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### Abstract

Operational procedure for artificial incubation of alligator eggs (Alligator mississippiensis) in controlled environmental chambers and methods of handling eggs were tested to determine hatching success and to evaluate mortality factors. Hatching success was determined for eggs collected from three sources; eggs produced from wild marsh nests, eggs from wild adult alligators brought into captivity, and eggs from alligators hatched and raised in captivity.

Hatching success for wild eggs collected prior to the fifth week of incubation was greater than that reported from wild eggs hatched in the marsh under natural conditions. Artificial incubation success for eggs collected from nests in the wild was superior to eggs collected from either wild captured adult alligators nesting in captivity or alligators hatched in captivity and raised to adulthood.

The stage of incubation when eggs are collected as well as environmental factors, and egg collection techniques were found to greatly influence hatchability. Biological information collected from wild adult alligators and their nests provided a basis for comparison of reproductive success under captive conditions.

### Introduction

A basic understanding of nesting, incubation, and embryology in the wild is a prerequisite for evaluating reproductive success

under artificial conditions. Artificial incubation of alligator eggs can improve on nature and increase hatchability as compared to nests which are allowed to hatch in their natural environment. Advantages of incubation include: the elimination of natural losses due to predation (Joanen 1969, Pooley 1973, Blake and Loveridge 1975, Fleming et al. 1975, Chabreck 1978), annulment of weather related mortality factors (Joanen and McNease 1977, Chabreck 1978), and especially for farm operations, the elimination of egg losses caused by competition for nest materials and destruction of nests by other alligators (Joanen and McNease 1977, Chabreck 1978). Additionally, rearing young alligators in controlled environments reduces hatching year mortality as compared to that experienced in the wild (Joanen and McNease 1979).

Artificial egg incubation studies began in 1964 to investigate the feasibility of rearing alligators in captivity. Culture operations also include propagating alligators in pens and rearing of artificially incubated offspring in controlled environmental chambers (Joanen and McNease 1971, 1974, 1975, 1976, 1977, 1979). Studies in the wild provided baseline data upon which to evaluate the success of our culture program (McIlhenny 1935, Joanen 1969, Joanen and McNease 1970, 1972, 1975, McNease and Joanen 1974). Studies by Pooley (1971), Blake and Loveridge (1974), Joanen and McNease (1977), and Chabreck (1978) investigated egg handling and methods of incubation.

During the past nine years artificial egg incubation techniques were evaluated to: determine hatching success, investigate aspects of embryology, study methodology and timing of egg

collections, and determine sex of hatchlings incubated under different temperatures. Eggs were collected from wild marsh nests and nests from two groups of captive alligators. The source of alligators used in the pen studies were captured wild adult animals and alligators hatched in captivity and raised to adulthood.

### Results and Discussion

#### Natural Incubation in the Wild

Joanen (1969) checked 266 wild nests over a 4-year period and found that 182 (68.3%) hatched successfully, 19 (7.3%) were partially infertile, 15 (5.8%) were infertile, and 50 (18.6%) were either destroyed by raccoons or lost to flooding. Hatching success was determined to be 58.2% for 154 nests followed during 1967 and 1968. Average clutch size was 38.9 eggs (Joanen 1969). Joanen found that each wild marsh nest produced an average of 22.6 hatchlings. Chabreck (1966) and Joanen and McNease (1980) determined that 68% and 63% of wild females nested each year. The ovulation rate varies slightly from year to year depending upon environmental parameters.

In one north-central Florida study, hatching success for wild nests was found to be 45%. Of 13 nests checked, 62% produced live young, 31% were destroyed by mammalian predators and 8% were lost to rising water. Mean clutch size for 14 nests was 30.3 eggs (Goodwin and Marion 1978). Dietz and Hines (1980) reported a hatching success of 67.9% for undisturbed nests and an average clutch size of 38.7 and 36.1 for 2 study areas in 111 nests observed in Florida. In South Carolina the mean clutch size for 17 wild nests was 41.9 eggs. Eight of 12 nests (66.7%)

produced viable offspring and 82% of the eggs hatched in good nests. Flooding and raccoon predation caused egg loss in 1/3 of the nests studied (Bara 1976). Metzen (1977) reported a 70% hatching success with an average clutch size of 30 eggs in 110 nests observed in Georgia.

#### Time of Egg Collections

We recommend that alligator eggs be collected either within 24 hours after laying or after the fourth week of incubation (Joanen and McNease 1977, 1980, Chabreck 1978, Ferguson 1981).

