**Title**

Examining Behavioral Sampling Schemes in Aviary-Reared Laying Hen Pullets



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Examining Behavioral Sampling Schemes in Aviary-Reared Laying Hen Pullets

By

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Approved:

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Committee in Charge

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Lastly, I would like to thank my family, especially my mom Jie Shao for her support and trust in me. Thanks must also go to my three adorable cats: Sam, Dudley, and Jellybean as well as my leopard gecko Saber for being silly and funny and bringing me daily joy.

## Abstract

In animal behavior studies, as in other fields, decisions about how data are collected directly affect the validity of the results and the interpretation of the findings. Thus, it is essential that the selected behavioral data collection strategy can accurately capture the behavior of interest. Within poultry science, a variety of behavioral observation sampling methods have been used. Although the method for determining the acceptability of a chosen sampling strategy should be provided, the reasoning behind the selection of a specific sampling strategy is seldom explained in publications. A previous study provided a framework for validating behavioral sampling schemes for adult laying hens. Rather than utilizing this framework, some researchers have used the results of the published sample validation to justify their sampling scheme. However, the results may not be generalizable due to sample size limitations. Application of previously validated sampling methods to new research contexts may be particularly problematic when a validation is completed on older birds but applied to other ages. This is due to developmentally relevant differences in activity levels and resource use. To emphasize the importance of sampling scheme validation and to investigate age effects on the sampling method, my thesis research examined the appropriateness of different interval scan sampling durations for estimating resource use by laying hen pullets reared in a single-platform aviary system. We predicted that resource use frequency changes as laying hen pullets age, and that different instantaneous scan intervals are needed to accurately capture platform/ramp, perch, and floor use at 6 and 12 weeks of age. The findings highlight the necessity of validating behavioral sampling schemes for each age and behavior independently, as well as reporting the validation process in research publications.

## 1. Introduction

Continuous sampling is considered the gold standard when collecting behavioral data. It allows researchers to precisely identify and record all occurrences of a behavior of interest. However, it is labor-intensive and time-consuming (Bateson and Martin, 2021). Instantaneous scan sampling, which provides a snapshot of the behavioral time budget based on a pre-determined time interval, is a more time-efficient method. The accuracy of this approach relies on the use of appropriate sampling intervals. Using long interval durations can, for example, underestimate transient and infrequent behaviors. Because the frequency of behaviors can vary by context (e.g., developmental state of the animals, aspects of housing design), the choice of time intervals used should be evaluated and justified whenever scan sampling is used (Makagon and Blatchford, 2017; Bateson and Martin, 2021). Although the type of behavior and the sampling method are usually reported in behavioral research, the reasoning behind the selection of specific sampling schemes is rarely stated. A preliminary review (Table 1) of 26 articles published between 2011 and 2021 in the Journal of Poultry Science, identified using the key phrase "laying hen behavior", revealed that less than 10% of the studies provided reasons behind selecting a specific sampling scheme. It is not clear whether this type of validation is not taking place, or is conducted but not reported out.

Daigle and Siegford (2014) previously published a framework for determining the appropriateness of different sampling strategies for quantifying the behavior of mature laying hens. Although Daigle and Siegford (2014) intended for their results from regression analysis to be used as a guide for evaluating different sampling schemes, some authors directly used the sampling scheme derived from Daigle and Siegford's sample validation, without independently validating the sampling method, (e.g., Riber and Guzman, 2016; Giersberg et al., 2019; Pufall et al. 2021). The results of the study by Daigle and Siegford (2014) may not be generalizable due to the limited sources of variation represented in the data. Their findings were based on a small sample of hens (6), of a single strain (Hy-Line brown) and age (20 weeks), all housed in a single pen replicate. While observations spanned 15 hours, they were conducted on a single observation day. Sampling method should be context and behavior specific (Makagon and Blatchford, 2017; Bateson and Martin, 2021) since behavioral time budgets can be affected by bird age, strain, and aspects of the housing system (e.g., availability of resources). For example, it is known that age can affect physical activity levels in laying hens. Younger pullets (10-16 weeks of age) showed a greater level of high-intensity physical activity that usually involves whole-body movement and a change in the location, and the activity level decreased with an increase in age (Kozak et al., 2016). Thus, using an instantaneous sampling interval that can capture behaviors in adult layers might not be sufficient to generate representative results for layer pullets, especially if they change activities more sporadically. An increased sampling frequency may be needed to correctly quantify at least some aspects of the behavioral time budgets of pullets. My thesis examined whether the duration of scan sampling interval impacts resource use estimates in layer pullets reared in a single-platform system at 6 and 12 weeks of age (woa).

## 2. Material and Methods

### 2.1 Housing and Management

All housing and management protocols were approved by UC Davis IACUC (Protocol #20307). Day-old Dekalb White pullets (N = 220) were obtained from a commercial hatchery and raised across identical 4 pens (3.05 x 3.05 x 2.74m, L x W x H; 55-56 pullets/pen) at Hopkins Avian Facility at the University of California, Davis. Each pen contained a single-platform aviary system (Figure 1) which included three elevated metal perches (121.9 L, 3.8 cm diameter) installed at the heights of 35.4 cm (two perches) and 64.7 cm (one perch) from the concrete floor, as well as one elevated (62.9 cm high) platform covered with plastic slats (121.9 x 61.0 cm, L x W; Dura-Slat Poultry and Kennel Flooring, South wear, Agri-Plastics, Inc., Addison, TX, USA). A metal floor perch identical to those installed on the single-platform aviary system was placed on the floor. The floor was lined with pine wood shavings (Mallard Creek Inc., Rocklin, CA). A wire mesh ramp connected the floor to the platform (96.5 x 31.8 cm, 40-degree angle; McNichols Wire Mesh, McNichols Co., Inc., Livermore, CA, USA). Water was provided ad libitum through automatic water lines (12 nipples/pen; Lubing USA, Cleveland, TN, USA), which ran along the back wall of the pen. Start and grow diets (Purina Start and Grow Medicated Crumbles, Purina Animal Nutrition LLC, Gray Summit, MO, USA) were provided ad libitum in two 13.6 kg round feeders (53 cm circumference/feeder) located on the floor of in each pen. Temperature and artificial lighting were maintained according to the Dekalb White Product Guide (Hendrix Genetics, N.D.).

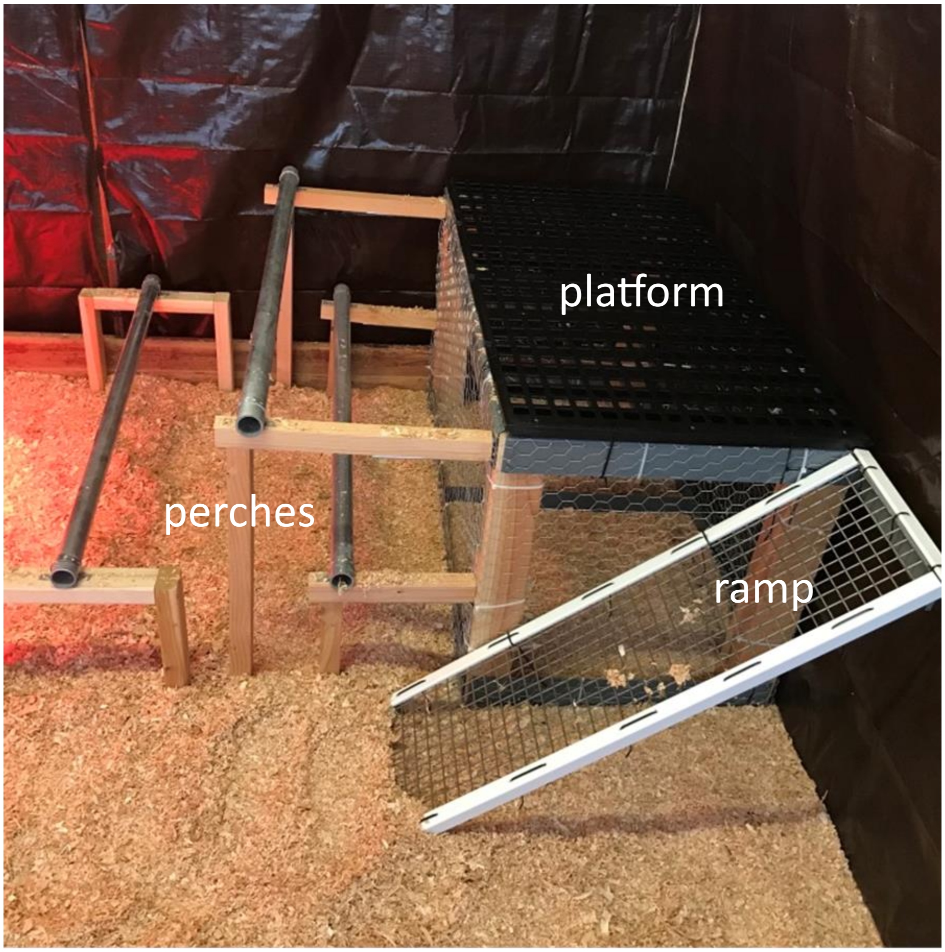


*Figure 1. Pullets were housed in pens furnished with a single-platform aviary system (pictured), a floor perch, two feeders and a nipple drinker line.*

## 2.2 Data Collection

Twenty focal pullets (5 birds/pen) were individually color marked using nontoxic livestock crayons (Markal® All-Weather Paintstik Livestock Marker, LA-CO Industries, Inc., Elk Grove Village, IL, USA), and observed from 8:00-11:00h and 13:00-16:00h on one of two consecutive weekend days at 6 and 12 weeks of age. Weekend days were selected for observation as no other research activities took place over the weekends, minimizing human disturbance of bird behavior. Two pens were sampled Saturday, and the remaining two on

Sunday, in order to include variations in resource use due to day effects. Videos were recorded using two cameras per pen (4K Ultra HD IP Security Camera, Lorex Corporation, Irvine, CA, USA) connected to a network video recorder with the pentaplex operation (4K Ultra HD Security NVR, Lorex Corporation, Irvine, CA, USA). We used a continuous sampling scheme to record each focal bird's movements between the ground, perches, and platform, recording the time of each transition and the amount of time each pullet spent at each location. A pullet was considered to have moved to a new location when both of its feet made contact with the new location, and it subsequently remained at the location for at least 1 second. Location transitions lasting less than 1 second (e.g., when a bird touched a perch as it moved from a platform to the floor) were not recorded. The pullet was considered to leave a location when one of its feet lifted off, and the bird immediately moved to another area of the aviary. Two trained observers reviewed the video, with the lead observer (CL) coding approximately 90% of the data, and the other coding the remaining 10%. Both observers coded some data from each pullet age. The inter-observer reliability, determined based on a review of 2 hours of video, was excellent (ICC = 0.99). To ensure the inter-rater reliability remained high, the lead observer randomly checked the work of the other observer throughout the data collection. Data collection was performed using the VLC media player (VideoLan, VLC media player) or Behavioral Observation Research Interactive Software (Friard and Gamba, 2016).

## 2.3 Statistical Analysis

To better assess overall resource use, data collected on the use of individual perches were merged together into a single "perch use" category. Ramp use lasting >1s was rare. Ramp and platform use were merged into one single group and categorized as platform use.

The raw data collected using continuous sampling was converted to 1-s interval samples using PROC EXPAND (SAS software version 9.4; SAS Institute Inc., 2016). From the 1-s intervals, we extrapolated instantaneous samples for 5 intervals (1, 5, 10, 15, and 30 min) and converted these to the number of times a resource use was observed under each sampling interval at each age using PROC MEANS. Frequency of resource use was calculated as the number of times a resource use was observed divided by the total number of observations using different sampling intervals.

We considered the 1-s interval samples to be representative of the continuous data (Daigle & Siegford, 2014; Chen et al., 2016), and conducted pairwise comparisons between the actual frequencies of resource use (1-s interval data) and the data generated using the 1 to 30-min sampling intervals ('ModEval' R package version 0.0.0.9000, Da Silva, 2023; R statistical software version 4.2.2, R Core Team, 2021). A scan sampling interval was considered accurate when 3 criteria were met: the slope of the regression between the actual and estimated values did not differ from 1 ($P>0.05$), the intercept did not differ from 0 ($P>0.05$), and the association among the actual and estimated values was strong. Following Ledgerwood et al. (2010) and Chen et al. (2016), we considered coefficients of association ≥0.9 to signify a particularly strong association, and very good accuracy. To account for multiple comparisons, a Benjamini-Hochberg (Benjamini and Hochberg, 1995) adjustment was applied to the P-values for the slopes and intercepts.

## 3. Results

Table 2 shows the average proportion of time that the pullets spent on each resource, calculated using the different sampling schemes. The intercept, slope, and coefficient of determination for resource use data collected using the different scan sampling intervals are shown in Table 3. The pairwise relationship between each tested interval and the continuous (1-s samplings) observation was graphed. Figures 2-6 illustrate the comparisons for instantaneous sampling schemes with one or more unmet criteria. The graphs for samplings schemes meeting all 3 criteria are shown in Appendix S1-S22.

### 3.1 Perch

The average (± SD) percent of time that pullets spent perching was 9.2 (± 9.3%) and 38.2 (± 24.7%) at 6 and 12 weeks of age. Sampling intervals up to 5 min met all 3 evaluation criteria when estimating perch use for 6-week-old pullets. The slope of the regression for data sampled at 10-min intervals was significantly lower than 1 ($p = 0.012$). The regression analysis of 15-min intervals (Figure 2b) met the criteria for both the slope and the intercept. However, the coefficient of determination was less than 0.9 ($R^2 = 0.813$), lower than for the ≤5 min samples. The regression line created from data obtained using 30-min intervals (Figure 2c) did not meet the slope ($p = 0$) or intercept criteria ($p = 0.003$), and had a coefficient of determination of less than 0.9 ($R^2 = 0.868$).

At 12 weeks of age, all 3 criteria were met when data were sampled using intervals of 15 min or less. The regression line resulting from data collected at 30-min intervals (Figure 3) met the slope and intercept criteria. The coefficient of determination was nearing 0.9 ($R^2 = 0.895$).

### 3.2 Platform and ramp

At week 6 and 12, the pullets spent 53.1 ± 21.7% to 41.6 ± 23.4% of observed time on the platform or ramp. At 6 weeks of age, all 3 criteria were met when the use of this resource was sampled at time intervals of 15 min or less. The slope and intercept criteria were also met when 30-min intervals were used (Figure 4). The coefficient of determination was high, but smaller than 0.9 ($R^2$ = 0.844).

At 12 weeks of age, all 3 criteria were met when scan sampling intervals of up to 15 min were used. The regression analysis of the data obtained using the 30-min intervals (Figure 5) had a coefficient of determination that was high, but smaller than 0.9 ($R^2$ =0.832).

### 3.3 Ground

At week 6 and week 12, the average (± SD) percent of time pullets spent on the ground was 37.8 (± 24.2) % and 20.2 (± 19.9) %, respectively. At 6 weeks of age, the data from all sampling intervals up to 30 min met all 3 evaluation criteria. At 12 weeks of age, all evaluation criteria were met when ground use was sampled at intervals of 10 min or less. Regression data from the 15- and 30-min sampling intervals (Figure 6a and 6b) met the slope and intercept evaluation criteria, and with the coefficient of determination at or nearing 0.9 ($R^2$ = 0.899; $R^2$ = 0.875).

