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RESEARCH ARTICLE

Captive-Rearing of Gunnison Sage-Grouse From Egg Collection to Adulthood to Foster Proactive Conservation and Recovery of a Conservation-Reliant Species

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Gunnison sage-grouse (*Centrocercus minimus*) are distributed across southwestern Colorado and southeastern Utah, United States. Their distribution has decreased over the past century and the species has been listed as threatened by the U.S. Fish and Wildlife Service. Reduced genetic diversity, small population size, and isolation may affect Gunnison sage-grouse population persistence. Population augmentation can be used to counteract or mitigate these issues, but traditional translocation efforts have yielded mixed, and mostly unsuccessful, results. Captive-rearing is a viable, although much debated, conservation approach to bolster wild conservation-reliant species. Although there have been captive-rearing efforts with greater sage-grouse (*C. urophasianus*), to date, no information exists about captive-rearing methods for Gunnison sage-grouse. Therefore, we investigated techniques for egg collection, artificial incubation, hatch, and captive-rearing of chicks, juveniles, subadults, and adults for Gunnison sage-grouse. In 2009 we established a captive flock that produced viable eggs. From 2009–2011, we collected and artificially incubated 206 Gunnison sage-grouse eggs from 23 wild and 14 captive females. Our hatchability was 90%. Wild-produced eggs were heavier than captive-produced eggs and lost mass similarly during incubation. We produced 148 chicks in captivity and fed them a variety of food sources (e.g. invertebrates to commercial chow). Bacterial infections were the primary cause of chick mortality, but we successfully reduced the overall mortality rate during the course of our study. Conservationists and managers should consider the utility in developing a captive-rearing program or creating a captive population as part of a proactive conservation effort for the conservation-reliant Gunnison sage-grouse. Zoo Biol. 34:438–452, 2015.

Keywords: artificial incubation; bacterial infection; captive population; *Centrocercus minimus*; Galliformes husbandry; wild and captive produced egg collection and hatch

INTRODUCTION

The Gunnison sage-grouse (*Centrocercus minimus*; GUSG) is a recently described species [Young et al., 2000] with a distribution in North America that has decreased over the past century concomitantly with a reduction in sagebrush (*Artemisia* spp.) distribution [Oyler-McCance et al., 2001; Schroeder et al., 2004]. Gunnison sage-grouse have distinct genetic [Oyler-McCance et al., 1999], morphological, and behavioral [Young et al., 1994,2000] differences from greater sage-grouse (*C. urophasianus*; GRSG; Hupp and Braun, 1991; Young et al., 1994, 2000]. Despite these differences,

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habitat requirements and diets for GRSG and GUSG appear similar [Young et al., 1994] because both species are sagebrush obligates [Connelly et al., 2000; Hagen et al., 2007; Patterson, 1952; Schroeder et al., 1999, 2004; Wallestad, 1975].

The Gunnison sage-grouse is designated as a species of concern by the Colorado Parks and Wildlife (CPW), a sensitive (Tier 1) species by the Utah Division of Wildlife Resources [GSRSC, 2005], and on the Red List of Threatened Species of the International Union of Conservation of Nature and Natural Resources (IUCN) as endangered [IUCN, 2013]. In 2000, the U. S. Fish and Wildlife Service (USFWS) designated the GUSG as a candidate species for federal protection [USFWS, 2000] and in 2014 issued a final rule listing the GUSG as threatened [USFWS, 2014].

Seven GUSG populations occupy southwestern Colorado and southeastern Utah [GSRSC, 2005]. Six populations are small and have <40 males counted in the spring during the breeding season on strutting grounds and occupy <40 468 ha of sagebrush habitat (CPW, unpublished results). The seventh population inhabits the Gunnison Basin and has 745–850 males counted on strutting grounds and occupy >202 342 ha of sagebrush habitat (CPW, unpublished results). Even though the Gunnison Basin population is relatively stable [Davis, 2012; Garton, 2005; GSRSC, 2005; Oyler-McCance et al., 2005], the persistence and viability [GSRSC, 2005] of the smaller populations are in question because they are vulnerable to demographic variability from environmental and demographic stochasticity [Caughley, 1994; Lande, 1988; Ruggiero et al., 1994; Shaffer 1987; Stiver et al., 2008; Williams et al., 2003] and low genetic diversity (compared to the Gunnison Basin population) [Oyler-McCance et al., 2005]. Gunnison sage-grouse also have fewer haplotypes per population (lower diversity) than more robust populations of GRSG and a preponderance of GUSG genetic diversity is found in the Gunnison Basin [Oyler-McCance et al., 1999, 2005].

There is genetic evidence suggesting a historic linkage facilitating demographic interchange between the small GUSG populations and the Gunnison Basin [Oyler-McCance et al., 2005]. Any current linkage is limited due to the physical distance among populations and anthropogenic barriers. Additionally, Oyler-McCance [1999] suggests the populations have not been isolated for a long enough period of time to develop genetic differences which facilitates outbreeding depression. Therefore, due to the lack of connectivity, there is a need to pursue conservation actions to increase small population size, support stable demographic rates, and maintain or enhance genetic diversity [Oyler-McCance, 1999, 2005].

A traditional approach to enhance small population demography and/or genetics involves augmentation of individuals from larger populations [Reese and Connelly, 1997] through conservation translocations [IUCN/SSC, 2013]. However, conservation translocations with grouse (primarily with GRSG) have yielded mixed results [Baxter et al., 2008; Musil et al., 1993; Reese and Connelly, 1997]. Since 2000, CPW has conducted conservation translocations of GUSG with ~300 individuals of varying ages classes (12-month apparent survival rate of 40–60%) from the Gunnison Basin to the smaller populations to bolster small population demography, enhance or retain existing genetic diversity [GSRSC, 2005; Oyler-McCance et al., 2005; CPW, unpublished data], and mitigate possible reproductive failures which could be linked to inbreeding depression [Stiver et al., 2008] or Allee effects [Stephens et al., 1999]. Traditional conservation translocations or "laissez-faire" conservation [Dreschsler et al., 2011] approaches are usually not sufficient conservation measures to support a conservation-reliant [Scott et al., 2010] species. We suggest that GUSG meet the definition of a conservation-reliant species [Scott et al., 2010] and conservation practitioners need to consider additional conservation options to initiate proactive conservation efforts including captive-rearing [GSRSC, 2005].

Captive-rearing approaches for egg collection, artificial incubation, and rearing of juvenile and adult wild-produced GRSG [Huwer 2004; Huwer et al., 2008; Johnson and Boyce 1990, 1991; Oesterle et al., 2005; Pyrah 1963, 1964; Thompson, 2012] and other Tetraoninae [Drake, 1994; Griffin, 1998; Jurries et al., 1998; Lockwood, 1998; McEwen et al., 1969; Smith, 1993; Watkins, 1971] have been investigated. Although captive-rearing techniques of all age classes have been investigated with varying degrees of success, techniques for GUSG are needed because some techniques may not be directly comparable. In addition, updated approaches are needed because artificial incubation techniques continue to evolve [Klimstra et al., 2009].

Captive-rearing programs have encountered varying challenges. Previous captive-rearing studies with Tetraoninae have documented mortality from bacterial [Drake, 1994; Griffin, 1998; Johnson and Boyce, 1991] or fungal infections (e.g. salmonellosis, *Pseudomonas aeuginosa*, and aspergillosis), vitamin E deficiency [Pyrah, 1963, 1964], and reticuloendothelial virus (REV) [Drew et al., 1998]. Post-hatch physical irregularities have also been documented with Attwater's prairie-chicken (*Tympanuchus cupido attwateri*) [Jurries et al., 1998; Smith, 1993] (e.g. toe [Griffin, 1998], leg, and digestive tract abnormalities) and GRSG (e.g. splayed legs, curled toes, seizures and curved backs) [Huwer, 2004; Thompson, 2012]. These irregularities may result from a combination of the incubation environment, inadequate nutrition, poor thermoregulation and/or stress from handling.

