae (*Drosophila*), and budding of yeast (*Sacchromyces*). Many professional organizations in academia outline or link to laboratory exercises of this sort on their websites (e.g. American Society for Cell Biology: www.ascb.org; American Physiological Society: www.aps.org; and Society for Neuroscience: www.sfn.org).

Many temperature control devices have been introduced in previous decades (Kingsley et al., 1976; Rose, 1983; Forsythe and Coates, 1988; Squalli-Houssaini et al., 1991; Correges et al., 1998). Though some have been dubbed inexpensive, their cost is reported in hundreds of dollars (Kingsley et al., 1976; Correges et al., 1998). Much of this expense resides in various forms of thermoelectric feedback control. Constructing a temperature control device has become more reasonable with the advent of affordable Peltier devices and pre-built controllers. The Peltier effect involves the transfer of heat energy between two joined semiconductors of varied

conduction properties upon current flow through them. Modern Peltier devices, such as those commonly used in physiology research, are made up of many pairs of semiconductors arranged in a flat, high surface area orientation. These can move considerable heat and provide impressive cooling capabilities. The Peltier devices, by themselves, are available for under \$50, but the requisite electronics to control current flow through them remains several times more expensive. An off-the-shelf controller with Peltier device can cost thousands of dollars (pricing via Fisher Scientific and VWR suppliers at time of publication).

Eliminating this component of the system attenuates expense; we describe a passive system that introduces no current source to the recording area. Cost is further reduced by accommodating lower precision: $\pm 1-2^{\circ}$ C rather than the $\pm 0.1^{\circ}$ C offered by more elaborate “inexpensive” systems. Utilizing components available at many national supercenters further supports the attainability of our system. Using only simple components, we can adjust temperature rapidly and elicit robust temperature sensitive biological phenomena.

The system described here changes the temperature of a preparation by circulating water from a reservoir to a heat sink beneath the preparation dish. The preparation temperature thus approaches the reservoir temperature, which can be easily set to a wide range of physiologically useful temperatures. The system offers several advantages: (1) It is inexpensive; about \$50.00. (2) Changes in temperature between 10° and 45° C occur rapidly. (3) No novel source of electrical noise is introduced by the system; no electrical components about the recording area. (4) Proficiency in electronics is unnecessary and no power supply is needed. (5) Sustaining lowered temperatures requires only an initial distraction from the exercise(s) and can substantially lengthen the period during which a preparation is effective for physiology experiments. (6) Changes in temperature are tractable and thus are convenient for student-run temperature sensitive experiments.

MATERIALS & CONSTRUCTION

Components of the system are pictured in Figure 1. An AquaTech submersible powerhead (pump) was purchased from Walmart® (AquaTech: Moordark, CA). AquaTech offered the least expensive pump per flow rate, retailing at just under \$19 and providing a flow of 170 gallons per minute. Comparable pumps are likely available at local pet stores nationally.