*Table 1. Overview of different types of sampling techniques utilized in previous research to assess behavior, location, and resource use of commercially housed chickens.*

| Author | Production System | Parameter assessed | Sampling Scheme Justification |
|---|---|---|---|
| Ali et al. (2020) | laying hens | resource use | None provided |
| Ali et al. (2019) | laying hens | resource use | None provided |
| Aranibar et al. (2020) | broiler pullets | behavior | None provided |
| Asensio et al. (2020) | broiler pullets | behavior | Referred to previous study |
| Cronin et al. (2018) | laying hen pullets | location and behavior | None provided |
| De los mozos et al. (2017) | broiler pullets | behavior | Referred to previous study |
| Eberle et al. (2018) | laying hens | behavior | Referred to previous study |
| Grebey et al. (2020) | laying hens | behavior | Analysis provided |
| Habinski et al. (2017) | laying hen pullets | location | None provided |
| Hartcher et al. (2015) | laying hens | behavior | Referred to previous study |
| Hester et al. (2014) | laying hen (pullets/adults) | behavior and location | None provided |
| Hu et al. (2016) | laying hens | behavior and resource use | None provided |
| Huber-eicher et al. (2013) | laying hens | behavior and location | Referred to previous study |
| Lalonde et al. (2021) | laying hen pullets | behavior | None provided |
| Louton et al. (2016) | laying hens | behavior and location | None provided |
| Mazzuco et al. (2011) | laying hen pullets | behavior | None provided |
| Pokharel et al. (2017) | laying hens | behavior | Provided |
| Qaisrani et al. (2013) | laying hens | behavior | None provided |
| Scholz et al. (2014) | laying hens | behavior | Provided |
| Scholz et al. (2011) | laying hens | behavior | Partially provided |
| Skånberg et al. (2021) | laying hen pullets | location | None provided |
| Stämpfli et al. (2013) | laying hens | behavior | None provided |
| Van der eijk et al. (2019) | laying hens | behavior | None provided |
| Villanueva et al. (2017) | laying hens | location | None provided |
| Wei et al. (2020) | laying hens | behavior | None provided |
| Winkel et al. (2016) | laying hens | behavior | None provided |

*Table 2. The average (± SD) proportion of time pullets (at 6 and 12 weeks old) spent on each resource based on the 1-s samples, and estimated using different scan sampling intervals. The proportion of time pullets spent on each resource use was calculated as the number of times a pullet was observed on that resource divided by the total number of observations made.*

| **Resource** | **Age (weeks)** | **1-s samples** | **Scan sampling interval** | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1 min | 5 min | 10 min | 15 min | 30 min |
| **Perch** | **6** | 0.092 ± 0.093 | 0.090 ± 0.094 | 0.086 ± 0.095 | 0.092 ± 0.105 | 0.088 ± 0.105 | 0.092 ± 0.135 |
| | **12** | 0.382 ± 0.247 | 0.383 ± 0.249 | 0.382 ± 0.247 | 0.386 ± 0.239 | 0.382 ± 0.253 | 0.364 ± 0.238 |
| **Platform** | **6** | 0.531 ± 0.217 | 0.533 ± 0.220 | 0.537 ± 0.222 | 0.532 ± 0.217 | 0.525 ± 0.242 | 0.508 ± 0.231 |
| | **12** | 0.416 ± 0.234 | 0.417 ± 0.235 | 0.418 ± 0.235 | 0.398 ± 0.219 | 0.417 ± 0.248 | 0.395 ± 0.247 |
| **Ground** | **6** | 0.378 ± 0.242 | 0.378 ± 0.242 | 0.379 ± 0.244 | 0.381 ± 0.243 | 0.392 ± 0.265 | 0.408 ± 0.253 |
| | **12** | 0.202 ± 0.199 | 0.200 ± 0.188 | 0.200 ± 0.191 | 0.216 ± 0.199 | 0.202 ± 0.200 | 0.241 ± 0.217 |

*Table 3. The intercept, slope, and coefficient of determination ($R^2$) for resource use data by pullets (at 6 and 12 weeks old) collected using the different sampling intervals (1 min, 5 min, 10 min, 15 min, and 30 min) compared to 1-sec intervals. Bold values indicate expected (intercept and slope) or preferred ($R^2$) criteria that were not met[1]. Benjamini-Hochberg adjustment was applied to all the p-value.*

| Resource | Age (weeks) | Scan interval | Intercept estimate | Intercept p-value | Slope estimate | Slope p-value | $R^2$ |
|---|---|---|---|---|---|---|---|
| **Perch** | **6** | 1 | 0.002 | 0.291 | 0.992 | 0.654 | 0.995 |
| | | 5 | 0.009 | 0.107 | 0.966 | 0.415 | 0.97 |
| | | 10 | 0.013 | 0.074 | 0.864 | **0.012** | 0.946 |
| | | 15 | 0.022 | 0.092 | 0.802 | 0.042 | **0.813** |
| | | 30 | 0.033 | **0.003** | 0.642 | **0** | **0.868** |
| | **12** | 1 | 0.002 | 0.431 | 0.993 | 0.255 | 0.999 |
| | | 5 | 0.001 | 0.91 | 0.997 | 0.888 | 0.991 |
| | | 10 | -0.01 | 0.662 | 1.015 | 0.753 | 0.963 |
| | | 15 | 0.014 | 0.448 | 0.964 | 0.391 | 0.97 |
| | | 30 | 0.052 | 0.138 | 0.907 | 0.236 | **0.895** |
| **Platform** | **6** | 1 | 0.006 | 0.154 | 0.986 | 0.051 | 0.999 |
| | | 5 | 0.01 | 0.455 | 0.97 | 0.202 | 0.991 |
| | | 10 | 0.01 | 0.704 | 0.98 | 0.648 | 0.966 |
| | | 15 | 0.074 | 0.018 | 0.871 | 0.017 | 0.946 |
| | | 30 | 0.093 | 0.073 | 0.862 | 0.132 | **0.844** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **12** | 1 | 0.001 | 0.797 | 0.996 | 0.542 | 0.999 |
| | | 5 | 0 | 0.975 | 0.995 | 0.772 | 0.994 |
| | | 10 | -0.005 | 0.791 | 1.057 | 0.17 | 0.976 |
| | | 15 | 0.032 | 0.194 | 0.921 | 0.125 | 0.954 |
| | | 30 | 0.074 | 0.11 | 0.866 | 0.175 | **0.832** |
| | | | | | | | |
| **Ground** | **6** | 1 | 0.001 | 0.738 | 0.997 | 0.465 | 1 |
| | | 5 | 0.004 | 0.719 | 0.987 | 0.57 | 0.99 |
| | | 10 | 0.005 | 0.778 | 0.979 | 0.617 | 0.969 |
| | | 15 | 0.029 | 0.193 | 0.891 | 0.027 | 0.956 |
| | | 30 | 0.005 | 0.867 | 0.913 | 0.196 | 0.916 |
| | | | | | | | |
| | **12** | 1 | 0.002 | 0.397 | 1 | 0.946 | 0.999 |
| | | 5 | 0.007 | 0.287 | 0.977 | 0.326 | 0.991 |
| | | 10 | 0 | 0.979 | 0.934 | 0.059 | 0.98 |
| | | 15 | 0.022 | 0.293 | 0.891 | 0.151 | **0.899** |
| | | 30 | 0.006 | 0.798 | 0.811 | 0.021 | **0.875** |

[1] Interval was considered accurate if the regression line met all three criteria: $R^2 \geq 0.9$: strong association between the estimated resource use and the actual resource use (based on 1-sec intervals); Slope did not differ significantly from 1 (P>0.05); Intercept did not differ significantly from 0 (P>0.05).

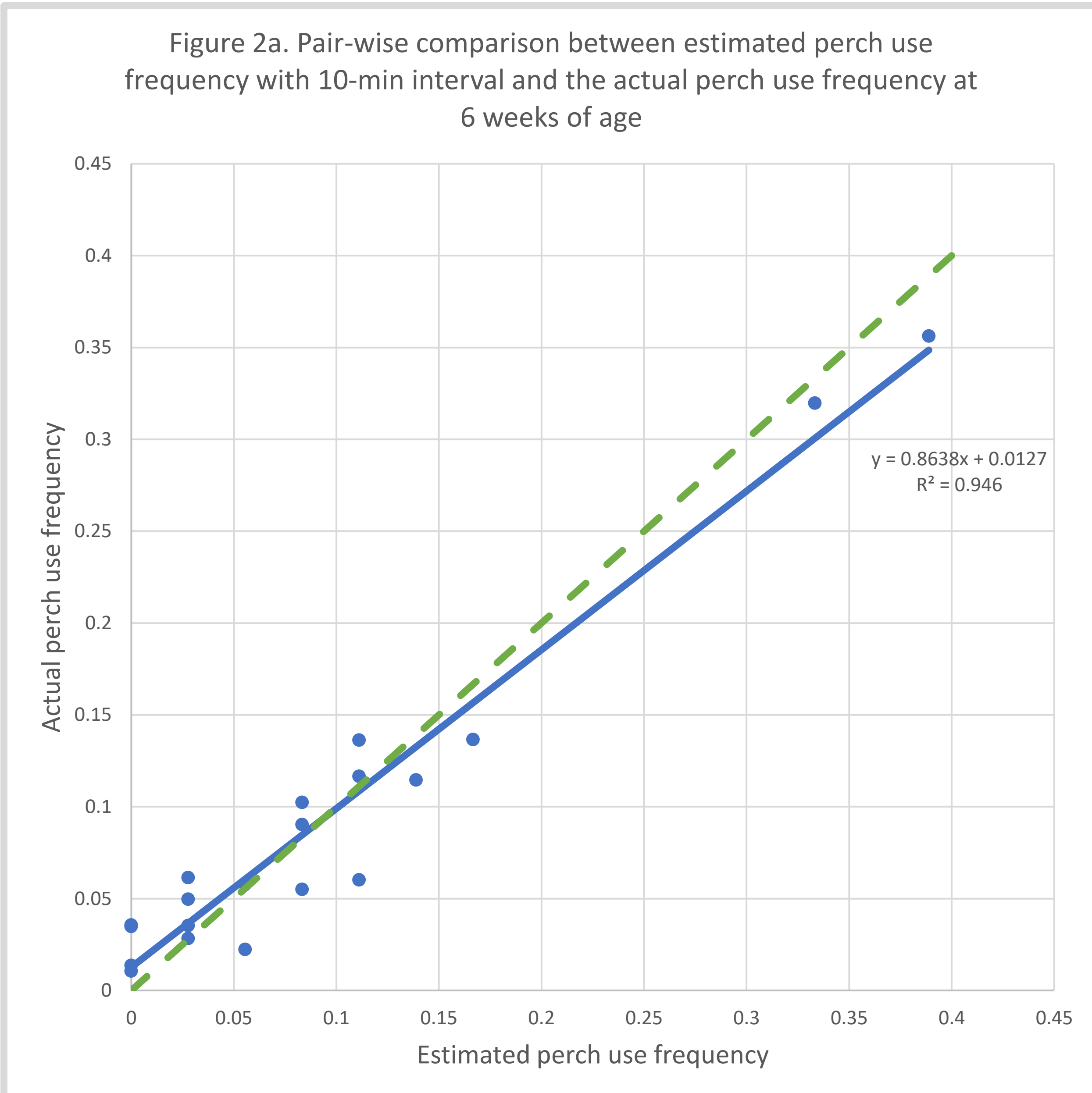
Figure 2a. Pair-wise comparison between estimated perch use frequency with 10-min interval and the actual perch use frequency at 6 weeks of age
Actual perch use frequency
Estimated perch use frequency
y = 0.8638x + 0.0127
R² = 0.946
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45

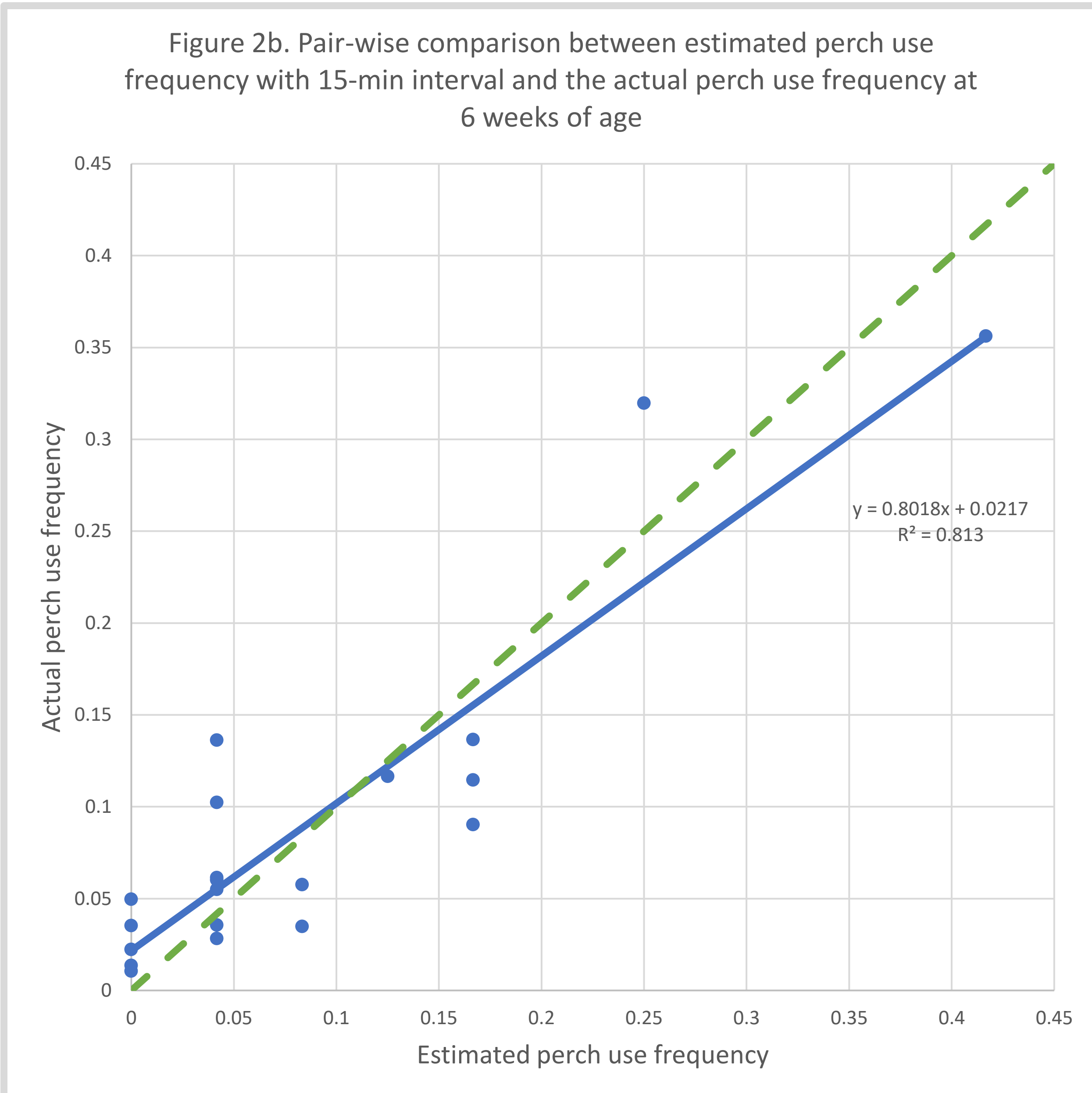
Figure 2b. Pair-wise comparison between estimated perch use frequency with 15-min interval and the actual perch use frequency at 6 weeks of age
y = 0.8018x + 0.0217
R² = 0.813
Actual perch use frequency
Estimated perch use frequency
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45

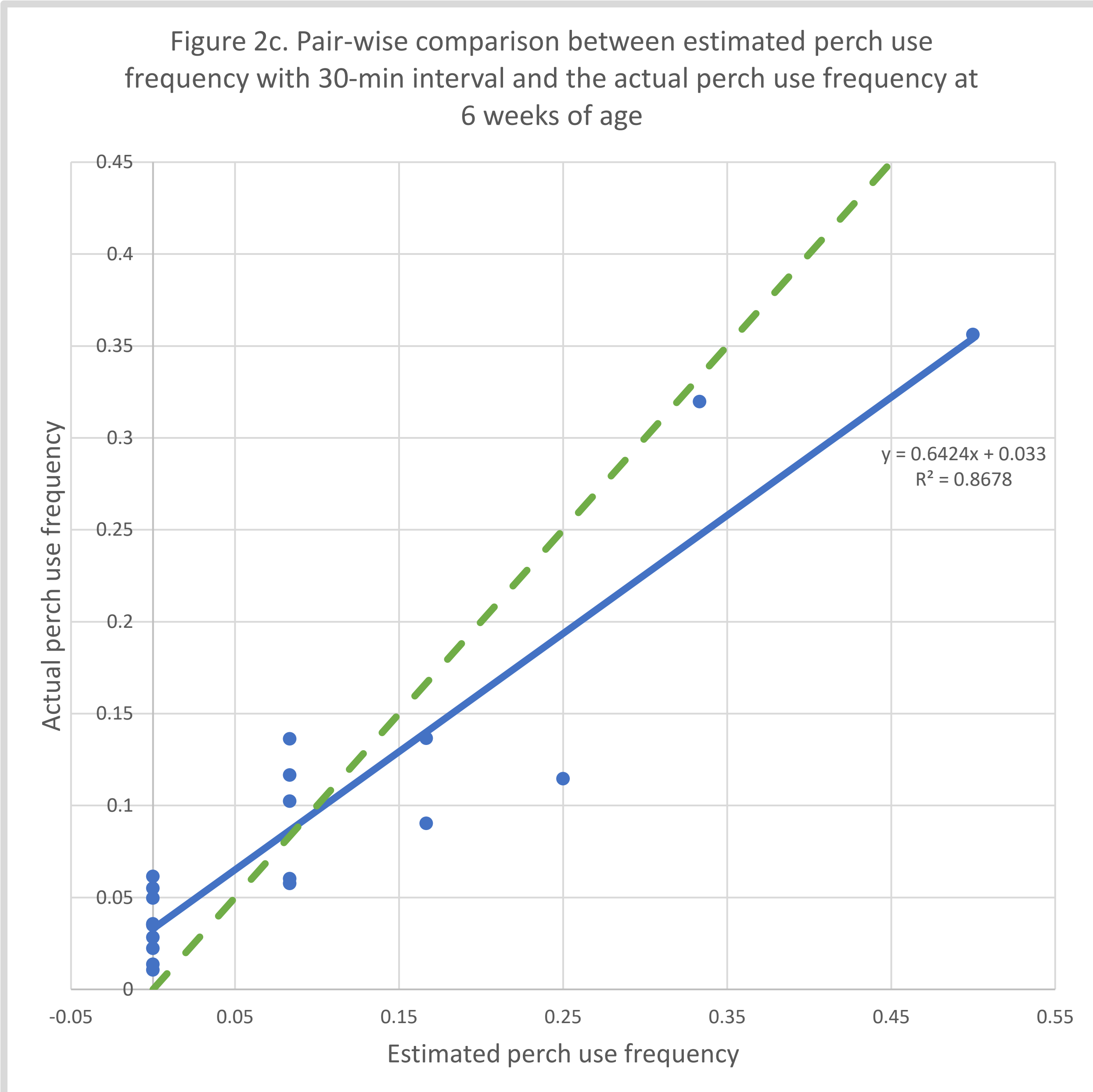


*Figure 2a-c. Pair-wise comparison between estimated perch use frequency using tested intervals (10, 15 and 30 min) and the actual perch use frequency derived from 1-s samples at 6 weeks of age. Green dash line: reference line indicating a 1:1 alignment between data from the 1-s sampling and tested intervals. Blue solid line: actual relationship between the results of 1-s samples and the tested intervals.*

