

1 **Detection of embryo mortality and hatch using thermal differences among incubated**  
2 **chicken eggs<sup>1</sup>**

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## 20 **Abstract**

21 Accurate diagnosis of both the stage of embryonic mortality and the hatch process in incubated  
22 eggs is a fundamental component in troubleshooting and hatchery management. However,  
23 traditional methods disturb incubation, destroy egg samples, risk contamination, are time and  
24 labour-intensive and require specialist knowledge and training. Therefore, a new method to  
25 accurately detect embryonic mortality and hatching time would be of significant interest for the  
26 poultry industry if it could be done quickly, cheaply and be fully integrated into the process. In  
27 this study we have continuously measured individual eggshell temperatures and the  
28 corresponding micro-environmental air temperatures throughout the 21 days of incubation using  
29 standard low-cost temperature sensors. Moreover, we have quantified the thermal interaction  
30 between eggs and air by calculating thermal profile changes (temperature drop time, drop length  
31 and drop magnitude) that allowed us to detect four categories of egg status (infertile/early death,  
32 middle death, late death and hatch) during incubation. A decision tree induction classification  
33 model accurately (93.3%) predicted the status of 105 sampled eggs in comparison to the classical  
34 hatch residue breakout analyses. With this study we have provided a major contribution to the  
35 optimization of incubation processes by introducing an alternative method for the currently  
36 practiced hatch residue breakout analyses.

37 **Keywords:** egg breakout, eggshell temperature, embryo status, hatching time.

## 38 **Introduction**

39 Hatchability is key for assessing incubation results. Thus, investigating hatching failures is an  
40 increasingly recognized concern for the modern poultry industry in attempting to uncover the  
41 basis of egg fertility and embryonic mortality (Sellier et al., 2006). Besides, Non-viable eggs also  
42 take up space that can be used for fertile eggs and a potential source of bacteria and/or fungi and

43 can thus cause contamination. During incubation, egg candling is normally carried out in the  
44 middle of the process (day 10) or during transfer (day 18), in order to identify infertile eggs and  
45 mortality. However, egg breakout analyses requires invasive intervention, destroys egg samples,  
46 kills embryos, risks contamination, is time and labour-intensive and requires specialist  
47 knowledge and training (Sellier et al., 2006, Liu and Ngadi, 2013). Detection of infertility and  
48 mortality is not the only issue, investigation of the hatch evolution is also very important to  
49 evaluate uniformity of the batch. In practice, the moment of hatch is examined by taking several  
50 hatchery baskets out of the incubator and checking the number of chicks hatched. However, this  
51 procedure may require the door of the incubator to be opened and is carried out several times  
52 during the process which may significantly affect the incubation conditions and interrupt the  
53 hatching process (Tong et al., 2015). Therefore, there is an increased interest in an alternative  
54 and less invasive method for the detection of egg fertility and monitoring of embryo mortality  
55 and hatch. This paper attempts to show the benefit of using eggshell temperature sensors during  
56 the whole incubation to quantify thermal profile differences among infertile eggs, eggs  
57 containing dead embryos at different developmental stages (early, middle and late) and eggs that  
58 succeed in hatching.

## 59 **Material and methods**

60 Ross 308 eggs (Henry Stewart & Co. Ltd., Lincolnshire, United Kingdom) were incubated and  
61 hatched in a custom small-scale incubator (Petersime NV., Zulte, Belgium) using a standard 21-  
62 day incubation program. Twenty out of six hundred eggs were randomly picked and individually  
63 labelled as focal eggs in each incubation trial to serve as samples for the current study. In total,  
64 120 focal eggs from six repetitions were analysed. Standard low-cost contact temperature sensors  
65 (Romanini et al., 2013) were attached to the equator of the focal egg eggshells. Another

66 temperature sensor was positioned 1 cm away from each focal egg to record the corresponding  
67 micro-environmental air temperature ( $T_{\text{air}}$ ). The eggshell temperatures ( $T_{\text{egg}}$ ) of each focal egg  
68 and  $T_{\text{air}}$  were recorded every minute throughout the entire incubation.

69 At the end of incubation, hatch residues were evaluated by an expert in breakout analysis  
70 (Petersime NV, Zulte, Bekgium). Egg status was determined according to the developmental  
71 stage of the dead embryo (Hamburger and Hamilton, 1992) and allocated into the following  
72 categories: infertile (INF), early death (ED), middle death (MD) and late death (LD). Cracked  
73 and contaminated eggs were excluded from the analyses. This study had an ethical approval from  
74 the Royal Veterinary College Ethics and Welfare Committee.