Captive-rearing as a conservation practice has been the subject of discussion [Lynch and O'Hely, 2001; Seddon et al., 2007; Snyder et al., 1996]. Unfortunately, conservation practitioners typically respond to ecological emergencies when populations are no longer viable in the wild [Scott et al., 2010; USFWS, 2010] and recovery requires substantial financial resources [Drechsler et al., 2011] and steep captive-rearing learning curves. Therefore, in an effort to be proactive and provide conservation practitioners better tools

to assist in GUSG recovery, the objectives of our study include: 1) evaluating and, if necessary, modifying existing GRSG egg collection, incubation, and hatch techniques, 2) developing chick, juvenile, and adult husbandry techniques, and 3) developing techniques to maintain a captive GUSG flock.

MATERIALS AND METHODS

Study Area

Our study occurred in two locations, the Gunnison Basin and Fort Collins, Colorado, USA (38°30'N, 106°54'W and 40°42'N, 104°54'W, respectively). The Gunnison Basin served as the source population of wild-produced eggs and wild birds for captive-breeding. The Gunnison Basin is an intermontane basin located in south-central Colorado including parts of Gunnison and Saguache Counties, Colorado, USA. Our study area is predominately a sagebrush-steppe plant community, with an average annual precipitation of 26.5 cm, and elevation ranging from 2290 to 2900 m.

The incubation, hatching, and captive-flock aviary were located in Fort Collins, Larimer County, Colorado, USA situated on the eastern slope of the Rocky Mountains. The average annual precipitation is 38.4 cm and elevation is 1525 m. The captive-rearing facilities were not located in current or historic GUSG distribution [Schroeder et al., 2004], but were located near the Colorado State University School of Veterinary Medicine, the CPW Staff Veterinarian, veterinary technicians, and avian husbandry experts at the Denver Zoological Gardens in Denver, Colorado, USA.

Captive-Rearing Facilities

Our incubation, hatching, and chick-rearing (<10 days post-hatch) facilities located at the CPW Foothills Wildlife Research Facility (FWRF) differed in 2009 from 2010 to 2011. In 2009 we used existing facilities. These facilities provided minimal control of room temperature and humidity and allowed non-project related human access. To correct these issues, in 2010 we constructed a new incubation, hatching, and brood-rearing building ($3.7 \times 6.1 \times 2.4$ m; W × L × H) allowing more efficient use of space, room temperature and humidity control, maintenance of sanitary conditions, and non-project oriented human access control. Our captive-rearing facility for all years for flight-capable juveniles (10 d–4 months), subadults (>4 months–2 years), and adults (>2 years) was at the National Wildlife Research Center (NWRC). The NWRC strictly controls human access preventing extraneous human contact or animal disturbance thereby controlling disease transmission.

The aviaries have removable net panels between them allowing the segregation and integration of differing genders and ages (Fig. 1; see Oesterle et al. [2005]). We added a 3.2-cm mesh knotless nylon net modification in front of the peripheral chain link fence. The netting extended from the ceiling to the ground to prevent grouse injury by striking hard

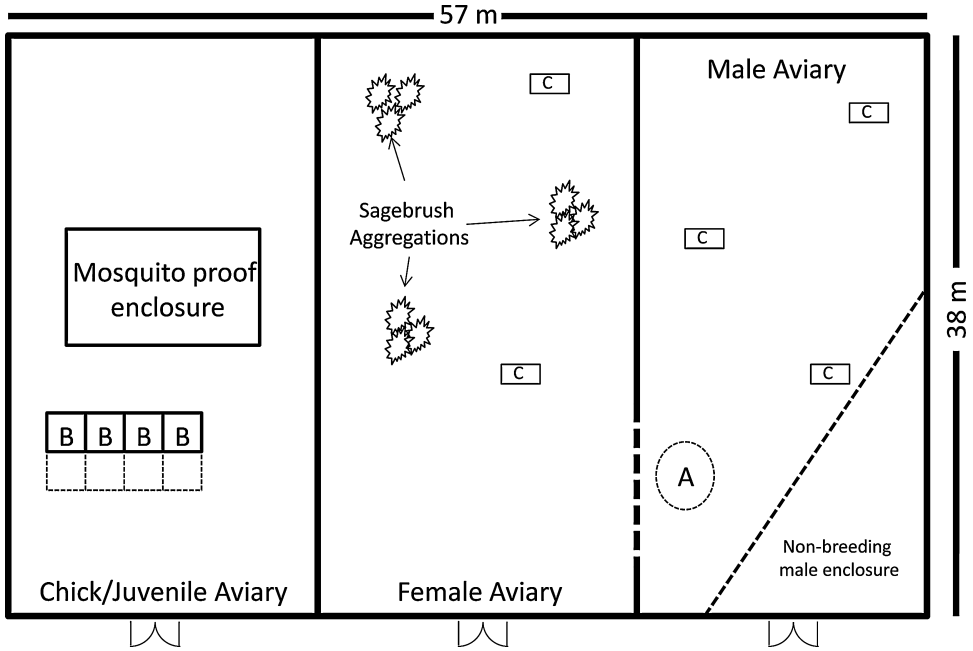

Fig. 1. National Wildlife Research Center aviary design, Larimer County, Colorado, 2009–2011. For additional details for the design of each aviary, see Oesterle et al. [2005]. Major aviary sections include the male aviary (individuals housed during the breeding season and mixed flocks post-breeding, the female aviary (individuals housed during the breeding season and mixed flocks post-breeding), the chick and juvenile aviary. A = established strutting ground in 2010 and 2011; B = chick and juvenile coops and brood pens (7–10 d post-hatch); C = wood shelters for cover and obstruction. The number and distribution of sagebrush aggregations and wood shelters changed over time.

surfaces during flight. We also provided eight wood shelters ($0.6 \times 0.6 \times 0.46$ m) and big sagebrush (*A. tridentata*) plants for *in situ* food and cover (Fig. 1).

Our captive-rearing research and techniques were approved by CPW Animal Care and Use Committee (ACUC) (Permit #03-2009) and the NWRC ACUC (Permit # QA-1625).

Wild Grouse Capture, Marking, and Transplant

From 2009–2011, we captured wild GUSG in the Gunnison Basin using night spotlighting [Giesen et al., 1982; Wakkinen et al., 1992]. We weighed, classified by age [Beck et al., 1975; Dalke et al., 1963; Eng, 1955], and fitted females with individually numbered aluminum leg band and 17-g necklace-mounted VHF radio transmitters (model A4050, Advanced Telemetry Systems; Isanti, MN). We released radio-marked females at the point of capture. In 2009, our study in the Gunnison Basin was conducted concurrently and cooperatively with Davis [2012] by using 10 previously marked adult females for egg acquisition. Additionally, we captured wild grouse for integration into our captive flock at the NWRC. We also marked translocated grouse with uniquely colored leg bands. We transported grouse to Fort Collins in small ($60 \times 40 \times 30$ cm; L × W × H) animal crates, equipped with floor padding to facilitate foot traction and minimize injury during transport. We released grouse into captivity $\leq$24 hr after capture.

Grouse Nest Monitoring and Egg Collection

From 2009–2011, we monitored radio-marked wild female movements and nesting activity every 1–2 d between 0700–1200 from the ground or the air using a fixed-wing aircraft. If we suspected a female was nesting, we returned for confirmation ($\leq$24 hr). Once we confirmed nesting, we monitored nest fate [Reardon, 1951] every 3–7 d at $\geq$75 m from the nest [Holloran and Anderson, 2005]. We differentiated between egg incubation and laying by returning to the nest at different times ($\geq$2 hr difference) to monitor female behavior and presence. If the female was present for two consecutive days, we concluded she was incubating, otherwise she was laying. We estimated the nest initiation date based on the female's previous non-nesting locations.