The benefits derived from collecting eggs within 24 hours of laying for a farm operation are superior to waiting until after the fourth week to collect. The egg can be gently moved during the 24 hours following laying without serious injury because the embryo (embryonic disc) is not attached to the eggshell membrane. The embryo can move around and become attached after the egg is set in an incubator. Early egg pick-up allows the farmer through careful observation of eggshell banding to separate good eggs from infertile ones or eggs where embryos have died or suffered from serious ailments (Fig. 1). Eggs which are laid upright in the nest, with the long axis perpendicular to the ground, die unless they are positioned correctly during artificial incubation in early development. Early egg collection will eliminate losses caused by predation and flooding. Early pick-up combined with proper incubation procedure (providing optimum temperature and humidity conditions) will also eliminate egg desiccation which is a serious factor in pen situations due to poor quality nesting media and in the wild during drought periods. Early egg pick-up

eliminates loss in pen situations when multiple clutches are laid in one nest; where one female in laying may break eggs laid previously by another or other females (Joanen and McNease 1977, 1980, Ferguson 1981).

The reason eggs can be safely moved after 4 weeks of incubation, if handled gently and correctly, is that the embryo along with the embryonic membranes have developed sufficiently and become strong enough to resist damage from being moved about and stressed inside the eggshell (Ferguson 1981).

#### Methods of Collecting and Transporting Eggs

Two very important steps in collecting eggs for artificial incubation are to handle the eggs very gently and to permanently mark the top surfaces of the eggs so as to preserve their original nest orientation. Eggs laid upright should be marked near the embryonic disc attachment (widest part of opaque band) and positioned correctly for transport and incubation.

After each egg is marked and packed in single layers completely enclosed with natural nest material or in cases (especially in pen situations) where nest media are not suitable water-soaked grass hay may be substituted. The nest material or hay serves many important functions; it protects the eggs from rolling around in the container, cushions the egg from shock and stress, insulates so that the temperature does not change dramatically, maintains egg moisture levels, and produces bacteria media for exterior shell degradation (Ferguson 1981).

Transport containers should be of a suitable size so that eggs are easily packed without being too crowded. We found 5

gallon (19 liters) plastic buckets to be ideal in a pen situation where a lot of walking is required or where thick cover must be traversed. We use 35 gallon (132 liters) capacity garbage cans when collecting out of airboats (Joanen and McNease 1977).

### Incubation Techniques

Within 3 or 4 hours of collection from nests, eggs were artificially incubated in environmental chambers, as described by Joanen and McNease (1976). Temperatures were maintained within a range of 29.4° C (85° F) to 32.7° C (91° F). The best hatching success was obtained at 31-31.7° C (88-89° F) (Joanen and McNease 1979). Temperatures of individual clutches of eggs within a tank varied by  $\pm 2^\circ$  F from the ambient tank temperature.

Eggs are set in trays, measuring 61 x 61 x 15 cm (2' x 2' x 6"), covered top and bottom with 1.7 cm (1/2") mesh hardware wire fabric for air circulation. We recommend using fresh natural nest material if it is suitable; e.g., composed primarily of grasses. Our best hatching results were obtained using wiregrass (Spartina patens). If natural nest materials are not available, we suggest soaking fresh hay made from wiregrass or wiregrass type grasses for about a week prior to setting of eggs. Alligator eggs must be incubated in their natural dirty state completely surrounded by nesting media. This aides in the breakdown of the eggshell, which naturally occurs in wild nests (Ferguson 1981). The relative humidity (moisture levels) should be maintained as high as possible (90+%) throughout the incubation period.

Hatching trays were set on shelves 7.5 cm (3") over water. Chamber doors were opened only once or twice a week for inspection

until hatching commenced. When necessary, incubation media was moistened by spraying warm tap water over the hatching trays.

Once hatching began the chambers were checked every second day. The hatchlings were retained in their hatching trays for at least 24 hours to allow time for them to separate from the shell and for the umbilical cord to break off (Joanen and McNease 1979).

#### Artificial Incubation and Reproductive Success

The most notable finding from our artificial incubation studies is that eggs collected from wild marsh nests hatch much better than do those produced in pen situations. Hatchability for 578 artificially incubated wild eggs averaged 94%. The hatching rate for 375 eggs produced by wild-caught captive adults was 72% (Joanen and McNease 1979). In an effort to increase productivity from captive animals, factors which effect egg production and hatchability are being investigated; e.g., diets, age of stock, stocking rates, sex ratios, stress situations, and vitamin and trace element additives. While considerable progress has been made much needs to be accomplished to enable farmers to reach the 90% hatching rates.

Age at first nesting for alligators raised in environmental chambers for 3 years and then placed in outside pens was 5 years 10 months as compared to 9 years 10 months for animals raised in outside enclosures (McIlhenny 1935, Joanen and McNease 1975). Wild alligators probably reach sexual maturity at 10 years of age also.