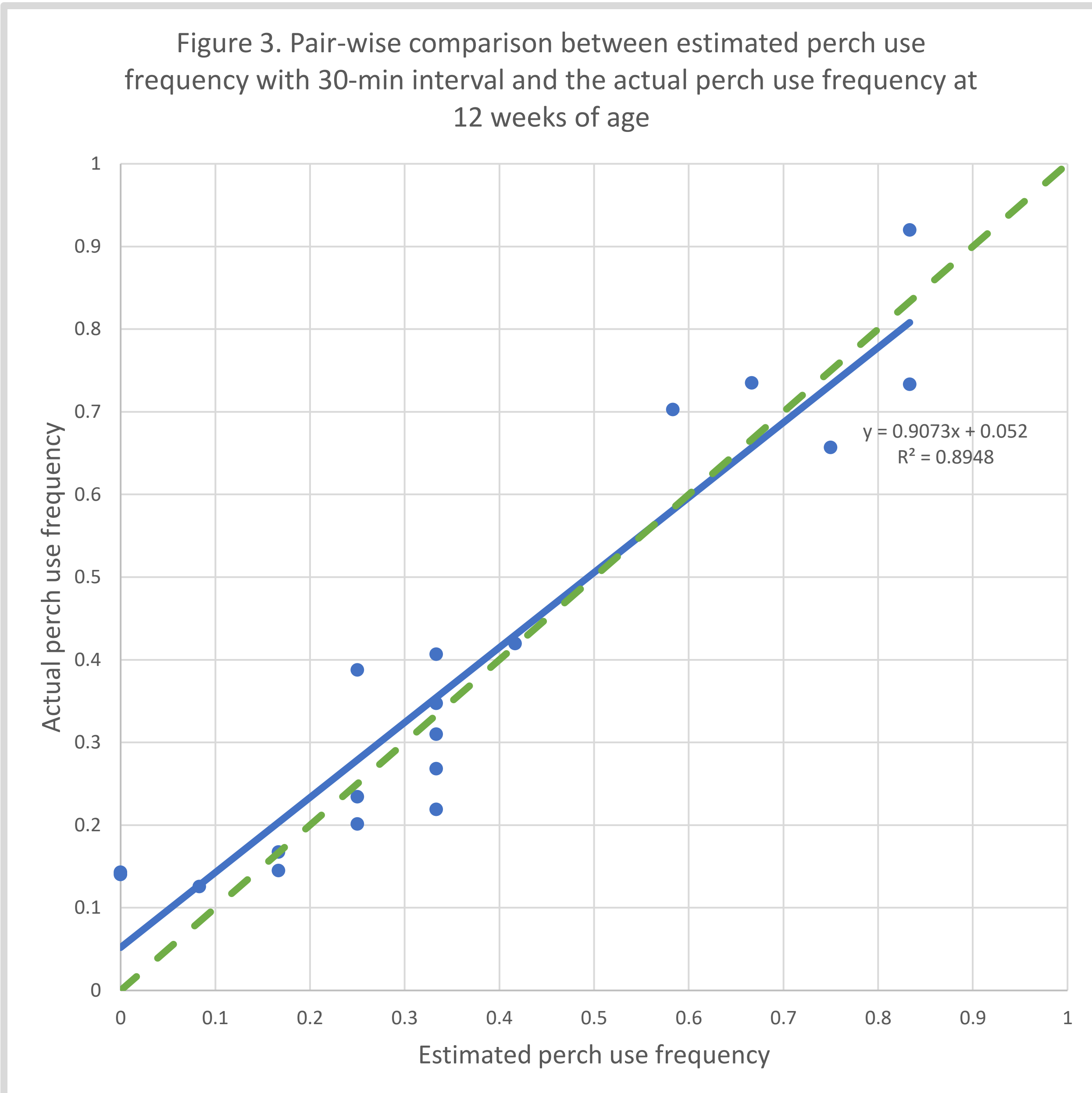


*Figure 3. Pair-wise comparison between estimated perch use frequency using tested intervals (30 min) and the actual perch use frequency derived from 1-s samples at 12 weeks of age. Green dash line: reference line indicating a 1:1 alignment between data from the 1-s sampling and tested intervals. Blue solid line: actual relationship between the results of 1-s samples and the tested intervals.*

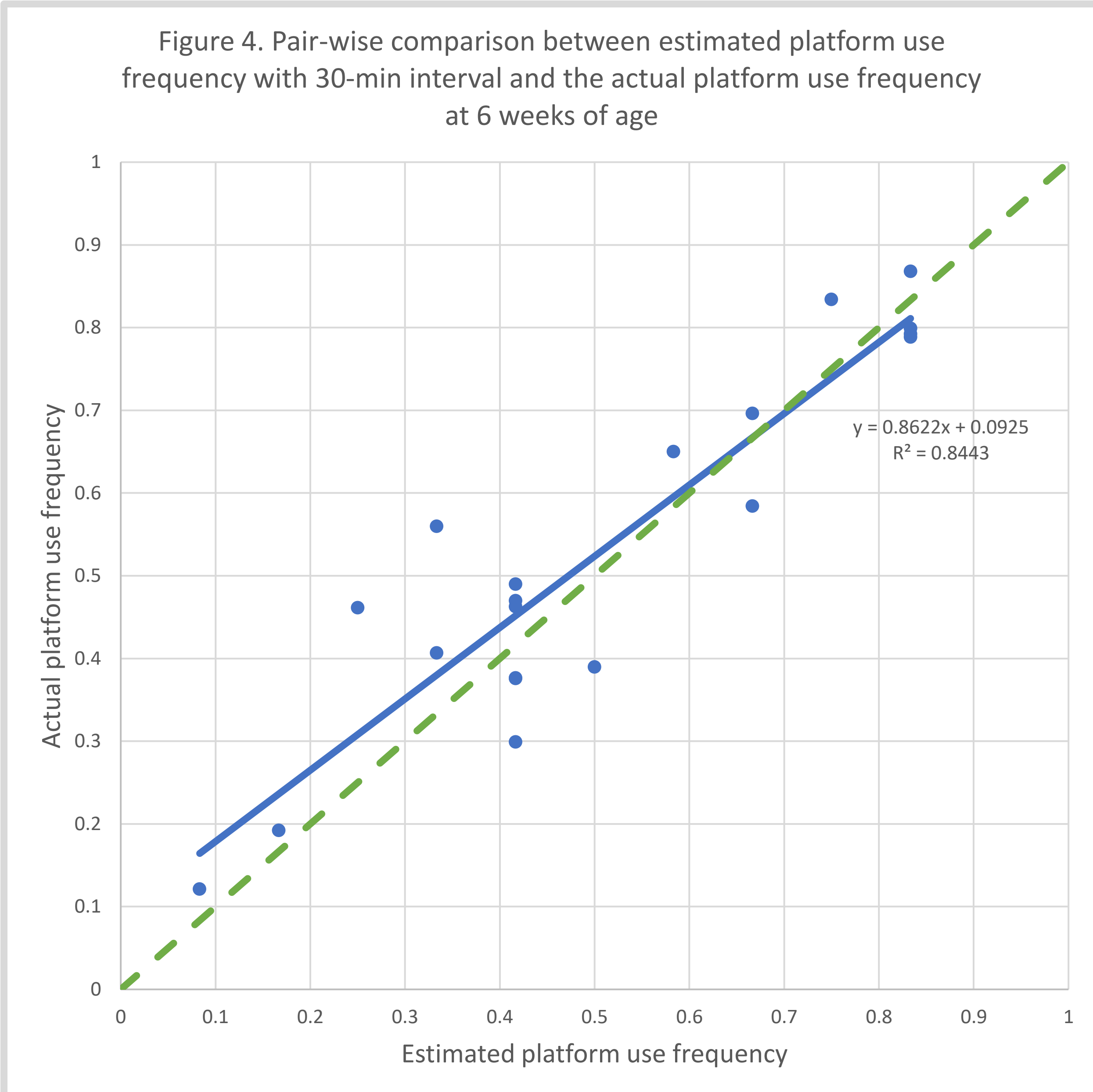


*Figure 4. Pair-wise comparison between estimated platform use frequency using tested intervals (30 min) and the actual platform use frequency derived from 1-s samples at 6 weeks of age. Green dash line: reference line indicating a 1:1 alignment between data from the 1-s sampling and tested intervals. Blue solid line: actual relationship between the results of 1-s samples and the tested intervals.*

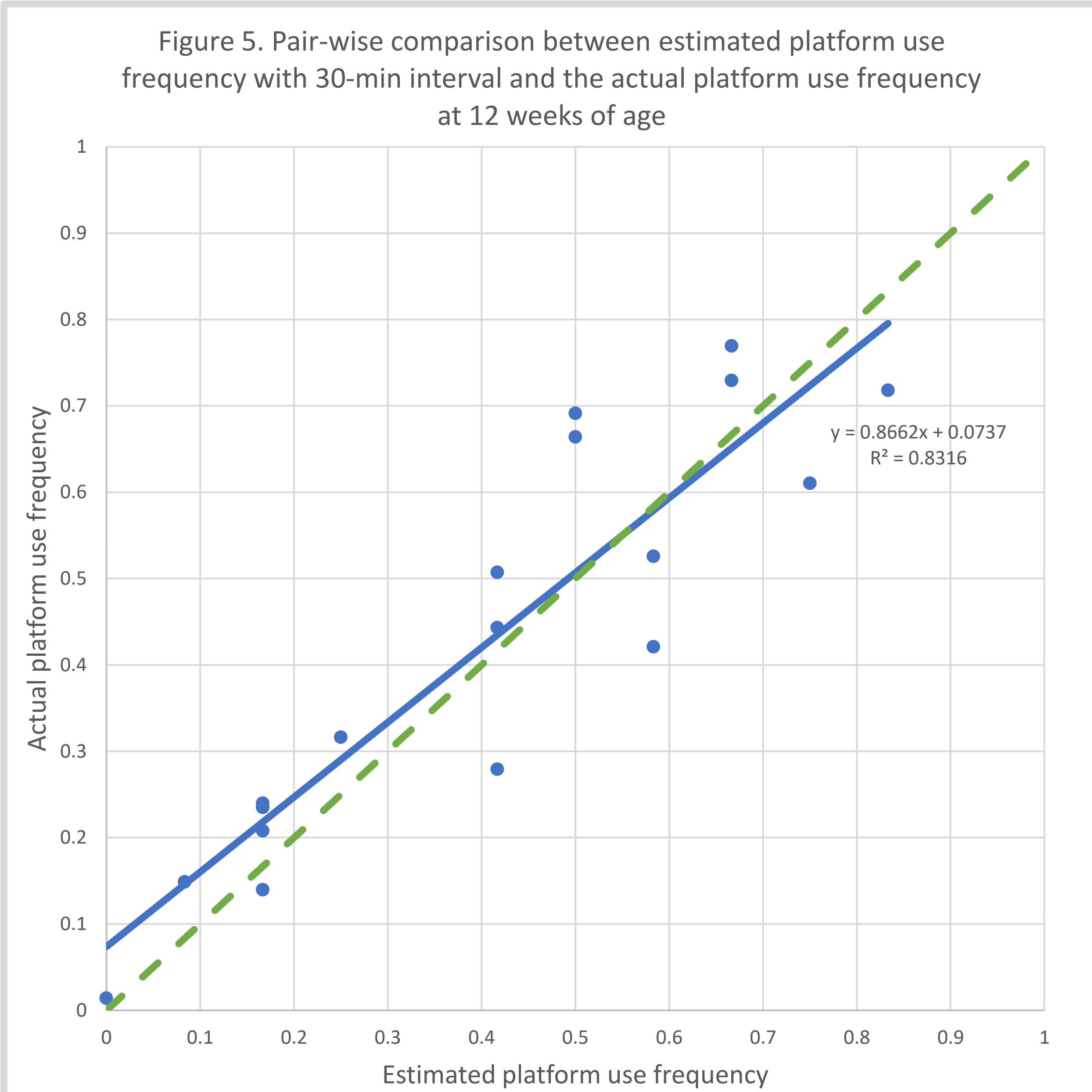


*Figure 5. Pair-wise comparison between estimated platform use frequency using tested intervals (30 min) and the actual platform use frequency derived from 1-s samples at 12 weeks of age. Green dash line: reference line indicating a 1:1 alignment between data from the 1-s sampling and tested intervals. Blue solid line: actual relationship between the results of 1-s samples and the tested intervals.*

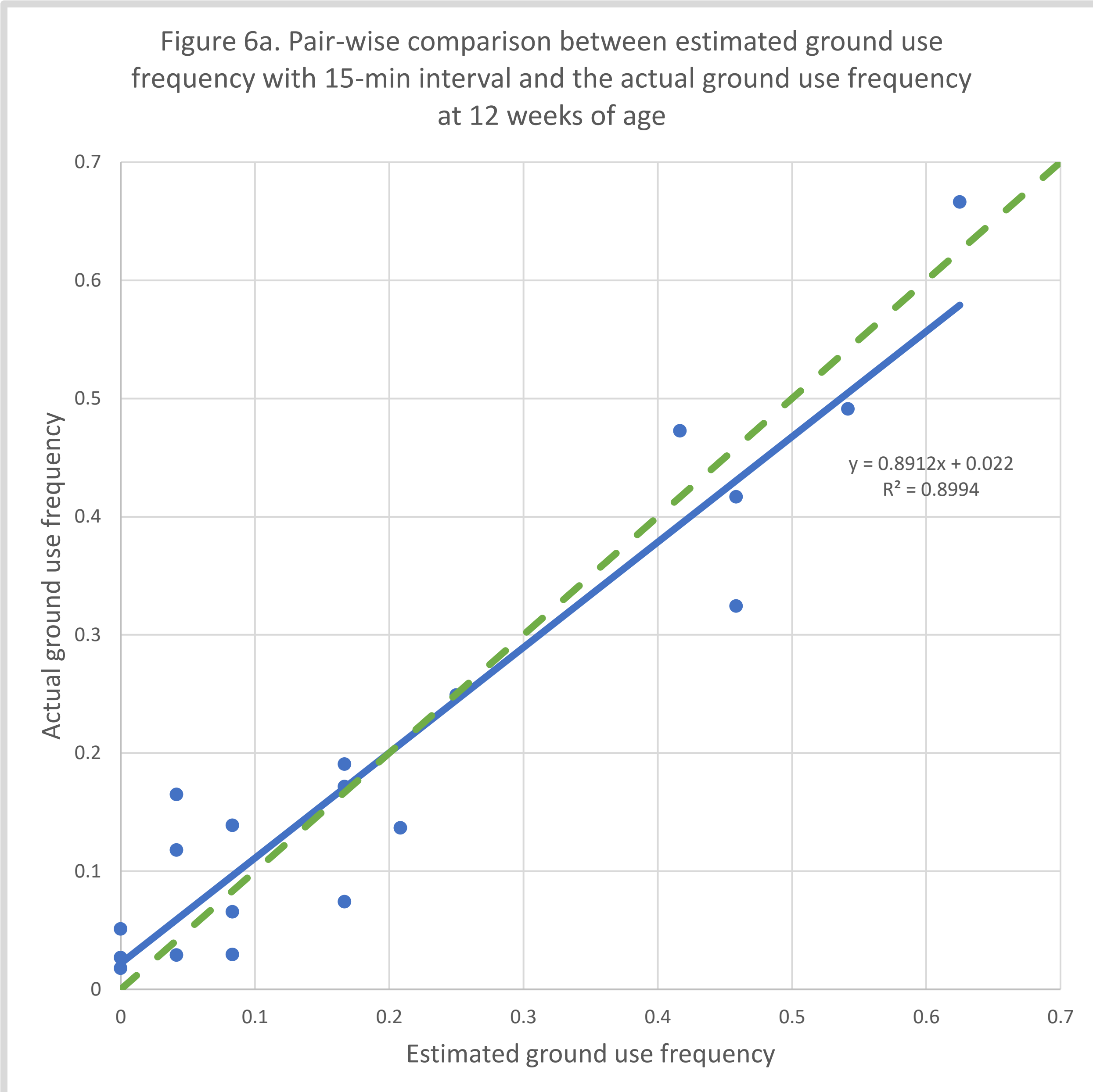
Figure 6a. Pair-wise comparison between estimated ground use frequency with 15-min interval and the actual ground use frequency at 12 weeks of age
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Actual ground use frequency
y = 0.8912x + 0.022
R² = 0.8994
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Estimated ground use frequency