75 Both  $T_{\text{egg}}$  and  $T_{\text{air}}$  time series data were processed in Matlab<sup>®</sup> (The Math Works, Inc., Natick,  
76 United States) using built-in codes and functions of the Captain Toolbox (Taylor et al., 2007).

77 The temperature difference ( $\Delta T$ ) between  $T_{\text{egg}}$  and  $T_{\text{air}}$  was calculated and filtered to produce the  
78  $\Delta T_{\text{filtered}}$  signal representing the final thermal profile. The  $\Delta T_{\text{filtered}}$  signal was further processed  
79 using a 10-hours average window approach to investigate differences in temperature. The  
80 following parameters of an identified temperature drop in  $\Delta T_{\text{filtered}}$  were quantified for the MD,  
81 LD and H focal eggs (Figure 1): 1) the time when the lowest drop occurred (drop time); 2) the  
82 duration of the drop from a maximum local value to the minimum local value (drop length); and  
83 3) the temperature scale of the drop (drop magnitude).

#### 84 **Insert Figure 1**

85 Statistics were performed using the statistical software package Minitab<sup>®</sup> (Minitab Inc., State  
86 College, United States). Initially, the thermal profiles of focal eggs were grouped into one  
87 category of egg status (INF, ED, MD, LD or H) according to the results of the hatch residue  
88 breakout analyses. The Anderson-Darling (Anderson and Darling, 1954) and the Bartlett's tests

89 (Ridgman, 1990) were used to test normality and homogeneity of variances, respectively. The  
90 parameters extracted from  $\Delta T_{\text{filtered}}$  were summarized using descriptive statistics (mean followed  
91 by the standard error of the mean). A single ANOVA followed by post hoc test, with a  
92 significance level of 0.05, was used to test the differences in drop length and drop magnitude  
93 among the egg categories.

94 The data set was classified using a decision tree induction model (Quinlan, 1986). Thermal  
95 profile derived parameters (temperature drop time, drop length and drop magnitude) were inputs  
96 towards the classification of the egg status (INF, ED, MD, LD or H) as output. The application  
97 WEKA 3.6.9 (University of Waikato, New Zealand) was used to develop a J48 decision-based  
98 classifier (Hall et al., 2009) with a 10-fold cross validation approach. Egg status classified by the  
99 decision tree model, was compared to the reference status from breakout analyses. The  
100 classification performance was expressed in terms of binary classification statistics (Olson and  
101 Delen, 2008): TP rate (rate of true positives); FP rate (rate of false positives); and ROC-curve  
102 (the ability of performing correctly classification).

### 103 **Results**

104 The 105 focal eggs were grouped according to the results of the hatch residue breakout analysis,  
105 as following: INF (n=15), ED (n=3), MD (n=12), LD (n=11) and H (n=64). The thermal profile  
106 interactions between  $T_{\text{egg}}$  and  $T_{\text{air}}$ , throughout the entire incubation time (512 hours), of two focal  
107 eggs from each egg status category are illustrated, as examples, in Figure 2.

### 108 **Insert Figure 2**

109 A common feature (notable temperature drops on Figures 2C, 2D and 2E) was found for all focal  
110 eggs categorized into MD, LD and H. Eggs in the status category INF/ED did not show the same  
111 pattern (Figure 2A and 2B). Those temperature drops were associated to the time of embryonic

112 mortality in the cases of MD and LD, or to the time which chicks emerge from their shells in the  
113 case of H eggs. Figure 3 shows the normal distribution curves of the temperature drop time for  
114 the MD, LD and H eggs. This result shows overlap between the temperature drop time registered  
115 for the egg categories MD and LD or, most clearly for LD and H.

### 116 **Insert Figure 3**

117 In addition, quantitative differences were identified in the thermal profiles (temperature drop  
118 length and drop magnitude) of MD, LD and H eggs (Table 1). The temperature drop length  
119 found in the H eggs (mean of 6.78 hours) was significantly lower than LD (15.82 h) and MD  
120 (18.75h) ( $P < 0.05$ ). Furthermore, differences in drop magnitude were found among the egg status  
121 categories. The highest temperature drop was obtained for the H eggs with a mean value of 0.73  
122 °C ( $P < 0.01$ ). MD and LD eggs showed smaller averaged drop magnitudes of 0.19 °C and 0.30  
123 °C, respectively.