We collected eggs from laying and incubating wild females. We monitored laying female movements while searching for nests near previous locations. We documented the UTM coordinates and clutch size of all nests. If <6 eggs were present, we assumed the female was laying and collected $\leq$50% of the current clutch, replacing them with artificial latex sage-grouse eggs (manufactured by J. Craig, Fort Collins, CO) to reduce the probability of nest abandonment due to a perceived nest depredation. We weighed and labeled all collected eggs and inconspicuously marked remaining eggs with a pencil to monitor for additional egg deposition returning 2–3 d later to collect additional unmarked eggs (never exceeding 50% of the clutch at the time of collection). We monitored female movement post-egg collection to determine re-nesting status, if applicable. If the female was still present and presumed to be incubating, we decided whether or not to collect the entire clutch. To collect the clutch we flushed the incubating female from the nest and collected all eggs. We collected clutches as early into incubation ($\leq$7 d) as possible in an effort to improve the likelihood of renesting and reduce nest exposure time to nest predators. We did not collect eggs from females incubating >7 d.

In 2010 and 2011, we collected captive-produced eggs by systematically searching the aviary daily during the morning, mid-day, and evening. In 2010 we collected and either stored or incubated all eggs (except for three captive female nests with 15 eggs total). In 2011, we collected and either stored or immediately incubated all eggs. We weighed, individually labeled, and marked the location of each egg on a grid map schematic.

Egg Transport, Storage, Incubation, and Hatch

We placed eggs from laying females in hard-sided coolers lined with foam and transported them by vehicle to the FWRF for storage or incubation. We stored laid eggs to synchronize hatch date with incubating wild females in the Gunnison Basin as part of an ancillary study (CPW unpublished data). In 2009, we stored laid eggs in a 0.03 m^3 refrigerator to maintain a storage temperature at 10–15°C. Our refrigerator temperatures exceeded the storage temperature goal by 8–22°C. In 2010 and 2011 we stored eggs in a temperature controlled cooler ($0.57 \times 0.83 \times 0.52$ m; D × H × W) to provide temperature consistency.

We transported eggs collected from wild incubating females in a temporary incubator (maintained at 35.0–37.5°C; Foam Hova-Bator, GQF Manufacturing Company, Savannah, GA) powered with a DC electricity convertor by vehicle from the Gunnison Basin to the CPW FWRF. We transferred all eggs from the temporary incubator or storage cooler to a permanent incubator (Model 1502 Sportsman, G.Q.F. Manufacturing Company, Savannah, GA) preconditioned and maintained at a temperature and relative humidity of 37.5°C and 58%, respectively. We monitored incubator temperature and humidity >6 times/d.

We stored 30 of 46 wild-produced laid eggs before incubation. In 2009, we stored two eggs for 1 d, and in 2010 and 2011, we stored six eggs for 3 d, and 22 laid eggs for a mean of 2.14 d ($\bar{x} \pm$ SE 0.18; range 1–3), respectively. In 2010 and 2011, we stored all captive-produced eggs for 6.60 d ($\bar{x} \pm$ SE 0.43; range 1–5; $N = 37$) and 25 of 32 eggs for 4.16 d ($\bar{x} \pm$ SE 0.41; range 1–9; $N = 25$), respectively. We manually turned eggs 2 times/d [Anderson Brown and Robbins, 2002; Harvey, 1993; Stromberg, 2001] and stored eggs $\leq$7 d prior to incubation [Anderson Brown and Robbins, 2002; Stromberg, 2001; Thompson, 2012], although in three instances we stored four eggs longer.

We weighed, examined, and candled eggs 2–4 times/week to monitor for mass loss and embryonic and vascular development [Huwer, 2004; J. Azua, Denver Zoological Gardens, personal communication]. We adjusted incubator relative humidity to achieve an 11–12% mass loss [Anderson Brown and Robbins, 2002; Harvey, 1993; Huwer, 2004; Thompson, 2012]. The incubator turned eggs automatically every 4 hr. During examination, if we detected no sign of development, or development ceased, we removed those eggs from the primary incubator placing them into a secondary incubator to prevent possible bacterial cross-contamination. We conducted egg necropsies, assessed the presence of an embryo, stage of development [Anderson Brown and Robbins, 2002; Hamburger and Hamilton, 1992], and tested all embryo mortalities to ascertain cause of mortality.

Failure to hatch is influenced by two primary factors during incubation: infertility and embryonic mortality [Kuurman et al., 2003]. Embryonic mortality can be influenced by damage (temperature, storage, poor incubation, and infection) late in incubation [Anderson Brown and Robbins, 2002; Klimstra et al., 2009], but rarely is embryonic mortality uniformly distributed throughout incubation. Thus, we classified embryonic mortality into two phases; phase I ($<$19 days into development) and phase II ($\geq$19 days into development) [Byerly, 1930; Kuurman et al., 2003; Moseley and Landauer, 1949; Payne, 1919]. Additionally we classified embryo malposition into class #1-7 [Anderson Brown and Robbins, 2002; Hutt, 1929; Sanctuary, 1925; Smith, 1930].

When we observed an internal (J. Azua, Denver Zoological Gardens, Denver, CO) or external pip, eggs were transferred to a hatcher (Model 1550 Sportsman, G.Q.F. Manufacturing Company, Savannah, GA) preconditioned and maintained at a temperature and relative humidity at 37.2°C and 80% until hatch, respectively. During this phase of incubation (approximately stage 45; [Hamburger and Hamilton, 1992]), we did not turn eggs [Anderson Brown and Robbins, 2002; Harvey, 1993] and they were placed into individually compartmentalized hatching trays [White and McMaster, 2005]. In 2009, we lined each hatching compartment floor with commercial paper towels, but in 2010 and 2011 we lined compartment floors with an enhanced commercial grip drawer liner. The liner better assisted with chick traction in an effort to reduce the incidence of curled toes or splayed legs.

We estimated hatch date of eggs collected during laying at 27 d after the egg was transferred to the incubator, and hatch date of eggs collected after the onset of incubation was estimated at 27—x_i days after collection; where x_i equals the mean number of days from the last recorded location of the wild female and the date of collection. Following hatch, we weighed, swabbed the umbilicus with Betadine® (providone-iodine 2% solution), applied an individually numbered leg band (Dunlap Hatchery, Caldwell, ID; size #4–6 [6–9 mm]), and confined chicks to the hatcher for 12–24 hr to dry [Apa et al., 2010a; Wiechman et al., 2011]. In 2010–2011, we wore and exchanged out sterile surgical latex gloves between handling individual chicks to reduce cross-contamination of potential contagions. With each exchange of a clutch or brood, we disinfected the entire incubator or hatcher and its contents with Tek-Trol®. Additionally, we monitored the wicking pads in the water reservoir between cleanings and replaced all pads at the first sign of discoloration or staining.

We monitored equipment continuously throughout incubation and hatching for electrical failure or interruptions. Redundant electrical back-up was available from a gas-powered generator.

Chick And Juvenile Husbandry

One to 10 days post-hatch

We weighed chicks daily ($\bar{x} = 1.19$ d SE 0.02, $n = 817$; range 1–5 d) through 10 d post-hatch. Following hatcher confinement, we transferred chicks to a chick brooder. Our chick brooder consisted of an inflatable decoy female (wild turkey (*Meleagris gallopavo*)), heated brooder-wing, and a heat lamp surrounded by a fence (1.3-m^2) [Apa et al., 2010b]. The triangular dimensions of our brood wing were $61 \times 25 \times 18$ cm. The roof was 23 cm from its base at its highest point and decreased in height to 10 cm. Our chick brooder wing had three heat sources located in the top, back and floor; a 110 volt heating pad, a 14 cm dia-75 watt-110 volt herptile lamp with an infra-red bulb, and a 28×28 cm 14 watt herptile heating mat, respectively. As the diurnal ambient temperature increased, we reduced or discontinued supplemental heat. We did not directly monitor brooder temperatures, but made adjustments based on chick behavior (e.g. moving away from or to heat sources). We monitored chicks for an additional 24 h for clinical signs suggesting other health issues or behavioral abnormalities (e.g. not feeding, unstable locomotion, solitary activity, etc.). To lessen the possibility of human imprinting [Huwer, 2004; Huwer et al., 2008], we outfitted staff with camouflage "ghillie" suits and they refrained from speaking when in contact with the chicks. We distributed chicks among brooders in groups of 4–10 individuals of similar age to simulate wild brood size and facilitate partial or complete clutches collected at differing times from wild females.

