# Using egg density and egg mass techniques for incubation stage assessment to predict hatch dates of Greater Flamingo *Phoenicopterus ruber roseus* eggs

**N. Jarrett[1], V. Mason[1], L. Wright[2] & V. Levassor[1]**

[1]The Wildfowl and Wetlands Trust, Slimbridge, Gloucestershire GL2 7BT, UK.
Email: nigel.jarrett@wwt.org.uk
[2]Centre for Ecology, Evolution and Conservation, School of Environmental Sciences, University of East Anglia, Norwich NR4 7TJ, UK.

Egg density and the egg mass techniques for incubation stage assessment were developed to predict the hatch dates of Greater Flamingo *Phoenicopterus ruber roseus* eggs laid in captivity at WWT, Slimbridge, UK. The accuracy of each technique was tested on 20 parentally incubated eggs by comparing actual hatch date with predicted hatch date. For the egg mass technique a strong positive correlation existed between actual and predicted fresh mass, suggesting that model accuracy was high. Both techniques predicted hatch dates within two days 80% of the time. These techniques were found to be useful for accurate incubation stage assessment of Greater Flamingo eggs and the authors encourage aviculturalists managing captive colonies to use them.

**Key Words: egg mass, egg density, incubation stage assessment, Greater Flamingo, *Phoenicopterus ruber roseus***

Each year at The Wildfowl and Wetlands Trust (WWT), Slimbridge, UK, up to 60 pairs of colonially breeding captive Greater Flamingos *Phoenicopterus ruber roseus* compete for nest sites. Breeding pairs usually construct nest mounds, often up to 0.3m high and just 0.2m apart, on and from the substrate of shallowly flooded islands. Single eggs are laid on these nest mounds, and nest-defence duties are shared by both sexes until a chick is hatched after 26-32 days' incubation. Fighting between nest mound occupants and their neighbours or pairs that intrude into the colony often results in eggs being displaced from nests. As flamingos do not attempt to retrieve displaced eggs, up to 25% of annual egg production is lost in this way (Pickering 1992).

Displaced eggs are usually replaced, sometimes within minutes, by eggs laid by intruding pairs. In 2000 there were five cases of eggs being laid on nest mounds by intruding females within two hours of the loss of the previous nest occupants' egg. In all cases, the 'new' eggs were adopted by the original nest occupants (N. Jarrett, pers. obs.). In order to free nest sites for other pairs to use, aviculturalists sometimes discard these 'new' eggs after 32 days' incubation, believing them to be beyond their hatch-by date, when, in fact, they are viable, although at an earlier stage of development (N. Jarrett, pers. obs.). Until now there has not been a reliable or safe field technique for aviculturalists to use when attempting to estimate the incubation stage of flamingo eggs.

Avian eggs lose approximately 16% of their initial mass at an almost linear rate during incubation, almost entirely due to diffusive water loss through the eggshell pores (Drent 1970; Rahn & Ar 1974). This has enabled field workers to develop several techniques, based on egg mass change over the incubation period, to determine the incubation stage of eggs of species with known incubation periods. Egg candling (eg Hanson 1954; Weller 1956) and egg flotation (eg Carroll 1988; Walter & Rusch 1997; Brua & Machin 2000) are the most commonly used techniques to estimate incubation stage of waterbird eggs of unknown lay-date, but neither are particularly suitable for flamingos.

The candling technique involves assessing egg air space development by shining a light source into the egg so that the size of the air space can be visualised and compared to known patterns of enlargement. However, the candling technique is difficult to perform on flamingo eggs, which are normally thick-shelled and, sometimes, marked on the surface with scratches or nest debris. Moreover, candling is a technique best practised in a low light environment, often not attainable in a field situation.

The egg flotation technique involves immersing the egg in a container of water so that the angle and height of the egg in the water column can be measured against patterns that are calibrated for different incubation stages. Although Alberico (1995) has demonstrated that this technique does not affect egg hatchability, immersion in water is known to carry a risk of damage to the developing embryo (Gabel & Mahan 1996). This risk factor, coupled with the impracticality of carrying an immersion vessel and uncontaminated water for flotation into the field, leads the authors to believe that the flotation technique is inappropriate for flamingo eggs.