### 124 **Insert Table 1**

125 Figure 4 shows the results of a binary decision tree and the thresholds used for classification into  
126 one of the four outcome egg status (INF/ED, MD, LD and H) according to the thermal profiles of  
127 the interaction between  $T_{\text{egg}}$  and  $T_{\text{air}}$ . At the top level of the decision tree there is the root node, at  
128 which the classification begins. It tests all focal eggs for temperature drop time  $\leq 455$  h of  
129 incubation. Instances that satisfy this condition are passed down to the left of the tree to the  
130 second root node. It corresponds to a new test (temperature drop time  $\leq 0$  h), meaning that there  
131 is no such notable temperature drop on the thermal profile ( $\Delta T_{\text{filtered}}$ ). If this is the case, this test  
132 passed once more down to the left reaching a leaf node INF/ED and no more tests are needed.  
133 Focal eggs are classified as MD if the temperature drop time  $\leq 383$  h, or as LD if the  
134 temperature drop time is  $> 383$  h but  $\leq 455$  h of incubation. From the top root node to the right,

135 focal eggs with a temperature drop time > 455 h are further tested at intermediate nodes  
136 (temperature drop length and drop magnitude) to be classified into one single egg status category  
137 (LD or H).

#### 138 **Insert Figure 4**

139 The performance of the decision tree classification model is shown in Table 2. The overall  
140 success rate of the classification model was 93%. Each element in Table 2 is a count of focal  
141 eggs. Rows represent the reference egg status categories classified by hatch residue breakout  
142 analyses and columns represent the predicted egg status by the decision tree model. A total of 98  
143 out of 105 focal eggs were correctly classified. The model was 100% precise at classifying  
144 INF/ED eggs. 10 out of 12 MD eggs were correctly classified and 2 were incorrectly categorized  
145 as LD. The highest error rate was in the LD eggs with 3 errors in 11 focal eggs due to the limited  
146 sample size. The classification model correctly classified 62 out of 64 H eggs. Detailed accuracy  
147 results for each status category are shown in Table 3. A ROC-curve value of 1 represents a  
148 perfect classifier while values approaching 0.5 indicate a classifier with reduced ability,  
149 comparable to random guessing. ROC-curve values were higher than 0.9 for each egg status  
150 category.

#### 151 **Insert Table 2**

#### 152 **Insert Table 3**

#### 153 **Discussion**

154 The quantification of the changes in the thermal profile interaction between  $T_{\text{egg}}$  and  $T_{\text{air}}$  for the  
155 whole incubation period allowed us to identify four categories of egg (fertility/early death,  
156 middle death, late death and hatch). As expected, the decision tree classification model indicated  
157 'drop time' as the criteria holding the greatest information. We cannot distinguish between an

158 infertile egg and an egg with early embryonic mortality because they showed similar thermal  
159 profiles throughout incubation. Furthermore, drop time alone seems unlikely to be enough to  
160 distinguish classes of embryo status in the overlapping regions when our results are extrapolated  
161 to a population level. Temperature drops were identified when embryos died or hatch as a  
162 response to an abrupt change in embryonic heat production. This allowed us to precisely  
163 determine the time for embryonic mortality and hatch. The quickest and largest temperature drop  
164 was observed for hatched eggs. After external pipping, the chick emerges from the egg and  
165 causes huge amount of heat release due to evaporation of the fluid left in the internal eggshell  
166 membrane by the recently hatched chick (Romanini, et al., 2015). Therefore, the temperature  
167 drop caused by hatch was different from the temperature drop caused by embryo death in terms  
168 of drop length and drop magnitude. We have developed a classification model with a small  
169 number of misclassification (7 errors out of 105 focal eggs), corresponding to an overall 93.3%  
170 accuracy. With the increase of egg samples, especially for late dead eggs, the classification  
171 model could be fine-tuned and yield even higher accuracy.

## 172 **Conclusion**

173 A method was proposed in this study to determine the egg status using the temperature sensors  
174 based on the different thermal profile changes throughout the incubation period. Accurate  
175 detection of embryo status and the moment of hatch in commercial hatcheries would be of  
176 significant interest to the poultry industry if it can be done quickly and cheaply. The results  
177 presented herein correspond to the absolute first step, distinction of thermal profiles of eggs from  
178 different status, towards the development of future automated monitoring systems for incubated  
179 chicken eggs.

