Performance of an Improved Temperature-Controlled Incubator

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Introduction

When dealing with artificial spawning of fish species, gametes must often be stored at specific temperatures to prolong fertilizing ability. Many studies have dealt with the issue of temperature and storage of sperm, eggs and embryos (e.g., Rothbard et al. 1996, Dinnyes et al. 1997). Storage of gametes can facilitate seedstock production and selective breeding, assist preservation of genetic diversity and expand research opportunities. Refrigeration of sperm also offers several advantages including hybridization and crossbreeding which can be performed in the hatchery. This particular study was part of a larger evaluation of the storage of eggs of koi carp *Cyprinus carpio* at different temperatures (Glenn 1998, Glenn and Tiersch 2002).

The purpose of this study was to modify general-purpose refrigerators to increase useable space and reduce spatial temperature variation to provide incubators for storage of gametes. Economics also played an important role. Commercially available incubators similar to the refrigerators in this study cost US $1000 or more. Other incubators with precise thermostat settings from 5 to 50 ^{o}C cost in the thousands of dollars. Information about the construction of incubators is limited, but a previous design was used as a guide for the use of an external hydraulic-action thermostatic controller (Tiersch and Tiersch 1993, Glenn and Tiersch 2000).

Modification Methods

A total of six incubators were constructed to maintain a constant temperature at different settings (0, 5, 10, 15, 20 and 25 ^{o}C). It required ~45 min to construct each incubator (Figure 1). Six dormitory refrigerators (0.06-m^3, Model 18SRCG, Gercor, Inc., Japan) were equipped with an external hydraulic-action thermostatic controller (Model 1609, White-Rodgers Division, Emerson Electric Co., St. Louis, Missouri, USA). This controller has a 2 ^{o}C differential setting that controls by opening and closing a refrigerant valve. Suction pressure controls the start and stop of the compressor through the motor starter. A small electric fan (115 V, Model 2412PS, Newark Electronics, Metairie, Louisiana, USA) was installed by exposing and separating the wires in the power cord for continuous operation. The interior door liner with built-in storage racks was removed by unscrewing existing tapping screws and replacing them with a 0.3-cm polyvinyl chloride (PVC) sheet (42 x 49 cm) to increase storage space. The original door gasket was removed carefully and replaced on the PVC sheet to ensure proper sealing of the door by using the original tapping screws in pre-drilled holes. In addition, plastic mesh (6-mm mesh size) was attached to the central shelf by use of plastic tie straps to provide a uniform surface for support of sample containers that would have otherwise passed between the shelf slats.

Installation of the thermostats involved exposing and separating the wires in the power cord. The 110-V (black) line was cut, the insulation stripped back, and the wire ends fitted with crimp-style terminals. The exposed portion of the cord was passed through the duplex connector at the bottom of the controller unit, and the line wire was connected in series with the controller. The exposed portions of the common (white) wire and ground (green) wire were left uncut and

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tucked inside the controller, and the unit was mounted on the side of the incubator with sheet metal screws (e.g., Tiersch and Tiersch 1993). During installation, the capillary tube sensor was passed through an existing hole in the back of the incubator, and the sensing bulb was fastened with plastic tie straps under the center shelf. The original thermostat was not disabled, but was placed at a setting ("off") that would not interfere with the operation of the external controller.

Evaluation of Performance

Prior to installation of the door liners and electric fans in all six refrigerators, a study was performed to determine temperature variation with and without improved air circulation. The purpose was to evaluate the benefits of replacement of the door liner and use of the fan to remove temperature gradients. The desired temperatures were set using the factory-calibrated dial settings on the controller, and incubators were allowed to equilibrate for 24 h in a room held at 27 ^{o}C. To record temperature, the incubators were outfitted with three type-T thermocouples and a data logger. The thermocouples were placed in three areas: Area 1 was located middle, right, on the shelf beneath the freezer compartment; Area 2 was located on the upper left shelf, and Area 3 was located in the bottom left (Figure 1).