The egg density (eg Furness & Furness 1981) and egg mass (eg Westerkov 1950; Lind 1991) techniques for incubation stage assessment have been adopted by relatively few workers but represent reliable and safe alternatives to egg candling and egg flotation (Jarrett & Warren 1996). Both techniques require egg size (maximum length and breadth measurements) and egg mass data to be collected so that fresh egg mass and egg density can be estimated. Furthermore, both techniques assume that all eggs lose the same proportion of their fresh mass, at a constant rate, over a defined incubation period with the implication that egg density and egg mass can be predicted for each day of incubation. For the egg density technique, incubation stage is indicated when the observed egg density is entered into a species-specific density loss equation. For the egg mass technique, incubation stage is indicated when observed egg mass is referenced to a model of mass loss for an egg of that particular egg's estimated fresh mass.

Here the usefulness of the egg density and the egg mass techniques for incubation stage assessment to predict the hatching dates of eggs laid by captive Greater Flamingo at WWT Slimbridge are described and discussed.

## Methods

Fresh egg mass and size measurements (maximum length and breadth) were recorded for 217 Greater Flamingo eggs laid in 2001, 2002 and 2003. Mass was recorded within four hours of oviposition to the nearest 0.1g using a portable, levelled electronic balance, and size was recorded to the nearest 0.1mm using vernier callipers.

### The egg density technique

Hoyt (1979) described egg volume (V) ($cm^3$) as:

$V=Kv.LB^2$ equation 1

where L is egg length (cm), B is egg breadth (cm) and *Kv* is a shape coefficient. Hoyt (1979) observed that the value of *Kv* was 0.509±0.008 (S.D.) for most species of birds except those laying very asymmetrical eggs. Equation 1 was used to calculate the egg volume of 217 Greater Flamingo eggs. Egg density (D) ($g/cm^3$) was then calculated for each egg using the equation:

$D=M/V$ equation 2

where M is egg mass (g). To calculate pre-hatch egg density, data were collected from parentally incubated eggs to estimate the proportion of initial egg mass lost over the course of the incubation period. Incubation period was defined as the number of days between the onset of incubation and the beginning of hatch (when the chick breaks into the air space and begins to vocalise and tap with its beak on the inner eggshell). Thus the mass of 18 eggs was recorded within two hours of oviposition and daily after day 24 until the hatching chick was audible within the air space of the egg. The average proportion of fresh mass lost was 14.5%±0.56 (S.E.) (range 10.4-19.5%) over an average incubation period of

28.0 days (range 26-30 days).

Thus pre-hatch egg density (Dp) ($g/cm^3$) for the sample of eggs was calculated using the equation:

$$Dp = Df.0.855 \qquad \text{equation 3}$$

where Df is fresh egg density ($g/cm^3$) and 0.855 is the average proportion of initial egg mass retained at the time the chick enters the air space. So that the hatch date of eggs of unknown incubation stage could be predicted, a species-specific equation describing egg density over the incubation period was developed using regression analysis.

General Linear Models were used to investigate how fresh egg density differed between years and first, second, third and fourth clutches (Norusis 2000). In the model chosen, fresh egg density was considered as the dependent variable with year and clutch number incorporated as main effects. Interactions between these independent variables were also investigated.

### The egg mass technique

Hoyt (1979) described the relationship between the fresh mass (Mf) (g) of a bird's egg and its linear dimensions, as:

$$Mf = Kw.LB^2 \qquad \text{equation 4}$$

where *Kw* is a species-specific mass coefficient. *Kw* was calculated for 217 Greater Flamingo eggs using the equation:

$$Kw = Mf/LB^2 \qquad \text{equation 5}$$

General Linear Models were used to investigate how *Kw* differed between years and first, second, third and fourth clutches (Norusis 2000). In the model chosen, *Kw* was considered as the dependent variable with year and clutch number incorporated as main effects. Interactions between these independent variables were also investigated.