## 180 **Acknowledgement**



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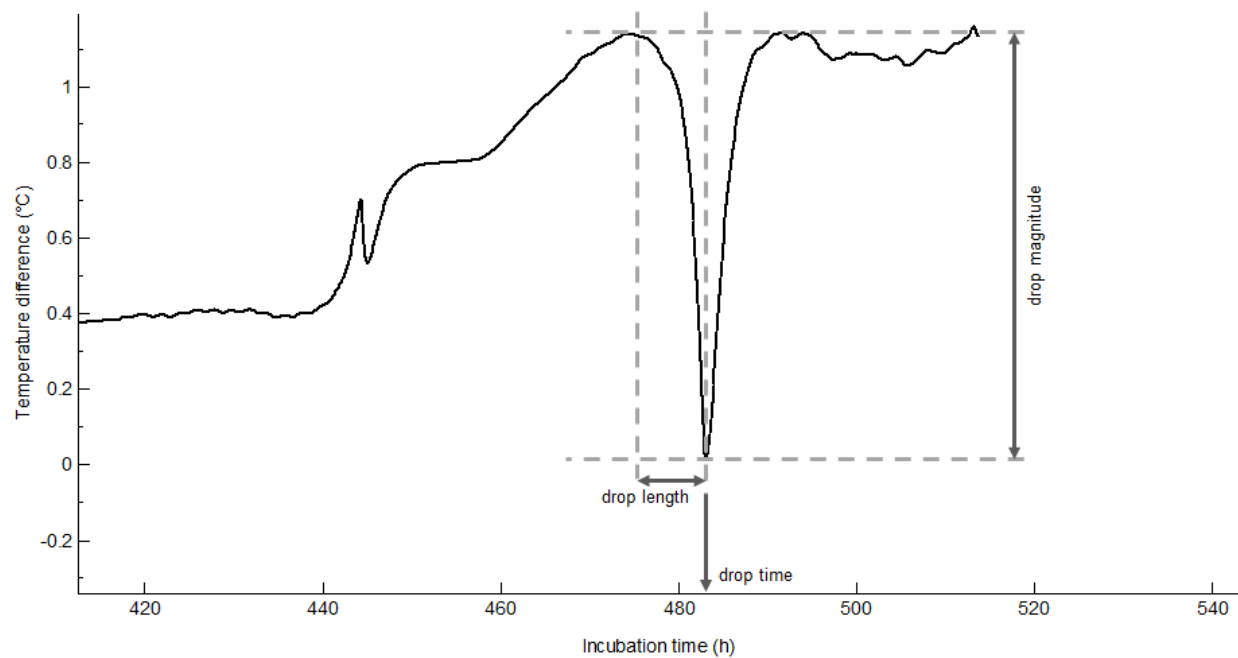
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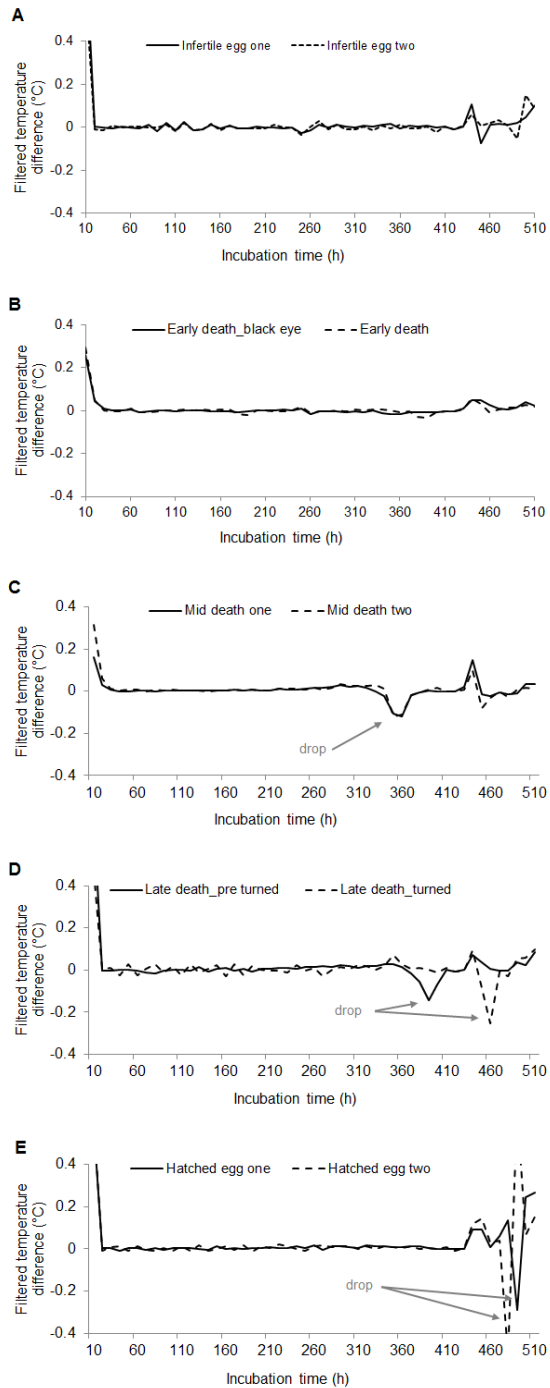
215 **Figure**



