MICROPROCESSOR-CONTROLLED MINI-ENVIRONMENTAL CHAMBERS CAPABLE OF SUBFREEZING TEMPERATURES IN CONSTANT OR TIME-VARYING TEMPERATURE REGIMES

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Abstract

A set of six microprocessor-controlled mini-environmental chambers (0.04 m³ each) was designed, built, and tested. Chambers are capable of subfreezing temperatures (less than -10°C) and can operate under constant or time-varying temperature regimes. Chambers are cooled by circulating ethyl alcohol from a reservoir chilled by an immersion cooler. Heat is provided by 17-W cartridge heaters. Temperatures are independently controlled by a single IBM 8088 computer instructing a data acquisition and control system. A single photoperiod is maintained by a commercial timer activating two miniature light bulbs in each chamber. Chamber temperatures were within 0.5 or 0.75°C of the set temperature 65 or 94% of the time, respectively, during a 39-d test period. Minimum temperature capabilities were estimated for a variety of chamber configurations by an examination of the thermodynamic characteristics of the system.

Introduction

Studies of the effects of temperature on insect development typically require several constant-temperature regimes. This is particularly true when temperature responses are anticipated to be nonlinear over the temperature range of interest. When the entire life cycle of a temperate insect is under investigation, this range may extend

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from −10 to +35°C. In addition, reported differences (Liu et al. 1995; Roltsch et al. 1990; Yeargan 1983; Tanigoshi et al. 1975) between developmental responses to fluctuating temperature versus a constant temperature equal to the mean of the fluctuating temperatures, the so-called Kaufmann effect (Worner 1992), require that some investigations include treatments of time-varying temperatures. Many available environmental chambers (ECs) are incapable of producing temperatures below ambient temperatures or of producing time-varying temperature regimes. The very high cost of a single EC with low-temperature and time-varying temperature capabilities can severely limit the number of treatments in an experiment.

Several researchers have encountered this problem and their solutions have resulted in systems of varying natures. The EC described by Nicholls and Grills (1968) produces constant temperature and humidity regimes, but is not capable of subambient temperatures. The system described by Wilson and Stinner (1981) can produce subambient temperatures, but its range is only 13–32°C. In addition, it can require considerable modifications to the room in which it is used because it uses an exterior 450-kg refrigeration unit. The microprocessor-controlled ECs of Taylor and Shields (1990) have a minimum temperature of 0°C. In their system a single data acquisition and control system (DACS), in conjunction with a computer-resident control program written in Microsoft quickBASIC, monitors and controls temperature and photoperiod in eight independent chambers. A maximum of 10 daily photoperiod and time-varying temperature regimes is possible. The system relies upon a commercial low-temperature EC to heat and cool each chamber. Total cost was Can$46 000 (Canadian dollars, March 1988), excluding the computer. The “Florida Reach-ins” (Walker et al. 1993) are the most sophisticated of those we describe. In those, temperature, relative humidity, and photoperiod are monitored and independently controlled in each of an octet of chambers by a single IBM XT computer running a 37 000 line (source code) Turbo Pascal program. Temperatures and relative humidities are controlled to ±0.1°C and 1% RH of constant or time-varying set points. However, minimum temperature is only 1–3°C above ambient. Total cost was Can$195 000 for 56 chambers, excluding labor to develop and test the chambers and software in 1992 (Walker et al. 1993).

The objective of this work was to design, construct, and test a set of six inexpensive ECs. The ECs were to be constructed from readily available material, require no sophisticated construction or electronics skills, and require no modification to the laboratory room. Our requirements were that each chamber be independently controlled for temperature and capable of a constant (≤51°C) or a time-varying temperature regime. The temperature range needed was at least −5 to +35°C. We required only a single photoperiod for the set of six ECs. Because gypsy moth eggs were the experimental material of interest, we did not require each chamber to have a large internal volume, but the design was to be suitable for larger units. Although never explicitly stated, we wished total cost to not exceed approximately Can$8000.