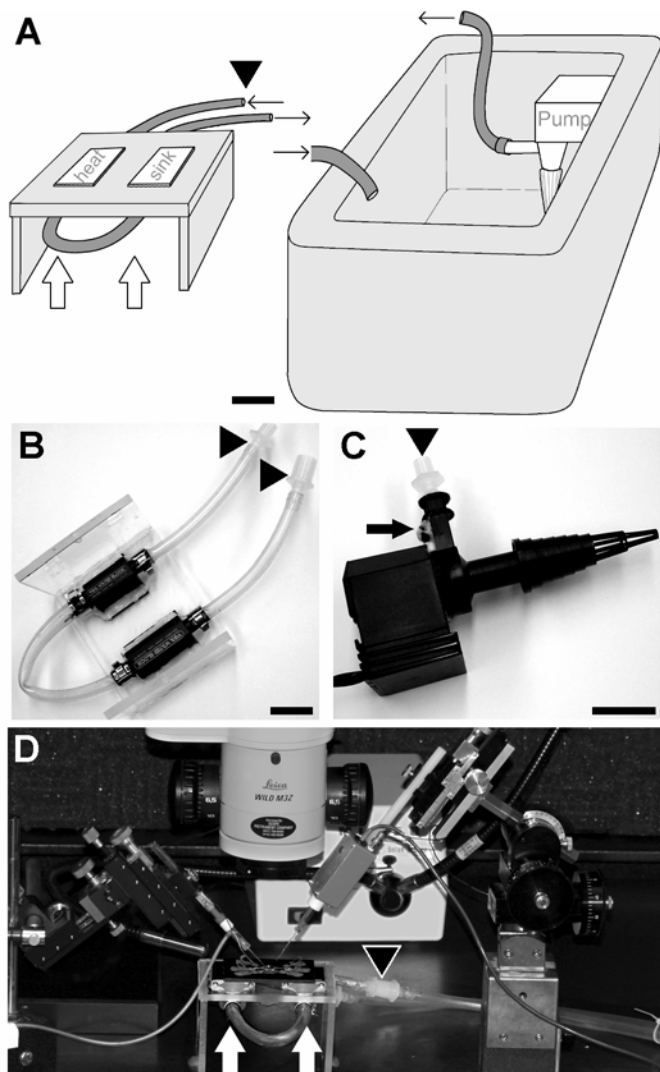


Figure 1. Components and construction of the system. *A*, Two principle components are shown in the line drawing: the heat sinks and the pump as it sits in a Styrofoam reservoir. The orientation of the two heat sinks within the Plexiglas stand, and the dimensions of the stand itself, can be modified to accommodate any size dish and/or illumination. *Open arrows* indicate the location of hose clamps in panels *A* and *D*. *Closed arrowheads* show the location of quick disconnect hose connectors throughout. *B*, Underside of the stand. *C*, The pump is shown with a filter on the intake port. *Arrow*: hot glue is used to close one of the outlets. *D*, The image shows the size of the stand, and one possible positioning scheme, relative to common physiological rig components. The dish used for larval fruit fly exercises is shown in panel *D*. Schematic line drawing by Kristin Gawera. Scale bars = 1.5 inches.

The pump has two outlet paths, one of them for an aeration tube; we closed that one using high temperature hot glue available from any craft store (arrow, Figure 1C). An epoxy would be appropriate in this capacity as well. Tube adaptors were mounted to the inlet and remaining outlet hole again using hot glue (arrowhead, Figure 1C). Tubing that is insensitive to temperatures from 10-90° C is sufficient for the project. An acceptable vinyl product can be purchased at just over \$1 per foot at Ace Hardware Stores (www.acehardware.com, Raleigh, NC). The Home Depot® also sells appropriate tubing and has retail locations throughout the United States. We purchased quick disconnect hose fittings from Scienceware® (Pequannock, NJ) in bulk for about \$20. Less expensive variations are available from this and other suppliers, the primary determinant in selection being that the connectors can be easily attached and detached.

We used water blocks as heat sinks. These were originally produced for VGA computer chip cooling (Zalman model ZM-GWB1, www.zalman.co.kr, supplied by www.frozenCPU.com). The ZM-GWB1 kit includes hose clamps and mounting hardware. The two water blocks were set into a piece of 3/8" thick clear Plexiglas about 1" apart by cutting rectangular holes in the Plexiglas (Figure 1A and 1B). We made these cuts with a household Dremel™ rotary cutting tool, though any small cutting device would suffice. Mounting the heat sinks slightly separated allows the preparation dish to sit across them, making a bridge. Illuminating preparations from beneath is often advantageous and this configuration permits that form of lighting. If lighting from beneath is unnecessary, a larger single water block can be purchased for about the same expense (e.g. Maze4 Copper Water Block: www.directron.com, ~\$35.00).

Temperature was assessed using standard laboratory thermometers as well as thermochromic liquid crystal strips with effective ranges of 12° to 44° C (Tip Temperature Products, Burlington, NJ). These are not an essential component of the system, but were used for documentation purposes and greatly simplified temperature monitoring. Similar products can be purchased from pet supply stores. Adhesive thermometers (also thermochromic crystals) intended for reptile and fish tanks have equivalent temperature ranges and cost from two to three dollars each.

COST

The total cost of this project was about \$50 (Table 1). Some components of the system can be constructed using simple tools, and this would reduce the project's cost. Specifically, a heat sink could be constructed by drilling a single hole through a block of aluminum. With appropriately sized holes, tubing will fit snugly into the holes at each end and an epoxy could be used to seal the juncture.

USE & FUNCTION

There are four main elements of this system: (1) a pump,

Table 1: Breakdown of component costs. Sources for each component are given in the Materials and Methods section.