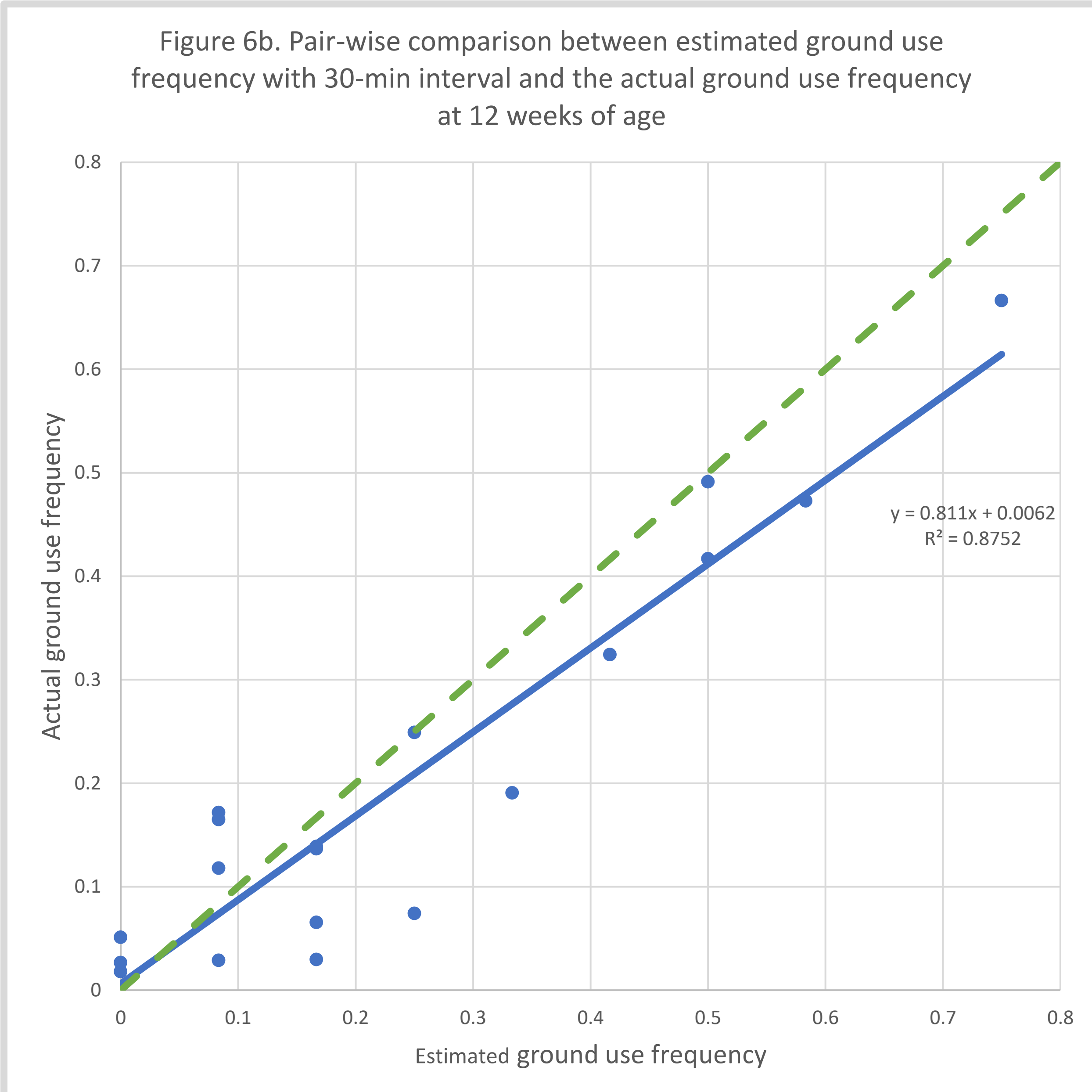


*Figure 6. Pair-wise comparison between estimated ground use frequency using tested intervals 15 (6a) or 30 min (6b) and the actual ground use frequency derived from 1-s samples at 12 weeks of age. Green dash line: reference line indicating a 1:1 alignment between data from the 1-s sampling and tested intervals. Blue solid line: actual relationship between the results of 1-s samples and the tested intervals.*

## 4. Discussion

Previously, Daigle and Siegford (2014) published a methodological framework using results from regression analysis to compare the accuracy of behavioral scan sampling schemes for quantifying laying hen behavioral time budgets. Similar approaches have been used to determine the appropriateness of behavioral sampling methods in other species across a variety of contexts, including for evaluating pain-related behaviors in pigs (Park et al., 2020; Robles et al., 2021; Wilder et al., 2021), to estimate behavioral time budgets of beef cattle (Mitlöhner et al., 2001; Madruga et al., 2016), dairy cattle (Chen et al., 2016; Downey et al., 2021), and feedlot lambs (Pullin et al., 2017). While these studies have emphasized the importance of validating behavioral sampling schemes for specific contexts, the reasoning behind the selection of specific sampling strategies is rarely reported in studies of pullet and laying hen behavior. Given that the behavioral time budgets changes with hen age (Liu et al., 2018), we predicted that different sampling schemes may be appropriate for different age groups. To test this, we evaluated the accuracy of five different scan interval sampling schemes (1, 5, 10, 15 and 30 min) for determining resource use by 6- and 12-week-old pullets.

Average perch use increased numerically from 6 to 12 weeks of age. Based on the reference data (1-s sampling over the 6-hour period) pullets were observed on the perch 9.2 % of the time at 6 weeks of age, while at 12 weeks of age, they were on the perch 38.2% of the time. The observed difference in perching time budgets reflects previously documented age effects: Chicks start perching as early as 1 week of age and it takes 5 to 7 weeks after first time exposure to the perch for the pullets to show consistent perching behavior (Heikkilä et al., 2006; Liu et al., 2018; Skånberg et al., 2021). Therefore, at week 6 pullets may still have been learning to use the perches.

Intervals up to 5 min reflected the actual perch use frequency at 6 weeks of age with high accuracy. Starting from 10-min intervals, one or more criterion was not met. Intervals of 10 min (Figure 2a) and 30 min (Figure 2b) could overestimate the frequency of perch use when perch use frequency was low and underestimate the frequency of perch use when perch use frequency was high. For interval of 15 min, both the intercept and slope met the criteria and the value of $R^2$ indicate a “good” accuracy. However, it is more conservative to use a smaller sampling interval, considering that accuracy concerns arose when a shorter (10-min) interval was tested.

At week 12, intervals up to 15 min resulted in highly accurate estimates of perch use. Meanwhile, 30-min intervals had a $R^2$ just shy of our stated cut-off, and were considered to have “good” accuracy. We selected the $R^2$ value cut-off based on recommendations from previous studies (Ledgerwood et al., 2010; Chen et al., 2016). The $R^2$ indicates how much variation is captured by the model, with higher values indicating a better fit. The most appropriate sampling interval is one that balances accuracy and efficiency, and the appropriate balance depends on the context of the study. It is probably more feasible to improve model fit, and overall accuracy of the data, by collecting more frequent data when group sizes are small, and birds are easily individually discernable. On the other hand, the effort and time investment needed to conduct more frequent observations of individuals in exceptionally large groups may not justify the small difference in fit.

Daigle and Siegford (2014) found that a 5-min scan sample interval would accurately capture perch use in layers at about 20 weeks of age. Their results differ from our findings, which could reflect methodological differences in terms of hen strain, age, housing design, and sample size. Studies have shown that both white and brown leghorn layers perch more as they age, and white Leghorn perch more compared with brown leghorn hens (Ali et al., 2019; Faure and Jones, 1982a, b). Daigle and Siegford's (2014) study focused on adult brown leghorn layers at 20 weeks of age, whereas data from white leghorn pullets at 6 and 12 weeks of age were used in our study. Moreover, Daigle and Siegford (2014) took into account the strength of the association as part of their evaluation criteria. Perch use was best represented with the 5-min interval in Daigle and Siegford's study (2014) due to the strongest association between the estimated and actual perching behavior. Following Chen et al. (2016), we set a threshold criterion for the coefficient of determination ($R^2$>0.9). Both the 5- and 15-min intervals reported by Daigle and Siegford (2014) would meet our three criteria, meaning that we would have considered both intervals to have a "very good" accuracy. Perch use can also be affected by other factors, such as stocking density (Newberry et al., 2001), perch length (Appleby, 1995), perch height (Brendler and Schrader, 2016), and spatial availability (Wichman et al., 2007). For instance, Daigle and Siegford's (2014) aviary system was consisted of three perches at different heights (53, 76 and 99 cm above the ground) and the size of the slat platform (100 cm x 61 cm) and perches (6 m long. ~ 5cm diameter) were also different. All these differences in study design could potentially contribute to the differences we observed between our results, which further emphasizes the importance of conducting behavioral sampling validations as part of the standard data collection and reporting processes.

A numerical decrease in platform use was observed as the pullets aged, but a sampling interval up to 15 min represented the data with high accuracy at both ages. At 6 and 12 weeks of age, platform use averaged 53.1% and 41.6% of the observation period. The high degree of platform use could reflect the pullets' overall preference of height (Schrader and Müller, 2009). Ramp use was very low at both ages (6 wks: 0.61 ± 0.45%; 12 wks: 0.62 ± 1.12%). Thus, we combined ramp and platform use and consider them together as platform use. When using 30-min intervals, both the slope and intercept met the criteria we set up, but $R^2$ for both ages were lower than 0.9 (6 wks: $R^2$ = 0.844; 12 wks: $R^2$ = 0.832). As discussed earlier, the value of $R^2$ here does not indicate that the platform use frequency was inaccurate. However, we do propose, that when feasible the sampling scheme that produces the best model fit be used.

Average ground use decreased numerically from 37.8% at week 6 to 20.2% at week 12. Our results do not correspond to previous reports that young chicks raised on the floor with access to perches or other enrichments for climbing spent the most amount of time on the litter (Heikkilä et al., 2006; Skånberg et al., 2021). Yet, it should always be kept in mind that the design of each study differed and could have an impact on resource use. The provision of ramp would allow earlier assess to elevated resources (Stratmann et al., 2022), which might explain why relatively low ground use was observed in our study. Ross et al. (2019) found that a 5-min scan interval would accurately capture ground use in young broiler pullets at 19 days of age, while results of our study suggested using longer intervals could provide a "very good" accuracy. Previous studies have shown that broilers have a different behavioral time budget from layers (Kozak et al., 2016; Bizeray et al., 2000; Weeks et al., 2000), which might explain why our sampling schemes for ground use were different from Ross et al.'s results.

## 5. Conclusion

This study examined the accuracy of different instantaneous scan intervals to capture the resource use frequency at two pullet ages. Based on the results, it is suggested that different instantaneous sampling schemes are needed to sample perch use at two ages. For platform/ramp and ground use, there was not an age difference between the needed sampling schemes. These results highlight the importance of validating the sampling scheme independently for each age. Compared with previous research, this study included more sources of variation that could affect the behavioral time budgets, including individual and pen-level variation in behavior, and day effects. Still, limitations exist since the study was conducted using a single strain of pullets, and a single aviary type. These limitations make it inappropriate for the results to be applied directly to other contexts. Thus, the information provided should be used to guide researchers' own sampling scheme validations based on the design of each study.

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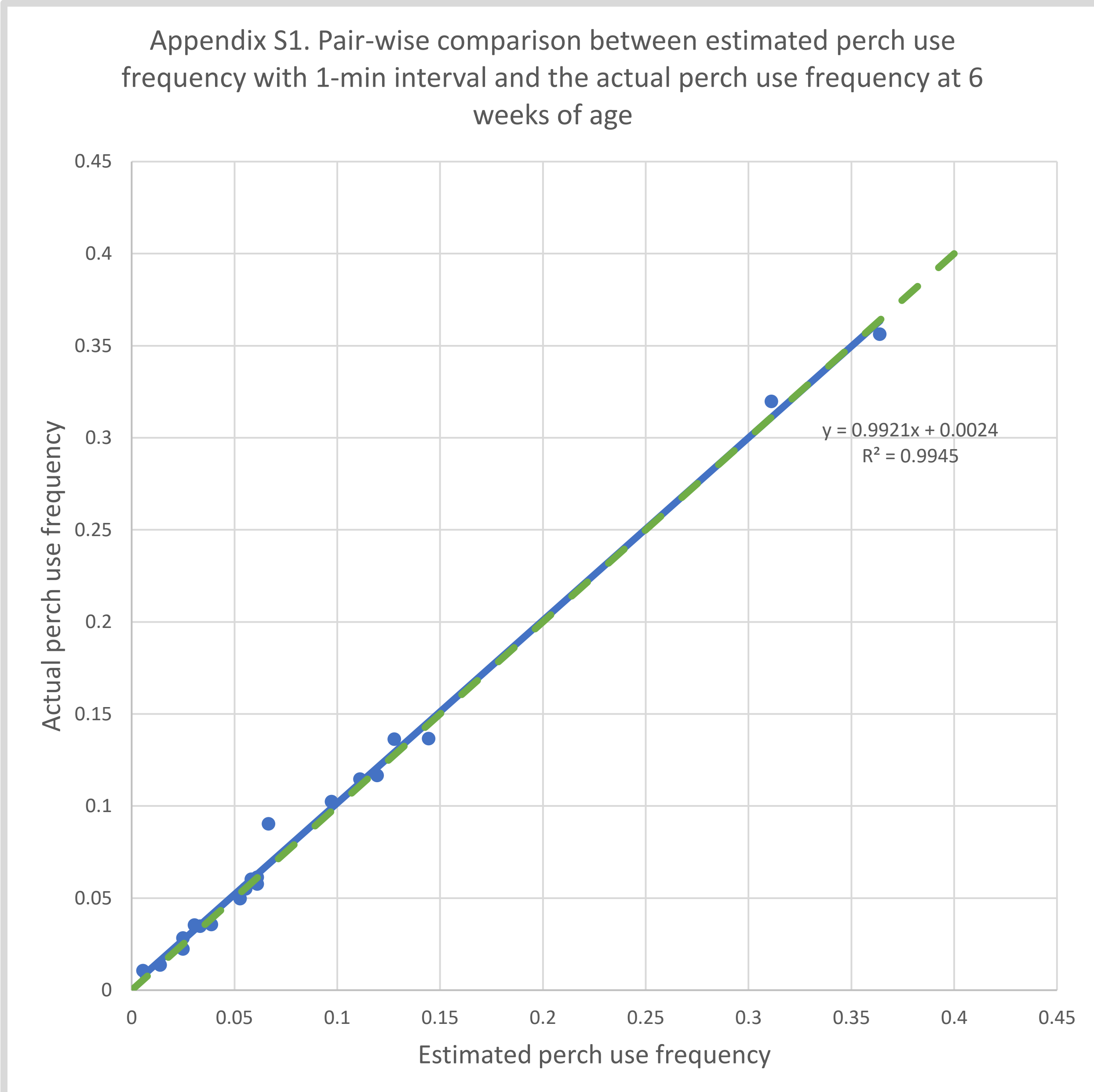
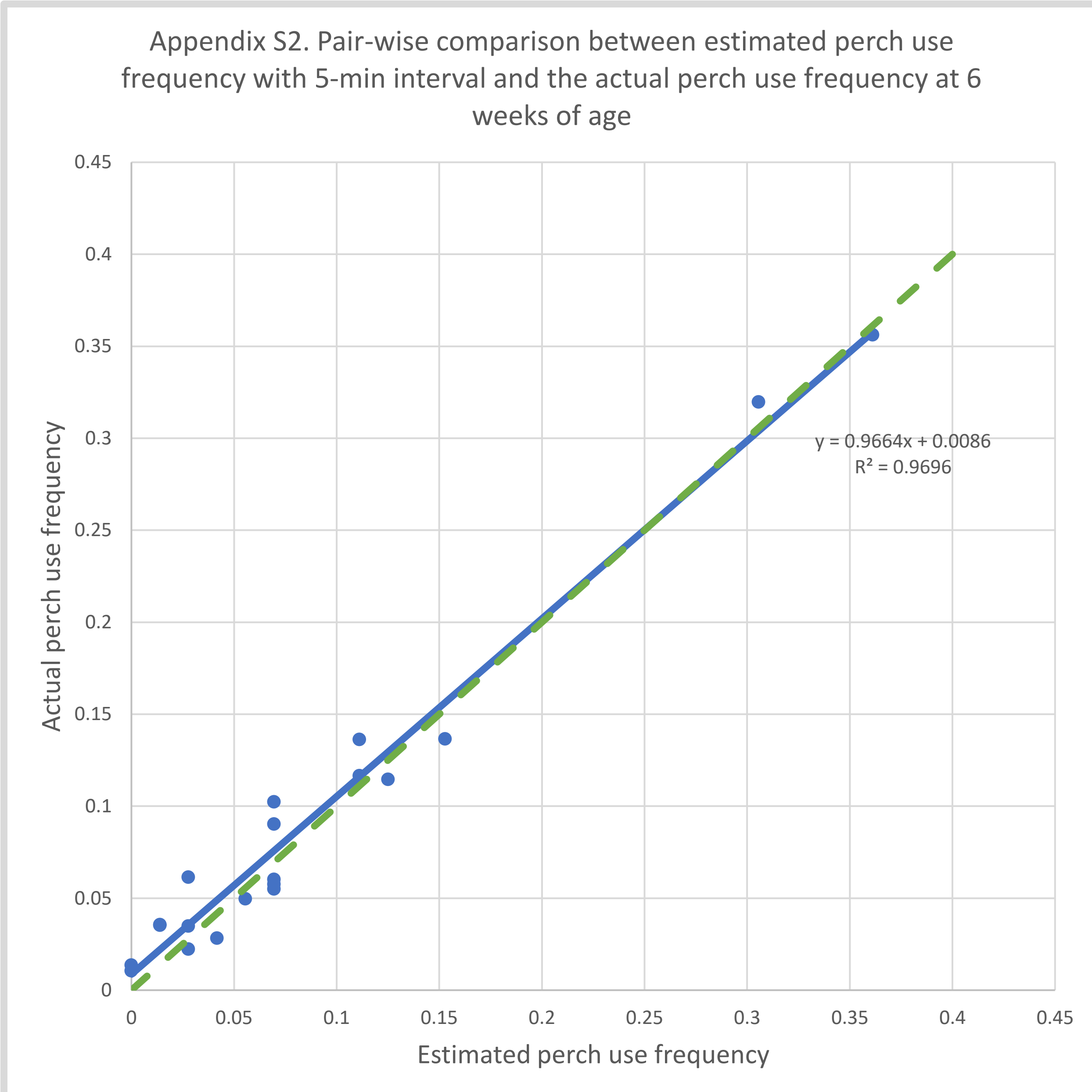
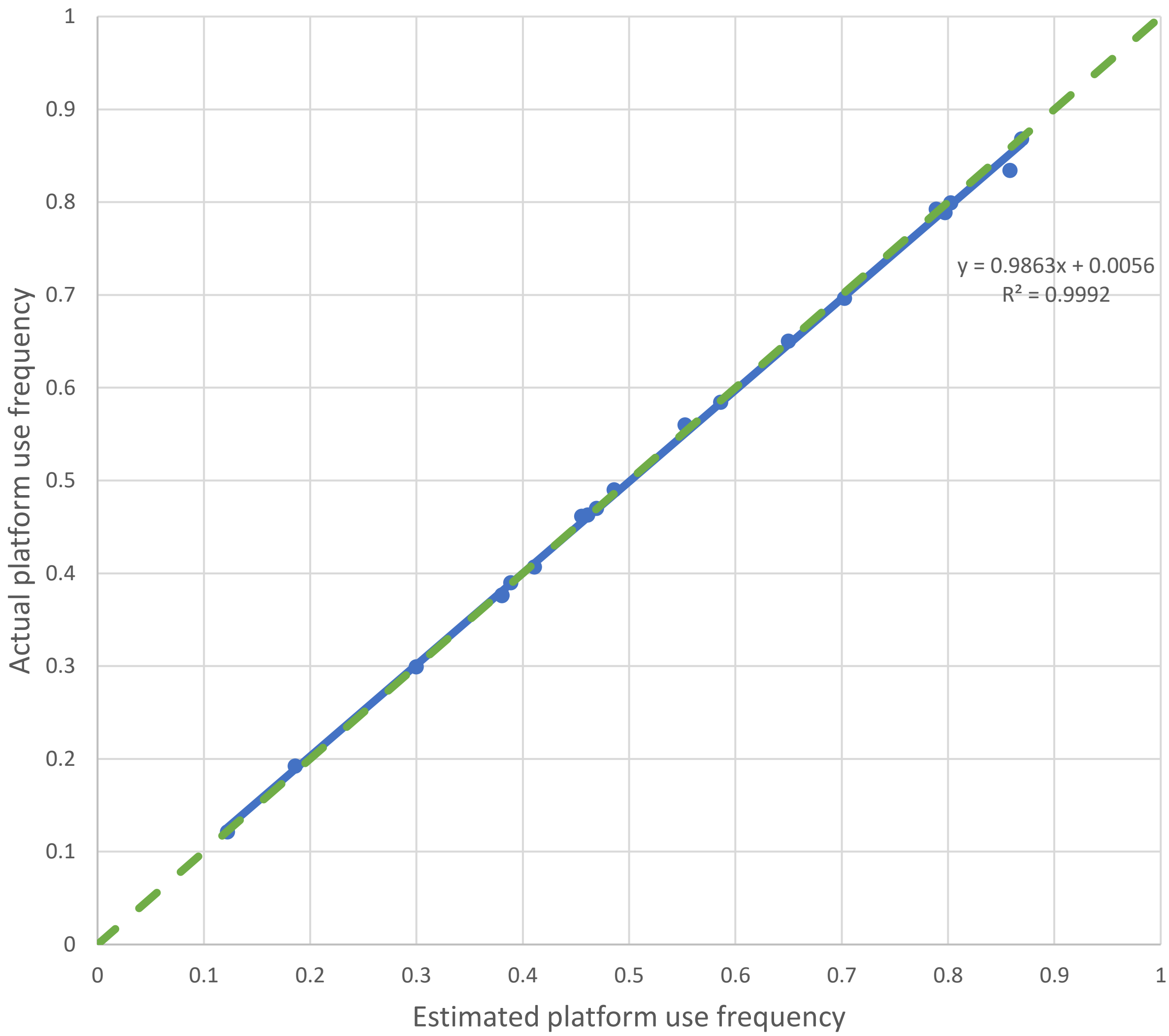
## Appendix