Every 1–2 hr (0600–2100) we provided 2-5 g of small ($<$13 mm) and 8–19 g of medium (13–24 mm) invertebrates [house crickets (*Acheta domesticus*), waxworms (*Galleria mellonella*), flightless fruitflies (*Drosophila melanogaster, D. hydei*)] per chick, vitamin supplemented water (Vitamax®) *ad libitum*, and $<$5 g of chopped (<1 cm^2) forbs [alfalfa (*Medicago sativa*), common yarrow (*Achillea millefolium*), white sweet clover (*Melilotus albus*), common dandelion (*Tarxacum officinale*)] per chick. In 2009 only, we provided 5 g per chick of a prepared mixture of commercial Purina Game Bird Chow® (Diet 1 and 2; Table 1).

TABLE 1. Commercial grades of Purina Mills Game Bird Chow® diet total weight proportion ratios used for captive Gunnison sage-grouse in Larimer County, Colorado, USA, 2009–2011

		Percent of Purina Mills Game Bird Chow® Bulk Ratio			
Diet Number	Bird age (days)	Layena® (20% protein)	Startena® (30% protein)	Flight conditioner® (19% protein)	Maintenance® (12% protein)
1	1–5	—	50	50	—
2	6–10	—	25	75	—
3	11–29	—	—	100	—
4	30–34	—	—	75	25
5	35–39	—	—	50	50
6	40–44	—	—	25	75
7	>45	—	—	—	100
8[a]	Seasonal	100	—	—	—

[a]Pre-breeding and breeding subadult/adult.

In all years, at approximately 7 d post-hatch, we moved chicks to juvenile brooders at the NWRC (Fig. 1). We distributed juveniles among brooders in a similar fashion to chicks. The juvenile brooder consisted of an enclosed "coop" and outdoor pen. The coop ($1 \times 1 \times 1.2$ m; L × W × H) is a factory manufactured domestic chicken (*Gallus gallus domesticus*) coop equipped with heat lamps and herptile heating pads. The outside pen (1.8×1.8 m) was bordered by 0.6–1.2 m tall 1.3 cm mesh wire fence. We allowed unobstructed movement between the pen and coop. From sunset to sunrise and during inclement weather, we provided supplemental heat in the pen (e.g. heat lamp) and confined juveniles to the coop.

We employed adaptive methods in an effort to control bacterial infections and reduce mortalities in chicks $\leq$10 d post-hatch. We continuously improved upon our levels of sanitation to minimize cross-contamination risk by using surgical gloves, non-organic brooding substrates (i.e., gravel), and by controlling unnecessary human contact for the first 5–7 d post-hatch. In 2009, we used reactive methods to respond to bacterial infections. Once we indentified and confirmed the bacterial infection, we isolated the affected chick(s) and administered erythromycin by injection as well as in their water. We found both approaches unsuccessful. In 2010, we isolated the affected chicks(s) at the first clinical sign of a bacterial infection and orally administered 10 mg enrofloxacin/kg of body mass for 7 d. We also treated water with 30 ml/3.78 L of sulfamethoxacole, but found that the bacteria responsible (see results) were resistant to sulfamethoxacole, and discontinued this treatment. In 2011, we instituted a proactive approach by administering the aforementioned dosage of enrofloxacin to all chicks from hatch to 5 d and because enrofloxacin is effective against a majority of the bacteria we encountered [Babaahmady and Khosravi, 2011]. A negative side effect of enrofloxacin use is its effectiveness against normal gut microflora [Johnson and Nicholson, 2004]. Therefore, in 2011, we attempted to counteract these negative side effects by adding naturally occurring probiotics (e.g. crushed GUSG fecal matter, feathers, and soil collected from strutting grounds in the Gunnison Basin) to the artificial brooder substrate.

We euthanized chicks with non-bacterial related conditions such as crooked neck or ruptured umbilicus [Anderson Brown and Robbins, 2002; Harvey, 1993; Stromberg, 2001]. These conditions are not atypical and can be related to incubator temperature and/or humidity extremes, genetic issues, or parent female nutrition [Anderson Brown and Robbins, 2002]. We provided a coarse hatching tray surface in an attempt to reduce the occurrence of splayed legs [Anderson Brown and Robbins, 2002]. We provided chicks with curled toes approximately 24 hr to self-correct. If the toes did not straighten, we applied white athletic tape to the affected toes dorsally and ventrally for 24 hr (J. Azua, Denver Zoological Gardens, pers. comm.).

Eleven days to 4 months post-hatch

We weighed juveniles periodically ($\bar{x} = 5.57$ d SE 0.13, $n = 229$; range 1–14 d) through 35 d post-hatch. At approximately 14 d post-hatch (age varied by year and mosquito (*Culex spp.*) presence), we transferred juveniles to a mosquito-proof enclosure (Fig. 1) protecting them from seasonally occurring (July-September; [CSU Extension, 2014]) West Nile Virus (WNV) infection (documented with GRSG [Murray et al., 2010; Walker et al., 2007]). The mosquito-proof enclosure is constructed using a PVC pipe (5-cm diameter) frame ($12 \times 4 \times 4$ m; L × W × H) covered by black mosquito-proof netting fabric (42 holes/cm^2). We implemented other levels of protection including a mosquito trap (Mosquito Magnet®), treatment of standing water with *Bacillus thuringiensis* [Becker, 1998], and managing vegetation height.

We fed juveniles every 1–2 hr from 0600–2000 and recorded the quantity and food type. We provided juveniles medium and large (>19 mm) crickets, mealworms, and waxworms, chopped forbs, commercial Game Bird Chow®

(Diets 3–7; Table 1), and vitamin supplemented water *ad libitum*. The quantity of medium invertebrates per individual ranged from 8–16 g at 11 d post-hatch. We increased large invertebrate quantity from 10–25 g per individual at 12 d post-hatch to 40–60 g per individual at ≥35 d post-hatch. We provided an increasing quantity of forbs with age ranging from approximately 2 g at 12 d post-hatch to 15–30 g per chick ≥35 d post-hatch.

Sub-adult And Adult Husbandry

Five months post-hatch to one year

Periodically we moved the mosquito-proof enclosure to avoid unsanitary conditions caused by the accumulation of fecal material. Additionally, juveniles and subadults actively consumed available herbaceous vegetation, which effectively denuded the ground cover. We confined juveniles in the enclosure (Fig. 1) until 1 October or when environmental conditions (two evenings below 0°C) effectively reduced mosquito activity [Reisen, 1995; Reisen et al., 2008]. Following the reduced threat we allowed juveniles full access to the aviaries (Fig. 1).

We provided big sagebrush plants throughout the aviaries for food *ad libitum* and cover (Fig. 1). We obtained plants from two sources, wild harvested plants and potted organic commercial nursery stock. We planted 215 individual big sagebrush plants, and rotated 717 of 853 potted plants through a greenhouse to re-foliate. Additionally, we provided 530 harvested plants. These plants eventually defoliated due to consumption or plant mortality. Overall, we provided an average of 30 sagebrush plants/week for food and cover.

We also provided commercial chow (Diet 7; Table 1), superworms (*Zaphobs morio*), and refreshed supplemental water twice daily. We changed the commercial chow diet to Diet 8 (Table 1) in February 2010 and 2011 to provide higher protein and calcium content for the breeding season. We weighed all food provided and varied the quantity of superworms and commercial chow by the breeding (1 February–31 May), summer (1 June–30 September) and winter (1 October–31 January) season, flock, and gender composition (Fig. 2).