The accuracy of the sample mean *Kw* was assessed by using equation 4 to estimate fresh mass of each egg and then using a correlation to determine the similarity between observed and predicted fresh egg masses. On the assumption that all Greater Flamingo eggs lost 14.5% of their initial mass at a linear rate over the 28-day incubation period, tables were compiled to show the predicted daily mass of eggs with fresh masses ranging those of the 217 eggs sampled. (**Appendix 1** provides an example of a table for eggs of fresh mass ranging from 150 to 165g).

### Evaluating the techniques

The egg density and egg mass techniques for incubation stage assessment were used to predict the hatching date of 20 Greater Flamingo eggs of known hatch date laid in 2000, using mass and linear measurement data collected at different stages of incubation. As flamingo chicks often break into the air space 24-36 hours before emerging from eggs (Studer-Thiersch

1975), hatch date was predicted to fall one day after the chick was expected to enter the air space. Mass and size measurement data were recorded in the same way as those of the sample. For each egg, egg density was estimated using equation 2. When this value was entered into the egg density equation determined for the species, an indication of incubation age (in days) was obtained and a prediction of hatch date was made. Also, for each egg, fresh egg mass was estimated using equation 4. This estimated value for fresh egg mass informed the authors which table of daily egg masses to consult. Actual (observed) egg mass indicated egg age (in days) when the table for that egg's estimated fresh mass was consulted. Thus, for each egg, a second indication of incubation age (in days) was obtained and a hatch date prediction made.

## Results

For 217 Greater Flamingo eggs, mean fresh mass was 157.6±0.90g (S.E.) (range 122.1-191.8g) (**Figure 1**); mean egg length was 8.88±0.026cm (S.E.) (range 7.93-9.74cm); mean egg breadth was 5.66±0.012cm (S.E.) (range 5.14-6.19cm).

### The egg density technique

For 217 Greater Flamingo eggs mean egg volume was 145.2±0.83cm$^3$ (S.E.) (range 112.9-174.5cm$^3$) and mean fresh egg density was 1.09 g±0.001g/cm$^3$ (S.E.) (range 1.023-1.152g/cm$^3$) (**Figure 2**). Fresh egg density did not vary