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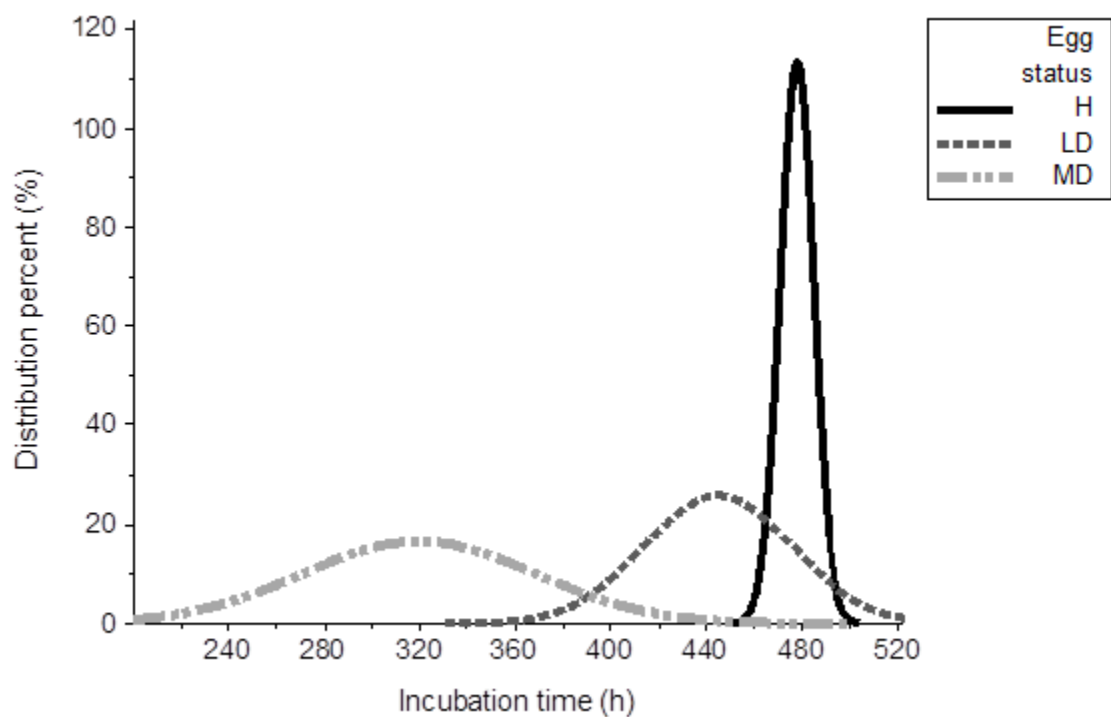
217 **Figure 1** An example of the quantitative characteristic (drop time, drop length and drop

218 magnitude) of the  $\Delta T_{\text{filtered}}$  time series data.