Materials and Methods

Chamber Design and Construction. Initial design was suggested partially by our budget limitations and currently available equipment: a Keithley System 570 DACS and Neslab CC-60 immersion cooler (see Table 1 for part numbers and manufacturers), and an IBM 8088 with color monitor and VGA card. The CC-60 was replaced by a Neslab PBC-75 in the final product. In the final design ethyl alcohol is chilled by the immersion cooler and then pumped through each of the six insulated plywood boxes. Each chamber is heated by a small cartridge heater (Fig. 1).

Chambers are constructed in two tiers of three chambers each. Each tier is constructed of 1.25 cm thick plywood and measures 121.5 × 38.51 × 43.5 cm (width ×
<table>
<thead>
<tr>
<th>Item</th>
<th>Manufacturer</th>
<th>Model no.</th>
<th>Supplier (if different from manufacturer)</th>
<th>Part no.</th>
<th>Quantity</th>
<th>Unit price (Can$)</th>
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<sup>a</sup> Neslab Instruments, P.O. Box 1178, Portsmouth, NH, USA 03802-1178.

<sup>b</sup> Replaces the Keithley model 570.

<sup>c</sup> Keithley, 440 Myles Standish Blvd., Taunton, MA, USA 02780.

<sup>d</sup> Omegalux, 1 Omega Dr., Stamford, CT, USA 06907.

<sup>e</sup> Automatic Switch Co., Florham Park, NJ, USA 07932.

<sup>f</sup> Cole-Parmer Instruments Co., 7425 North Oak Park Ave., Niles, IL, USA 60714.

<sup>g</sup> People’s Electronics, P.O. Box 6067, Roanoke, VA, USA 24016.

<sup>h</sup> American Power Conversion Corp., 132 Fairgrounds Rd., P.O. Box 278, West Kingston, RI, USA 02892.

<sup>i</sup> Fisher Scientific, 711 Forbes Ave., Pittsburgh, PA, USA 15219-4785.

<sup>j</sup> Newark Electronics, North Ravenswood Ave., Chicago, IL, USA 60640-4496.
depth \times \text{height}) externally. Five centimetres of high-density Styrofoam® insulation on all inside surfaces and chamber partitions of 5 cm thick Styrofoam® create three $33 \times 26 \times 31$ cm (width \times depth \times height) chambers. Inside each chamber 17 W of heat is supplied by a 6.03 \times 1.27$ cm (length \times diameter) cartridge heater mounted 2 cm above the floor. Light is provided by a 7.5 V, 0.22 A light bulb placed at the top of each side wall; air is circulated by a box fan (7.9 \times 7.9 \times 3.8$ cm) attached to the ceiling; and temperature is sensed by a copper–constantan (type T) thermocouple placed 10 cm from the ceiling, 13 cm from the back wall, and 5 cm from the side wall. Within each chamber coolant circulates through a $19 \times 17 \times 1.5$ cm (height \times depth \times width) aluminum block that serves as a heat exchanger.

A 7.5-L Nalgene tank serves as a reservoir for the ethyl alcohol coolant. The reservoir is housed in a plywood box above the chambers and is insulated with 5 cm of high-density Styrofoam® on all sides. Openings in the reservoir enclosure are provided for the cooling probe of the immersion cooler and the coolant feed and return lines. The single gravity feed line from the reservoir fills a $20 \times 2.5$ cm (length \times diameter) manifold made of copper tubing which in turn feeds each chamber (Fig. 1). Coolant flows to each chamber through a low-temperature solenoid valve located on the outer, back wall of each chamber between the manifold and chamber. Coolant then flows to a single return line outside the chambers and is returned to the reservoir by a peristaltic pump (Fig. 1). C-Flex® tubing (0.64 cm i.d.) is used for feed and return lines. A 2.5 cm thick layer of high-density Styrofoam® insulation covers the back (outside) of the sextet of chambers, and the coolant lines and manifold are embedded in channels cut in the insulation. An outer layer of 5 cm of Styrofoam® insulation covers the lines and manifold (but not the solenoid valves), and is removable to permit inspection and replacement of lines and valves.