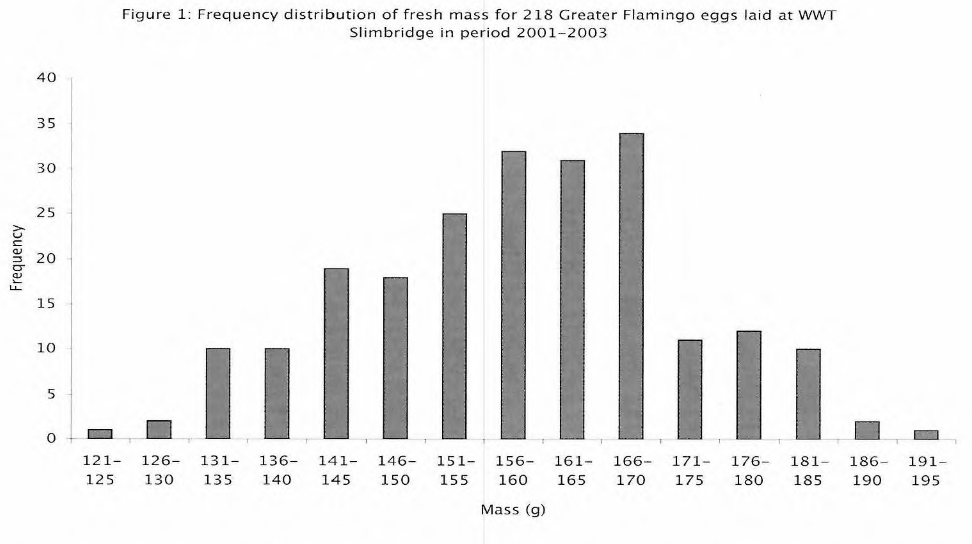


**Figure 1.** Frequency distribution of fresh mass for 217 Greater Flamingo eggs laid at WWT, Slimbridge, in 2001-2003.

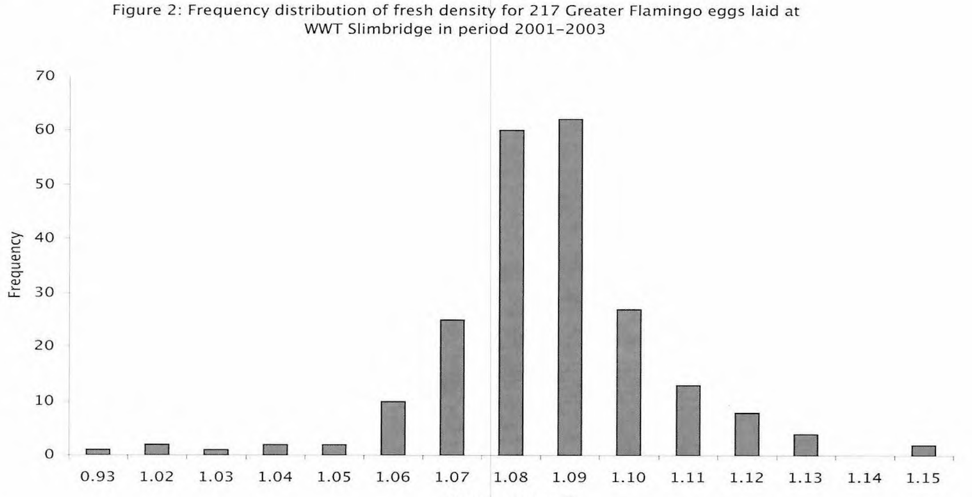


**Figure 2.** Frequency distribution of fresh density for 217 Greater Flamingo eggs laid at WWT, Slimbridge, in 2001-2003.

significantly for eggs of different clutches ($F_{4,161}$=1.658, $P$>0.05) or eggs laid in different years ($F_{2,161}$=0.24, $P$>0.05), nor were there any significant interaction effects between these variables. Mean pre-hatch egg density was calculated as 0.928g/cm$^3$. Equation 5 describes the relationship between egg density (D) (g/cm$^3$) and stage of incubation (I) (days) for Greater Flamingo eggs of any size.

$$I=193.1-177.9.D \qquad \text{equation 5}$$

## The egg mass technique

For 217 Greater Flamingo eggs, the mean value of $Kw$ was 0.553±0.0006 (S.E.). There were no significant differences between $Kw$ values of eggs from different clutches ($F_{4,161}$=1.658, $P$>0.05) or eggs laid in different years ($F_{2,161}$=0.024, $P$>0.05), nor were there any significant interaction effects between these variables. There was a strong positive correlation between predicted fresh egg mass and actual fresh egg mass (Pearson product-moment correlation coefficient, $r$=0.982, n=217, $P$<0.0001).

## Evaluating the techniques

On average, the egg density technique for incubation stage assessment over-estimated actual hatching date by 1.04±0.45 days (S.E.) (the range of prediction was two days before and five days after hatch date). Thus, eggs hatched approximately 25 hours earlier than expected. Within two days of the predicted date, (two days before or after actual hatch date), 16 of 20 (80%) of eggs hatched.

On average, the egg mass technique for incubation stage assessment under-estimated actual hatching date by 0.75±0.43 days (S.E.) (the range of prediction was four days before and four days after hatch date). Thus eggs hatched on average 18 hours later than expected. Within two days of the predicted date (two days before or after actual hatch date), 16 of 20 (80%) of eggs hatched.

## Discussion

Both the egg density and egg mass techniques for incubation stage assessment that were developed for Greater Flamingo eggs were useful in indicating hatch date. The egg mass technique, using the *Kw* value that was generated, was slightly more precise at estimating the incubation stage of eggs than was the egg density technique, using the *Kv* value provided by Hoyt (1979) for birds' eggs generally. However there was some error associated with the mean predicted hatch dates for the techniques. Some of this error may have been due to the assumptions that were made about Greater Flamingo eggs.