Item Description	Cost
Aquarium water pump: AquaTech powerhead	\$19
Water blocks: Zalman ZM-GWB1	\$22
(or)	(or)
Water blocks: custom aluminum block(s)	\$5
Tubing (5') + Fittings (x2 pair)	\$10
Plexiglas (6"x12" @ 3/8" thick)	\$5

(2) a heat sink, (3) a preparation stage, and (4) various tubing and hose connectors. The pump circulates fluid of varying temperature and is positioned external to the physiology rig (Figures 1A and 1D). Tubing connects the outflow of the pump with heat sinks set into the preparation stage. Aluminum water blocks act as heat sinks directly abutting the physiological dish (Figure 1A and 1B). Tubing returns fluid to a reservoir where it is re-circulated. The temperature of the stage thus approaches the temperature of the reservoir without introducing any electrical components to the physiology rig. Any water-tight container large enough to submerge the pump could act as a reservoir. We used a Styrofoam shipping box (Figure 1A). For cooling exercises, ice chips were added to water in the reservoir. For warming exercises, hot water was used. This arrangement gave sufficiently rapid temperature changes (Figure 2). For heating exercises, a large glass beaker and a hotplate was also effective in managing temperature. A conventional water bath device provides even greater precision. Neither of these was functionally different from a simple container (Styrofoam) in physiology exercises.

The components of the system, connected and functioning in a physiology rig, are depicted in Figure 1D. Positioning of the pump is entirely flexible since it is extrinsic to the recording area. A pump with a flow rate of 100 gallons per minute or better, and appropriate inlet and outlet ports, would suffice in this application. The pumps that we tested were submersible, and required priming before pumping effectively. This was accomplished by momentarily submerging the whole pump, establishing flow, and then attaching the outlet hose via a quick release fitting (Figure 1C). This type of connector utilizes friction between the male and female fittings and thus offers a rapid means of connecting system components. This was useful after priming the pump and when moving the setup in and out of rigs.

The placement of heat sinks should be adjusted according to the dimensions of the dish chosen. This system was developed with respect to the larval fruit fly preparation. We chose a previously described and inexpensive dish for the preparation (Figure 1D; Bellen and Budnik, 2000). For our purposes, the blocks were placed into a Plexiglas stand about one inch apart. In this configuration there was no observable difference between the temperature of the heat sinks and the temperature of

the preparation dish. This may vary in other configurations using other dishes.

Maximum and minimum sustainable temperatures (~10° and ~42° C) were achieved in about two minutes (Figure 2A). We were unable to sustain temperatures further from ambient. Any temperature in this range is attainable with simple calibration. Target temperatures that are closer to ambient require less extreme reservoir temperatures, are maintained for longer periods, and are more quickly reached. Larger dishes containing more Sylgard (Dow Corning Silicones, Midland, MI) required greater time to reach target temperatures. Sylgard is a silicone elastomer that is often used in physiological dishes as a pinning substrate.

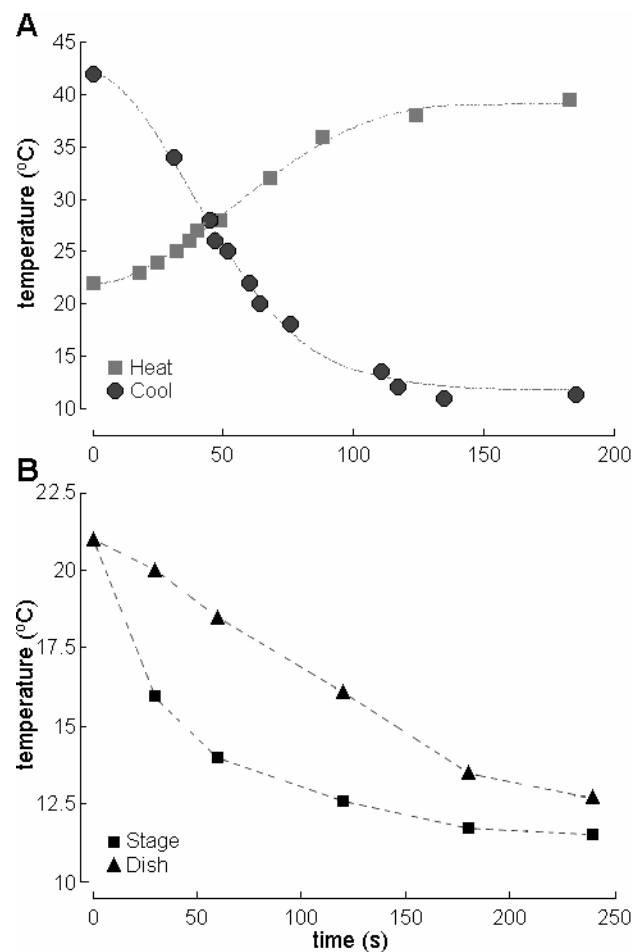


Figure 2. Rate of temperature change. Temperature was measured using thermochromic liquid crystals ($\pm 1^\circ \text{C}$). *A*, These were adhered to the surface of a fruit fly preparation dish: a 3"x2", 1/8" thickness glass plate and two magnetic sheets of comparable dimensions. *Squares*: Preparation temperature was increased from ambient by circulating $\sim 60^\circ \text{C}$ water. *Circles*: Ice chips were added to the reservoir to cool the preparation. Cooling was initiated from a stage temperature of about 42°C , modeling a relatively large temperature step. *B*, Temperature was measured on the heat sinks as well as in a small Sylgard lined glass dish suitable for submersed preparations.

