

Incubation and Hatching

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This chapter compares crane egg incubation techniques and provides troubleshooting guidelines. For an expanded treatment of this subject see Stromberg (1975), Brown (1979), and Jordan (1989). The simplest approach to incubation is to permit a pair of cranes to incubate and hatch their own eggs (see Chapter 6). Circumstances may, however, require alternative methods. Genetically valuable birds may be unreliable parents that cannot be trusted with their own eggs. Eggs may also be removed from valuable birds to induce laying of multiple clutches for maximum production. Inclement weather, the threat of predators, or the unexpected death of a parent may all require that eggs be removed from their original nest. In each case, dependable substitutes for parental incubation are needed.

In planning an incubation program, consider the following: (1) size of the breeding flock, (2) reliability of electrical power, and (3) availability of space, manpower, incubation equipment, and supplies. To evaluate an incubation program, benchmarks must also be established. A reasonable hatch rate of fertile eggs is 80-85%, whether incubation is natural or artificial. Hatch rates below 80% indicate a need for improvement.

Generally, the best hatching success has been achieved for cranes using natural or a combination of natural and artificial incubation (Sullivan 1994 unpubl.). Chickens have been successfully used as surrogate incubators, although hatching rates can be lower (Mahan 1992). Chicken incubation may be the best method when crane parents and artificial incubators are not available or when power supplies are undependable.

A good hatchery manager is familiar with embryo mortality patterns, weight-loss profiles, and other egg, embryo, and hatchling characteristics that are indicative of improper temperature or humidity, or other incubation problems. This information is available in the avicultural and poultry science literature, from which many of the references for this chapter were taken.

Several factors can decrease hatchability: disease, behavioral anomalies, improper nutrition, inbreeding, and other genetic defects (see Kuehler and Good 1990; Kuehler and Loomis 1992). Consultation with veterinarians and professionals in other relevant disciplines can greatly enhance an incubation program.

Natural Incubation

The natural parents, foster parents of the same or a related species, or even unrelated species (e.g., domestic chickens) may be used to incubate crane eggs. Choose birds that are reliable incubators and whose reproductive cycle can be synchronized with that of the natural parents (see Chapter 5).

Natural incubation has several **advantages**, and hatchability for some cranes can be improved if at least the first 7-10 days of incubation are natural (Brown 1979; Erickson and Derrickson 1981; Heck and Konkel 1983; Mahan 1992). First, variation in nest temperature due to environmental temperature changes, incubation exchanges by the parents, and a temperature gradient from top to bottom of the egg are lacking in conventional artificial incubation and are believed to affect hatchability (Gee et al. 1995). Second, separate facilities are not needed for incubation and rearing if eggs and chicks remain with the parents or foster parents. Third, naturally incubated eggs are not threatened by an interruption in electrical service or mechanical failure. Finally, natural incubation, as well as subsequent chick rearing, may enhance pair bonds between birds and promote higher reproductive rates in the future (Derrickson and Carpenter 1987).

Natural incubation also has associated **difficulties and risks**: (1) contamination of eggs by feces, soil, nesting material, or other debris; (2) disease transmission from parent to egg or chick; (3) accidental or deliberate breakage of eggs by parents or foster parents; (4) predation; (5) nest abandonment (even if the

parents or foster parents have previously incubated successfully); and (6) reduced ability to monitor embryo development and egg condition. Lower production may also result if parents are used to incubate rather than recycle and produce additional clutches of eggs in the same season (Derrickson and Carpenter 1982). In addition, if foster parents are used, natural incubation involves higher costs for facilities and staff because several pairs of foster parents must be maintained year-round to care for the eggs and young of each pair of birds whose eggs are fostered. Similarly, incubation under chickens requires maintaining several hens so that at least one will be broody when each clutch of crane eggs is laid. Chickens also require a facility where the photoperiod can be controlled to stimulate egg laying and incubation to coincide with that of the cranes. A backup incubation system is needed in any event to incubate thin-shelled or cracked eggs, eggs deserted by parents, or eggs endangered by severe weather.

Parental Incubation

Each potential layer is observed two to four times each day. As the caretaker walks through the colony, he or she reviews a previously prepared form (Fig. 10.8) showing the presence and condition of eggs during the last visit and records his or her own observations. In pens with unreliable parents, new eggs, especially eggs of endangered species, are immediately removed when found. Sometimes these are exchanged for dummy eggs to stimulate incubation (see Chapter 3).

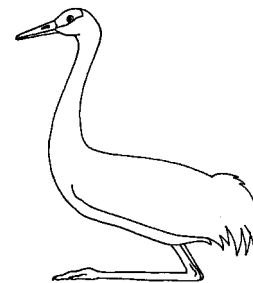
When first handling eggs, mark each with an identification (ID) number, weigh, and measure (length and width). Patuxent **disinfects** each egg by dipping in a 10% Betadine or similar povidone-iodine solution, or in quaternary ammonia or other non-toxic disinfectant at 43° C (110° F), as soon as possible after laying. ICF does not disinfect and has 77-84% hatchability (Sullivan 1994 unpubl.). Disinfection is particularly important when breeding birds are in enclosures that have been used for several years and therefore may have a burden of soil pathogens.

During incubation, observe parents from a blind or at a distance to determine nest attendance. If the parents are frequently off the nest, especially in cold weather, consider removing or replacing the eggs and using another method of incubation until conditions improve. Determine egg fertility by candling, if possible, 5 to 7 days after oviposition (see Fertility Determination section, this chapter). This is also a

good time to remove the eggs if the parents are to lay another clutch (see Chapter 3). Weight loss of the egg should also be monitored throughout incubation. Around the 20th day of incubation, Patuxent uses **flotation** to check the viability of eggs remaining with the parents (technique described later). ICF candles a second time and only uses flotation if candling is unsuccessful or embryo death is suspected. Once you are certain an embryo is not developing, remove infertile or nonviable eggs and open them for bacterial culture and examination of contents. The eggs may be replaced with artificial eggs or other viable eggs to keep the pair incubating.

One or two days before **hatching**, the chick becomes active, punctures the air cell, and becomes vocal (Hartman et al. 1987). The incubating parents communicate with the hatching chick by purring frequently, and they spend more time hock-sitting rather than lying in the nest. More frequent nest checks are advisable at this stage if the adult birds are not unduly agitated. Make at least one close inspection as soon as possible after the chick pips (i.e., when the first break in the eggshell occurs, usually indicated by noticeably louder cheeping from the nest) to see if the pip is in the correct position (at the large end of the egg). Additional inspections can be made with binoculars from a distance of 15-30 m. If an egg has been pipped for more than 48 h but has not yet opened, enter the pen to see if the inner eggshell membranes have dried and are adhering to the chick, thereby preventing hatching progress. **Chick deformities** can also prevent hatching. Problem eggs are best dealt with by moving them to the artificial incubation facility (see Hartman et al. 1987; also Assisted Hatches section in this chapter).