First, it was assumed that the incubation period for Greater Flamingo eggs was 28 days, and this was defined as the interval between the onset of incubation and the beginning of hatch (that is, when the chick breaks into the air space). Previously, Pickering (1992) had observed that Greater Flamingos at WWT Slimbridge hatched eggs after 26-32 days incubation. Similar ranges have been reported elsewhere (eg Allen 1956; Studer-Thiersch 1975). As chicks usually enter the air space 24-36 hours prior to hatch (Studer-Thiersch 1975), chicks may be expected to enter the air space at any time between 24.5-30.5 days of incubation, that is, an interval of six days. This range may be related to differences in parental incubation efficiency, in turn affected by environmental, physiological, behavioural or individual factors that produce variable incubation times thus leading to hatch date prediction error. The assumption that incubation time is 28 days is clearly not appropriate for all eggs.

The second assumption was that mass loss over the incubation period amounted to, on average, 14.5% of initial fresh mass, for all eggs. This was based on observations of 18 eggs, hatched by parents, which showed a mass loss ranging from 10.4% to 19.5%. This wide range is probably attributable to variation in individual eggs' porosity to water vapour: eggs with greater porosity would be expected to lose more water than eggs with lower porosity. Furthermore, over the period of incubation, flamingo eggs become increasingly marked with scratches from contact with the parents' toenails, and, sometimes, soiled with nest debris. Scratched or debris-covered shells are likely to have increased or decreased water vapour porosity respectively. Debris-covered eggs may also show increases in egg mass (due to the mass of debris) with the result that the egg mass and egg density techniques lose precision. Therefore, although 14.5% is a reasonable estimate for mean mass loss, there is variation around this value linked to egg shell structure and the nest environment.

The third assumption was that the rate of mass loss was linear over the incubation period. This assumption was made since there are no published data available describing the pattern of mass loss in flamingo eggs. However, it is known that the daily rate of water loss from eggs generally increases in a non-linear manner in some avian species and is affected by incubation temperature and humidity, which in the nest are affected by parental behaviour and climate (eg Kendleigh 1940; Carey 1980; Woodall & Parry 1982). Swart *et al*. (1987) observed that in the Ostrich *Struthio camelus* var. *domesticus* the increased rate of daily water loss over the incubation period was associated with a gradual increase in embryonic metabolism and temperature, which in turn raised the water vapour pressure in the egg, increasing the vapour pressure difference between the egg and the nest. Although fresh and pre-hatch masses of Greater Flamingo eggs were recorded at WWT, too few observations were made to investigate changing rates of mass loss over the incubation period. An attempt will be made to improve the techniques for assessing incubation stage of Greater Flamingo eggs by collecting data on rates of mass loss in future.

The fourth assumption was that all eggs had identical composition ratios of egg shell, yolk and albumen. However, since there was a degree of variation in fresh egg density for the captive-laid eggs sampled (**Figure 2**), it is likely that egg composition varied between different eggs.

Finally, only fertile eggs were assessed, that is, eggs containing a viable and developing embryo. Ar (1991) reports that non-fertilised eggs lose mass at a lower rate than do fertilised eggs. As neither the egg mass nor the egg density techniques for incubation stage assessment indicate egg fertility, they are likely to detect infertile eggs as being incubated for a shorter time than they actually have been. As Pickering (1992) observed a fertility rate of 57% for Greater Flamingos laying at Slimbridge, over 40% of eggs might be expected to be infertile. Thus managers using these egg-ageing techniques may inadvertently determine that infertile eggs are recently laid fertile eggs. That is, when these egg-ageing techniques are used on eggs that have been incubated beyond the normal incubation period but that are infertile the techniques are likely to indicate that they have been incubated for a shorter period. Consequently, the manager may surmise that the egg is not the original egg but a replacement laid by another female. The authors will assess rates of water loss from infertile Greater Flamingo eggs by collecting mass data over the course of natural incubation in future.

