

### FREQUENTLY ASKED QUESTIONS: ANIMAL REARING

# What should I be looking for in a diurnal lighting cycle? More to the point, do I even need one?

All terrestrial, and most aquatic animals rely on an internal circadian rhythm for timing of eating, sleeping, resting, and reproducing. The exact day/night proportion varies from species to species, and can also be treated as an adjustable experimental variable. Most small mammals only require low light levels, and many actively avoid high illuminance zones.

While many animal rearing facilities rely on existing artificial and natural room lighting to provide a circadian trigger, there are good reasons to independently control this variable within the chamber. The main reason to house animals in a small-scale environment, versus a large-scale room-based cage or tank rack system, is to model unique conditions on a subset of the general research population. Since varying light/dark cycles may be needed, as part of that study, an automatic chamber-based lighting system is preferable to requiring staff to remember to manually turn the room lights on and off at specific times of the day.

Look for a chamber that produces in-cage light levels of 400 lux or less, and has a light-tight door, to prevent bright room lighting from interfering with cycle parameters. The cycle should be easy to program and change, to reduce the risk of cycle errors when altering experimental parameters or changing out host populations.

#### What temperature and humidity conditions are ideal for my animals?

Each species has slightly different needs. Most small mammals, however, thrive between 18 and 26°C, and a Relative Humidity (RH) between 50 and 70%. Select a chamber that is capable of reliably maintaining temperature within this range. Typically, for most building ambients (20-25°C), and given that unit components generate waste heat and most small animals are endothermic, some form of cooling or refrigeration system will be required.

Depending on the rearing environment, additive humidification may or may not be required. The ambient humidity level recommended for lab spaces (50%) is within the range of most insects, but airflow throughout the chamber can cause evaporation and desiccation in smaller or drier working volumes. Depending on your experimental conditions, you may need to specify a humidity control system. A system capable of controlling the chamber RH at a preset level is recommended, since simple uncontrolled evaporative systems can produce chamber humidity levels in excess of 95%, creating condensation and resulting in mold and fungal growth.

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#### Why is airflow important in a large reach-in chamber?

Big chambers have big air volumes, which are usually conditioned using heat and refrigeration (if applicable) elements within the chamber. Small incubators, by comparison, typically have heated walls, and very small working volumes. Since air has farther to go in a reach-in, these larger units are more dependent on forced-air convection (moving air around mechanically) than is the case for their smaller counterparts, which typically use either gravity convection (warm air rising and spreading out), or a mix of the two technologies.

In addition, animal rearing reach-in's are often used to incubate large cages, which can cover shelves and obstruct vertical airflow. Reach-ins need directed airflow though pressurized ducts or air gaps that can't be obstructed. Otherwise, a single heavily loaded shelf can completely stop airflow to the area above or below the blockage, disrupting chamber temperature uniformity and creating "cold spots/regions" within the box.

Directed airflow is, however, more expensive to manufacture than non-directed, requiring more metalwork and higher capacity blower systems. Non-directed airflow units often show up in the insect rearing market, due to suppliers selling lightly-adapted commercial/ foodservice units. These converted food/flower cases come from an application where a temperature variation of several degrees is acceptable. These suppliers often use wire shelves to increase airflow from the top to the bottom of the chamber, and produce artificially attractive unloaded uniformity specifications. Standard wire shelves are a giveaway of this lower-end unit origin and are predictive of resulting airflow problems when used fully loaded.

### What is the purpose of these "fresh air" ports? Shouldn't we want to keep the warm chamber air in, and the cooler outside room air out?

Ordinarily, this would be good advice. Small animals, however, can create high levels of ammonia and CO<sub>2</sub> in their cages and in the chamber environment. For both mice and rats, ammonia levels above 25 Parts Per Million (PPM) are not recommended. Pulling in a small amount of room air will dilute out this potentially toxic chemical, without overwhelming the chamber's air conditioning systems with large volumes of outside air.

Look for units that provide at least 10-15 Air Changes per Hour (ACH), a level which meets most animal rearing room HVAC recommendations. Make certain that the system you select has ports that are easy to find, and intuitive to adjust.

#### How can I select a chamber that's easy to keep clean?

Big chambers have lots of surface area, which can hide spilled bedding, food, and water from view. That said, certain design features can make chambers easier or more difficult to clean than comparable units. Interior surfaces made of stainless steel will resist corrosion better than painted or coated steel or aluminum. Corrosion is a physical process that pits and roughens materials, making a unit nearly impossible to wipe down. Interior components such as duct sheets, fan shrouds, or reinforced shelves that are attached to the chamber with screws or bolts are extremely time-consuming to remove, clean, and reassemble.

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Look for a chamber that requires few or no tools to disassemble for cleaning. It should take no more than 5 minutes to remove all chamber components.

If using an additive water system to boost chamber RH, it's important to carefully evaluate the humidification source. Simple evaporative water pans, while inexpensive to buy, tend to collect dead insects, and serve as a chamber-wide source for mold contamination. Select a humidification system with an external water source; it will be naturally easier to keep clean, be more contamination resistant, and won't require regular refilling by lab personnel.

# Can I run a cycle to automatically kill mold, fungus, and other pathogens in my large reach-in?

While certain types of incubators/chambers (namely cell culture CO<sub>2</sub> incubators) often feature some kind of periodic contamination elimination cycle, few large chambers have this functionality. There are currently no manufacturers, other than Caron, offering a decontamination or sterilization system for large chambers with proven efficacy versus parasites, mold, fungi, bacteria, and viruses.

Caron's 7350 series reach-ins now offer an optional validatable two-hour hydrogen peroxide  $(H_2O_2)$  sterilization cycle. This process provides a statistically significant kill ( $10^{12}$  reduction) of all organisms and microorganisms in the chamber, and can be proven to work through the use of biological indicators, the same methodology used to validate autoclave (steam sterilizer) cycles.

## Why are there two parts to Caron's reach-In sterilization system? What is the "prep" portion all about?

Not all reach-in customers want or need a sterilization cycle, which requires special operational components. Rather than force customers to buy parts they don't want or need, we've broken them out into a special "prep" kit just for sterilization-ready units. The "prep" consists of an electromechanical door lock, which prevents chamber access during the cycle, a power/data connector cable to the sterilization module, and unique programming to run it.

# You show a narrow spectrum "blue" lighting option in your literature. What's that all about?

Recent research (Billamizar, N, Vera LM, Foulkes, N, and Sanchez-Vazquez, F Effect of Lighting Conditions on Zebrafish Growth and Development Zebrafish, Apr 1; 11(2) 173-181; DOI:10.1089/zeb.2013.0926 (2014)) shows that Zebrafish, a species commonly used in developmental biology, hatches develops and grows best when exposed only to blue spectrum light.. Exposure during the study to other spectral bands resulted in lower hatching rates, a higher proportion of malformations, and reduced body weight. This study hypothesized that this is due to longer wavelengths being filtered out by the water column, causing the essential zebrafish circadian rhythm to only be triggered by short-wavelength light. By providing an optional 440-500 nanometer narrow-spectrum light source, Caron can tailor its animal rearing chambers to the unique needs of the zebrafish rearing community reducing the risk created by a conventional wide-spectrum light source.

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