



Using Weight Loss Method to Determine the Ages of Chinese Grouse Nests Found During the Egg Laying and Incubation Periods

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ABSTRACT

With a decreasing population trend, the endemic Chinese grouse (*Tetrastes sewerzowi*) is categorized as class I national protected animal species in China. Lower breeding performance has been suggested as a main factor influencing the population viability of Chinese grouse. Nest predation, which might be time-varied, is a main contributor to the nest failures of Chinese grouse. Therefore, it is urgent to estimate nest age of Chinese grouse accurately before taking appropriate conservation actions. In this study, we estimated the nest age of Chinese grouse using the weight loss method, which has been found to be easily conducted and also has a high accuracy compared with other methods, such as egg candling and floating. Our results confirmed the convenience and accuracy of the weight loss method in estimating nest ages of Chinese grouse. We further discussed some cautions when applying the weight loss method. We recommended investigators to use the weight loss method rather than the candling or egg floating methods to determine avian nest ages in future field studies.

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Authors' Contribution

JMZ and YHS conceived the idea. JMZ and YF conducted the field work and analyzed the data. JMZ, YF and YHS wrote the manuscript.

Key words

Weight loss method, Fresh egg weight, Incubation period, Nest age, *Tetrastes sewerzowi*

INTRODUCTION

Distributed along the east margin of the Tibetan plateau and with a decreased population trend (IUCN, 2017), the rare and endemic Chinese grouse (*Tetrastes sewerzowi*) is listed as a category I national protected animal species by the Chinese government (Zheng and Wang, 1998). Habitat fragmentation has been proposed as a main threatening variable in a landscape scale (Sun, 2000; Sun *et al.*, 2003), however, the lower breeding success has been identified as the most important factor which affecting the population viability in a local scale (Lu and Sun, 2011). The most practical and simple conservation management tool would be to increase breeding performance of Chinese grouse (Lu and Sun, 2011; Sun *et al.*, 2003).

Egg laying and incubation period is a vulnerable stage for both eggs and incubating parents in birds

(Dinsmore *et al.*, 2002; Rotella *et al.*, 2004). Due to the activities of predators, the whole clutch loss of eggs and/or the death of incubating females (nest predation) have been identified as the main reasons of nest failures in Chinese grouse (Sun *et al.*, 2003). Nest predation of Chinese grouse shows a time-varying pattern possibly because of the activity rhythms of predators and breeding females or other confounding factors, such as the growth of vegetations around the nests (Zhao *et al.*, 2020). Therefore, daily nest survival (DNS) is correlated with nest age. It is a prerequisite to accurately determine the nest ages of Chinese grouse in order to identify the key variables affecting DNS and take proper conservation measures to increase its breeding performance.

There are four main methods to determine ages of bird nests that has been widely applied in field researches. The original method to determine nest age was through the investigation of embryos' development status, necessitating the opening and thus the destruction of the investigated eggs, requires rather great skills on the part of the investigators, reference tables for the species concerned, and description, measurements and weights of the embryos (McCabe and Hawkins, 1946). It was not desirable for investigators to destroy the incubation eggs, especially for the endangered species. Lately egg floating, candling and measure of weight loss were invented by

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researchers (Westerskov, 1950), with candling method and floating method being widely applied in later studies (Walter and Rusch, 1997; Rizzolo and Schmutz, 2007; Ackerman and Eagles-Smith, 2010; Uherkoch *et al.*, 2015). However, some drawbacks of these two methods have also been identified by researchers. Candling of eggs has to be conducted in a dark room, or a special candling box must be carried around. And candling method is practical only with large sized eggs. Effective candling is difficult when checking thick shelled or mottled eggs (Lokemoen and Koford, 1996). Egg floating method gave high variable results for eggs incubated for equal lengths of time after clutch competition (Nol and Blokpoel, 1983; Liebezeit *et al.*, 2007). Moreover, using floating method and candling method require reference tables which are only existing in relatively a few bird species, precluding investigators to estimate nest age accurately when applying these methods (Ackerman and Eagles-Smith, 2010).

The use of weight loss method to determine nest age is based upon an assumption that there is a constant water loss throughout the whole period of incubation (Rahn and Ar, 1974). The weight loss of eggs can be ascribed almost exclusively to the loss of water vapor since the embryo has a typical respiratory quotient near 0.72 where the exchanging mass of O₂ and CO₂ molecules are equal (Drent, 1973). Despite Drent (1970) has shown that optimal egg temperature is not reached during the first few days of incubation, and thus a somewhat smaller water loss during that time; while after pipping, the eggs undergo a larger water loss. On balance, an average constant water loss throughout the whole period of incubation is a valid approximation (Rahn and Ar, 1974). In another word, a linear relationship between nest age and loss of egg weight is expected during incubation. Despite that the weight loss method has been introduced by investigators for a long time and have the merits of easily being conducted and a high accuracy (Collins and Gaston, 1987; Demongin *et al.*, 2007), it has been seldom applied in empirical researches.