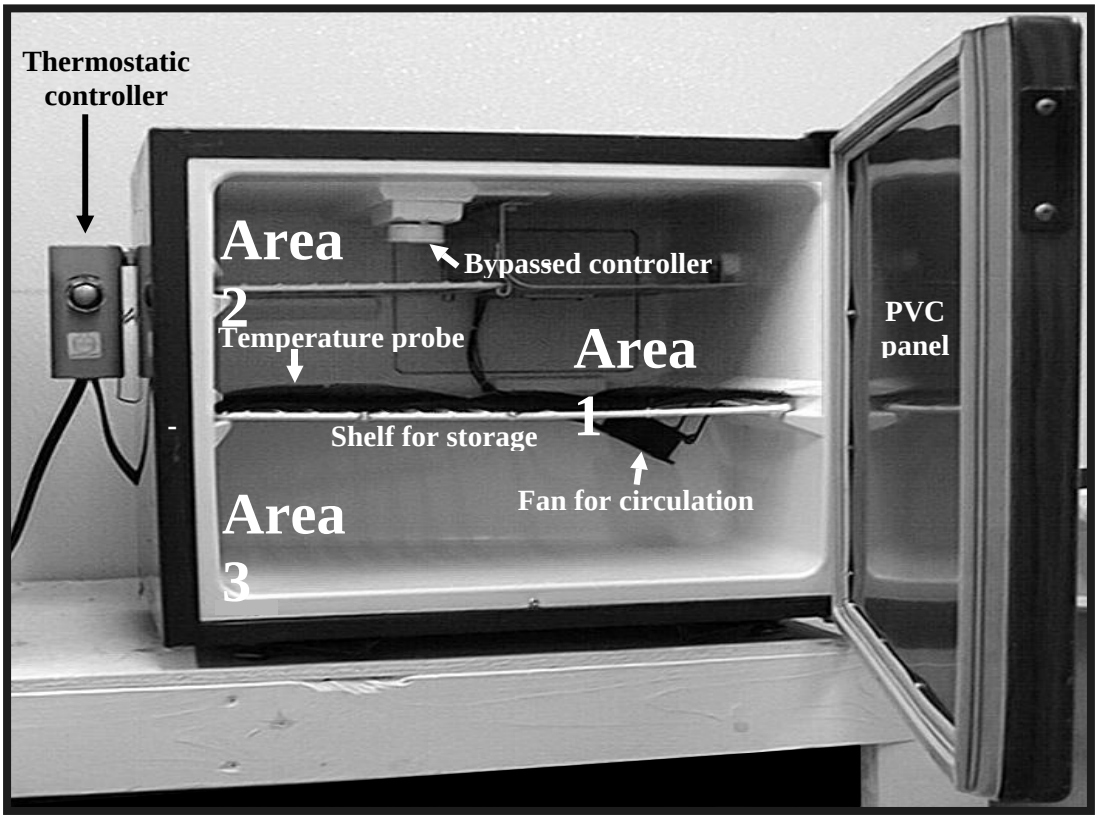

Figure 1. Front view of a modified incubator equipped with external hydraulic-action thermostatic controller mounted on the side. A sensing bulb and capillary tubing were inserted through a hole in back, and attached above the center shelf. The three areas indicated were used to measure temperature variation.

At a setting of 5 ^{o}C, the unmodified incubator showed significant spatial variation (P = 0.0001) in temperature (Table 1).

Table 1. Time and temperature relationships during a 5-hr test at a setting of 5 °C for modified and unmodified incubators constructed from dormitory refrigerators. The mean (± SD), and minimum and maximum values were obtained for three areas (Figure 1) within the incubators. The percentage of time is indicated when recorded temperatures were above, at, or below the set temperature during the test period. Area means sharing letters within an incubator type were not significantly different.

Area Monitored	Temperature (°C)			Deviation from temperature (% time)		
	Mean	Min.	Max.	Above	At	Below
Unmodified:						
1	1 ± 3^{d}	-3.4	5.9	12	0	88
2	6 ± 1^{e}	3.7	8.4	70	0	30
3	3 ± 2^{f}	0.2	6.9	27	0	73
Modified:						
1	5 ± 2^{a}	2.3	8.4	41	0	59
2	6 ± 2^{b}	3.4	8.7	60	0	40
3	5 ± 2^{c}	2.7	8.6	47	0	53

Although the temperatures in the three areas were stable, the lower areas were colder, and the coldest area (Area 1) was below the freezer compartment (Figure 2).