![Fig. 1. Schematic drawing of the coolant components and plumbing (only one chamber shown for simplicity). Cutaway of environmental chamber shows heat exchanger and heater.](image-url)
The DACS is equipped with an analog input module with a cold-junction reference, permitting direct connection of the chamber thermocouples. A separate circuit connects each of the six heaters and coolant solenoid valves and the pump to a 114-V power source via a solid-state relay (Fig. 2). The 13 relays are in a 16-channel relay board, which is connected to the DACS. A separate circuit connects each fan via a toggle switch to a 114-V power source. A single circuit connects the lights to a 114-V power source via a transformer and is controlled by a household timer. Each fan, coolant valve, and heater circuit is equipped with a 10-, 10-, or 1-A quick-burning fuse, respectively. The light circuit is equipped with a 1-A fuse. Standard lamp cord (14 gauge) was used for wiring. A 450-W uninterrupted power supply maintains power to the computer and controlling program for approximately 45 min in the event of a power failure.

Each chamber is lined with Plexiglas® panels on the bottom and sides. Eight removable Plexiglas® shelves in each chamber are supported by machined grooves in
the heat exchanger and by Plexiglas runners glued to the vertical surfaces of the Plexiglas liners. High humidity is maintained by a 10 × 20 × 3 cm (width × length × depth) tray of water in the bottom of each chamber.

Two programs were written in interpretative basic (BASIC) for temperature control. Constant-temperature regimes are produced in all chambers by running "constant.bas" and supplying at run time the starting date and time and the set temperature for each chamber. Individual chambers can be disabled by a set temperature of 999. Time-varying temperature regimes are produced by running "sinwave.bas". Prior to start-up the user must load six files containing the Julian date and daily maximum and minimum set temperatures for each chamber. Each file may contain up to 365 entries. File entries are loaded into arrays of daily maximum and minimum set temperatures. At start-up the user is asked for the starting Julian day and time. A modified sine wave routine (Allen 1976) regularly alters the set temperature to produce a time-varying temperature regime. A maximum set temperature of 999 turns off the chamber for the day. A minimum set temperature of 999 turns off the chamber at noon of the previous day for 24 h. Equal maximum and minimum set temperatures result in a constant-temperature regime, thus allowing a mixed pattern of constant and time-varying regimes within the six chambers. The control programs are available from the first author.

A status screen digitally displays the maximum and minimum set temperatures, or the constant set temperature, and actual temperature of each chamber. The on-off status of heating and cooling devices (solenoid valves) in each chamber is also displayed. At the start of each 5-s cycle the DACS is instructed by the control program to return chamber temperatures to the controlling program where each is compared with the appropriate set temperature. Differences between actual and set temperature in excess of tolerances result in an instruction to the DACS to close the appropriate relay, or relays, which will turn on a heater or open a valve and turn on the pump. Differences within tolerances result in the opening of the relay and the deactivation of the heating and cooling devices. To avoid conflict between heating and cooling devices within a chamber we use uneven tolerances around the set point temperature. Our tolerances are 0.25°C above and 1.0°C below set temperature. Actual temperatures in each chamber and the reservoir are displayed graphically for a 6-h period. Chamber and reservoir temperatures are averaged for each 15-min period and stored in random-access memory (RAM). At the end of the 6-h period the 15-min average temperatures are written to the computer hard drive, the screen is refreshed and updated with a new 6-h time scale and date (if necessary), and RAM is cleared for the next 6-h period.

The ECs are within a 2.5 × 4.3 m room with ambient temperature maintained at approximately 20°C by an air conditioner.

Chamber Testing. We examined the performance of our chambers under constant-temperature regimes over a 39-d period at set temperatures of 5, 10, 15, 20, 25, and 30°C. Frequency distributions in 0.25°C classes were calculated for the 4320 recorded 15-min averages for each chamber. Within each chamber, photophase and scotophase temperatures were compared by a two-sided *t* test (Zar 1984).

We also examined chamber performance under a mixed regime of time-varying and constant temperatures over a 33-d period. Three chambers operated with daily maximum–minimum temperature files collected during the summer (14–32°C), autumn (−7 to +26°C), and winter (−14 to +16°C) in Blacksburg, Virginia. The pattern of 15-min averages for each chamber was compared graphically with the time-varying set temperature for the regime. A fourth chamber was programmed to maintain a
constant temperature of 15°C. Frequency distributions in 0.25°C classes were calculated for the 3168 recorded 15-min averages for the constant-temperature chamber.