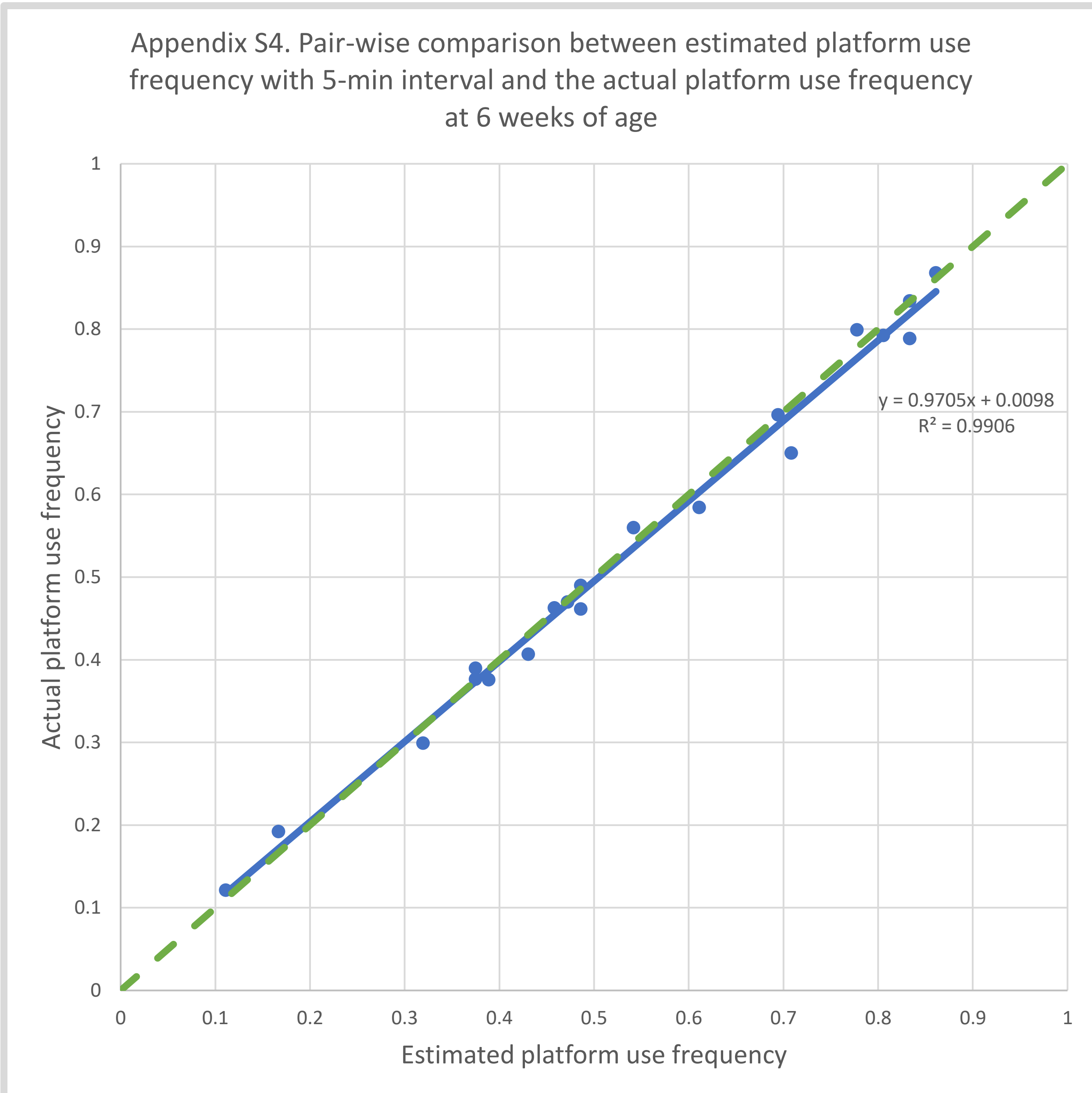

Appendix S1. Pair-wise comparison between estimated perch use frequency with 1-min interval and the actual perch use frequency at 6 weeks of age
y = 0.9921x + 0.0024
R² = 0.9945
Actual perch use frequency
Estimated perch use frequency
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45

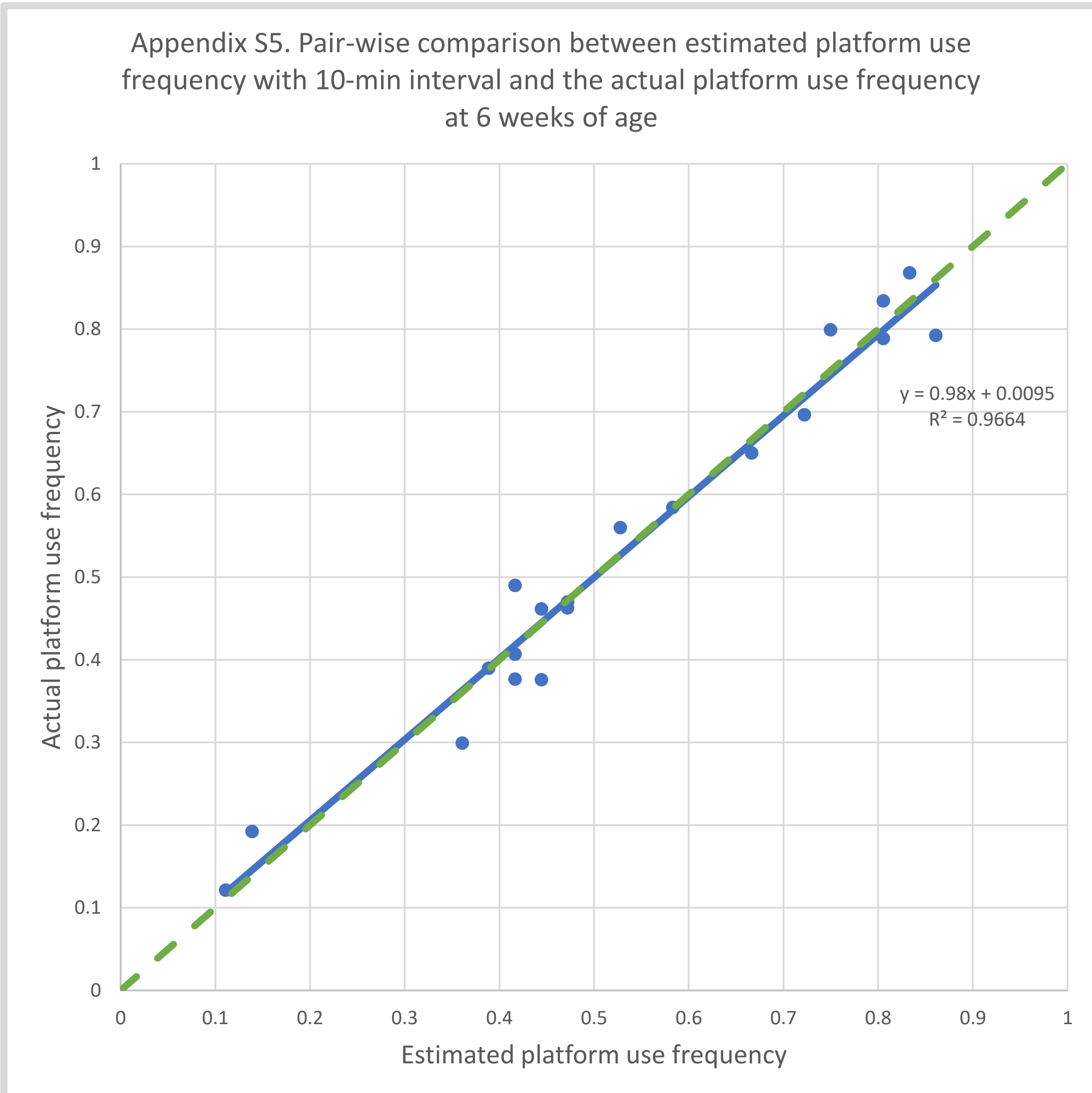


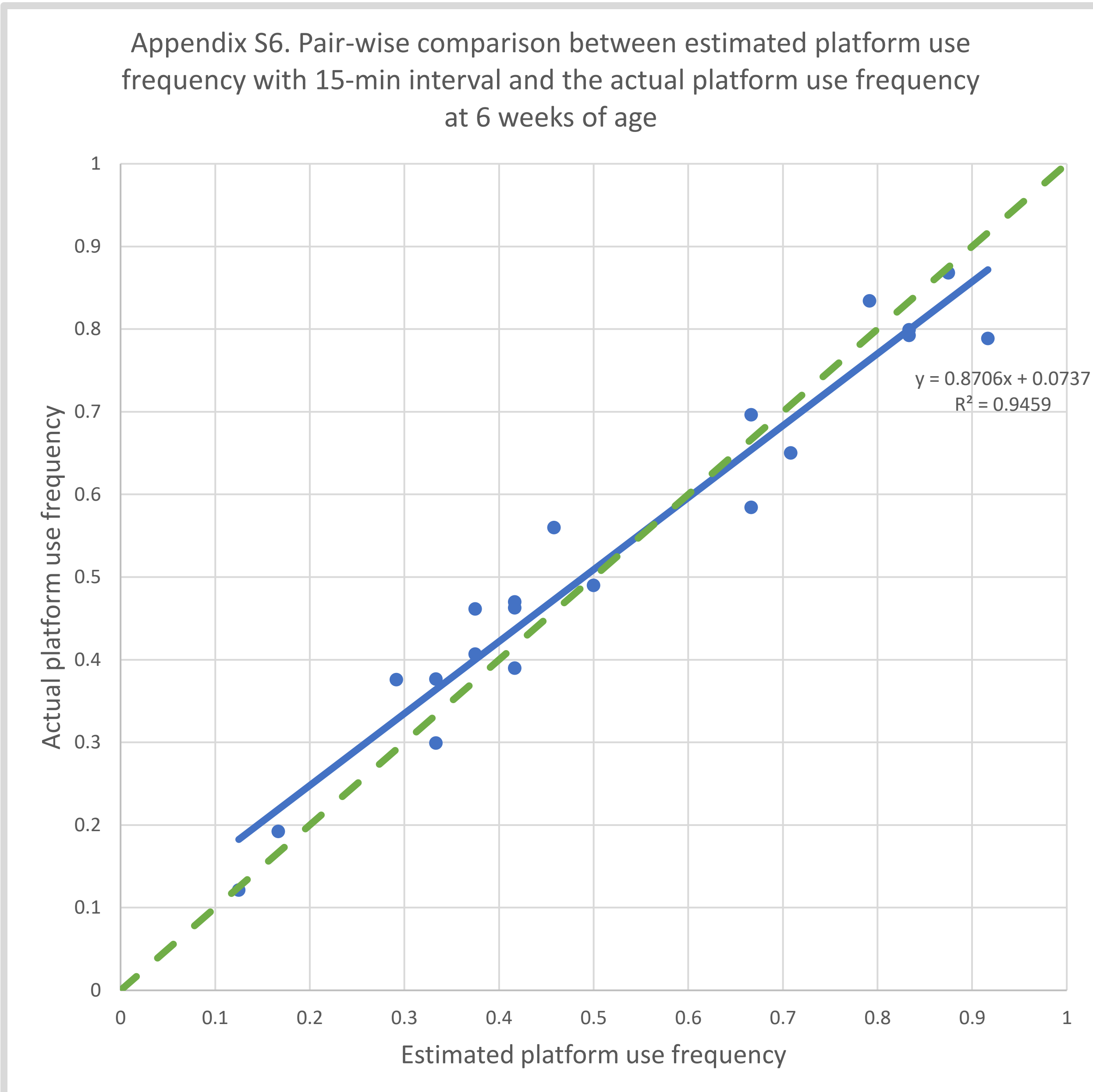

*Appendix S1-2. Pair-wise comparison between estimated perch use frequency using tested intervals (1 and 5 min) and the actual perch use frequency derived from 1-s samples at 6 weeks of age. Green dash line: reference line indicating a 1:1 alignment between data from the 1-s sampling and tested intervals. Blue solid line: actual relationship between the results of 1-s samples and the tested intervals.*

Appendix S3. Pair-wise comparison between estimated platform use frequency with 1-min interval and the actual platform use frequency at 6 weeks of age