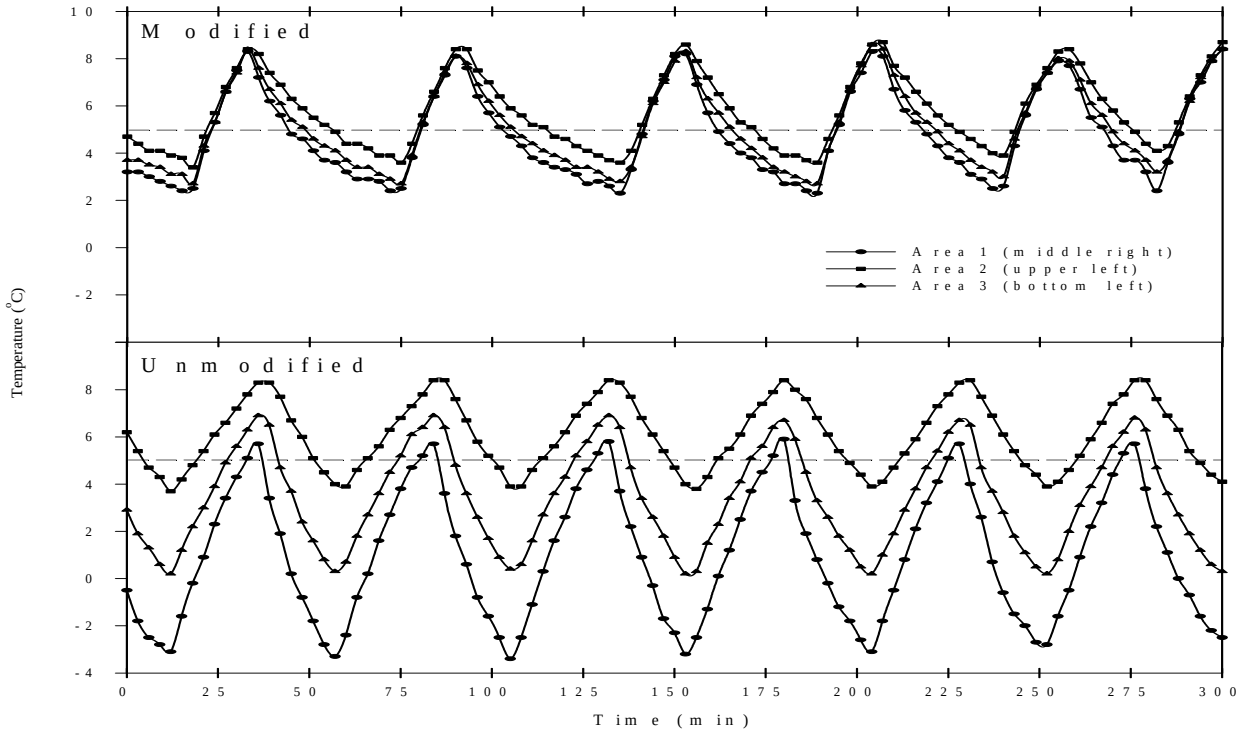

Figure 2. Comparison of spatial variation in temperature in modified and unmodified incubators set at 5 °C. Temperatures were recorded for three areas within the incubator: Area 1, middle, right (below the freezer compartment); Area 2, upper left, and Area 3, bottom left.

The values in Area 1 were below the set temperature for 88% of the test period and were at or below 0 °C for more than 2 h (43%) of the 5-h test period, posing the possibility of inadvertent freezing of samples held in this area. The results for the modified incubators were also stable in each area, although significantly different from one another ($P = 0.0003$). It was concluded that the modified incubators had improved airflow, reduced temperature variation and increased available space. Accordingly, all six incubators were modified with a PVC door liner and fan at a total cost of ~US $150 each in 2008 (including the cost of purchasing the used refrigerators from the LSU Housing Office at US $100 each).

Upon completion of the conversions, six desired temperatures (0-25 °C) were set using factory-calibrated dial settings on the controllers, and the modified incubators were allowed to equilibrate for 24 h. After equilibration, temperature inside the incubators was recorded for 5 h with the thermocouples positioned at the center of the middle shelf. The set temperatures agreed with the measured temperatures (Figure 3).