In this study, we determined the nest age of Chinese grouse using the weight loss method. We verified that this method could be used in avian studies where nests were found throughout the breeding season (egg laying and incubation period) and eggs were measured only once (usually on the founding date). We also discussed some cautions when applying the weight loss method to determine nest age.

MATERIAL AND METHODS

The general use of weight loss method

Suppose that investigators find nests throughout the nesting season, with some nests during egg laying

and others during various incubation stages. We have no difficulties to estimate the ages of nests found during egg laying. Nests which found during incubation but finally successfully hatched are also could be estimated by subtracting average incubation days from nests been monitored. Therefore, we only need to estimate those nests that have been found during incubation but fail before successfully hatching.

According to the linear change of egg weight with nest age, the age of nests can be calculated by a linear equation: $Age = a + b*(W - W_f)$ (1), where W is fresh egg weight, W_f is egg weight at found date. If we could draw this linear equation, we could use it to calculate the nest age for all nests in a focal bird population.

Fresh egg weight W is both related to egg shape and density. Hoyt (1979) described the weight coefficient (K_w) to calculate fresh egg weight by equation: $Weight = K_w * LB^2$ (2), where L is egg length and B is egg breadth. This method is efficient in calculating fresh egg weight with an error rarely over 2% (Hoyt, 1979). K_w can be obtained from eggs found during incubation. The values of constant a and b could be estimated by least square method using nests with known ages, which could be obtained through mean incubation days minus days under monitoring of successful nests. Finally, we could use equation 1 to calculate ages of nests found during incubation, especially those nests being found and predated during incubation.

Nest searching and monitoring

We used nests of Chinese grouse to verify weight loss method. Chinese grouse is distributed in alpine conifer forests at altitudes between 2400 m and 4300 m along the eastern edge of the Tibetan Plateau in China (Sun, 2000). Chinese grouse is monogamous (Sun *et al.*, 2003). During the breeding season, males occupy territories and females select males to copulate with and construct nests at the base of trees within mated males' territories (Sun *et al.*, 2007). Females begin breeding at one year of age and all females take part in reproduction during a breeding season (Sun, 2004). Due to the relatively short summer at high elevations, females do not re-nest if their nests failed during incubation (Sun *et al.*, 2003). Field work was carried out at Lianhuashan Nature Reserve, Gansu, China (34.6853° N, 103.5233° E), during 2009-2012. More detail informations about study site could be found in (Sun *et al.*, 2003; Klaus *et al.*, 2001; Zhao *et al.*, 2018).

We searched nests during the whole egg laying and incubation periods, so nests were of different ages at found date. Eggs were only weighed and measured once at the found date. We weighed eggs (W_f) using an electronic balance with an accuracy of 0.1g and measured eggs' maximum length (L) and maximum breadth (B) using a

caliper with an accuracy of 0.02 mm. Nests were checked every 2-5 days until its final fate were determined. A nest was considered successful when above one egg was hatched and failed when eggs all disappeared, egg shells crashed or egg shell punched (Zhao *et al.*, 2018). We recorded the dates of found and dates of final fates being determined.

Nest age determination method and accuracy estimation

At the first step, we calculated the egg weight coefficient (K_w) of Chinese grouse from nests found during egg laying period using the transformed formula of equation 1: $K_w = W/LB^2$. We did not take weight loss during egg laying period into account because weight loss at this period was not really measurable and had a negligible influence on weight loss (Westerskov, 1950). Then the weights of fresh eggs W found during incubation could be calculated with equation 1. At the second step, we applied the linear regression to calculate the coefficient a and b in equation 2, $Age = a + b * (W - W_p)$, using two thirds of randomly selected successful nests ($n = 22$) found during incubation, where Age was determined by subtracting the monitoring days from the mean 28 incubation days (Sun, 2004). At a third step, we applied this our equation with other 11 remaining selected successful nests whose ages at founding date were also determined by the method introduced above. We compared the predicted nest age and real age using paired t test. We used 22 nests to estimate the coefficients and 11 nests to examine the accuracy because sample size is modest and also to increase our accuracies on estimations of the coefficients.