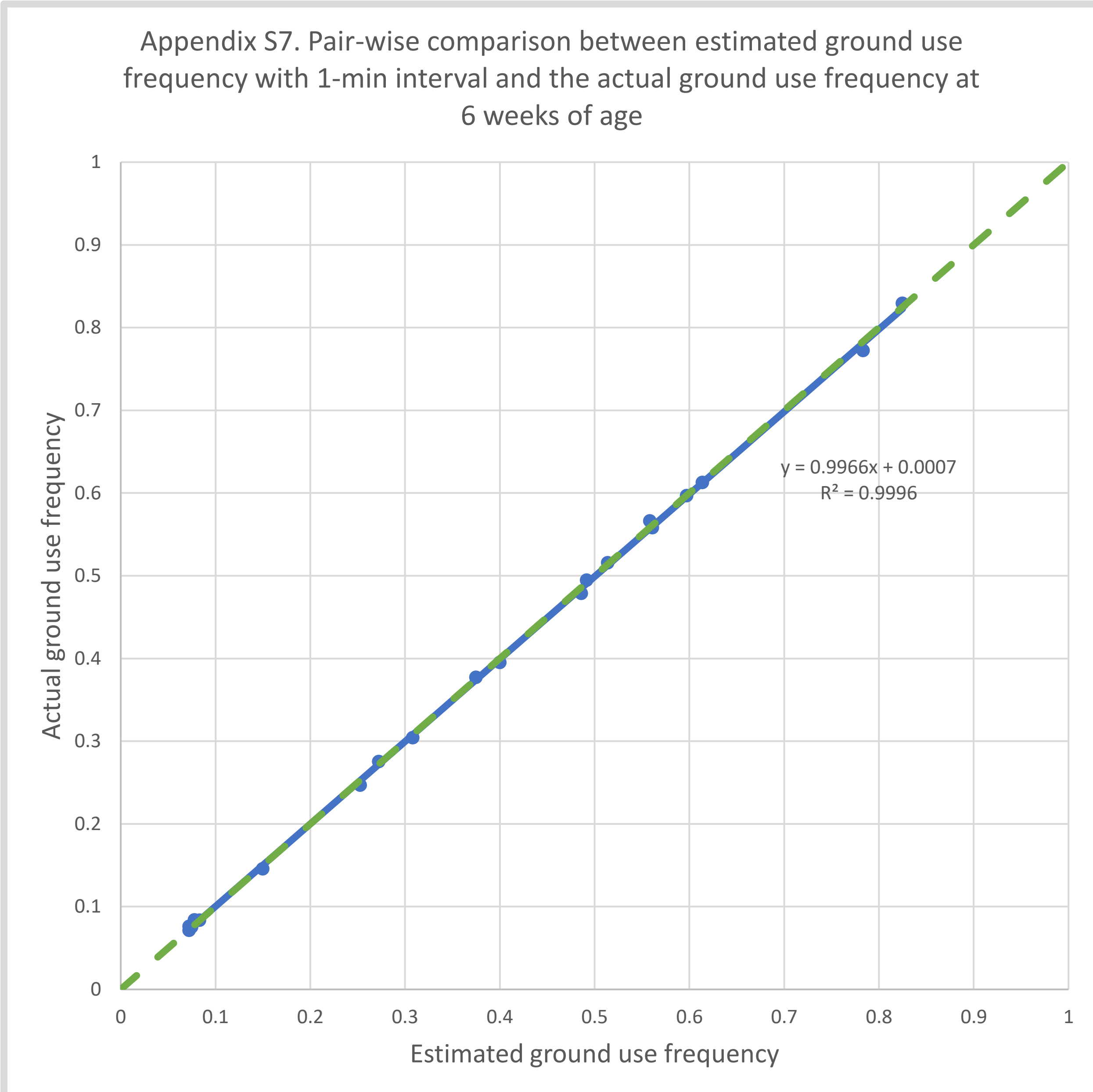


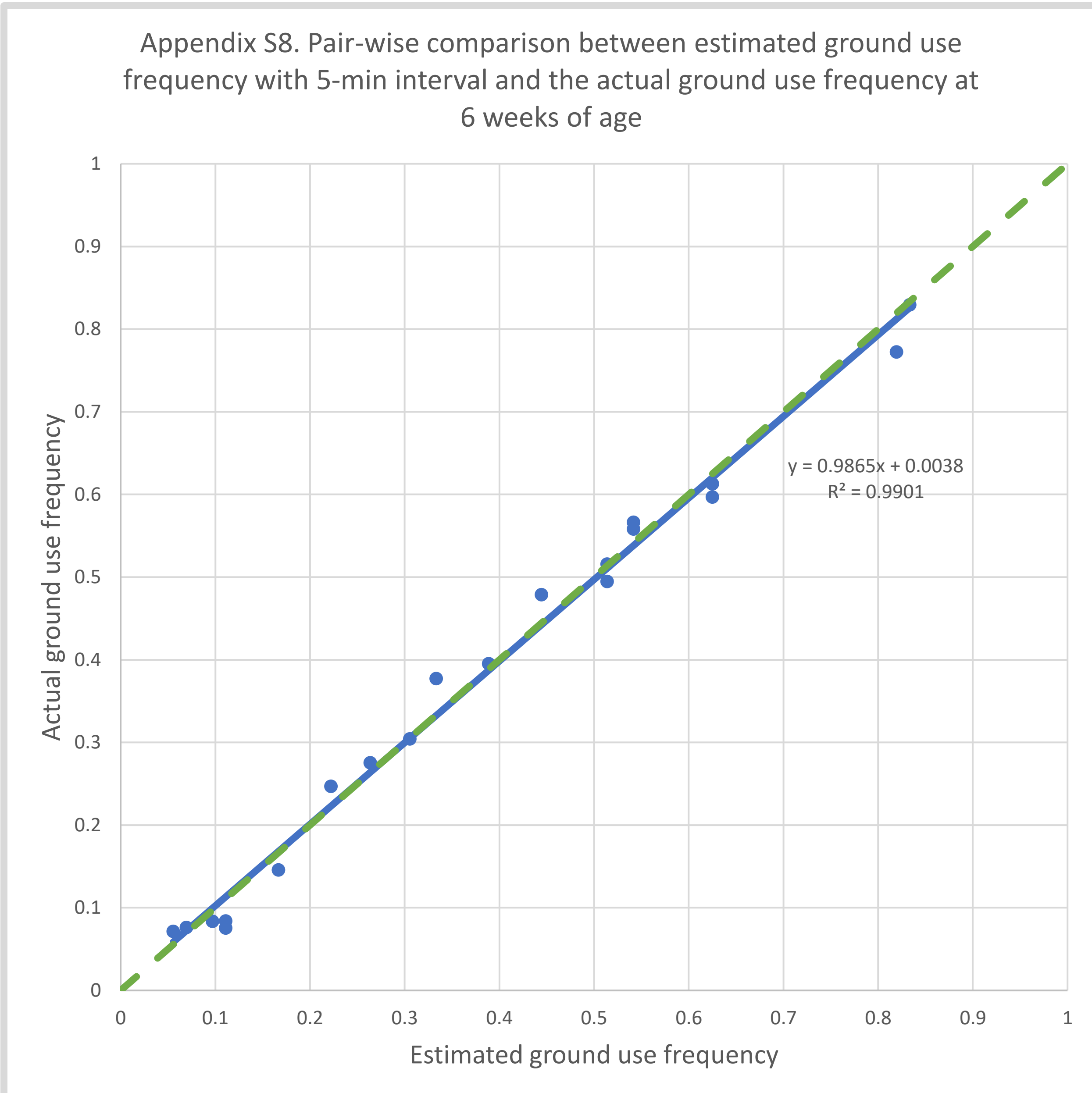

Appendix S4. Pair-wise comparison between estimated platform use frequency with 5-min interval and the actual platform use frequency at 6 weeks of age
y = 0.9705x + 0.0098
R² = 0.9906
Actual platform use frequency
Estimated platform use frequency
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

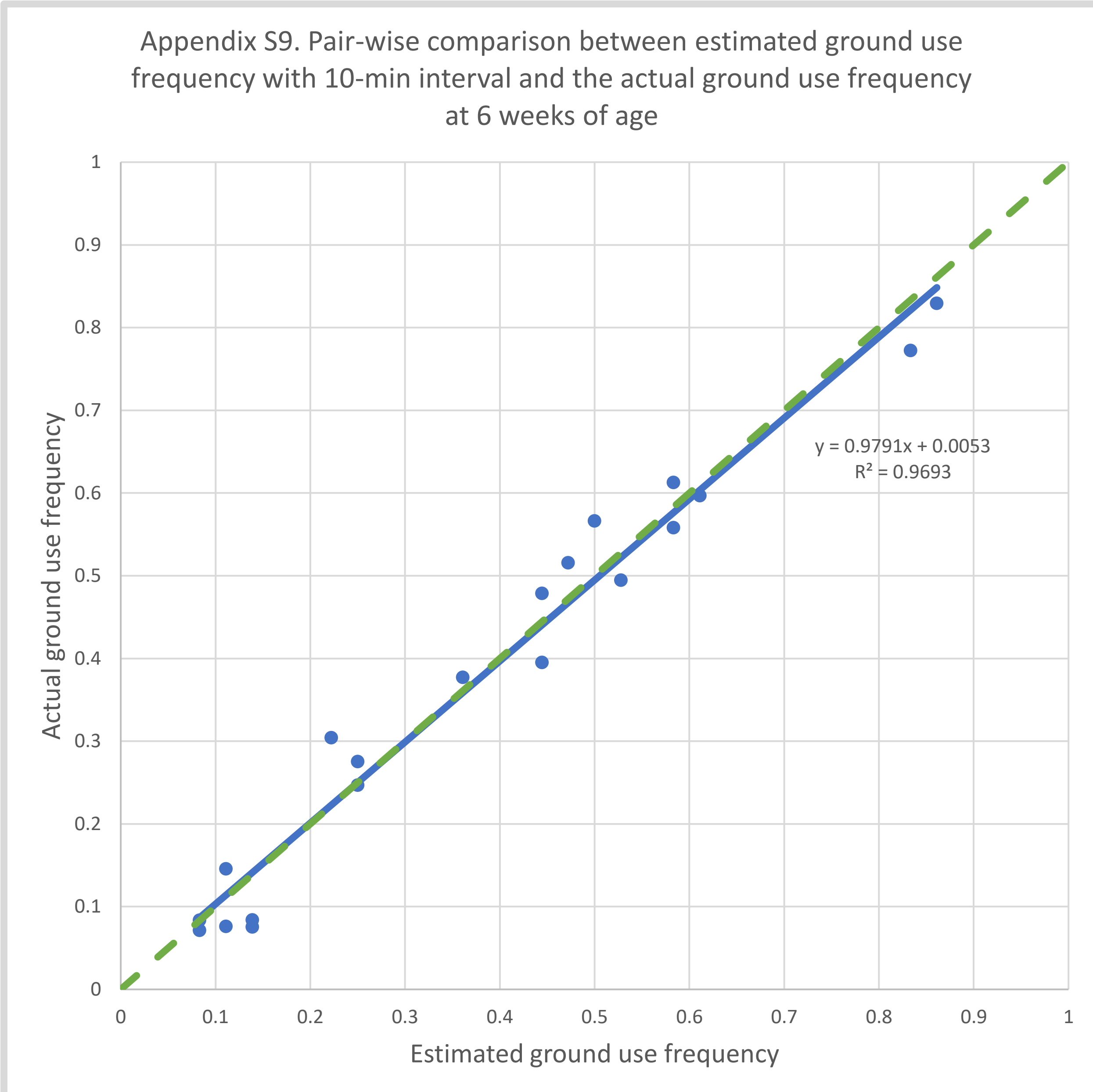

Appendix S5. Pair-wise comparison between estimated platform use frequency with 10-min interval and the actual platform use frequency at 6 weeks of age
Actual platform use frequency
Estimated platform use frequency
y = 0.98x + 0.0095
R² = 0.9664
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

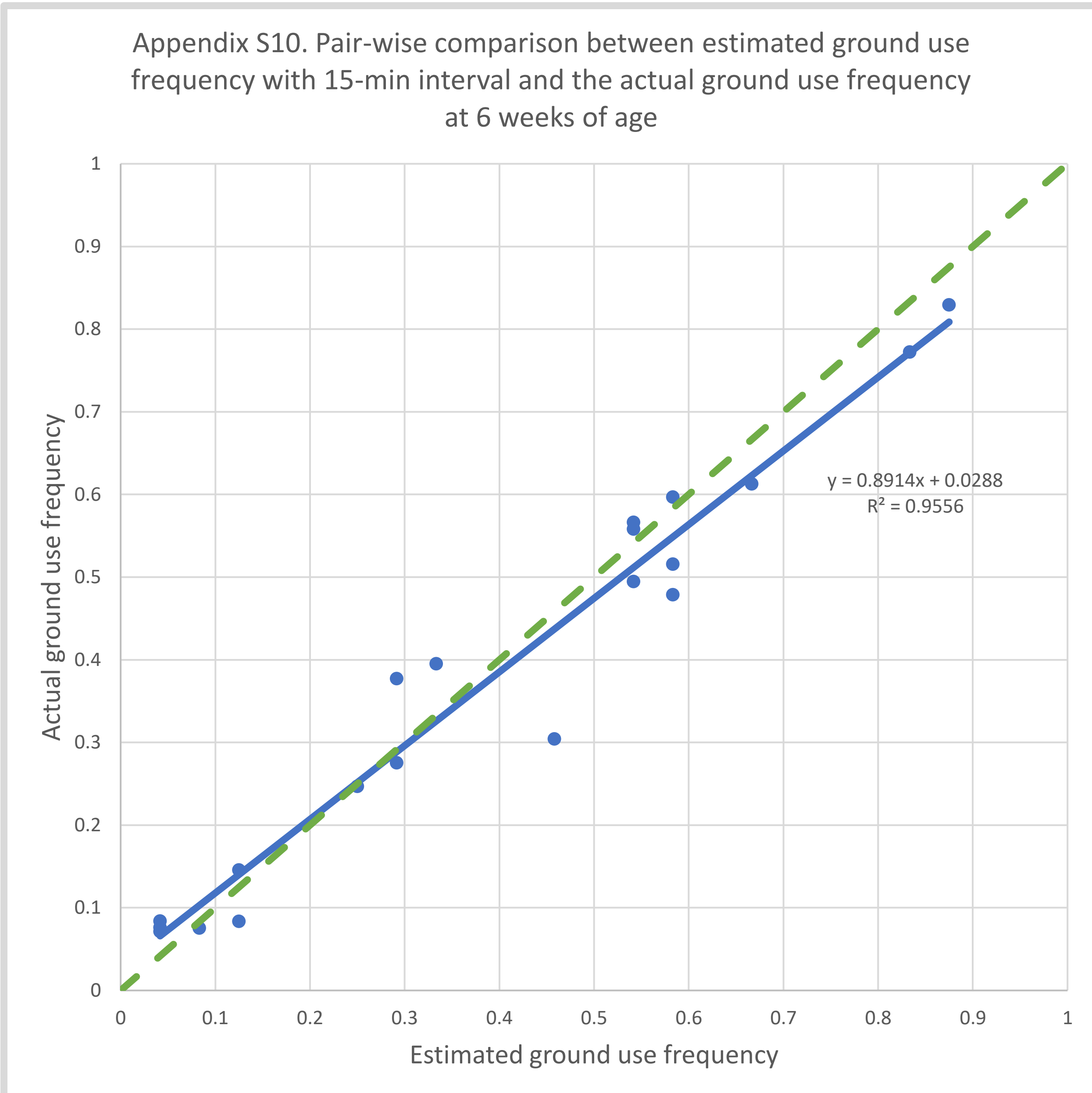


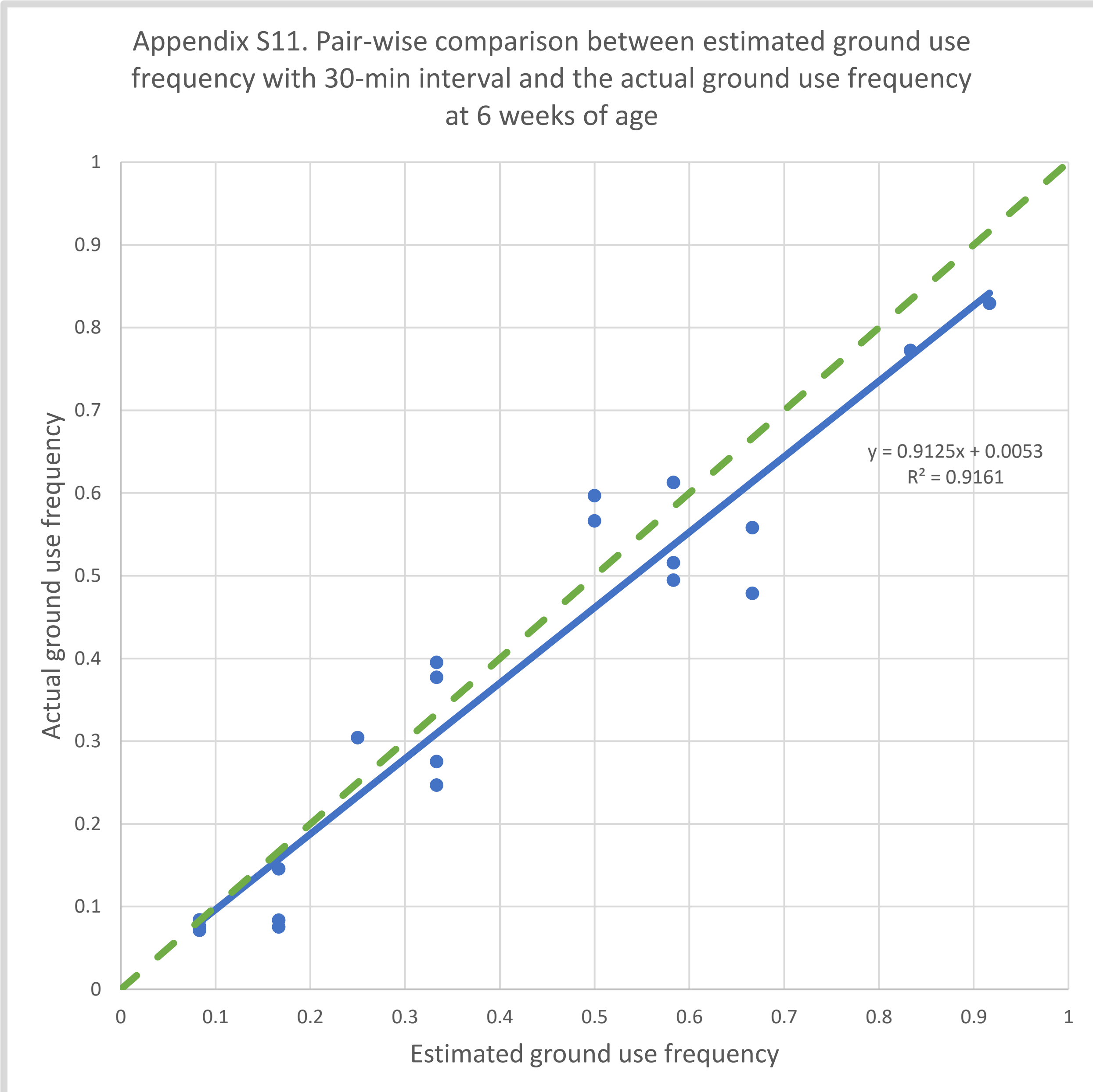

*Appendix S3-6. Pair-wise comparison between estimated platform use frequency using tested intervals (1, 5, 10 and 15 min) and the actual platform use frequency derived from 1-s samples at 6 weeks of age. Green dash line: reference line indicating a 1:1 alignment between data from the 1-s sampling and tested intervals. Blue solid line: actual relationship between the results of 1-s samples and the tested intervals.*