Results and Discussion

System Testing and Use. The chambers performed extremely well when operated for extended periods under either constant, time-varying, or mixed regimes. During the 39-d test of constant-temperature regimes, means (±SE) of the 15-min average temperatures in the 5, 10, 15, 20, 25, and 30°C chambers were 4.91 (0.0058), 9.75 (0.0053), 14.76 (0.0056), 19.82 (0.0053), 24.73 (0.0047), and 29.68°C (0.0053), respectively. These minor differences between set and mean temperatures were not
excessively large for our purposes. In addition, the choice of any particular set temperature is often arbitrary in insect development studies. It is more important to have an accurate record of the actual temperatures than to have the actual temperatures equal the set temperatures.

The consistently negative difference between the set temperature and the 15-min average temperatures suggests that greater accuracy could be achieved by a shorter cycling period. This would result in a weaker cooling response when actual temperatures exceed set temperatures. Increasing the positive tolerance on control would also improve temperature accuracy, but would adversely affect temperature precision.

Approximately 94% of the recorded 15-min average temperatures were within 0.75°C of the set temperature, 65% were within 0.5°C of the set temperature, and less than 0.2% differed from the set temperature by >1.0°C (Fig. 3).

The 15-min average temperatures were significantly different (p < 0.05) between scotophase and photophase in the 5, 10, 15, and 20°C chambers. However, mean differences between the phases were less than 0.05°C. There were no significant
differences between photophase and scotophase temperatures in the 25 or 30°C chambers.

In the 33-d test of mixed time-varying and constant-temperature regimes, the pattern of 15-min average temperatures within the three chambers programmed to simulate Virginia temperatures conformed to calculated set temperatures very closely (Fig. 4). Daily temperature ranges of greater than 20°C were successfully duplicated. A closer examination of chamber performance under time-varying regimes (Fig. 5) reveals minor deviations from set temperatures. Deviations were greatest at temperature maxima and minima, but never exceeded 0.75°C. The precision of a constant-temperature chamber was not adversely affected by operating it in combination with time-varying
temperatures. Eighty-three percent of the 15-min average temperatures were within 0.5°C of 15°C when a combination of constant and time-varying regimes was used (Fig. 6).

After the testing described above we used these six temperature chambers for a 6-month experiment during which time they operated without failure. During that time they produced constant-temperature regimes from -5 to +35°C (25 and 15°C below and above room temperature, respectively). The small internal volume, together with good air circulation resulted in temperature variation of less than 0.1°C within each chamber.

Although some ice accumulated on heat exchangers in chambers with subzero temperatures, it was not a serious problem. The hard, smooth surface of the heat exchanger permitted quick removal of accumulated ice with a heated paint scraper every 2–3 weeks.

**System Modifications and Minimum Temperature Capability.** The small internal volume of our ECs may not be practical for some prospective users. However, any change to the configuration described here would affect the minimum temperature capability. Nonetheless, desired temperatures may still be attained in larger units if sufficient compensating modifications are also made. The most significant changes in minimum temperature capability would result from an increase or decrease in EC internal volume, changes to insulation, and use of a different cooling apparatus; smaller changes would result from changes in ambient temperature and the use of different heat exchangers or fans. Estimating the minimum temperature capability of ECs with a new configuration is recommended prior to construction. The estimation is done in the following manner.

The temperature within the EC reaches its minimum when equilibrium exists between the rate of energy added to the system ($E^+$), the rate of energy transferred

![Fig. 6. Proportional frequency distributions of 15-min average temperatures in 0.25°C accuracy classes for a chamber operating under a constant-temperature regime while three companion chambers operated under time-varying regimes. Values on the x axes are temperature extremes of the classes.](image-url)
between the components of the system \( (E^e) \), and the rate of energy removed from the system \( (E^-) \). In the system described here, energy is added to the interior of the chambers across the walls of the ECs \( (E^{+EC}) \) and from the fans and lights \( (E^{+F}) \). For simplicity, energy transfer within the system can be considered to occur only between the air inside the chamber and the heat exchanger \( (E^{+EC} - E^{X-C}) \), and between the heat exchanger and the coolant reservoir \( (E^{X-C}) \). Additional energy is added to the coolant reservoir across its walls \( (E^{+EtOH}) \). Energy is removed from the system \( (E^-) \) by the immersion cooler. Energy flow is illustrated in Fig. 7. Equilibrium exists when

\[
E^{+EC} + E^{+F} = E^{X-C} = E^{X-C} - E^{+EtOH}
\]  