We used nest as a sample unit and mean values of eggs in the same nest were used in all calculations. Data used for paired t-test and linear regression in this study were all examined and fit the assumptions of normal distribution and equality of variance. Results were presented as means \pm SE and two-tailed alpha level was set to 0.05. All statistical analyses were carried out on SAS 9.1 (SAS Institute Int., 2001).

RESULTS

Totally 68 nests were found during 2009-2012. Of this, we removed 27 nests from our calculations because of various reasons (9 nests' fate determined at found date, 17 nests failed during incubation period, 1 nest egg unmeasured). Finally, we used 8 nests found during egg laying period to calculate weight coefficient K_w , and 33 successful nests found during incubation were randomly selected to construct linear equation of weight loss and nest age ($n = 22$ nests, 11.7 ± 1.4 days of age at found date), or used to test our results ($n = 11$ nests, 13.5 ± 2.2 days of

age at found date). There was no difference in found dates between these two groups (two-sample t-test, $t_{31} = -0.70$, $P = 0.491$).

The weight coefficient of Chinese grouse eggs was 0.543 ± 0.002 (Table I). Fresh egg weight (W) of the 22 nests was estimated. Weight loss was then calculated by subtracting weight at found date (W_p) from estimated fresh egg weight. Our final equation for calculating nest age found during incubation was $Age = 2.511 + 5.005 * (W - W_p)$ (22 nests, $F_{1,21} = 263.146$, $P < 0.001$, adjusted $R^2 = 0.926$, Fig. 1). Mean daily weight loss for an egg during incubation is about 0.1495g. So, we also got another equation between nest age and weight loss (W_l): $W_l = 0.1495 * Age$. And total weight loss during the whole incubation period was $28 * 0.1495$, which is 4.159g and constitute 19.0% of a mean fresh egg weight of 21.9g.

Table I. Eight nests found during egg laying period at Lianhuashan, Gansu, China, 2009-2012.

Year	CSAF	FCS	Length	Breadth	Weight	Kw	P_weight
2009	4	7	4.23	3.00	20.6	0.541	20.534
2010	7	8	4.28	3.07	22.1	0.548	21.903
2010	4	7	4.49	3.15	24.3	0.545	24.191
2010	3	7	4.22	3.00	20.9	0.550	20.623
2010	6	7	4.41	3.08	22.5	0.538	22.716
2011	1	6	4.34	3.09	22.2	0.536	22.501
2011	3	9	4.15	2.99	20.1	0.542	20.146
2011	4	7	4.39	3.12	23.1	0.541	23.204
Mean	4.2	7.2	4.31	3.06	21.9	0.543	21.991
SE	0.7	0.3	0.04	0.02	0.5	0.002	0.509

CSAF, clutch size at found date; FCS, final clutch size; Length, egg length in centimeter (cm); Breadth, egg breadth in cm; Weight, fresh egg weight in gram (g) before incubating; Kw, gravity coefficient; P_weight, predicted fresh egg weight in g.

We tested the efficiency of our methods with 11 nests. Predicted age of the 11 nests at found date was 12.6 ± 2.0 days (range 4.3-25.4 days). And the differences between predicted ages and real ages were not significantly (0.8 ± 0.5 days, range: -1.8 to 3.2 days; paired t-test, $t_{10} = -1.37$, $P = 0.200$, Fig. 2).

DISCUSSION

The weight loss method showed a high accuracy in determining the nest age of Chinese grouse, where differences between predicted age and real age were between -2 and 4 days. The deviation of our results was smaller than those based on floating method, which was usually about 7 days (Liebezeit *et al.*, 2007). Data

required for estimating nest age was easy to collect and the calculation procedure was simple. Investigators did not need to have much experience or rely on a reference table on the embryo development status at different ages, but weigh and measure the eggs at the found date. The weight loss method may also reduce the potential adverse effects from personal experience. The most popular least squared method was used to calculate the regression parameters, which could be easily performed by hand or by any statistic software. Nest initiation date and hatching date could also be reckoned out. The application of weight loss method could improve the accuracy of nest survival estimates, help to ascertain nest fate, and reduce the need for frequent nest visits to check hatching date (Liebezeit *et al.*, 2007).