In spite of some error, these egg-ageing techniques proved to be practical and reliable for determining the incubation stage of captive-laid fertile Greater Flamingo eggs. The authors encourage aviculturalists managing captive Greater Flamingo colonies to use the techniques to assess the incubation stage of 'overdue' or abandoned eggs before these are discarded or subjected to artificial incubation conditions, respectively. Used in this way, the techniques will hopefully lead to improved productivity in captive flocks of Greater Flamingos.

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**Appendix 1**. Predicted daily mass of Greater Flamingo eggs with fresh mass 150-165g, assuming an incubation time of 28 days and a total mass loss of 14.5%.

| Fresh Mass | Mass on each day of incubation | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| 150 | 149.1 | 148.3 | 147.4 | 146.6 | 145.7 | 144.9 | 144.0 | 143.1 | 142.3 | 141.4 | 140.6 | 139.7 | 138.9 | 138.0 |
| 151 | 150.1 | 149.3 | 148.4 | 147.5 | 146.7 | 145.8 | 145.0 | 144.1 | 143.2 | 142.4 | 141.5 | 140.6 | 139.8 | 138.9 |
| 152 | 151.1 | 150.3 | 149.4 | 148.5 | 147.7 | 146.8 | 145.9 | 145.1 | 144.2 | 143.3 | 142.4 | 141.6 | 140.7 | 139.8 |
| 153 | 152.1 | 151.3 | 150.4 | 149.5 | 148.6 | 147.8 | 146.9 | 146.0 | 145.1 | 144.3 | 143.4 | 142.5 | 141.6 | 140.8 |
| 154 | 153.1 | 152.2 | 151.4 | 150.5 | 149.6 | 148.7 | 147.8 | 147.0 | 146.1 | 145.2 | 144.3 | 143.4 | 142.6 | 141.7 |
| 155 | 154.1 | 153.2 | 152.3 | 151.5 | 150.6 | 149.7 | 148.8 | 147.9 | 147.0 | 146.1 | 145.3 | 144.4 | 143.5 | 142.6 |
| 156 | 155.1 | 154.2 | 153.3 | 152.4 | 151.5 | 150.7 | 149.8 | 148.9 | 148.0 | 147.1 | 146.2 | 145.3 | 144.4 | 143.5 |
| 157 | 156.1 | 155.2 | 154.3 | 153.4 | 152.5 | 151.6 | 150.7 | 149.8 | 148.9 | 148.0 | 147.1 | 146.2 | 145.3 | 144.4 |
| 158 | 157.1 | 156.2 | 155.3 | 154.4 | 153.5 | 152.6 | 151.7 | 150.8 | 149.9 | 149.0 | 148.1 | 147.2 | 146.3 | 145.4 |
| 159 | 158.1 | 157.2 | 156.3 | 155.4 | 154.5 | 153.5 | 152.6 | 151.7 | 150.8 | 149.9 | 149.0 | 148.1 | 147.2 | 146.3 |
| 160 | 159.1 | 158.2 | 157.3 | 156.3 | 155.4 | 154.5 | 153.6 | 152.7 | 151.8 | 150.9 | 149.9 | 149.0 | 148.1 | 147.2 |
| 161 | 160.1 | 159.2 | 158.2 | 157.3 | 156.4 | 155.5 | 154.6 | 153.6 | 152.7 | 151.8 | 150.9 | 150.0 | 149.0 | 148.1 |
| 162 | 161.1 | 160.1 | 159.2 | 158.3 | 157.4 | 156.4 | 155.5 | 154.6 | 153.7 | 152.7 | 151.8 | 150.9 | 150.0 | 149.0 |
| 163 | 162.1 | 161.1 | 160.2 | 159.3 | 158.3 | 157.4 | 156.5 | 155.5 | 154.6 | 153.7 | 152.8 | 151.8 | 150.9 | 150.0 |
| 164 | 163.1 | 162.1 | 161.2 | 160.3 | 159.3 | 158.4 | 157.4 | 156.5 | 155.6 | 154.6 | 153.7 | 152.8 | 151.8 | 150.9 |
| 165 | 164.1 | 163.1 | 162.2 | 161.2 | 160.3 | 159.3 | 158.4 | 157.5 | 156.5 | 155.6 | 154.6 | 153.7 | 152.7 | 151.8 |