Statistical Analyses

We used a general linear model (GLM) procedure [SAS Institute, 2006] with an unbalanced analysis of variance (ANOVA) design to assess the dependent variable of egg mass in relation to independent variables of egg source (wild or captive) and year of collection (2009, 2010, and 2011). Logistic regression was used to evaluate the dichotomous dependent variable of egg fate (hatch; yes or no) and its relation to independent variables of egg source and mass. We report hatch success (percentage of total eggs produced that hatch) and hatchability (percentage of fertile eggs that hatch). Additionally, logistic regression was used to evaluate hatch success of stored eggs with the independent variable of the number of days eggs were stored prior to incubation. We used likelihood ratio χ^2 (G-statistic or Fischer exact test for cells ≤5) to assess the relationship of enumerated hatch success count data with years, egg collection type (laid or incubated), and egg source. To evaluate if we influenced chick mortality rates through the adaptive approaches we employed during our study to control bacterial infections, we regressed chick survival as the dependent variable against age and year as independent variables.

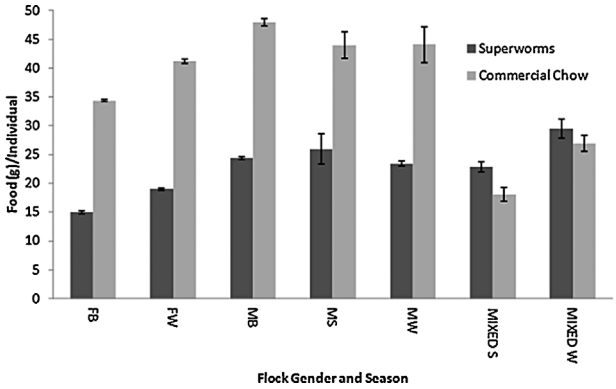

Fig. 2. Quantity of superworms and commerical chow provided per individual ($\bar{x} \pm$ SE) to captive Gunnison sage-grouse flocks by gender (F = female, M = male, Mixed = mixed gender flock) and season (B = breeding, W = winter, S = summer) in Larimer County, Colorado 2010–2011.

We used a multilevel model with longitudinal data [Singer and Willett, 2003] analyzed with PROC MIXED [Little et al., 2006] to estimate egg mass loss (within and between-subject) throughout incubation. We selected a multilevel model over simple regression because our data incorporates a level 1 submodel that described individual egg mass loss during incubation and a level 2 submodel that described the change in mass during incubation varies across eggs. Our fixed effects include the egg source, egg fate and the day of incubation. Random effects included the intercept and each unique egg identification nested within clutch (multiple eggs/female). The clutch identification was unknown for captive-produced eggs. The random error associated with the egg mass for an egg at each incubation day by source was modeled using an unstructured covariance structure [Singer and Willett, 2003].

Similarly, we used a multilevel model with longitudinal data to assess chick/juvenile mass gain (within and between-subject) with age. Fixed effects included chick source (chick from a captive or wild-produced egg), fate (chick did or did not survive to release in an ancillary study), age-related pattern of mass increase (linear, quadratic, and cubic), and the associated interactions. Random effects included the intercept and the unique chick identification nested within a brood (multiple chicks/female). As previously mentioned, brood

identification was unknown for chicks from captive-produced eggs. The random error associated with chick mass for each age and it's associated with source was modeled using an unstructured covariance structure [Singer and Willett, 2003]. Typically, logistic functions are used in growth models (e.g. logistic, Richards' and Gompertz, etc.) but because our study concluded before chick mass plateaued, we used a cubic polynomial function for our growth model [Huwer, 2004]. We used Akaike Information Criteria (AIC; [Akaike, 1973]) to assess the relative goodness-of-fit [with the small sample correction factor (AIC_c; Burnham and Anderson 2002)] of each model. We conducted all analyses using SAS 9.3 [SAS Institute, 2006].

RESULTS

Wild Grouse Transport, Capture, and Marking

In 2009, we monitored 10 (10A:0Y) wild females for egg acquisition. In 2010 and 2011, we captured and marked 35 (19A:16Y) and 14 (11A:3Y) additional wild females, respectively. Additionally, during 2009-2011 we captured, transported, and integrated 7 (5M:2F) GUSG from the Gunnison Basin into our existing captive-hatched flock of 11 GUSG (3M:8F) at the NWRC. We integrated 4 (2M:2F) in September and October 2009, two males in September 2010, and one adult male in March 2011.

Of the seven wild-reared GUSG introduced into captivity, one male was returned to its initial capture site in the Gunnison Basin and six died in captivity. One female died in 2009, 49 d post-introduction (body mass loss of 40%). The second female died 196 d post-introduction after a collision with the aviary ceiling. Of the four remaining males, one died (in 2010) 189 d post-introduction into captivity from injuries suffered during a territorial dispute during the breeding season (with the released wild male), and two died from a fungal (*Aspergillus sp.*) infection 55 and 81 d post-introduction in 2010. The last wild male died in 2011, 35 d post-introduction into captivity following a collision with the aviary ceiling.

Grouse Nest Monitoring and Egg Collection

In 2009, 2010, and 2011, we collected a total of 137 eggs from 6, 3, and 14 wild females, respectively. We collected eggs from 11 laying and 13 incubating females (one female was used for egg collection twice; Table 2). All females were adults. Nine of 11 laying females maintained nests following egg collection, and two abandoned and renested post-egg-collection. Nest success of the original nests (when eggs were collected during laying and the female continued nesting) was 56% (5/9). Eight of 13 wild incubating females renested after egg collection, and nest success was 50% (4/8). In 2010 and 2011, we also collected eggs from 9 and 5 captive females, respectively (Table 2). In 2010 one female was a wild-reared adult and eight were captive-reared first-year birds from the captive-flock established in 2009. In 2011, all five females were captive-reared adults from the 2009 cohort.

Egg Storage, Incubation, and Hatch

The probability that an egg hatched was not influenced by egg storage ($\beta = -0.03$; 95% CI -0.15, 0.10; Wald $\chi^2 = 0.17$, df = 1; $P = 0.68$). No effect of cooler storage time length was supported by the adjusted odds ratio point estimate (0.97) 95% CI, which included 1 (0.86, 1.10).

Overall egg hatch success was 74.3% (Table 2), with success by year declining from 90.0% (36/40) in 2009 to 78.0% (46/59) and 66.4% (71/107) in 2010, and 2011, respectively ($G_2 = 10.02$, $P = 0.01$). Hatch success differed between captive—(56.5%) and wild-produced (83.2%) eggs (Table 1; $G_1 = 16.45$, $P < 0.00$). Hatch success of eggs from wild laying females (76.1%) and wild incubating females (86.8%) was comparable ($G_1 = 2.42$, $P = 0.12$), but hatch success of wild laid eggs was higher (76.1%) than captive laid eggs (56.5%; $G_1 = 5.41$, $P = 0.02$). The time from external pip to hatch averaged 38.3 hr (SE 1.8) ($n = 117$, 95% CI = 26.9, 49.7), and did not vary by source ($F_{1,16} = 0.20$; $P = 0.66$).

In total, 34% (18/53) of the eggs we collected did not hatch due to egg infertility (visual inspection) and embryonic mortality (Table 2). Egg fertility for captive-produced eggs was lower than wild-produced eggs [4.4% (6/137) and 17.4% (12/69); $G_1 = 9.10$, $P = 0.00$], respectively. We found no relationship between our wild-produced egg infertility rate for laid and incubated eggs [6.5% (3/46) and 3.3% (3/91); $G_1 = 0.72$, $P = 0.40$], respectively. Fifty-five percent (17/31) of our embryonic mortalities were <19 d into development and 45% (14/31) were ≥ 19 d into development. Twenty-six percent (8/31) of the embryos were malpositioned and unable to hatch. We observed embryo malposition class #2 ($n = 2$), #4 ($n = 4$), and #6 ($n = 2$).