Check the chick as soon as possible after it hatches to determine if the yolk sac has been completely absorbed into the body cavity (see Chapter 5). If the umbilicus is closed (with the yolk sac in the abdomen), apply iodine solution to the site to prevent infection. Weigh the chick to establish a reference point for early growth evaluations.



Surrogate Incubation by Cranes

The surrogate pair must have eggs of their own that are at approximately the same stage of incubation as the fostered eggs (usually within 10 days of synchrony). This is easiest to arrange if the foster species breeds near the same time as the donor. Otherwise, the breeding cycles of one or both species must be altered by manipulating the photoperiod with artificial light, recycling the foster parents, or both. If surrogate parents are in short supply, move eggs to artificial incubators after 7-10 days. This allows pairs to incubate up to three sets of eggs in a single season (see Egg and Chick Adoptions in Chapter 5).

Surrogate Incubation by Chickens

Incubation of rare or exotic species by chickens is standard practice in aviculture, especially for the production of game birds (pheasants, quail, etc.; Brown 1979; Heck and Konkel 1983). Chickens receiving crane eggs for surrogate incubation must be in reproductive synchrony with the crane pairs. Unlike cranes, which have an incubation period of about 30 days, the normal incubation period for chickens is 21 days. Although a chicken's incubation period may be extended to 40 days, the behavior and physical condition of the hen should be closely monitored to prevent nest abandonment and to avoid impairing the health of the hen.

Use large or "standard" chicken breeds rather than bantams (which may be unable to cover the eggs completely) as surrogate incubators for cranes. Because crane eggs are much larger than chicken eggs, it is unlikely that chickens can adequately turn crane eggs. As a result, eggs under a chicken should be turned by hand at least four times per day (see Turning under Mechanical Incubation). Suggested chicken breeds for crane egg incubation are Brahmas, Langshans, and especially Cochins. Other breeds of large-bodied chickens may also be used, but commercial strains of these breeds are unlikely to be good incubators. Particularly good incubating strains are available from poultry hobbyists. Day-old chicks of these exhibition-type breeds (some of which are good incubators) are available from commercial hatcheries. Obtain chicks hatched in spring or summer for use as incubators ("broodies") the next year. All chickens should be quarantined and health tested before introducing them into the collection.

Males are needed for breeding, but are not necessary for hens to incubate. Only a few males are needed because one male can easily inseminate 7-10 hens. Producing your own replacement chicks avoids the risk of introducing disease and allows for selection of stock with good incubating qualities. Although unrelated stock must be obtained periodically to minimize inbreeding, such additions need not be frequent because healthy hens can be used for 5-7 years or longer. Indeed, experienced, older hens are the most valuable birds in a surrogate incubation program.

Maintain chickens in a well-ventilated enclosure that provides about 1 m² of floor space per adult bird (enough space for the birds to move about freely and remain unsoiled). Use hardwood chips, shavings, or other dry, relatively dust-free bedding. Prevent bedding from becoming wet or damp; immediately remove damp bedding to avoid growth of fungi and bacteria. If possible, provide an outdoor yard for exercise and a more interesting environment. This practice is believed to provide the birds opportunities for normal behavior and prevent feather plucking, cannibalism, or other destructive activities. Keep exercise areas clean and tightly enclosed to prevent the entry of predators, vermin, or wild birds that could introduce disease. Test and treat for internal parasites before the chickens are put into an outdoor enclosure so the ground does not become contaminated and serve as a source of reinfection.

Chickens are able to withstand fairly cool and warm temperatures, but should be protected from temperature extremes. Provide shade and good ventilation during warm weather and enough heat during extreme cold to prevent frostbite and freezing of drinking water (preferred minimum temperature ca 10° C [50° F]).

Controlled lighting can be used to bring hens into production and incubating condition to coincide with crane egg production. About 4 months before hens are needed for incubation, adjust the **photoperiod** to 8 h light:16 h dark/day for 8 weeks. Thereafter, change the lighting regime to 14 h light:10 h dark/day to stimulate egg production. Egg production should begin about 3 weeks later, and some hens should become broody about 2-3 weeks after the onset of laying. There is considerable variation among birds, however, and some may never become broody. Whenever the natural day length (i.e., light period) exceeds 8 h/day, eliminate access to any natural light or artificial short-day photoperiods will be ineffective. If broody hens are needed over more than 1-2 months, it may be

necessary to maintain two or more groups of chickens on different lighting schedules in separate enclosures. Prevent light leakage between such enclosures.

To prevent competition for nest sites, provide **several nests** for each group of hens. Line nest bottoms with felt-type indoor-outdoor carpeting, and fill them with chopped straw or similar material (e.g., clean, fine grass) to a depth of 5-7 cm. Hens arrange this into a cup shape for laying. Change nesting material monthly, or immediately if it becomes damp or soiled with feces or broken eggs.

Maintain the chicken flock on a high-quality **diet**, readily available from commercial livestock supply stores. Follow the manufacturer's instructions regarding the appropriate diet to feed at each age or stage of production (e.g., layer diet for hens in production). Provide feed *ad libitum* along with clean, fresh water. Scratch (i.e., cracked corn, wheat, or other grain) may be offered as a treat so that the birds become tame and are easily approached. However, because commercial poultry diets are designed to be nutritionally complete, feeding more than small amounts of scratch can lead to a dietary imbalance. Layer diets generally contain elevated levels of calcium, but crushed oyster shell can be provided as a calcium supplement for eggshell production (Brown 1979).

After laying begins, remove eggs daily to prevent breakage. Broken eggs soil the nest and hen, and may lead to habitual egg-eating. Place two or three **dummy eggs** in each nest before the onset of laying to encourage hens to use the nests and to stimulate incubation.

The combs of young and nonlaying hens are small and dull orange, whereas the comb of a laying hen enlarges and becomes bright red. In addition, the abdomen of the laying hen becomes enlarged, the vent becomes large and moist, and the spread between the pubic bones increases from about the width of one finger to two to three finger widths. Laying hens will also crouch in a sexually receptive position when approached.

Birds change in both appearance and behavior when they become broody. Their combs and other physical characteristics return to the condition of a nonlaying hen. They remain in the nest almost continuously and only leave briefly to eat, drink, defecate, and exercise, whereas laying hens are usually found in the nest only in the morning, when most laying occurs. Laying hens will generally leave the nest readily when disturbed, whereas broody hens leave the nest reluctantly and may become extremely defensive. This behavior varies with each hen, so become

familiar with each bird's idiosyncrasies to be certain that a hen is broody. When removed from the nest for egg examinations, broody hens often sit where they are placed and refuse to walk about. They elevate their hackles and squawk when approached by another bird or a caretaker, and emit an almost continual cluck when moving on their own or when disturbed.