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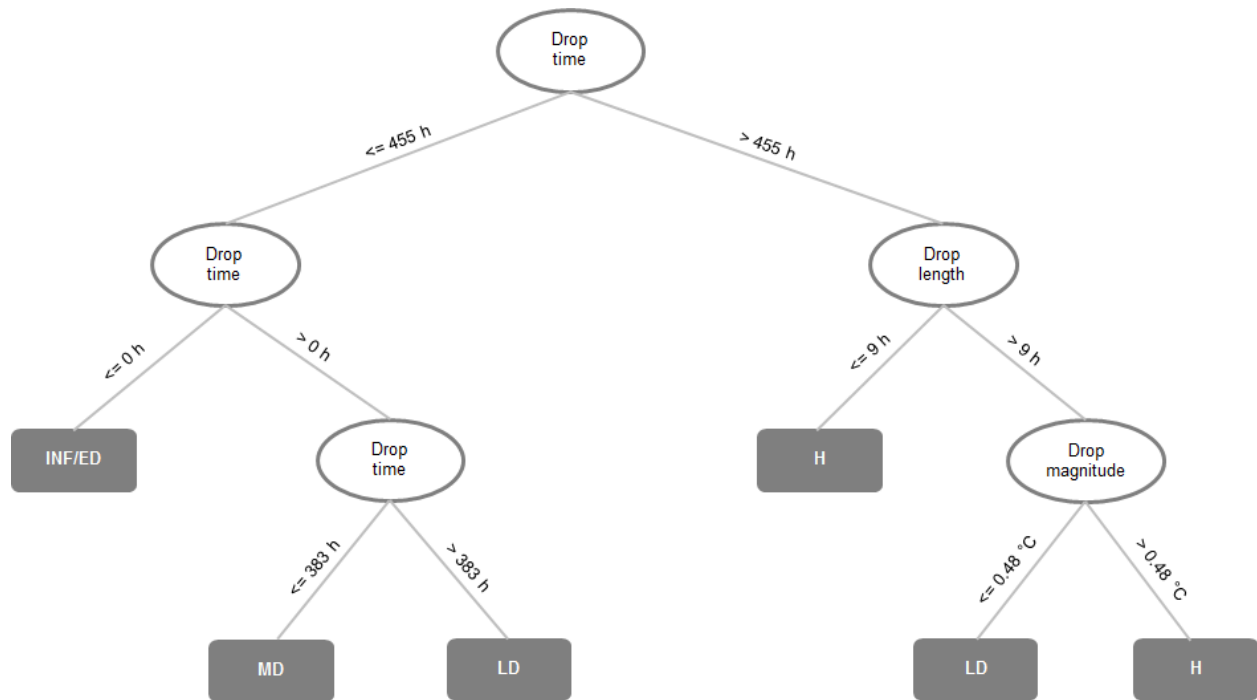
220 **Figure 2** The corrected  $\Delta T_{\text{filtered}}$  profiles throughout incubation of: A) Two early death eggs; B)  
 221 Two mid death eggs; C) Two late death eggs; D) Two hatched eggs. The variation between 430-  
 222 450 hours of incubation was caused by the temperature change during transfer of the eggs from  
 223 incubation trays to hatching baskets.



224

225 **Figure 3** Fitted normal distribution curves for the time of temperature drop (drop time) occurred  
 226 in  $\Delta T_{\text{filtered}}$  for MD, LD and H embryo status categories in the function of incubation time. MD =  
 227 middle death (n = 12), LD = late death (n = 11), H = hatched eggs (n = 64).

228



229

230

**Figure 4** J48 decision tree classification model for egg status into the INF/ED, MD, LD and H

231

categories with the specification of the classification rules thresholds. INF/ED = infertile or early

232

death, MD = middle death, LD = late death, H = hatched eggs.

233

234 **Table Captions**

235 **Table 1** The comparisons of the drop length and drop magnitude among MD, LD and H focal  
236 eggs categories.

Item	Sample size	Drop length (h)	Drop magnitude (°C)
INF/ED	18	/	/
MD	12	18.75 ± 1.80 <sup>a</sup>	0.19 ± 0.02 <sup>b</sup>
LD	11	15.82 ± 1.90 <sup>b</sup>	0.30 ± 0.03 <sup>b</sup>
H	64	6.78 ± 0.14 <sup>c</sup>	0.73 ± 0.03 <sup>a</sup>

237 INF/ED = infertile or early death, MD = middle death, LD = late death, H = hatched eggs;

238 <sup>a-c</sup> means within the column with different superscripts differ significantly (P<0.05).

239 **Table 2** Results of the classification tree in a confusion matrix form using a 10-fold cross-  
 240 validation.

Reference status from hatch residue analyses	Classified by the decision tree model			
	INF/ED	MD	LD	H
INF/ED (n = 18)	18	0	0	0
MD (n = 12)	0	10	2	0
LD (n = 11)	0	1	8	2
H (n = 64)	0	0	2	62

241 INF/ED = infertile or early death, MD = middle death, LD = late death, H = hatched eggs, n =  
 242 sample size of focal eggs.



243 **Table 3** Detailed accuracy results of the decision tree model by category of egg status

Egg status Category	INF/ED	MD	LD	H	Weighted Average
TP rate	1	0.83	0.73	0.97	0.933
FP rate	0	0.01	0.04	0.05	0.035
ROC-curve	1	0.99	0.91	0.99	0.985

244 INF/ED = infertile or early death, MD = middle death, LD = late death, H = hatched eggs, TP

245 rate = rate of true positives, FP rate = rate of false positives, ROC-curve = classification ability.