TABLE 2. The number and perentage of wild- and captive-source Gunnison sage-grouse eggs that hatched or did not hatch due to infertility or embryonic mortality and were collected from laying and incubating females in Gunnsion and Larimer Counties, Colorado, USA, 2009–2011

	Wild source				Captive source				
	Laid	%	Incubated	%	Laid	%	Incubated	Total	% of Total
Hatch	35	76	79	87	39	57	—	153	74
Not hatch	11	14	12	13	30	43	—	53	26
Total	46	100	91	100	69	100	—	206	100

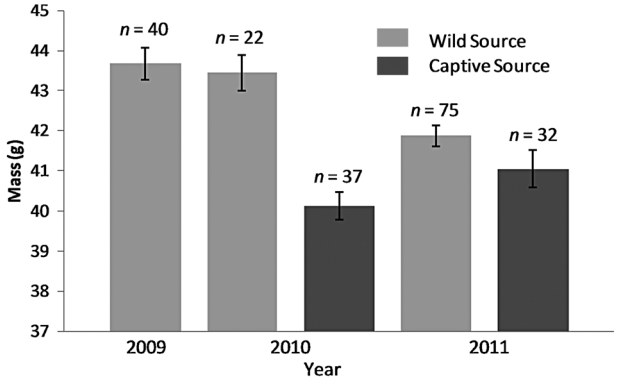

Fig. 3. Captive- and wild-produced Gunnison sage-grouse egg mass ($\bar{x} \pm$ SE) by year of study (2009, 2010, and 2011) and egg source (wild or captive) in Larimer and Gunnison Counties, Colorado, USA, 2009–2011.

Egg mass varied among years and by egg source ($F_{2,205} = 9.85$, $P = 0.00$). Eggs from captive females in 2010 were smaller than eggs from wild females in 2009 ($t_{201} = 6.69$, $P < 0.0001$), 2010 ($t_{201} = 5.32$, $P < 0.00$) and 2011 ($t_{201} = 3.77$, $P < 0.00$) (Fig. 3). In addition, eggs produced by captive females in 2011 were smaller than eggs produced by wild females in 2009 ($t_{201} = 4.75$, $P < 0.00$) and 2010 ($t_{201} = -3.72$, $P < 0.00$), but not in 2011 ($t_{201} = -1.66$, $P = 0.09$) (Fig. 3). Eggs produced by captive females in 2010 and 2011 were similar ($t_{201} = -1.66$, $P = 0.10$) in mass (Fig 3). Logistic regression indicated that source was a better predictor of egg hatch ($\beta = -0.64$, df = 1; 95% CI [−1.00, −0.29, $P < 0.00$) than egg mass ($\beta = 0.03$, df = 1; 95% CI [−0.11, 0.17, $P = 0.68$) with the probability of hatch increasing with egg mass (Fig. 4).

To obtain the best estimate of the rate of egg mass loss during incubation, given our set of candidate models (Table 3), we found that egg mass loss was linear throughout incubation while controlling for the effect of whether the egg had a captive or wild origin. This is not an unexpected result due to the constant incubation environment. The next plausible model includes egg fate (egg hatched or not) as an additional effect but not as part of an interaction. Eggs, regardless of source, lost mass during incubation ($\beta = -0.60$, $F_{1732} = 1725.04$, $P < 0.00$). Captive-produced eggs, although smaller initially, lost mass at a different rate compared to wild-produced eggs ($\beta = 0.19$, $F_{1732} = 61.45$, $P < 0.00$; Fig. 5). The Least Square Mean (LSM) percent mass lost for captive produced eggs was 14.21% (95% CI 13.80, 14.63) and 19.8% (95% CI 19.51, 20.17) for wild produced eggs (Fig. 5).

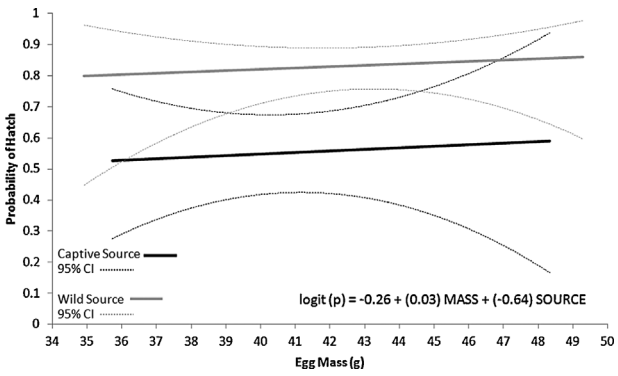

Fig. 4. Egg source (captive or wild) hatch probability and 95% CI for Gunnison sage-grouse in Larimer and Gunnison Counties, Colorado, USA, 2009–2011.

Chick And Juvenile Husbandry

One to 10 days post-hatch

We collected 2389 mass measurements from 148 (110 from wild egg source and 38 from captive egg source) GUSG individuals hatched and raised in captivity. Mass was not collected on five individuals because mortalities occurred during or immediately after hatch. Most (91%; 2161/2389) of the mass measurements were collected from hatch (0 d) to 10 d post-hatch. The remaining mass data were collected from 11–35 d post-hatch.

Given our set of candidate models (Table 4), the model with a cubic age function, while controlling for the effect of surviving to release and incorporating source only at the initial status, provided the best explanation of chick growth. Chick and juvenile growth was indiscernible until the fourth mass measurement sequence (e.g. 4 d post-hatch) when surviving chick mass diverged from chicks that did not gain mass and ultimately died (Fig. 6). Therefore, when controlling for whether a chick survived, the estimated difference in mass of chicks from captive-produced eggs resulted in chicks that were 1.59 g smaller than their wild egg source cohort at the time of hatch. Ultimately, when we accounted for chick origin, chicks that died were approximately 4.0 g heavier at hatch, but their average rate of mass loss per unit of time per chick were negative ($\beta = -0.07$) when compared to surviving juveniles ($\beta = 0.24$) ($F_{2,1029} = 49.80$; $P < 0.00$).

Our overall chick mortality rate was 36% (56/154) from hatch to 10 d post-hatch with 52% (29/56) of the mortalities occurring from 1–5 d post-hatch and 48% (27/56) between 6-10 d post-hatch. Bacterial (*Proteus vulgaris*, *Enterococcus spp*., *Klebsiella spp*., *Clostridium perfringens, Escheriachia coli*, *Burkhoderia spp.*, *and Staphylococcus spp.*) infections were the leading cause of mortality (57%; 32/56) followed by euthanasia due to deformities (20%), unknown causes (16%), and accidents or trauma (7%; 4/56). The rate of mortality (negative survival rate slope) was $\beta = -0.07$ (SE 0.01) in 2009 ($F_{1,9} = 68.60$, $P < 0.00$), $\beta = -0.03$ (SE 0.00) in 2010 ($F_{1,9} = 240.60$, $P < 0.00$), and $\beta = -0.02$ (SE 0.00) in 2011 ($F_{1,9} = 125.36$, $P < 0.00$). The rate of mortality differed among years ($F_{2,27} = 19.73$, $P < 0.00$), and although the slope differed between 2009 and 2010 ($F_{1,27} = 21.30$,

TABLE 3. Candidate models for factors influencing mass loss of captive- and wild-produced Gunnison sage-grouse eggs during incubation time (INCDAY) including the −2 log-likelihood (deviance), number of parameters (*K*), Akaike's Information Criteria correction for small sample size (AIC_c), Akaike's Information Criteria compared to the best model (ΔAIC), and Akaike weight (w_i) in Gunnison and Larimer Counties, Colorado, USA, 2009–2011

Model description[a]	−2 Log(*L*)	*K*	AIC_c	ΔAIC	w_i
SOURCE + INCDAY + SOURCE*INCDAY	90.8	8	106.8	0.0	0.56
SOURCE + FATE + SOURCE*INCDAY	90.5	9	110.5	1.7	0.24
SOURCE + FATE + SOURCE*INCDAY + FATE*INCDAY	90.5	10	108.5	2.0	0.21
INCDAY	152.2	6	164.2	53.7	0.00
HATCH + HATCH*SOURCE	357.7	7	371.7	207.5	0.00
INCDAY	418.5	10	398.5	234.3	0.00
NO GROWTH	7738.8	3	7744.8	7346.3	0.00

[a]SOURCE (captive or wild egg source), INCDAY (linear mass loss change trajectory through incubation), $INCDAY^2$ (quadratic mass loss change trajectory through incubation), FATE (egg hatched or not), NO GROWTH (no change trajectory with a polynomial function resulting in INCDAY raised to the 0th order).