If possible, move incubating hens to a separate room or building. Maintain this area at light and temperature conditions to which the hen is accustomed (or slightly warmer, i.e., 15° C [60° F]). Place each broody hen in a **lock-box nest** (see Fig. 4.1) where she will remain most of the day. Place an indoor-outdoor carpet liner in the nests similar to the laying nests, and form a cup-shaped straw or grass nest by hand before the hen is placed in the nest. When a hen is first moved to the broody house or room, put dummy eggs under her for 2-3 days to make sure that she continues incubating after the move. Let the hens out every morning for 30-45 min to eat, drink, defecate, and exercise. Observe the group of hens for aggression if they have not been previously housed together, and deal with serious encounters by separating the birds. Aggressive encounters may be reduced by providing the birds with multiple feeders and waterers separated by 2 m or more.

Observe broody hens in the morning when they are released into the exercise yard and again in the afternoon. These observations are especially important for hens that have just started incubating or that have been sitting for more than 3-4 weeks. Hens that defecate in their nests, or that do not sit tightly and appear eager to leave the nest, are no longer broody. If hens do not sit tightly after they are moved to the broody area, move them back to the laying house until they develop stronger incubation tendencies.



FIG 4.1. Lock-box nest for chickens used to incubate crane eggs.

PHOTO PATUXENT

Special care is necessary to prevent egg breakage and to avoid injury when removing the hen. Lift the broody hen from her nest by gently sliding one hand under her breast and placing your other hand on her back. Slowly lift the hen up and out of the nest and set her on the floor. Be careful to prevent her from kicking eggs out of the nest. Close nest box doors while the hens are out to prevent them from flying into their own or another nest, thereby accidentally damaging the eggs. When returning hens to the nests, gently lift them to the edge of the nest and allow them to step in and settle onto the eggs. Hens often become so accustomed to this routine that they may approach the caretaker to be returned to the nest. As you handle each hen, evaluate her general body condition, and return any hen that is becoming too thin to the laying house or, ideally, to a separate area where she can lose her broodiness and regain weight.

Although crane eggs may remain under hens for the entire incubation period, the benefits of hen incubation are realized by about the tenth day of incubation. Thereafter, the eggs may be incubated artificially. Some hens recycle and are available for incubation a second time within the same season, although others molt after the first incubation cycle and do not return to production. Factors controlling these different cycles probably include ambient temperature, other environmental conditions, and the genetic make-up of the hen.

Mechanical Incubation

Critical Components

TEMPERATURE CONTROL. Reliable and consistent incubator operation requires a system with dual temperature controls consisting of primary and secondary thermostats. The primary thermostat controls temperature during normal functioning of the incubator. The secondary thermostat assumes control if the primary thermostat fails. To monitor temperature, place calibrated thermometers inside the incubator. These thermometers must be readable from outside the closed incubator.

Small temperature variations affect embryo survival because normal development occurs within a very narrow temperature range. Detrimental effects of incorrect temperatures, however, depend on duration, direction (too high or too low), and stage of development. Sharp

temperature increases of only several degrees can be almost immediately lethal. A temperature increase of only 1-1.5° C (2-3° F) may not be immediately fatal, but embryos are likely to die after only 4-5 days. Low temperatures (but still within rather narrow limits) slow development and delay hatching, but do not increase mortality substantially (Brown 1979).

Minor temperature variations exist within an incubator, especially among egg trays at different levels. Measure temperatures in different parts of the incubator annually with thermometers accurate to within 0.1° C (0.2° F). By moving the thermometers to different places in the incubator, you can actually map temperature variation within the machine (Heck and Konkel 1983). The stability of temperature conditions inside the incubator depends on ambient conditions, and therefore requires a stable temperature inside the incubator room or building. Remove or add eggs relatively quickly, but carefully, because prolonged or repeated opening of the incubator door can cause the temperature to drop significantly (Burnham 1983).

HUMIDITY. The humidity level inside the incubator controls egg weight loss. Incubator humidity is controlled by the addition of water through evaporation from a reservoir, by misting, and by regulating air flow through the incubator or through the incubator room from outside. For incubators that are humidified by evaporation of water from a reservoir (tray), higher humidity is achieved by increasing the surface area of the water (i.e., by increasing the length and width of the tray, using rotating fins, or placing sponges in the water reservoir). Placing the fan so air blows directly across the water surface also increases evaporation. Use distilled water to avoid mineral buildup, although large incubators often have flow-through humidifying systems for which the use of distilled water would be costly.

Vent openings control air flow into the incubator and thereby reduce (vents open) or increase (vents closed) relative humidity. Except during fumigation, do not completely close the vents because developing embryos need a constant influx of fresh air. Humidity is also lost when the incubator door is opened, so open the machine only when necessary and for as brief a time as possible. Humidity losses are even more important than temperature decreases, because desired humidity levels are not restored as quickly as temperature. To minimize this problem and to rapidly restore humidity in the incubator, especially large incubators, lightly spray the incubator floor with water before closing the door.

Before eggs are set in an incubator, thoroughly clean all inside surfaces of the incubator, including egg trays, with a bactericidal and fungicidal disinfectant. The electronics and wiring are cleaned with compressed air or a light spray with the disinfectant. After cleaning, allow electrical components to dry, then turn the incubator on to raise the inside temperature to 26-37° C (80-100° F) and increase relative humidity to 50-60%.

After establishing operational conditions, the incubator may be fumigated with formaldehyde by combining 35 mL of 40% formalin with 17.5 g of potassium permanganate per 2.83 m³ (100 ft³) of incubator volume. Although it is very effective for disinfection of incubators, fumigation with formaldehyde gas requires great caution and good ventilation because formaldehyde is carcinogenic and a strong irritant to human eyes and respiratory tracts. For this procedure, we recommend the use of goggles and a mask (respirator). Be sure that the incubation facility is vented to the outside to keep fumes from escaping to other indoor areas. If appropriate handling of formaldehyde is not possible, the use of commercially available spray disinfectants is advisable to avoid health risks.

Use only porcelain, earthenware, or heat-tempered glass with the fumigation reagents because these chemicals cause a violent exothermic reaction. The reagents interact with some metals and melt or burn plastic and other flammable materials. Before fumigating, close the incubator door and vents. Place the potassium permanganate in the container first. Then place the container inside the incubator, add the formalin, and immediately close the incubator. After 20 min, remove the fumigant and leave the incubator open for a few minutes to air out. The machine is then ready to receive eggs. For incubators with a recent history of poor hatches from disease or that have had rotten eggs break or explode inside the machine, use a double-strength mixture of the chemicals.