Figure 2B shows the difference between the temperature of the heat sinks and the temperature of saline within a larger preparation dish than that used for fruit fly larvae. A 50 mm diameter glass dish, lined with Sylgard, was used as a model of larger dishes sometimes utilized in undergraduate laboratories. Reaching maximum and minimum temperatures required about twice as long using this type of dish and sustainable temperatures were less deviant from ambient ($\sim 13^{\circ}$ to $\sim 38^{\circ}$ C).

DISCUSSION

Temperature control can be used to illustrate many biological phenomena. Although we developed this device for the *D. melanogaster* preparation, it has more general applications. Fundamental concepts such as the rate of enzymatic reactions and reversal potential of ion conductance across biological membranes are examples of functions affected by temperature. Illustrating the temperature dependence of intrinsic neural parameters is particularly salient in undergraduate neuroscience courses. One simple exercise that stands out is the investigation of temperature dependent change in axonal conduction velocity. Large axons of the ventral nerve cord of many preparations show a robust change in conduction velocity over the range of temperature made available by our system (unmyelinated: Hille, 1977; myelinated: Paintal, 1965). Data from this type of exercise are easily quantified and relate directly to concepts discussed in formal texts, providing students the opportunity to link concept with observation.

In a companion paper, we discuss the value of this cooling device to preparation longevity (Krans et al., 2005). Moderate cooling extended the lifespan of the larval *D. melanogaster* preparation considerably, and this phenomenon applies to many preparations. Most crustacean preparations used in teaching laboratories benefit from cooling. These preparations typically make use of larger dishes. Moreover, these dishes typically contain a pinning substrate of low thermal conductivity. To address the efficacy of our system in this facility we cooled a 9 cm diameter glass dish containing ~ 1 cm of Sylgard and ~ 80 ml of standard insect saline. A temperature of 18° C was reached in 15 minutes and 13.5° C, the lowest sustainable temperature, was attained within one hour (ambient = 23.5° C). Changes in temperature were thus slower than described for the fruit fly preparation dish, but the temperature modulation system remained effective.

Temporal precision of temperature change using our device may not be as good as other published devices claim (Forsythe and Coates, 1988; Sqalli-Houssaini et al., 1991; Correges et al., 1998). More expensive cooling systems that utilize feedback control can require several minutes to reach set temperatures in a comparable range (Correges et al., 1998). We used thermochromic liquid crystals to measure temperature. These are designed for flat surface-contact applications. These may not accurately represent the temporal component of temperature change; they require some time to change color and display the temperature. We were unable to find

documentation on this delay, but estimate it at less than 20 seconds.

Though changes in temperature can occur rapidly, there is an inverse relationship between speed and precision. We were unable to realize resolution greater than about $\pm 2^{\circ}$ C during fast temperature changes. In order to reach the resolution of the thermochromic liquid crystals ($\pm 1^{\circ}$ C), slightly more attention and time is required. An additional consideration is the distance dependent loss in temperature from the reservoir to the heat sinks. In contrast, there is very little loss in heat exchange between the heat sinks and the dish. It is difficult to predict the absolute loss in temperature because of its dependence on the type of reservoir (controlled water bath, hotplate and beaker, or Styrofoam container), tubing type and distance, and dish size. Use of the reservoir temperature as the target temperature thus requires some user calibration. In our configuration, setting the reservoir temperature to about 40° C yielded preparation dish temperatures around 34° C (using a Styrofoam box). This was appropriate for activation of a temperature sensitive phenotype, as described in a companion paper, published in this issue (Krans et al., 2005). Moreover, this is an easy reservoir temperature to hold; it is similar enough to room temperature that its decay to ambient is very slow.

We endeavor to provide practical technology that offers novel educational value and is accessible to any budget. We are unaware of a less expensive means of temperature control that meets the uncomplicated construction standards described. However, feedback controllers may be purchased at greater cost from several sources. These are available in applications of computer processor / chip cooling, and in system design applications. One particular source of this technology is TE Technology, Inc. (Traverse City, MI; www.tetech.com/temp), which offers temperature control packages in the hundreds of dollars range. Our system is simple to construct, requires no calibration or electronic construction, can be easily employed by students, and makes temperature sensitive physiological experiments accessible at very low cost.

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