**Appendix 1.** Continued

| | Mass on each day of incubation | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fresh Mass | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
| 150 | 137.1 | 136.3 | 135.4 | 134.6 | 133.7 | 132.9 | 132.0 | 131.1 | 130.3 | 129.4 | 128.6 | 127.7 | 126.9 | 126.0 |
| 151 | 138.1 | 137.2 | 136.3 | 135.5 | 134.6 | 133.7 | 132.9 | 132.0 | 131.2 | 130.3 | 129.4 | 128.6 | 127.7 | 126.8 |
| 152 | 139.0 | 138.1 | 137.2 | 136.4 | 135.5 | 134.6 | 133.8 | 132.9 | 132.0 | 131.2 | 130.3 | 129.4 | 128.5 | 127.7 |
| 153 | 139.9 | 139.0 | 138.1 | 137.3 | 136.4 | 135.5 | 134.6 | 133.8 | 132.9 | 132.0 | 131.1 | 130.3 | 129.4 | 128.5 |
| 154 | 140.8 | 139.9 | 139.0 | 138.2 | 137.3 | 136.4 | 135.5 | 134.6 | 133.8 | 132.9 | 132.0 | 131.1 | 130.2 | 129.4 |
| 155 | 141.7 | 140.8 | 139.9 | 139.1 | 138.2 | 137.3 | 136.4 | 135.5 | 134.6 | 133.7 | 132.9 | 132.0 | 131.1 | 130.2 |
| 156 | 142.6 | 141.7 | 140.8 | 140.0 | 139.1 | 138.2 | 137.3 | 136.4 | 135.5 | 134.6 | 133.7 | 132.8 | 131.9 | 131.0 |
| 157 | 143.5 | 142.6 | 141.7 | 140.9 | 140.0 | 139.1 | 138.2 | 137.3 | 136.4 | 135.5 | 134.6 | 133.7 | 132.8 | 131.9 |
| 158 | 144.5 | 143.6 | 142.7 | 141.7 | 140.8 | 139.9 | 139.0 | 138.1 | 137.2 | 136.3 | 135.4 | 134.5 | 133.6 | 132.7 |
| 159 | 145.4 | 144.5 | 143.6 | 142.6 | 141.7 | 140.8 | 139.9 | 139.0 | 138.1 | 137.2 | 136.3 | 135.4 | 134.5 | 133.6 |
| 160 | 146.3 | 145.4 | 144.5 | 143.5 | 142.6 | 141.7 | 140.8 | 139.9 | 139.0 | 138.1 | 137.1 | 136.2 | 135.3 | 134.4 |
| 161 | 147.2 | 146.3 | 145.4 | 144.4 | 143.5 | 142.6 | 141.7 | 140.8 | 139.8 | 138.9 | 138.0 | 137.1 | 136.2 | 135.2 |
| 162 | 148.1 | 147.2 | 146.3 | 145.3 | 144.4 | 143.5 | 142.6 | 141.6 | 140.7 | 139.8 | 138.9 | 137.9 | 137.0 | 136.1 |
| 163 | 149.0 | 148.1 | 147.2 | 146.2 | 145.3 | 144.4 | 143.4 | 142.5 | 141.6 | 140.6 | 139.7 | 138.8 | 137.9 | 136.9 |
| 164 | 149.9 | 149.0 | 148.1 | 147.1 | 146.2 | 145.3 | 144.3 | 143.4 | 142.4 | 141.5 | 140.6 | 139.6 | 138.7 | 137.8 |
| 165 | 150.9 | 149.9 | 149.0 | 148.0 | 147.1 | 146.1 | 145.2 | 144.3 | 143.3 | 142.4 | 141.4 | 140.5 | 139.5 | 138.6 |