Fumigate incubators at least every 2 weeks, even during incubation. If eggs are added to the machine frequently (every few days or less), fumigate weekly to eliminate contamination from new eggs. Only eggs in very early incubation (5 days or less) or whose stage of incubation is unknown should be removed and placed in another machine during fumigation. If another incubator is not available, any heated container that maintains the eggs at 35.0-37.2° C (95-99° F) will suffice for a short time. Otherwise, fumigate fresh (unincubated) eggs and eggs incubated more than 5 days to kill any pathogens on the shell. Clean and fumigate hatch-

ers after each use, even if only used for one chick, according to the same procedure described above. If two or more eggs are in a hatcher, fumigate after all eggs are hatched and all chicks removed. If fumigation is not used, employ other disinfectants, but be sure to determine efficacy and safety for use with eggs.

The use of sterile surgical gloves or clean disposable (plastic, vinyl, or latex) gloves to handle eggs, dipping of eggs before they are placed in the incubator (see Egg Handling section below), and regular cleaning and disinfection of incubator water trays also helps to ensure good sanitation.

RECORD KEEPING. Incubator conditions (temperature, humidity, tray position, etc.) should be closely monitored and recorded at least two or three times daily. This will ensure that any trends, such as increases or decreases in temperature or humidity, are detected early and corrected. It is important that incubators with automatic turners be checked at different times during the day to determine if they are actually turning the egg. These records serve as a basis for making adjustments in incubation conditions if hatching problems arise.

Egg Handling

After an egg is collected, any dirt or fecal material adhering to the shell is wiped off with a soft cloth. Fine-grade sandpaper can be used to remove stubborn materials, but be sure not to damage the shell. To prevent the introduction of pathogens into the incubator, fumigate incubated eggs according to the procedures described previously. Do not fumigate eggs unless they are fresh or have been incubated for at least 5 days. As an alternative disinfection procedure for incubated eggs or those whose stage of development is unknown, dip them in 10% povidone-iodine solution at 43.3° C (110° F; Ernst 1975) and then allow them to dry at room temperature before setting them in the incubator.

Artificial Incubation Conditions

For all crane species, the proper dry-bulb temperature is 37.6° C (99.5-99.75° F; Putnam 1982). Preliminary research on regular **cooling** of crane eggs during incubation showed no significant effect on hatchability (Putnam 1982; Russman 1987). We recommend leaving eggs in the incubator continuously except for candling, fumigation, etc., as discussed elsewhere in this chapter.

The wet-bulb temperature used for all but two species of cranes is 30.0° C (85-87° F). For White-naped Cranes, the wet-bulb temperature used is 27.0° C (80.0° F; Putnam 1982), and Wattled Crane eggs have been successfully incubated at a wet-bulb temperature of 28.0° C (82-84° F; Carol Hesch, Memphis Zoological Garden and Aquarium, Memphis, Tennessee, personal communication). At higher altitudes with lower air pressure, wet-bulb temperatures should be adjusted slightly (1° C, 1-2° F) upward or in areas with high relative humidity, downward to achieve the desired rate of weight loss.

During incubation (natural or artificial), carefully monitor the progress of the developing embryo by candling and flotation (see Determination of Fertility). Weigh the most valuable eggs twice weekly to determine **weight loss** during incubation. Record egg weight, and track weight loss. Optimally, eggs lose 15% (range, 13-17%) of their fresh weight over the incubation period (Rahn and Ar 1974; Ar and Rahn 1978), although eggs that lose considerably more or less than this amount often hatch, either independently or with assistance (see Problems and Remedies). Conditions that can reduce egg weight loss include high humidity, low temperature, a thicker-than-normal shell, or blocked pores in the shell. Conditions that can increase weight loss are low humidity, high temperature, or an abnormally thin or porous shell.

Candle eggs one to three days before the scheduled hatching date to determine viability and locate the air cell. Mark the lowest point of the air cell (i.e., the point that extends farthest toward the small end of the egg). The egg is placed with the marked spot up (Putnam 1982). If positioned correctly, the chick will pip near this point. Turning of the egg is no longer necessary during the last 2 days of incubation, so the egg may be placed in a depression on a styrofoam pad on the bottom of the incubator.

ALARMS. An important component of the artificial incubation system is an alarm that notifies personnel of temperature extremes and power outages. Set the alarm to sound at temperatures above 38.3° C (101° F) or below 35.6° C (96° F), and when the power fails. In addition to an audible alarm (bell or siren) in the vicinity of the incubation facility, install an alarm with a telephone autodialer that notifies personnel of problems when they are off duty or away from the incubators. A flashing light can also be added to the system to serve as a visual alarm.

Artificial Hatching Conditions

Crane chicks normally hatch after about 30 days of incubation (see Table 4.1). After an egg pips, move it to a hatcher (Fig. 4.3) maintained at 37.2° C (98.5-99.0° F; Putnam 1982). A hatcher is a modified incubator maintained at a higher humidity to facilitate hatching. Use a separate hatcher for each chick, or if two or more chicks are hatching at the same time, they may occupy the same hatcher if the chicks can be separated inside the machine. Do not place additional pipped eggs in the used hatcher until it is cleaned and disinfected. Debris from hatched chicks provides a growth medium for bacteria and other pathogens that thrive in the heat and humidity of the hatcher. Hatching eggs in a machine separate from the incubator prevents contamination of eggs that are at earlier stages of incubation.

During hatching, eggshell membranes may become dry and adhere to the chick. Therefore, maintain the hatcher at the **highest humidity possible**. Generally, humidity inside the hatcher should yield a wet-bulb temperature of 32° C (90° F) or higher (Putnam 1982).

The chick may be heard scratching and cheeping inside the egg for up to 24 hours or more before pipping. The vigor of these activities is a good indicator of the chick's strength. Vocalization begins after the chick breaks through the inner membrane into the air cell and begins to breathe air. The egg may be moved to the hatcher at this time or after the chick has pipped. Move eggs with chicks in the air cell to the hatcher if they will not be checked again for several hours (e.g., overnight). Otherwise, the chick may hatch in the incubator and die from trauma if struck



FIG 4.3. Dan Sprague demonstrates hatcher. PHOTO DAVID H. ELLIS

TABLE 4.1.