$P < 0.00$) and 2009 and 2011 ($F_{1,27} = 35.99$, $P < 0.00$), there was no difference between 2010 and 2011 ($F_{1,27} = 1.91$, $P < 0.18$).

Sub-Adult And Adult Husbandry

Five months to one year

In 2009, we retained all ($N = 14$) of the captive-reared subadults to establish our captive flock. In 2010 no new captive-reared birds were retained, but in 2011 we retained one new subadult female due to a wing amputation following an injury. We maintained 11 (8F:3M) and 8 individuals (5F:3M) during the winters of 2009/10 and 2010/11, respectively. In total, over the 3 years of our study, our captive flock size varied (8–14 subadult and adult GUSG) and we maintained a captive flock for 694 d.

DISCUSSION

We have developed and report the first captive-rearing methods for collecting, incubating, and hatching GUSG eggs, and the feeding and maintenance of GUSG chicks, juveniles, subadults, and adults. We successfully collected viable eggs during laying from our captive GUSG flock and from wild free-ranging females during laying and incubation. Although egg size has been correlated with offspring performance [Williams, 1994; reviewed by Krist, 2011], we found no reports where authors have compared egg size between captive and wild populations of precocial birds. Our captive-produced GUSG eggs averaged 8% smaller mass than wild-produced GUSG eggs, except for eggs collected in 2011.

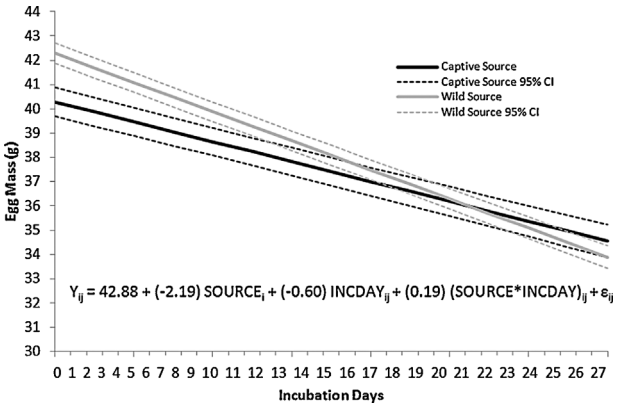

Fig. 5. Predicted mass loss (±95% CI) by source (wild or captive) through artificial incubation in Larimer County, Colorado, USA, 2009-2011.

We successfully stored laid eggs prior to incubation and storage did not negatively affect hatchability in contrast to numerous studies with domestic poultry reporting declining egg quality when increasing storage time [Alsobayel and AL-Miman, 2010; Butler, 1991; Mayes and Takeballi, 1984; Meijerhof, 1992; Petek et al., 2003; Petek and Dikman, 2006; Proudfoot, 1969; Reis et al., 1997]. We stored eggs an average of 7 d in 2010 and 3 d in 2011, but recommend egg storage not exceed >7 d [Anderson Brown and Robbins, 2002; Thompson, 2012] and preferably ≤3 d [Navarro and Martella, 2008; Stoleson and Beissinger, 1999; Veiga, 1992].

Only two studies [Huwer, 2004; Thompson, 2012] have successfully collected eggs from free-ranging wild GRSG during different phases of nesting. The reported hatch success from the eggs collected from laying females is 82% and 68% for each of two years [Huwer, 2004], and Thompson [2012] reported an average hatch success of 59% over three years. We found a similar hatch success for eggs collected from laying GUSG females (69%). Thompson [2012] reported an 89% hatch success rate for eggs collected from incubating GRSG females which is similar to our results (91%). Thompson [2012] also reported that eggs collected from laying females had lower hatch success, but we did not detect a similar relationship with GUSG. We found that egg source (captive or wild) was most important in predicting hatch success.

Gunnison sage-grouse egg hatchability was 90% for all eggs collected, 83% for wild-produced eggs collected during laying and 93% for wild-produced eggs collected during incubation. Huwer [2004] reported 84%

TABLE 4. Candidate models for factors influencing captive-reared Gunnison sage-grouse growth from hatch to 34 d post-hatch, including the -2 log-likelihood (deviance), number of parameters (K), Akaike's Information Criteria correction for small sample size (AIC_c), Akaike's Information Criteria compared to the best model (ΔAIC), and Akaike weight (w_i) in Gunnison and Larimer Counties, Colorado, USA, 2009-2011

Model description[a]	$-2\ \mathrm{Log}(L)$	K	AIC_c	ΔAIC	w_i
FATE*TIME^3 + SOURCE + FATE	8581.3	14	8619.3	0.0	0.91
SOURCE*TIME^3 + FATE*TIME^3 + SOURCE + FATE	8579.9	22	8623.9	4.6	0.09
SOURCE + TIME^3	8595.7	18	8631.7	12.4	0.00
TIME^3	8606.5	14	8634.5	15.2	0.00
TIME^2*SOURCE	9737.8	9	9761.8	1142.5	0.00
TIME^2	9745.3	9	9763.3	1144.0	0.00
FATE*TIME + SOURCE*TIME + TIME + SOURCE	11 475.7	10	11495.7	2876.4	0.00
TIME + SOURCE	11 490.4	8	11506.4	2887.0	0.00
TIME	11 507.8	6	11519.8	2900.0	0.00
NO GROWTH	12 927.5	3	12933.5	4314.2	0.00

[a]SOURCE (chick resulting from a captive or wild egg source), TIME (linear mass gain change trajectory post-hatch). TIME^2 (quadratic mass gain change trajectory post-hatch). TIME^3 (cubic mass gain change trajectory post-hatch), FATE (chick survived or not to be released in ancillary study) NO GROWTH (no change trajectory with a polynomial function resulting in TIME raised to the 0th order).

and 68% hatchability for eggs collected from laying GRSG over two years. Thompson [2012] reported 65% and 97% hatchability for laid and incubated GRSG eggs, respectively. Our hatchability results are also similar to expected values in previous GRSG research [Thompson, 2012] and incubation and hatching manuals [Anderson Brown and Robbins, 2002].

We achieved the highest artificial hatchability and hatch success by collecting eggs from incubating wild females. Although collecting eggs using this approach is more efficacious, there could be impacts to wild populations caused by the disturbance of birds or removal of potential individuals (eggs) from the source population. Therefore, the establishment and maintenance of a captive-flock could ameliorate or avoid these potential impacts, but at the cost of reduced hatch rates and reduced genetic variation. A captive-flock could also reduce the financial expense of collect ing eggs from marked wild birds in the field (e.g. radio-telemetry and associated field expenses), but those financial considerations must be weighed against captive-flock development and maintenance.

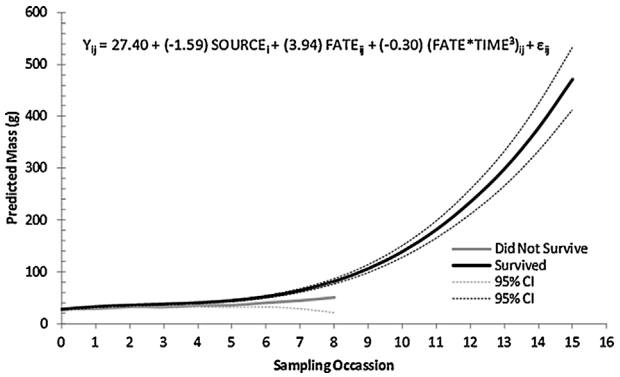

Fig. 6. Predicted mass (g) (least square mean ± 95% CI) of captive-reared Gunnison sage-grouse that survived or died before release in an ancillary study from hatch to 35 d post-hatch (presented in 16 sequential time periods) in Larimer County, Colorado, 2009–2011.