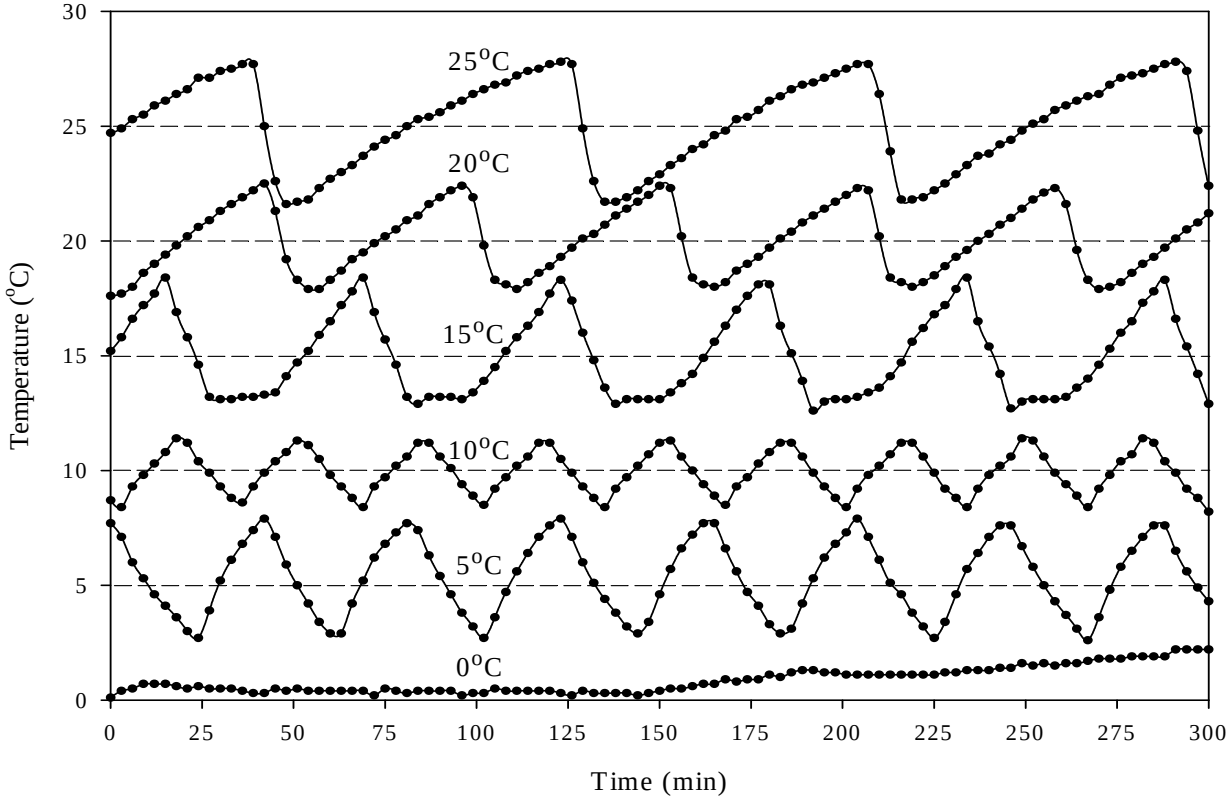

Figure 3. Temperature variation in modified incubators at six temperature settings (0, 5, 10, 15, 20, and 25 °C). A type-T thermocouple and data logger were used to record temperatures at the center of the middle shelf. Ambient room temperature (AT) was also recorderd.

The measurements for the six temperature settings were significantly different (Table 2). The time the temperatures were above or below the set temperature was approximately equal for the 5-h test period except for 0 °C which showed a longer cooling cycle having not reached temperatures below 0 °C.

Table 2. Time and temperature relationships during a 5-hr test at six temperature settings for modified incubators. The mean (± SD), minimum, and maximum values were measured at the center of the middle shelf (Figure 1). The percentage of time when recorded temperatures were above, at, or below the set temperature were calculated. Cooling rate refers to the periods of temperature decline, and cooling time is the percentage of the total 5-h period during which the incubators were actively cooling. Values sharing letters were not significantly different.

Set Temp.	Mean ± SD	Min.	Max.	Deviation from temperature			Cooling rate	Cooling time (%)
				Above	At	Below	(°C/min)	
25 °C	25 ± 2[a]	21.6	27.8	56	2	42	-0.57	12
20 °C	20 ± 1[b]	17.6	22.5	50	1	49	-0.31	30
15 °C	15 ± 2[c]	12.6	18.4	47	0	53	-0.33	18
10 °C	10 ± 1[d]	8.2	11.4	46	1	53	-0.18	54
5 °C	5 ± 2[e]	2.6	7.9	55	2	43	-0.25	45
0 °C	1 ± 1[f]	0.1	2.2	100	0	0	---	48

In all, these inexpensive incubators were useful in the storage of eggs of common (koi) carp while maintaining constant environmental conditions with minimal temperature variation, and providing sufficient space within each incubator for treatment replications. Incubators of this type would be useful for a wide variety of studies that require control of temperature, especially where numerous incubators are required for the purposes of experimental design. Approximately ten such incubators could be constructed for the cost of a single commercially available incubator (US $1,500).

Acknowledgments

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