Incubation Periods of Crane Eggs

SPECIES	NUMBER OF DAYS (RANGE; MEAN) ¹	REFERENCES
Black and Gray Crowned	26-31; 28	Carthew 1966; Walkinshaw 1965; Walkinshaw 1973; Urban et al. 1986; ICF records
Wattled	32-40; 33	Conway and Hamer 1977; Johnsgard 1983; Urban et al. 1986; Breiby 1994 unpubl.; ICF records
Blue	29-33; 30	Van Ee 1966; Johnsgard 1983; Urban et al. 1986; Hartman et al. 1987; ICF records
Demoiselle	27-30; 28	Stehlik 1970; Johnsgard 1983; Urban et al. 1986; ICF records
Siberian	26-32; 29	Johnsgard 1983; Hartman et al. 1987; Breiby 1994 unpubl.; ICF records
Sandhill	27-35; 30	Johnsgard 1983; Hartman et al. 1987; ICF and Patuxent records
White-naped	30-33; 30	Johnsgard 1983; Hartman et al. 1987; ICF records
Sarus	31-36; 31	Johnsgard 1983; Archibald and Swengel 1987; Hartman et al. 1987; ICF records
Brolga	28-36; 30	Johnsgard 1983; Archibald and Swengel 1987; ICF records
Eurasian	28-31; 30	Glutz von Blotzheim 1973; Cramp and Simmons 1980; Johnsgard 1983; ICF records
Hooded	28-30; 29	ICF records
Black-necked	30-33; 30	Johnsgard 1983; Liao 1987; Breiby 1994 unpubl.; ICF records
Red-crowned	29-36; 32	Johnsgard 1983; Hartman et al. 1987; ICF records
Whooping	28-34; 29	Johnsgard 1983; Kuyt 1987; Breiby 1994 unpubl.; ICF and Patuxent records

¹ Extremely long incubation periods (e.g., 39-40 days reported for Wattled Cranes) may represent erroneous field observations or eggs exposed to extreme environmental conditions or improper incubation conditions.

by moving parts. **Place** eggs in the hatcher with the **pip up**. This position is believed to facilitate hatching by the chick (Burnham 1983). In addition, this position allows observation of hatching progress through the glass lid or door of the hatcher.

If a chick's cheeping becomes less audible or its movements less vigorous, the chick may be weakening and unable to hatch. Check the egg four times daily and record the chick's progress and condition. The chick can be stimulated by tape recordings of crane brooding calls. Also, human imitations of this purring or gargling sound may stimulate the chick to vocalize or move. Crane chicks usually hatch between 24 and 36 h after they begin cheeping or scratching (Hartman et al. 1987). Consider helping the chick from the egg if it fails to hatch within 48 h or becomes noticeably weak (see Problems and Remedies).

Compartmentalize the hatcher to prevent mixing of chicks. Vinyl-coated wire dividers are nonabrasive to chicks and allow air to circulate. Line the bottom of each compartment with a removable piece of indoor-outdoor carpeting. This provides a good substrate and prevents the chick from catching its toes in the wire floor. Be sure that the dividers are tall enough to prevent injury or chicks mixing together.

Determination of Fertility

It is important to determine whether eggs are fertile or infertile for several reasons. First, consistent infertility may indicate improper management, disease, inbreeding depression or other genetic disorders, or

physiological or behavioral problems in the breeder flock. Second, early embryo mortality attributable to pathogens, egg handling, or incubator management may be mistaken for infertility and remain uncorrected. Third, infertile eggs can be removed from nests or incubators and replaced with fertile eggs so that incubator space or incubating birds are used to full advantage. When parents incubate their own eggs, early determination of infertility allows the eggs to be removed so the birds can lay another, hopefully fertile, clutch of eggs (Brown 1979; Erickson and Derrickson 1981).

Candling

Candling is an established technique that is especially useful for determining fertility and monitoring the development of embryos in eggs with lightly pigmented or unpigmented (white) shells. It requires a lamp inside a box or tube, and a dark room. To use a candler, hold the egg with the large end slightly above the horizontal and against a hole in the box or at the end of the tube so that light from the lamp passes through the egg (Fig. 4.4). For dark-colored eggs in particular, the candler hole should fit tightly around the egg to prevent light leakage. Light that illuminates the outer surface of the egg obscures egg contents. Even with the proper equipment, some crane eggs are too heavily pigmented for candling.

Candling is only useful for determining whether or not an embryo is developing inside the egg. It is not useful for distinguishing between infertile eggs and fertile eggs that fail to develop, or for detecting embryos that die very early. **Embryo development** is detectable by candling as early as 4 days after the onset of incubation in lightly pigmented eggs, but may not be detectable until 5-7 days in some eggs. Development is first recognized as a faint, weblike network of fine blood vessels radiating from a central focus, which is the embryo. The embryo will float to the uppermost position in the egg. At this stage, check to see if the embryo moves freely by **gently** rotating the egg; if it does not move freely, it has adhered to the shell membrane and may die. Initially, the blood vessels appear in a circular pattern about 2-3 cm in diameter. As the embryo grows, the blood vessels become larger, extending around the yolk and eventually throughout the egg. As the embryo enlarges, it blocks light passage through the egg. In light-colored eggs, you may be able to detect movement, and the head, wings, and legs may be

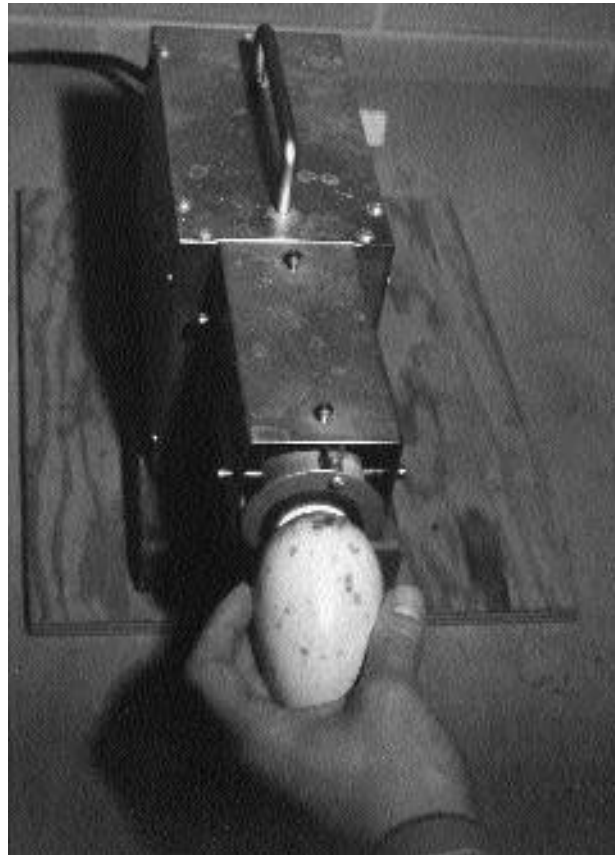


FIG 4.4. *Candling to determine fertility and viability of an egg.*

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discernible as they develop. In heavily-pigmented eggs, only light and dark areas within the egg may be distinguished, and increased opacity may be attributable to a viable, growing embryo.

Coloration of the egg contents during candling is often diagnostic for fertility and viability. A nearly clear egg with a large orange central area (the yolk) is either infertile or not incubated long enough to detect development, or contains an embryo that died in the very early stages of development. An egg with an overall reddish or pinkish hue is fertile, and the embryo is probably alive. Splotchy yellow and brown egg contents indicate a dead embryo or infected egg. For eggs in early incubation, a dark red ring indicates early embryo death or the imminent death of the embryo.