Captive breeding stock, raised in environmental chambers



and then outside pens, were hatched in 1972 (referred to as 1972 age class) and 1973 (1973 age class). These alligators are the first 'cultured' group that we have worked with; animals artificially hatched and hand-reared. Since trawl remnant fish, primarily Atlantic croaker (Micropogon undulatus), and nutria (Myocastor coypus) are inexpensive and readily available along the Louisiana coast, those were the 2 primarily food sources chosen for study. A multi-purpose vitamin premix, manufactured by Dawe's Laboratories Ltd., Chicago Heights, Illinois, was added to both nutria and fish.

Maximum stocking rate for the 1972 age class was 32 alligators:1 acre (0.4 ha). The sex ratio was 1 male:7.7 females. The low incidence of males was due to our hatching process favoring females but nevertheless should give useful information relative to number of females a male can service. The two acre (0.8 ha) pen was constructed with a 70:30 land/water ratio.

Maximum stocking rates for the 1973 age class was 63 alligators:1 acre (0.4 ha), twice the density for the 1972 age group. The sex ratio was 1 male:2 females. The reason for the ultrahigh stocking rate was to determine maximum stocking densities for cultured alligators and to investigate unusual stress/social conditions which arose. This 1.5 acre (0.6 ha) pen has a 70:30 land/water ratio.

The 1972 age class nesting for the first time (fish fed), produced 12 clutches of eggs in 1978, and 18.2% nesting rate. Nineteen clutches were laid in 1979 for a 33.3% nesting success. Nesting success in 1980 dropped off to 18.2% the same as for

their very first nesting effort. Nesting success in 1981 increased to 38.6%, the highest for their first 4 years of sexual maturity (Table 1).

The 1973 age class group (fish fed through 1979; changed over to nutria in 1980) produced 20 clutches of eggs in 1979, a 25% nesting rate in their very first year. In 1980, the 1973 age class showed a 36.2% nesting effort (29 clutches), corresponding to the change in diet to whole nutria carcasses. Nesting success in 1981 increased to 53.7%, the best in their short 3 year history of reproduction.

Chabreck (1966) and Joanen and McNease (1980) determined that 68% and 63% of wild females nested each year. The average nesting rate of 27.1% for young fish fed alligators and 38.3% for nutria fed during their early years of sexual maturity was considerably below the 48-50% reported for wild captured-pen cultured alligators (Joanen and McNease 1971, 1975, 1979). Only in 1981 did the nutria fed stock, with a 54% nesting effort, surpass that reported for wild captured pen stock.

Egg hatchability for the 1972 age class (fish fed) averaged 12, 32, 37, and 52%, respectively for the years 1978, 1979, 1980, and 1981. Fertility for this group of females averaged 33, 20, 36, and 52%. The service rate for each male in 1981 was 3 females.

Egg hatchability for the 1973 age class which were fed fish in 1979 and nutria thereafter was much higher than for the animals fed a fish diet. Hatchability averaged 36, 50, and 57% respectively for 1979, 1980, and 1981. Egg fertility was likewise much higher for the nutria fed alligators. Fertility jumped from 29%, their

first nesting year (1979), and on a fish diet to 73% and 72% for the years 1980 and 1981 when nutria were fed.

The number of eggs per clutch increased with age. Clutch size averaged 27, 28, and 37 eggs in successive years. The only year when appreciable differences in clutch size were noted was in 1980 when fish fed females averaged 24.2 eggs/clutch and nutria fed averaged 32 eggs/nest.

The very young age of sexual maturity for cultured alligators complicates the comparison of reproductive success to that achieved in the wild. Nevertheless, for crocodilian farm programs to be truly successful, reproduction should be above that experienced in the wild. While the age of sexual maturity can definitely be reduced by several years through intensive culture, it is important to note that it may take 10+ years before reproductive success reaches anywhere near acceptable limits.

Joanen (1969) reported an average clutch size of 39 eggs in the wild. Cultured known age American alligators approached this level in their third and fourth years of reproduction.

Egg fertility and hatchability from cultured stock are the two aspects of reproduction which are below that determined for either wild or wild-captured pen stock during their first 3 years of productivity. Egg fertility for young captive reared brood stock fed nutria averaged 58% for 3 years, much lower than the 87.5% determined for wild alligators and 75.4% for wild alligators held in pens (Joanen and McNease 1979). Hatchability averaged 47.6% for the 3 year study, again lower than the 58% reported by Joanen (1969).