These rates are a function of temperature gradient and the resistance to energy movement (Kreith 1973; ASHRAE 1985)

\[
E = \frac{\Delta C}{R}
\]  

where the gradient \( \Delta C \), is in °C, and the resistance \( R \) is in °C/W. The minimum temperature capability is estimated by determining \( \Delta C \) (across the walls of the EC) of Eq. [2] under equilibrium conditions. The EC temperature is lowered when the energy rate in the equilibrium is increased, thus increasing the temperature gradient across the walls of the EC.

Resistance associated with \( E^{+EC} \) and \( E^{+EtOH} \) is estimated by

\[
R = \frac{L}{kA}
\]  

where \( L \) is the thickness of insulation (in m), \( k \) is the thermal conductivity of the insulation [in W/(m·°C)], and \( A \) is the surface area (in m²) of the walls. Thermal conductivity of insulation is related to the R factor of construction terminology by
The rate of energy removal from the system (\( E^- \)) is a characteristic of the cooling unit and coolant and is approximated by

\[
E^- = \frac{\Delta C_{\text{C-PBC}}}{R_{\text{C-PBC}}}
\]

where \( \Delta C_{\text{C-PBC}} \) is the temperature gradient between the coolant returning from the heat exchanger and the cooling probe of the PBC-75 (approximately \(-30.4^\circ\text{C}\)), and \( R_{\text{C-PBC}} \) is a nonlinear function given by
TABLE 2. Environmental chamber characteristics

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<th>Characteristic</th>
<th>Value</th>
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<tr>
<td>Chamber dimensions (width × depth × height)</td>
<td>0.33 m × 0.26 m × 0.31 m</td>
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<tr>
<td>Coolant reservoir dimensions (width × depth × height)</td>
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<td>Insulation thermal conductivity (k)</td>
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<td>EtOH specific gravity</td>
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<td>EtOH specific heat</td>
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<td>Coolant flow rate</td>
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\[ R_{C-PBC} = 0.0205 \exp\left(0.0083\Delta C_{C-PBC}\right) \]  

which describes the limited capacity of the PBC-75. Figure 8 illustrates the cooling characteristics of the PBC-75 and ethyl alcohol.

In the system described herein (see Table 2 for specifications), equilibrium is reached when the energy flow rate is approximately 168 W and the temperatures of the EC \((T_{EC})\), heat exchanger \((T_X)\), and coolant \((T_c)\) are approximately \(-14.8\), \(-18.4\), and \(-26.1^\circ\text{C}\), respectively; a substantial increase in EC volume \((75 \text{ cm} \times 41 \text{ cm} \times 61 \text{ cm})\) increases the equilibrium energy flow rate to approximately 261 W and the temperatures to approximately \(-6.5\), \(-12.2\), and \(-24.1^\circ\text{C}\), respectively. The minimum temperature can be lowered in this larger EC by, for example, doubling the insulation of the ECs \((T_{EC} = -12.7^\circ\text{C})\), doubling the size of the heat exchanger \((T_{EC} = -9.4^\circ\text{C})\), doubling the flow rate of coolant \((T_{EC} = -10.5^\circ\text{C})\), doubling the size of the heat exchanger and doubling the flow rate of coolant \((T_{EC} = -14.1^\circ\text{C})\), or lowering ambient temperature to 15°C \((T_{EC} = -8.4^\circ\text{C})\).

Our total expenditure for the six chambers, excluding the DACS which we had on hand, was Can$8637. Table 1 lists the necessary items, manufacturers, suppliers, and costs. Where models have been discontinued by the manufacturer, we have supplied the replacement model and part number. We have not included a cost for the computer and monitor because we feel they are readily available at little or no cost.

The ECs described here performed within our requirements. Their reliability, flexible temperature regimes, low cost, wide temperature range, and ease of construction should make them useful for a variety of experiments. They require only readily available materials and, with the exception of the heat exchangers, require no special equipment for construction. The effect of modifications such as increased volume and (or) insulation can be estimated before construction and the utility of the planned system judged.

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References


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