Candling can also be used to monitor the size and position of the **air cell**. The air cell should enlarge during incubation as water is lost through evaporation and respiration (Brown 1979). If eggs cannot be weighed, changes in the size of the air cell can be used to indicate weight loss indirectly. The rate of weight loss and size or shape of the air cell are not indicators

of fertility. However, an irregular or poorly defined air cell often indicates an infertile egg or an early dead embryo. Malpositioning of the air cell or a floating air cell (i.e., one that moves as the egg is turned) are often caused by jarring of the egg and may cause difficulties in development or hatching (see Problems and Remedies). Late in incubation, the margin of the air cell shifts. Near hatching, movement of the embryo in the air cell is evident.

Flotation

Flotation (Fig. 4.5) is an alternative to candling for dark-colored eggs or where an adequate light source is unavailable. This method can be used to determine fertility and viability of eggs after about 21 days of incubation, and may also be used to determine age of eggs (Fisher and Swengel 1991). At this stage, eggs float nearly vertically, with the large end up. From 21-23 days, only a slight rotational movement of the egg is noticeable. When they first appear, these movements can be obscured by movement of the flotation vessel or even a slight breeze. Nearer to hatching, stronger, twitching movements are apparent.

Float eggs in a mild **disinfectant** solution (10% povidone-iodine or equivalent) at 43° C (110° F; Ernst 1975). Observe the egg for movement for 1 min or less. Floating the egg longer risks asphyxiation or overheating of the embryo. Do not float eggs in cool water lest the egg contents contract and draw bacteria through the shell. Avoid floating eggs frequently because of potential damage to the embryo or the protective cuticle on the eggshell. Fresh disinfectant solution should be used each time eggs are floated. An initial determination of fertility and viability can be attempted after about 20-21 days of incubation. If no movement is detected, continue incubating the egg and float it again in 1-2 days. Embryos can become quiescent and fail to move when floated, so a lack of movement is not a definite indicator of embryo death. Float the egg again after a day or two if pipping does not occur when expected to determine if the chick is still alive. From about day 25, purring to the egg while it is being floated or while on a flat, sterile surface may stimulate movement.

Opening and Examining an Egg

The only way to accurately determine the fertility of an unincubated egg or one that fails to develop is to open a hole in the shell or break the egg into a dish to

reveal the germinal disk on the yolk. Do this carefully so that the yolk remains intact. In infertile eggs, the **germinal disk** appears as a small whitish spot on the yolk, about 2 mm in diameter (Fig. 4.6). In fresh fertile eggs (Fig. 4.7), this spot is a 4-mm-diameter hollow ring (donut-shaped), darker in the center. The disk enlarges as the egg is incubated. By 3 days of incubation, the heart and blood vessels become evident, even though they may be indiscernible by candling (Fig. 4.8). If non-viable eggs are to be examined more than a few hours after termination of incubation, refrigerate at 4-10° C (40-50° F).

For embryos determined to be dead by candling or flotation, opening the egg can often reveal the probable cause of death or at least the stage of development at which the chick died. Figures 4.9 and 4.10 are included for reference in evaluating embryo developmental stages. It is also useful, in evaluating necrotic tissue, to know that feather follicles first appear around day 15. Consistent mortality at a given stage of development can be diagnostic of improper incubator conditions. For further discussion of **egg necropsy** techniques, see Langenberg (1989) and Joyner and Abbott (1991).



FIG 4.5. *Egg flotation to determine viability.* PHOTO DAVID H. ELLIS

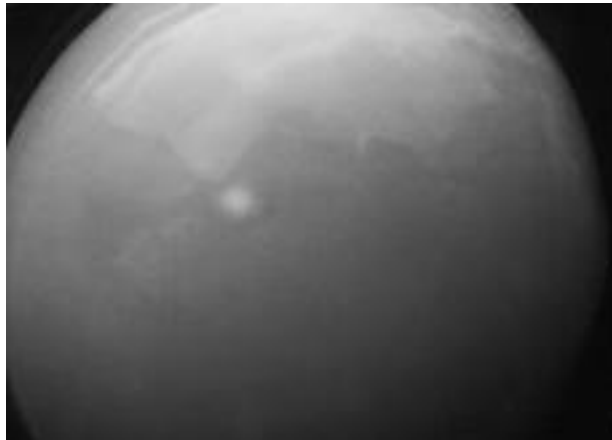


FIG 4.6. *An infertile chicken egg.*

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FIG 4.9. *A 12-day-old crane embryo.*

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FIG 4.7. *A fertile, unincubated chicken egg.*

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FIG 4.10. *A 23-day-old crane embryo.*

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FIG 4.8. *A 3-day-old chicken embryo.*

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Problems and Remedies

The response to incubation and hatching problems often requires innovation, creativity, and familiarity with the techniques used for other species. The following summarizes the most common incubation problems and established remedies.

Malpositions

Normally at hatching, the chick positions itself with its back parallel to the long axis of the egg, its tail in the small end of the egg, and its head turned underneath the right wing so the beak points into the air cell (Fig. 4.11). Failure of the chick to orient properly interferes with normal hatching and can lead to death of the chick.

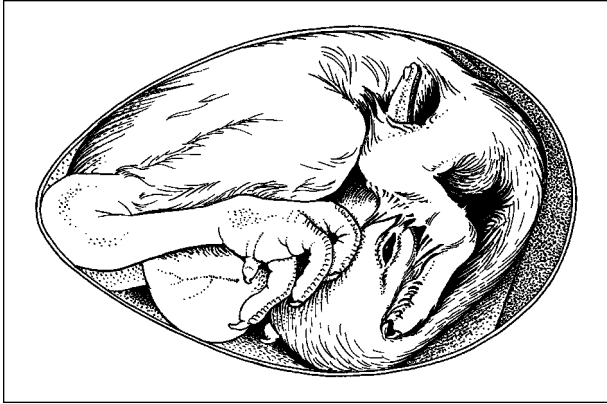


FIG 4.II. *A crane chick in normal hatching position.*

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In chickens, malpositions have been estimated to cause 50-55% of mortality in the last 3 days of incubation and 25% of total embryo mortality (Sanctuary 1925). Embryo malpositions and their effects on hatchability have been well characterized in the poultry literature (see Landauer 1967). Although some types of malpositions are attributable to other causes, frequent occurrence of certain malpositions may be related to **egg position** and **turning**.

In chickens, elevation of the large end of the egg during incubation has been associated with an increased incidence of certain types of malpositions: (1) failure of the chick to tuck its head, (2) tucking of the head under the left wing, and (3) tucking of the head with the beak over the wing. Elevation of the small end of an egg or incubation in a horizontal position can cause orientation of the chick away from the large end so it fails to pip into the air cell (Hutt and Pilkey 1934; Talmadge 1977). The incidence of malpositions can generally be decreased by increasing the frequency of turning, especially during the last third of incubation. However, if certain types of malpositions persist, consider changing the position of the eggs.