Appendix S7. Pair-wise comparison between estimated ground use frequency with 1-min interval and the actual ground use frequency at 6 weeks of age
Actual ground use frequency
Estimated ground use frequency
y = 0.9966x + 0.0007
R² = 0.9996
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

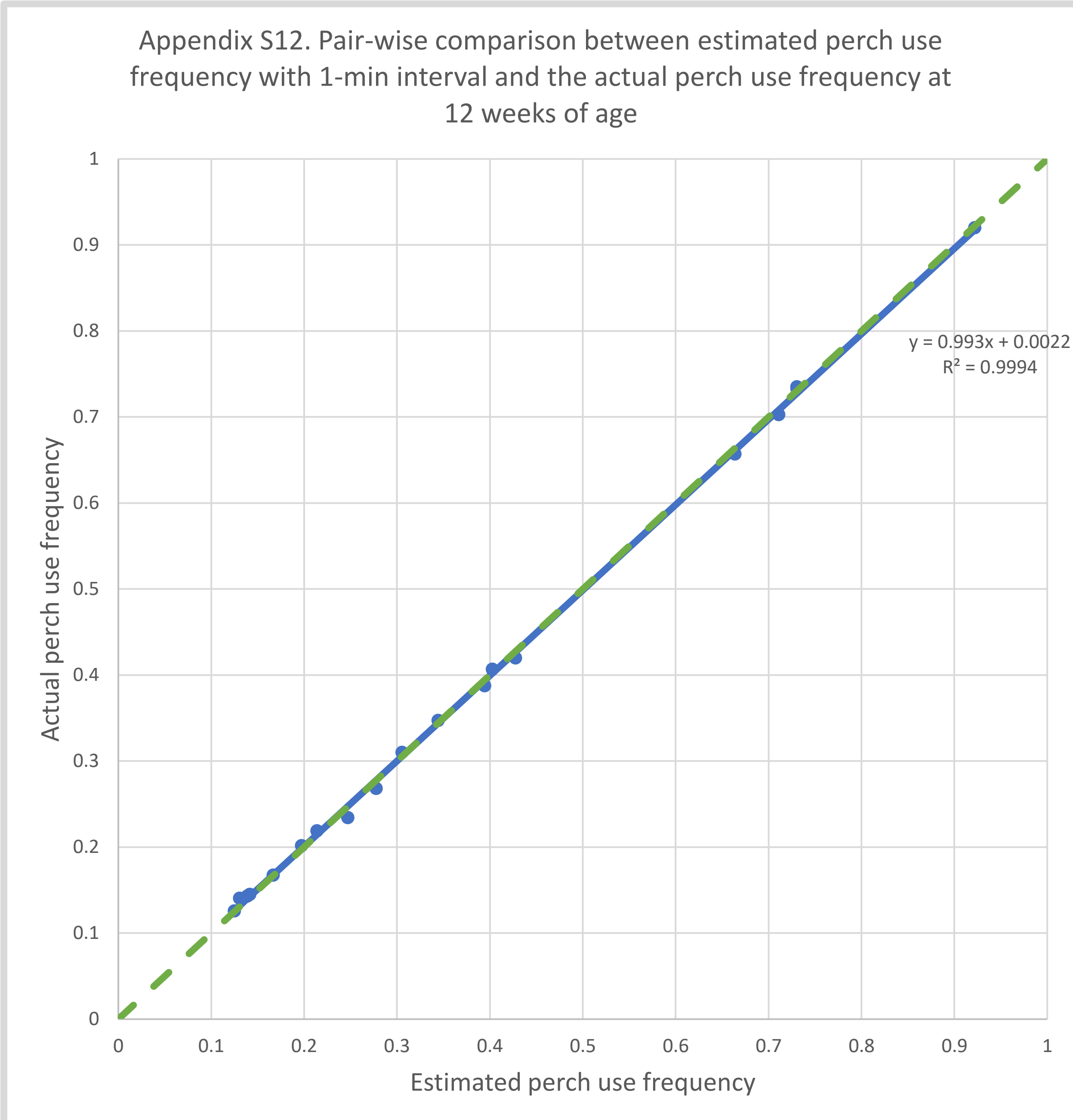

Appendix S8. Pair-wise comparison between estimated ground use frequency with 5-min interval and the actual ground use frequency at 6 weeks of age
Actual ground use frequency
Estimated ground use frequency
y = 0.9865x + 0.0038
R² = 0.9901
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

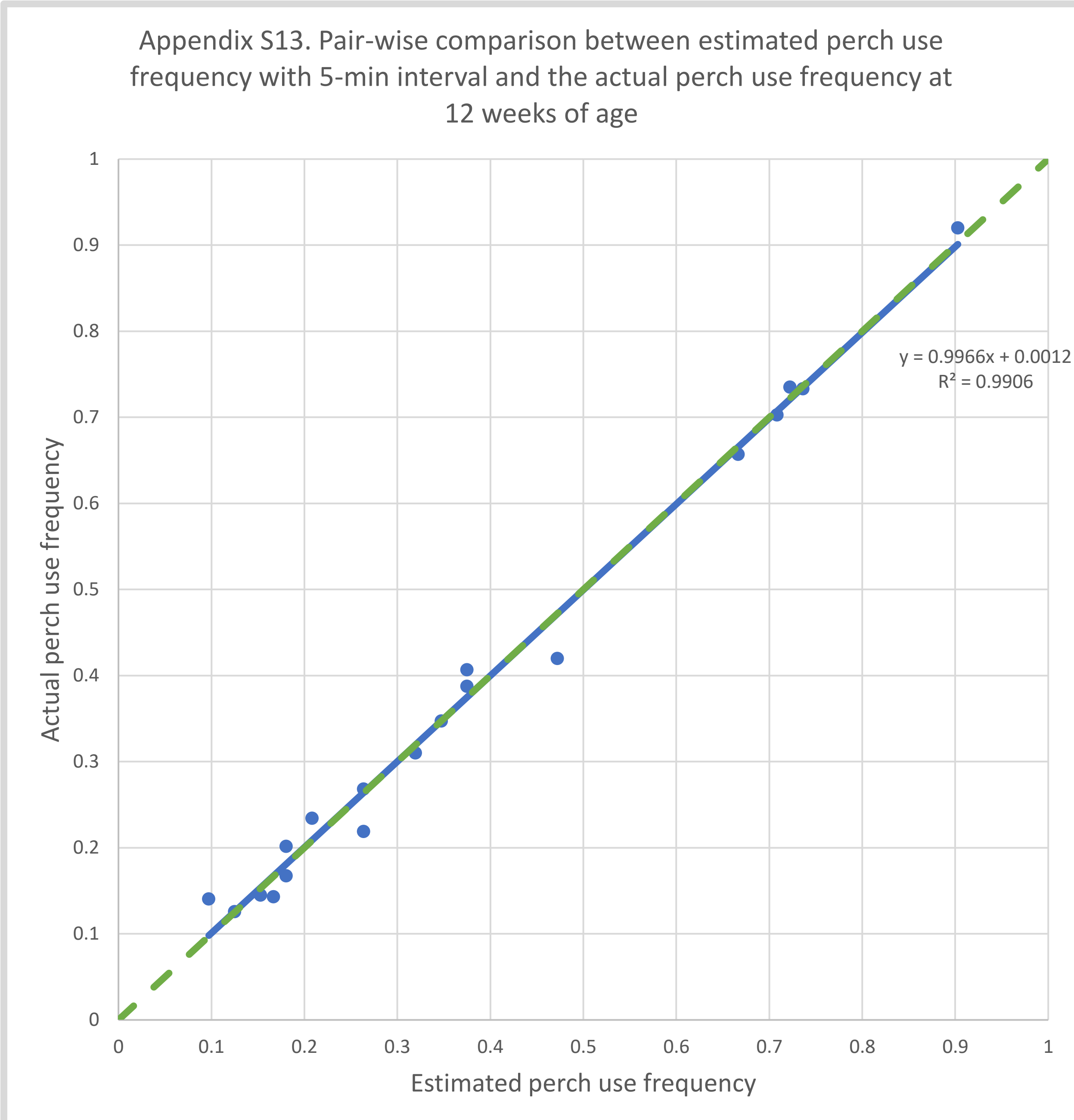

Appendix S9. Pair-wise comparison between estimated ground use frequency with 10-min interval and the actual ground use frequency at 6 weeks of age
y = 0.9791x + 0.0053
R² = 0.9693
Actual ground use frequency
Estimated ground use frequency
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

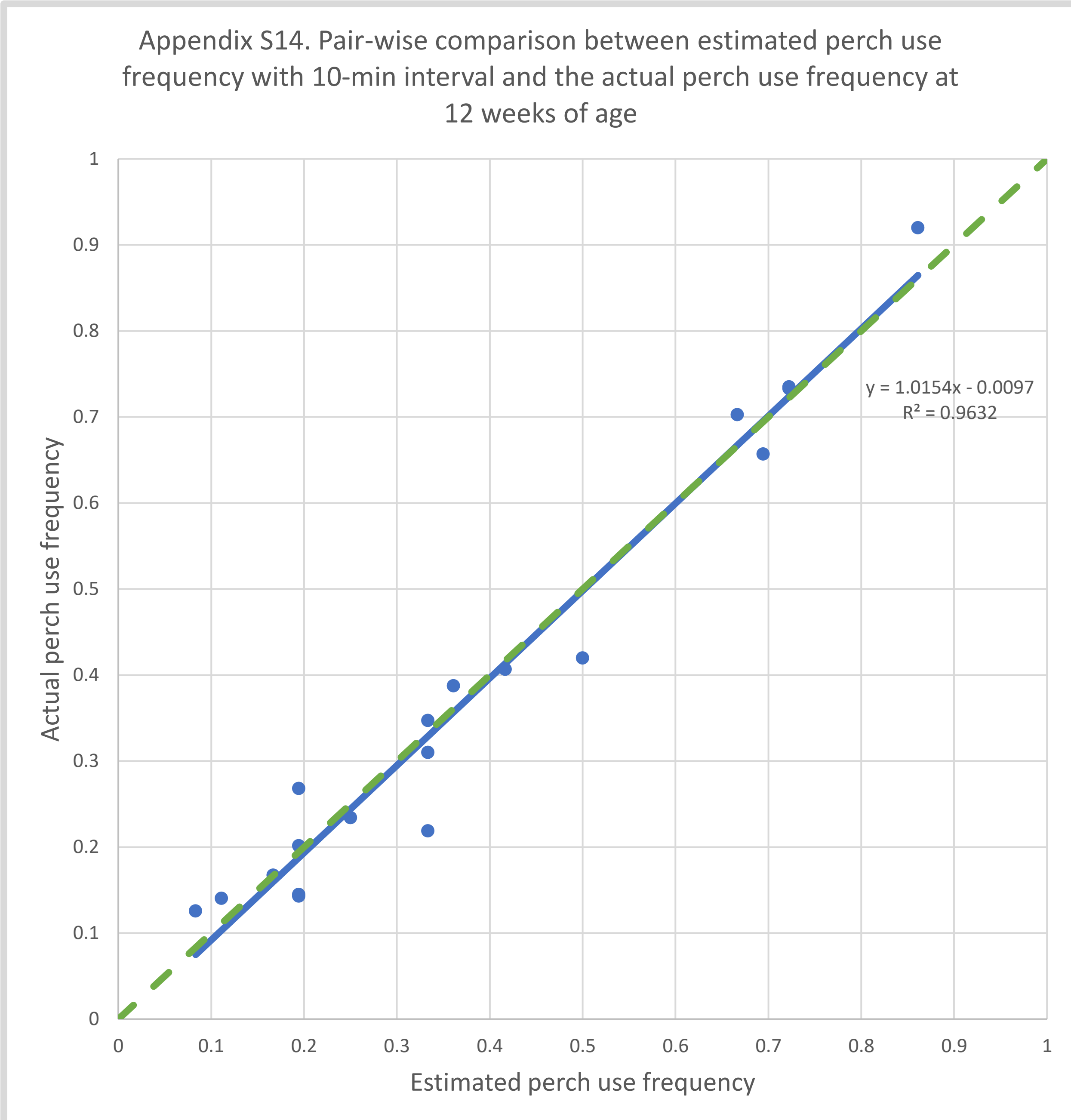

Appendix S10. Pair-wise comparison between estimated ground use frequency with 15-min interval and the actual ground use frequency at 6 weeks of age
Actual ground use frequency
Estimated ground use frequency
y = 0.8914x + 0.0288
R² = 0.9556
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1



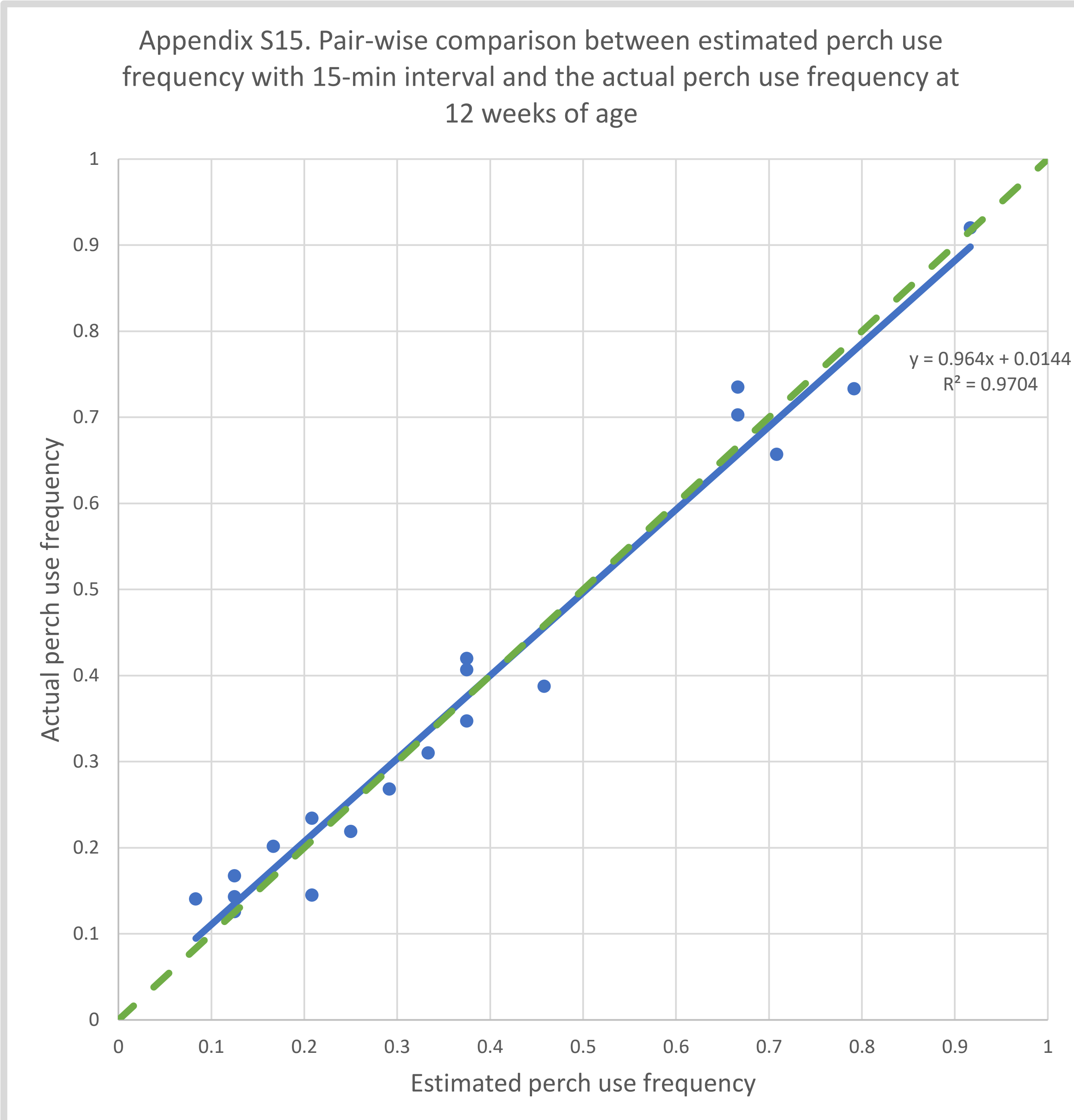

*Appendix S7-11. Pair-wise comparison between estimated ground use frequency using tested intervals (1, 5, 10, 15 and 30 min) and the actual ground use frequency derived from 1-s samples at 6 weeks of age. Green dash line: reference line indicating a 1:1 alignment between data from the 1-s sampling and tested intervals. Blue solid line: actual relationship between the results of 1-s samples and the tested intervals.*