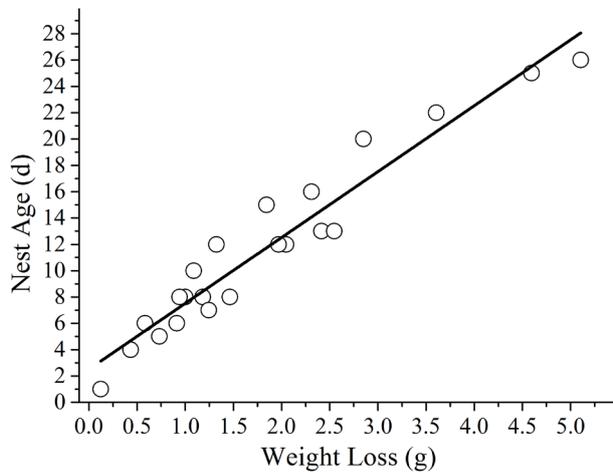


Fig. 1. Correlation of nest age and egg weight loss during incubation of Chinese grouse, Lianhuashan, Gansu, China, 2009-2012.

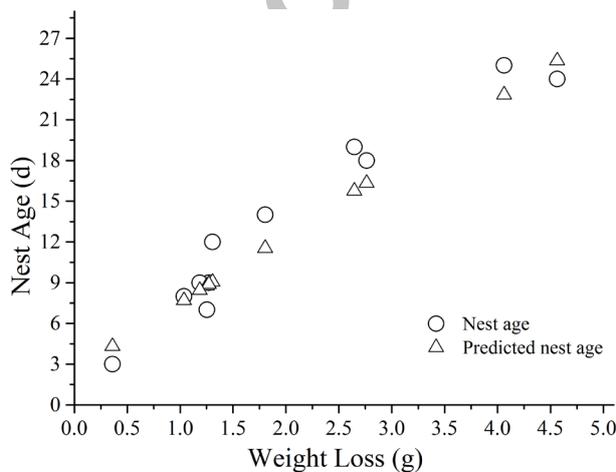


Fig. 2. Comparison of predicted nest age and real age of Chinese grouse at Lianhuashan, Gansu, China, 2009-2012.

The constant term in our linear equation of Chinese grouse was 2.511, which signified that the minimal age of nest found was 2.5 days after incubation started. This might be caused by the modest sample size. A large sample size might make the constant term close to zero. A further refinement could be achieved by examining mean daily weight loss in our study. When a negligible weight loss or no weight loss occurred, a nest could be classified as incubation started close to the found date. Another question to be put forward was that investigators needed to check the egg in a nest with unusual lower weight loss. For example, an infertile egg lost much less weight during incubation compared with fertile eggs (Paassen *et al.*, 1984). The differences of weight loss between fertile and infertile eggs could become more and more obvious as incubation progressing and inclusive of infertile eggs could thus underestimate nest age, especially during the late incubation period. Therefore, these infertile eggs should be removed when estimating nest age, which could be achieved by excluding the eggs that lost obvious less weight than the rest eggs in a nest.

Some prerequisites had to be aware of before using the weight loss method. Firstly, nests have to be searched and found during the whole egg laying and incubation periods. Nests found during egg laying periods are used to determine weight coefficient, which was useful in determine the fresh egg weight found during incubation. And successful nests found during incubation period was used to construct linear equations. Secondly, mean incubation period was used to determine nest age of successful nest before constructing linear equations. Mean incubation period could be obtained by monitoring successful nests found during egg laying periods or through literatures (if possible). Thirdly, if the date of first egg laying date was needed, which was of interest in quite a number of avian researches (Gibson *et al.*, 2015; Grant, 2015; Joos *et al.*, 2015), egg laying rhythm has to be known. This could also be observed from nests found during egg laying when such date could not be obtained from literatures.

Water vapor loss of eggs during incubation depended upon the pore geometry of the shell and diffusion constant of water vapor in air on the one hand and the water vapor pressure difference on the other hand which is set up between the inside of the shell and the microclimate of the nest surrounding the egg (Rahn and Ar, 1974). Therefore, we suggest developing species/ population specific regression equations in field researches. Moreover, the weight loss method would be suitable for species with relative regular incubation rhythm. For species with irregular incubation rhythm, for example, blue petrel (*Halobaena caerulea*) and some other seabirds occasionally neglect their nests during incubation (Gaston and Powell, 1989; Astheimer,

1991; Chaurand and Weimerskirch, 1994), whose water loss is not constant, utility of daily water loss should be used as a surrogate to calculate days under incubation, because development of embryos suspend when ambient temperatures is low during egg neglect periods (Zhao *et al.*, 2017).

Our study confirmed the convenient and accuracy of weight loss method in estimating the nest age of Chinese grouse. Considering the importance of estimating nest age in many avian studies, we recommended investigators to use the weight loss method rather than egg floating and candling methods to estimate nest age in future avian nest survival studies when a time-varied DSR is of interest.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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