Not all malposition types are lethal (Hutt and Pilkey 1934). Do not modify incubation methods if chicks are only occasionally malpositioned (<2%). However, the occurrence of a consistent type of malposition frequently calls for evaluation of the incubation protocol and appropriate modifications. Relatively few malpositions of crane chicks have occurred at Patuxent or ICF under the conditions specified earlier. Crane eggs may be less susceptible to malpositioning than other species due to their elongate shape. Research on chicken eggs has shown that more elongate eggs with easily distinguishable large

and small ends have higher hatchability and a lower incidence of malpositions than do eggs with indistinguishable ends (Benoff and Renden 1980). At Patuxent, eggs are positioned in incubator trays at a 20-30° lateral angle when the trays lie flat. When the tray is tilted forward, the large end of the egg is elevated 20-30°; when the tray tilts toward the back of the machine, the small end is elevated 20-30°. At ICF, eggs are incubated lying horizontally, so that the large and small ends of the egg are at the same level.

Shell Abnormalities and Weight Loss

Eggs with abnormally **thin shells** normally lose water too rapidly during incubation, whereas **thick-shelled** eggs retain too much water. Changes in egg weight can be regulated by increasing the humidity in the incubator to lower weight loss or by decreasing humidity to increase weight loss. Extremely thin-shelled eggs may also be dipped in sterile water at intervals of a few hours, or daily as needed, to maintain normal weight loss. The dip should be cooler than the egg (ca 10° C; 50° F) so the egg contents contract and draw water into the egg. Putnam and Wentworth (1986) used this technique on a Whooping Crane egg and were able to reduce the projected weight loss by 22%. Although they did not begin until two-thirds of the way through the incubation period, they slowed and even reversed the weight loss by dipping for 5 min/day in sterile water. For the first dip, 3 mg tylosin tartrate were added per liter of dip solution to prevent infection of the egg. For this procedure, adjust the duration and frequency of dipping according to the degree of weight loss and stage of incubation. Duration should be 5 min or less (shorter is preferable) to avoid stressing the embryo. Continually monitor the weight loss of eggs treated in this manner and make further adjustments in the treatment as needed.

Cracked and Damaged Eggs

Eggs that have been cracked or damaged can often be repaired. Although repaired eggs must be artificially incubated, they often develop normally and hatch.

Repair eggs with fine hairline cracks simply by applying surgical-grade cyanoacrylate (e.g., Nexaband; see Appendix) or candle wax along the crack to seal and strengthen the shell. Other non-toxic adhesives may also be suitable, but should be tested on expendable eggs, and should only be applied to the damaged

part of the shell. Extensive sealing of the shell can result in asphyxiation of the embryo. Apply wax by melting and dripping it from a burning candle. Also use candle wax to seal over larger cracks and shattered or crushed shells. If large areas of the shell have been crushed, bone cement may be used for repairs by applying a thin layer over the affected area.

To repair holes in the shell, a piece of sterilized eggshell may be glued over the hole; otherwise, parafilm, tissue, or gauze may be layered over the hole with glue (see Stoodley and Stoodley 1983; Jordan 1989). Restrict repairs to the affected area so that large areas of the shell do not become sealed and impermeable to gas exchange. In addition to asphyxiation of the embryo, sealing the egg can lead to malpositions by causing embryos to orient away from the sealed area (Byerly and Olsen 1931). Hatchability is greatly reduced in eggs with holes that penetrate the shell membranes. These eggs are likely to have been contaminated by pathogens introduced to the egg's interior, or may have received physical injury to the yolk, embryo, or blood vessels.

If a pipped egg has been damaged in some way but still contains a live chick, move it immediately from the nest to a hatcher. If it is likely that such an egg has become contaminated by soil, feces, or other material, the egg may be fumigated (see earlier precautions) to kill pathogens that could enter the yolk sac or ruptured blood vessels, or be aspirated by the chick. Although the fumigant may irritate the chick, the need to prevent infection outweighs these considerations. Studies with chickens have shown that chicks may be fumigated while still in the hatching stage (Taylor 1949). This procedure has been used for cranes at Patuxent. Alternatively, the chick may be given prophylactic antibiotic treatment while in the egg or after hatching (see Calle et al. 1989).

Floating or Malpositioned Air Cell

Occasionally, the air cell forms in locations other than the large end of the egg. In other cases, a definite air cell does not form, but bubbles form in the albumen and float loosely in the egg. Eggs with a floating air cell or bubbles are unlikely to hatch and may have bacterial or fungal infections that were introduced through holes.

Chicks hatching from eggs with stationary, but displaced, air cells can die at hatching because either the chick still orients to hatch from the large end or the chick orients to the air cell but is malpositioned

and cannot emerge from the egg. The air cell serves as a breathing space until the chick hatches, and if the chick does not pip into it, the chick must break directly out of the shell and may drown or suffocate. Immediate assistance may help such chicks (Jordan 1989), but they are often lost. Incubating these eggs in a vertical position (large end up) and hand-turning may result in successful hatching (C. M. Kuehler, Peregrine Fund, Volcano, Hawaii, personal communication).

Contaminated Eggs

Egg contamination by pathogens may occur in the oviduct before laying, in the nest, or in the incubator. Little can be done once pathogens enter an egg: the embryo dies or the egg begins to deteriorate. However, dipping the egg in a disinfectant solution or injecting antibiotics directly into the egg is sometimes effective in preventing infection (Kuehler and Loomis 1992). To avoid contamination of additional eggs, immediately remove eggs from the nest or incubator if they contain known dead embryos or show signs of infection (odor or discoloration of egg contents). Due to gases generated during decomposition, eggs can actually explode, contaminating the incubator and other eggs with debris.

Attempt to culture pathogens from obviously contaminated eggs and some or all eggs with dead embryos or eggs that are presumed infertile (i.e., show no development). If microbial infection is a problem, investigate the cause or origin of infection. Persistent egg contamination warrants obtaining cultures from incubators, egg handling equipment, and birds (cloacal swabs). To minimize the incidence of infection, wear sterile surgical gloves to handle eggs and scrupulously disinfect all equipment and surfaces that eggs contact. If cultures of dead embryos are negative for bacteria and fungi, more sophisticated testing for viruses, nutritional problems, and genetic problems may be needed.

Assisted Hatches

If an egg fails to pip when expected, or takes longer than 48 h to hatch after the initial pip, consider assisting the chick in emerging from the egg. If an egg has not pipped the inner membrane when expected and there are signs of weakening, a malposition should be suspected. **Malpositions** can be diagnosed by three methods: (1) candling may reveal the bill tip near the

air cell, (2) radiographs may indicate the position of the embryo (Ensley et al. 1994), and (3) opening the shell and moistening the membrane with sterile saline may reveal the bill tip.