Unhatched fertile eggs are common in domestic poultry [Kuurman et al., 2003] and wild bird species [Anderson Brown and Robbins, 2002; Klimstra et al., 2009]. Embryonic mortality of GUSG was similar to a study of GRSG [Thompson, 2012] where 55% of embryonic mortality occurred ≤21 d and 45% occurred >21 d. We believe that the embryonic mortality we observed was not caused by damage inflicted during egg storage because temperatures were within industry standards (14–15°C) [Alsobayel and AL-Miman, 2010; Holland, 2007; Petek and Dikmen, 2006], although industry standards may not apply directly to native bird species because storage temperatures can be as high as 18°C for quail (*Coturnix coturnix*) [Petek et al., 2003]. Amer [1962] reported that 29.8% of domestic chicken embryo mortalities were due to malposition. This is comparable to our rate of malpostioned embryos.

We also have no evidence that embryonic mortality was caused by inappropriate incubator environmental conditions (e.g. temperature or humidity) resulting in abnormal mass loss. Bird eggs lose mass during natural and artificial incubation [Ar and Rahn, 1980], but mass loss varies by bird species and order [Klimstra et al., 2009]. Drent [1970] predicted a 14% incubation mass loss for GRSG (0.21 mg/d mass loss; [Patterson, 1952]) using a 38 g average egg mass. Although the aforementioned egg mass is smaller than our wild GUSG egg mass, recent reports with GRSG [Thompson, 2012], previous recommendations for mass loss during incubation for Galliformes (14-18%; [Drent, 1970] and other avian species (15–16%; [Klimstra et al., 2009]), are ≥14%. We suggest a 14–19% egg mass loss during artificial incubation is an appropriate percentage, but it is higher than the recommendation by Thompson [2012] (11.6–13.1%), Stromberg [2001], and Anderson Brown and Robbins [2002] who suggested a minimum of 11% and an optimal loss of 15%.

The most complicating factor we encountered with GUSG chick captive-rearing was controlling bacterial infections from hatch to 10 d post-hatch, ultimately losing nearly 40% of chicks hatched. Although we anticipated bacterial infections [Drew et al., 1998; Friend, 1999; Johnson and Boyce, 1991; McEwen et al., 1969], the severity and high mortality rate was inconsistent with previous GRSG research [Huwer, 2004; Thompson, 2012]. Because of the adaptive approach we used to control bacterial infections, we reduced the rate of mortality due to these infections, but did not eliminate them.

The mortality of wild adults introduced into captivity was problematic. All of the mortalities were likely associated with stress-related failure to habituate to captivity [Xavier et al., 2007] and we experienced two mortalities from complications related to fungal infection reported in a previous study [Oesterle et al., 2005]. Therefore, we suggest when wild-reared birds are introduced into captivity that hatch-year juveniles rather than adults would habituate to captivity more readily [Oesterle et al., 2005]. An additional concern to captive sage-grouse flocks is WNV. We experienced one mortality from a WNV infection over the course of our study, but following our study, the captive flock experienced 4 additional mortalities from WNV in 2012 and 2013. Therefore, we suggest future captive-rearing programs have a vigilant WNV prevention and protection protocol.

We found that GUSG raised in captivity from hatch to their first breeding season produced viable eggs and readily consume native and non-native food items. Our findings may be used for refining previously reported captive-rearing methods for GRSG [Johnson and Boyce, 1990, 1991; Huwer, 2004; Oesterle et al., 2005; Thompson, 2012].

Because the timing of hatch can vary greatly depending on the incubation duration and species [Anderson Brown and Robbins, 2002; Kuehler et al., 2000], we recommend flexible and well-staffed artificial incubation programs throughout all phases of artificial incubation and hatch to accommodate irregular hatching schedules.

Over two decades, captive-breeding programs have been used to bolster or reestablish declining wildlife populations [Araki et al., 2007; Redford et al., 2011; Snyder et al., 1996]. Snyder et al. [1996] expressed concerns about captive-breeding programs, suggesting that captive-breeding efforts should be used sparingly with a limited number of endangered species recovery programs and used as a last resort when other conservation alternatives are unavailable or have been exhausted. We agree with the concerns outlined by Snyder et al. [1996], and suggest our findings can be informative in providing a foundation for the development of a proactive and alternative conservation and management approach for GUSG. Our findings are even more salient if a captive-rearing program is developed early in the recovery and conservation planning [Tear et al., 1993] process rather than waiting until captive-rearing is the only approach available to sustain wild populations [Scott et al., 2005]. Therefore, we encourage conservationists and wildlife managers to consider the conservation value of captive-rearing program in GUSG (and small isolated GRSG populations) conservation, because a laissez-faire conservation approach versus a proactive approach could result in increased costs [Drechsler et al., 2011] or problems with crisis management and population (species) viability [Silvy et al., 1999]. Our results provide important information in the recovery of this conservation-reliant species [Goble et al., 2012; Scott et al., 2010]. If a captive flock is used to produce stock for augmentations or reintroductions [Seddon et al., 2007], conservation practitioners should consider novel approaches to augmentation [Thompson, 2012] because of issues with similar species [Lockwood et al., 2005].

A captive-breeding program is not the only conservation solution, but should be considered in the suite of conservation approaches. Captive-rearing is only the first step in multifaceted recovery approach. If a captive-breeding program is implemented, we recommend caution because captive populations created through artificial selection can alter natural selection processes resulting in unintended consequences to gene flow, fitness-related traits [Lynch and O'Hely, 2001; Navarro and Martella, 2008], or disease issues [Drew et al., 1995]. A possible solution to these genetic issues could be the inclusion of artificially hatched wild-produced eggs into a captive population. This will help maintain a captive flock as a demographic or genetic reservoir [Navarro and Martella, 2008] to counteract possible issues related to inbreeding depression [Stiver et al., 2008].

CONCLUSIONS

If conservationists and managers choose to implement artificial incubation as part of a GUSG captive-rearing program, we offer the following conclusions and general protocol:

1. Collecting eggs from wild incubating rather than laying females results in higher hatchability, although collecting from wild females during laying may have less impact on the source population.
2. Collecting eggs from adult versus yearling wild females may result in higher likelihood of renesting.
3. Collecting eggs early in incubation ($\leq$7 d) rather than later can encourage renesting and provide a source of eggs from nests that would be ultimately destroyed by predators.
4. If egg collecting occurs during laying, storing eggs in advance of incubation can assist in hatch synchronization. Store eggs in a temperature controlled cooler for $<$7 d.
5. Establishing a captive flock can reduce the impact of egg collection on wild populations, but expect lower egg hatchability when compared to wild-produced eggs.
6. Pre-condition incubators and hatchers for a minimum of 14 d to a temperature and humidity of 37.5°C and 58% and 37.2°C and 80%, respectively.

7. Separate clutches and broods to reduce cross-contamination of contagions.
8. Maintain a high degree of cleanliness and institute rigorous handing protocol to reduce cross-contamination of harmful contagions.
9. Achieve 14–19% mass loss during incubation.
10. Transfer eggs from the incubator to hatcher when candling reveals an internal pip.
11. Anticipate and plan an artificial hatching program for a 10–15% hatch failure rate.
12. Provide sufficient staffing to support continuous monitoring of incubators and hatchers.
13. Use gravel or existing substrates (non-organic supplements) in artificial brooders.
14. Provide antibiotics to chicks for the first 5 d post-hatch. Supplement the diet with naturally occurring probiotics (e.g. crushed GUSG fecal matter, feathers, and soil collected from strutting grounds). Evaluate the success and effectiveness of such an approach.
15. Plan for 10% of artificially incubated and hatch chicks to succumb to physical abnormalities.
16. Provide a diversity of food sizes and types (dominated by invertebrates up to 10 d post-hatch) to captive-reared GUSG.
17. Provide native food types (e.g. sagebrush), although GUSG also consumed non-native commercial poultry food products.
18. Provide protective measures against WNV infection.

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