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TABLE 1. Reproductive success for Alligator mississippiensis, Rockefeller Refuge 1978-81

Year of Nesting	Type of Diet	Hatching Year of Brood Stock	Average Number Eggs/Clutch	Percent of Females Nesting	Percent Fertile Eggs	Percent Eggs that Hatched
1978	Fish	1972	25.8	18.2	33	12
1979*	Fish	1972	28.8	33.3	20	32
1979*	Fish	1973	25.3	25.0	29	36
1980	Fish	1972	24.2	18.2	36	37
1980	Nutria	1973	32.0	36.2	73	50
1981	Fish	1972	36.3	38.6	52	52
1981	Nutria	1973	38.3	53.7	72	57

\*Began using a vitamin supplement.

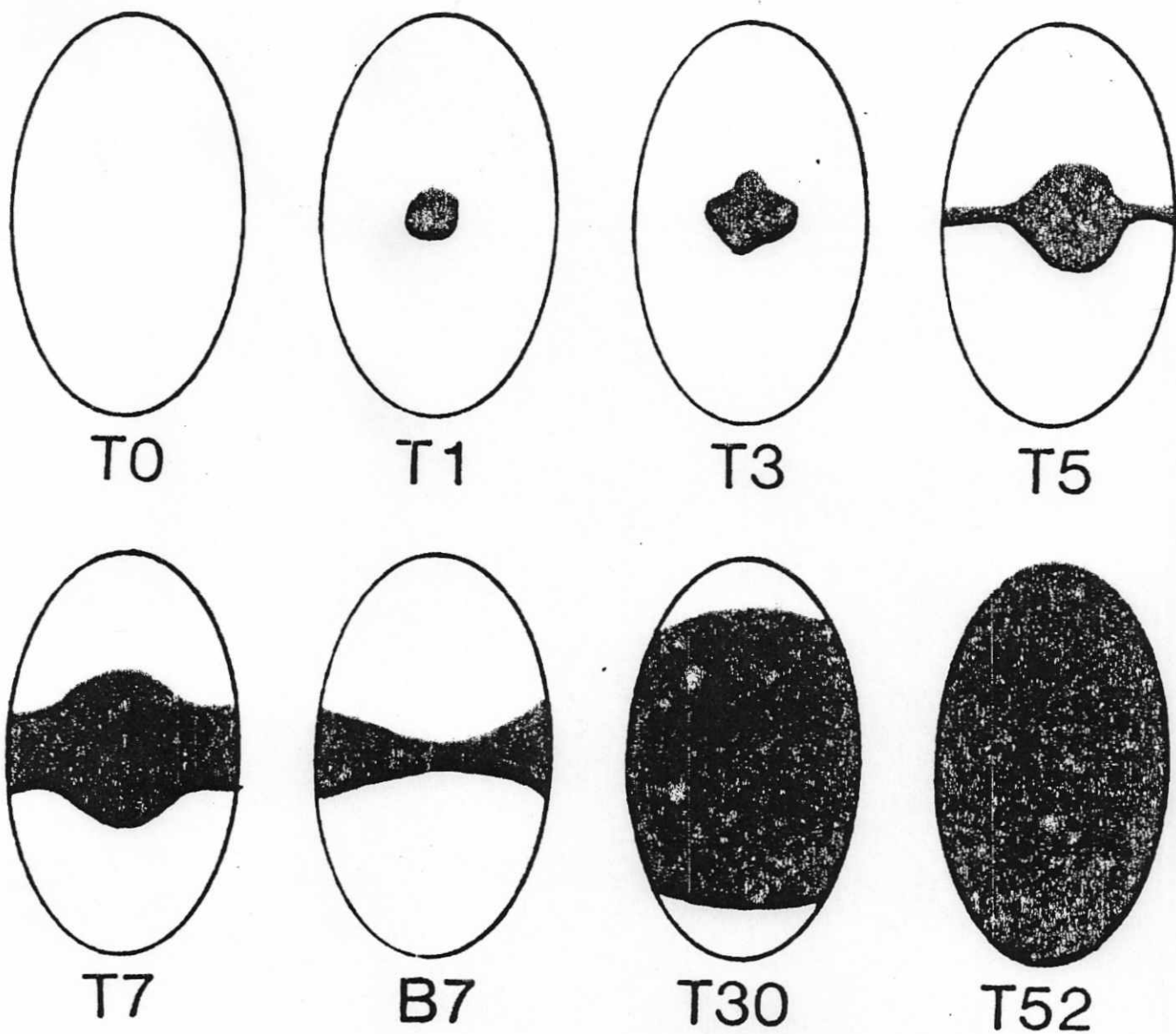


FIGURE 1. Opaque banding for the American alligator egg from day laid through day 52 of incubation. From Ferguson (1981).

T - Top view.

B - Bottom view.

Numerals - Stage of incubation in days.