If the chick's head is pointing away from the air cell, carefully peel away the shell without disturbing the inner membrane. When the bill is located, select a small area free of active blood vessels, make a small incision, and pull the bill through far enough to expose the nares and allow respiration. Fluids may need to be removed from the airway of the chick. Once the chick is breathing, allow a day or two for the blood vessels to dry and the yolk sac to retract. Keep humidity high, cover the missing shell loosely with plastic or tape, and moisten membranes to keep them from drying. The chick will likely need assistance to free itself when ready to hatch.

Base your decision to assist on the strength of the chick's vocalization and movements. A tape recording of an adult crane's brooding call (purring), cheeping of other chicks, or an imitation of these sounds by the aviculturist usually elicits loud cheeps (if the air cell has been entered) and struggling from the chick. Although chicks enter a quiescent period prior to hatching (Hamburger and Oppenheim 1967), a chick that remains quiet and motionless may be too weak to finish hatching on its own.

Failure to hatch after pipping may result from malposition, dehydration of the shell membranes (so the chick adheres to the membranes), an abnormally thick shell, or weakness of the chick due to improper incubation conditions. Assist the chick cautiously in case the yolk sac is still exposed (see below) or the external blood vessels are still functional. Remove the eggshell over the air cell to expose the chick without disturbing the membranes around the chick. Moisten the membrane with sterile water or saline to release the chick and reveal blood vessels. If the blood vessels appear empty (i.e., pale and small in diameter), gently and slowly peel the membrane away from the chick's bill a little at a time. Do this gradually to allow the chick to emerge by itself, if possible. Membrane blood vessels can also be tied off surgically. A chick that continues to be weak, shows edema around the back of the head and neck, and does not progress on its own should be carefully pulled from the egg. **Weak chicks** can be treated with fluids, glucose, and steroids by injecting either while still in the egg or immediately after assisted hatching. Additional information on time intervals between hatching events and assistance of chicks is provided by Hartman et al. (1987).

Exposed Yolk Sac

An exposed yolk sac (i.e., one that has not been retracted within the abdominal cavity) usually results from early hatching (caused by high incubation temperature, excessive behavioral stimulation, or an assisted hatch). Handle such chicks carefully because rupture of the yolk sac can increase susceptibility to infection and bleeding, and deprives the chick of a nutrition source immediately posthatching. See Chapter 5 for further discussion and information on the treatment of this condition.

Transporting Eggs

Fresh, unincubated eggs can be transported relatively easily as long as they are protected from physical damage (cracked shells, freezing, or overheating). For incubated eggs, a rapid transfer (less than 5 min) between nests and/or incubators at the same site requires no special equipment except that eggs should be cushioned to prevent damage from sudden or rough movement. The amount of cooling that occurs in the egg in a few minutes has no appreciable effect on the egg unless the weather is extremely cold or the egg is exposed to rain or chilling winds.

To transfer over substantial distances (e.g., from a wild nest to a captive-rearing facility), a portable **suitcase incubator** (Fig. 4.12) was developed at Patuxent (Erickson 1981). Hatchability of transported eggs is usually very high with these incubators, which are constructed by lining a reinforced cardboard suitcase with polyurethane foam. Supply heat using hot-water bottles filled with water at 51.7° C (125° F) and placed in the bottom of the suitcase. Above the hot-water bottles, place polyethylene foam inserts that will contain the eggs. Egg shaped cut-outs (pockets, one half of which is in the lid and half in the base) are interconnected with air channels running between egg pockets and extending to the ends of the inserts to allow convection and to equilibrate temperature throughout the incubator. When the incubator suitcase is filled with eggs, close the cover and insert a thermometer through a hole in the side or cover so that it extends into the area near the eggs (i.e., into one of the air channels between eggs). Monitor the temperature continuously, and regulate it within 34.4-37.2° C (94-99° F) by keeping the incubator lid closed to retain heat and by opening and fanning the

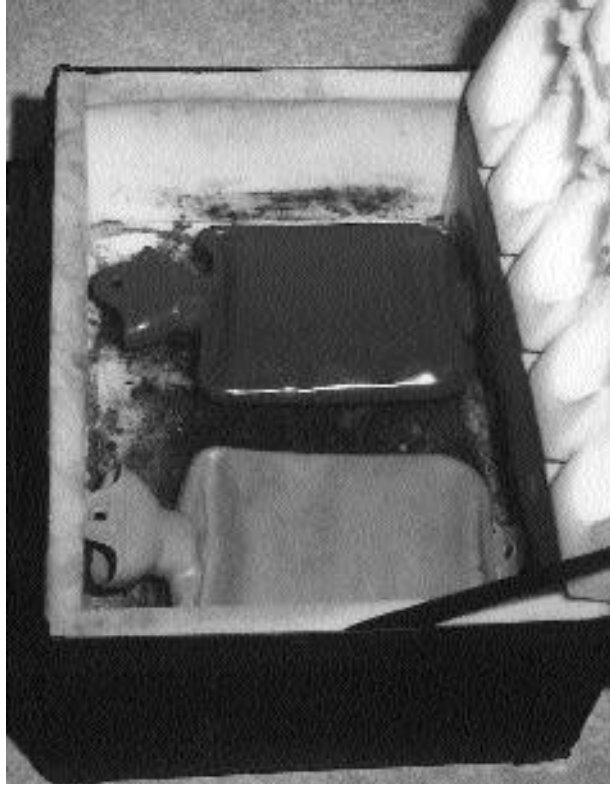


FIG 4.12. Portable incubator suitcase: Left, hot water bottles now empty but in place; Right, polystyrene liner with cavities for eggs and channels for convection and thermometer.

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lid to release heat if the temperature is too high. Do not allow eggs to enter or remain in an incubator hotter than 37.2°C (99°F). Replenish the hot-water bottles at approximately 2-h intervals or when the incubator temperature falls below 34.4°C (94°F). Hold the incubator suitcase in your lap or lift it above your lap to protect eggs, especially those in early stages of incubation, from sudden movements or stops, bumps, or vibrations, which would otherwise cause blood vessels or embryonic membranes to rupture.

For international shipments lasting 24 h or more, ICF has developed a wooden box with separate egg and water bottle compartments so eggs are not disturbed when changing water. Temperatures are kept between 36.1 and 37.2°C (97 and 99°F).

Closing Statement

Incubation is part art and part science. Understanding the principles of incubation alone will not guarantee success. The aviculturist must become experienced in recognizing subtleties revealed during candling

and general handling of eggs, and in dealing with problems with the incubators, whether they are machines or birds. In the beginning, experiment with less valuable eggs, and expect to lose a few of these, but learn from your losses and avoid them. The information presented here and in the references cited provide a basis for conducting an incubation program, but the novice should also seek the advice of experienced persons when problems arise.

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