Appendix S12. Pair-wise comparison between estimated perch use frequency with 1-min interval and the actual perch use frequency at 12 weeks of age
y = 0.993x + 0.0022
R² = 0.9994
Actual perch use frequency
Estimated perch use frequency
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1


Appendix S13. Pair-wise comparison between estimated perch use frequency with 5-min interval and the actual perch use frequency at 12 weeks of age
y = 0.9966x + 0.0012
R² = 0.9906
Actual perch use frequency
Estimated perch use frequency
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

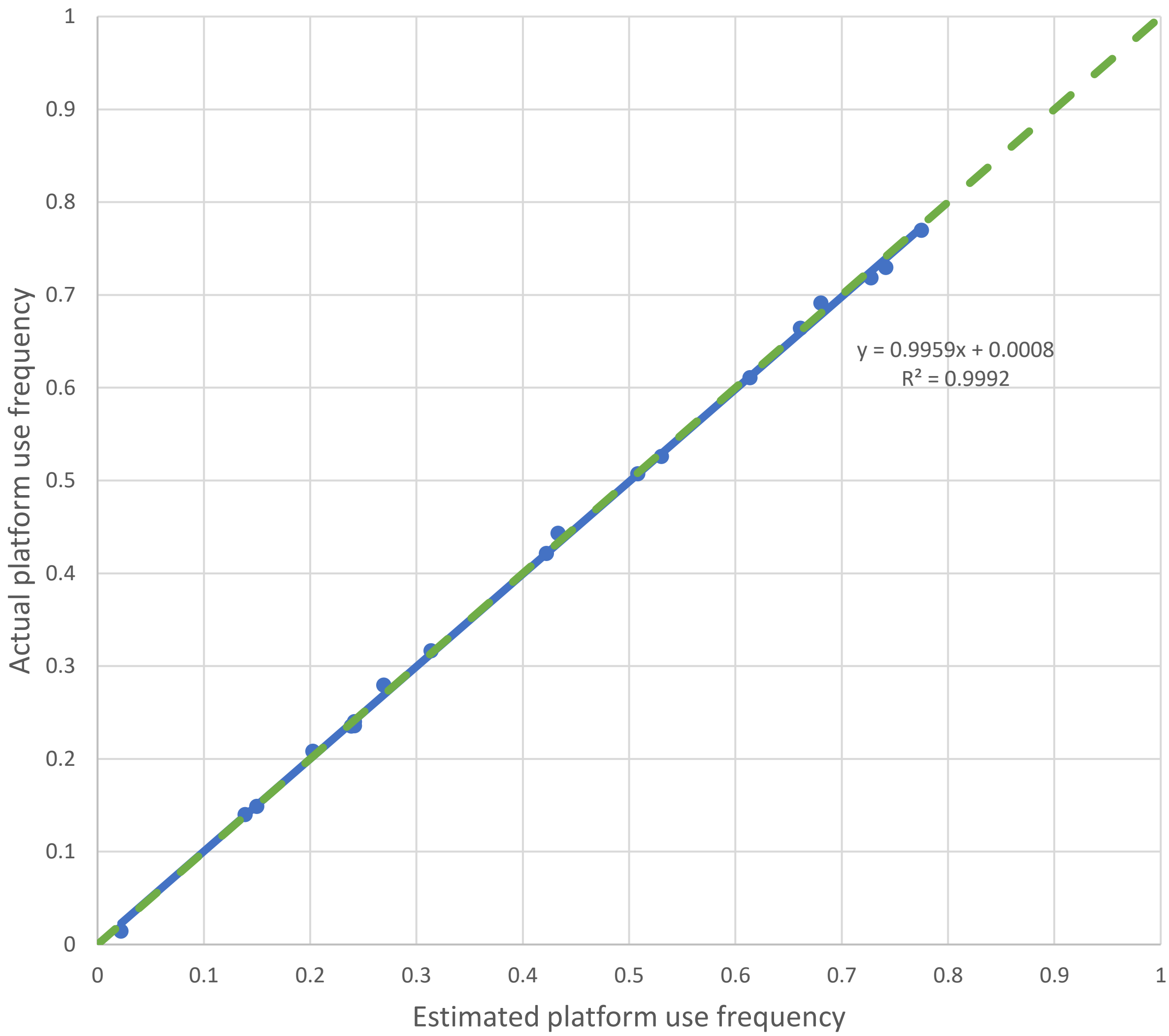


Appendix S14. Pair-wise comparison between estimated perch use frequency with 10-min interval and the actual perch use frequency at 12 weeks of age
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Actual perch use frequency
Estimated perch use frequency
y = 1.0154x - 0.0097
R² = 0.9632

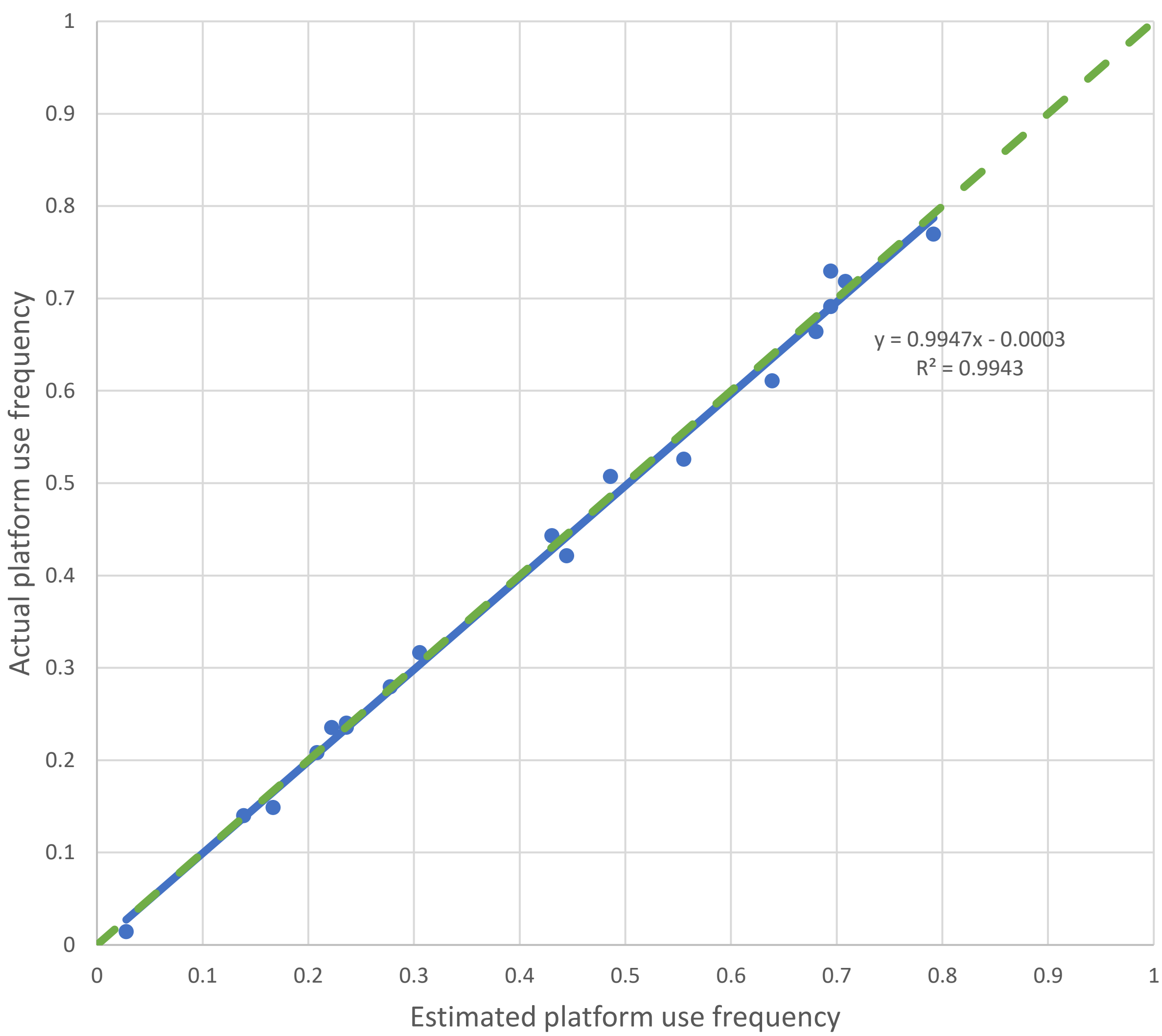


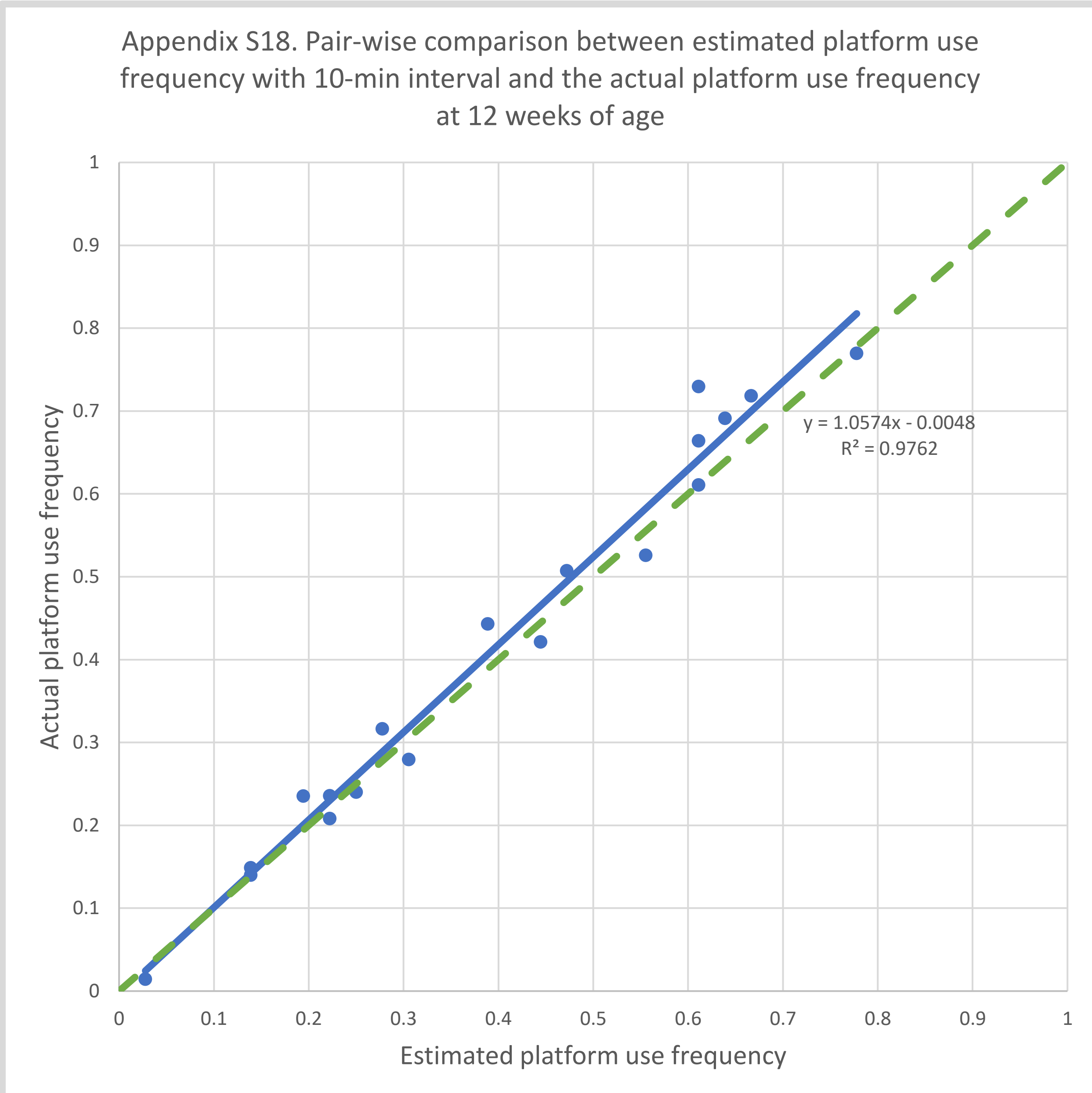

*Appendix S12-15. Pair-wise comparison between estimated perch use frequency using tested intervals (1, 5, 10 and 15 min) and the actual perch use frequency derived from 1-s samples at 12 weeks of age. Green dash line: reference line indicating a 1:1 alignment between data from the 1-s sampling and tested intervals. Blue solid line: actual relationship between the results of 1-s samples and the tested intervals.*

Appendix S16. Pair-wise comparison between estimated platform use frequency with 1-min interval and the actual platform use frequency at 12 weeks of age

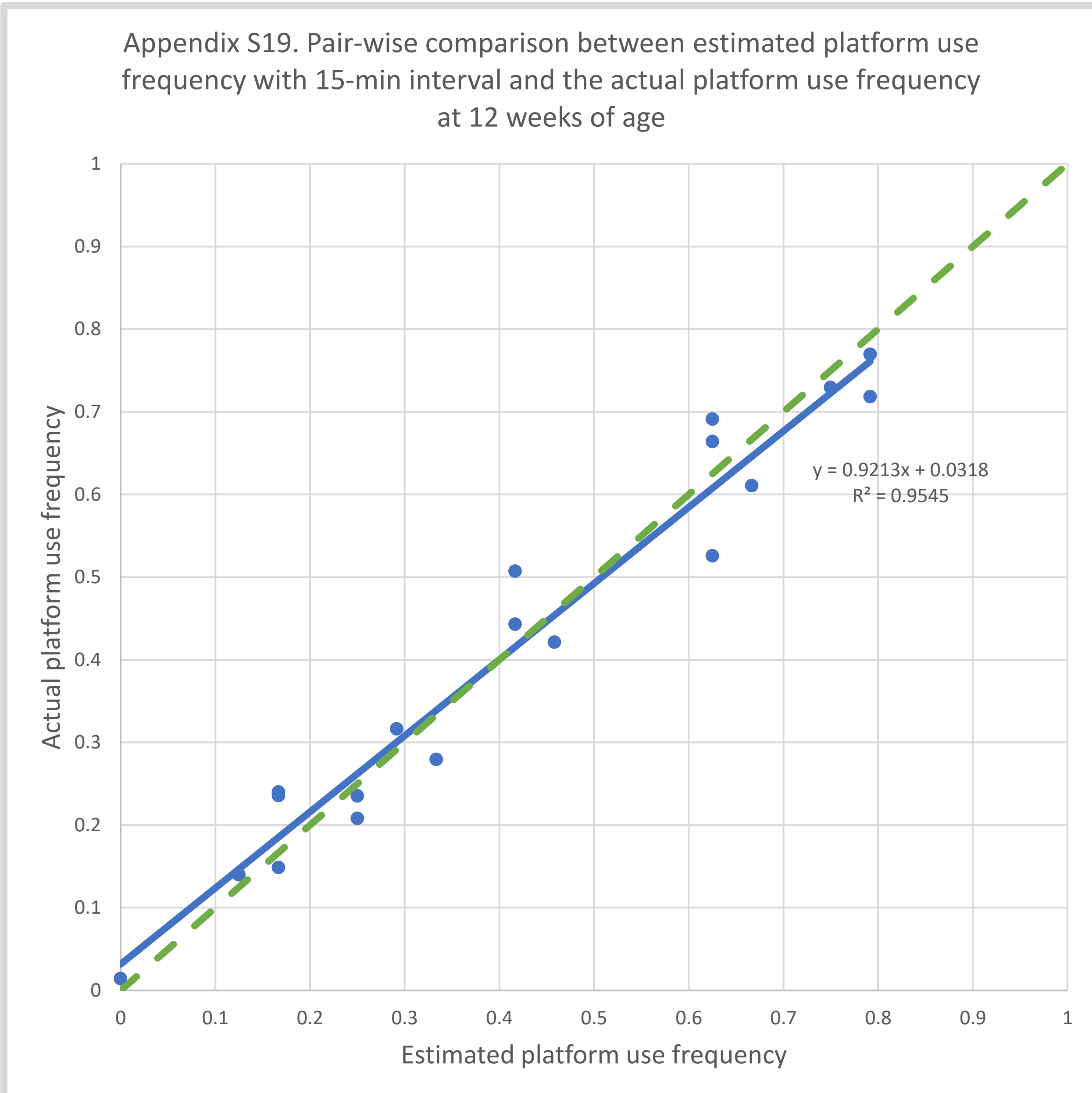


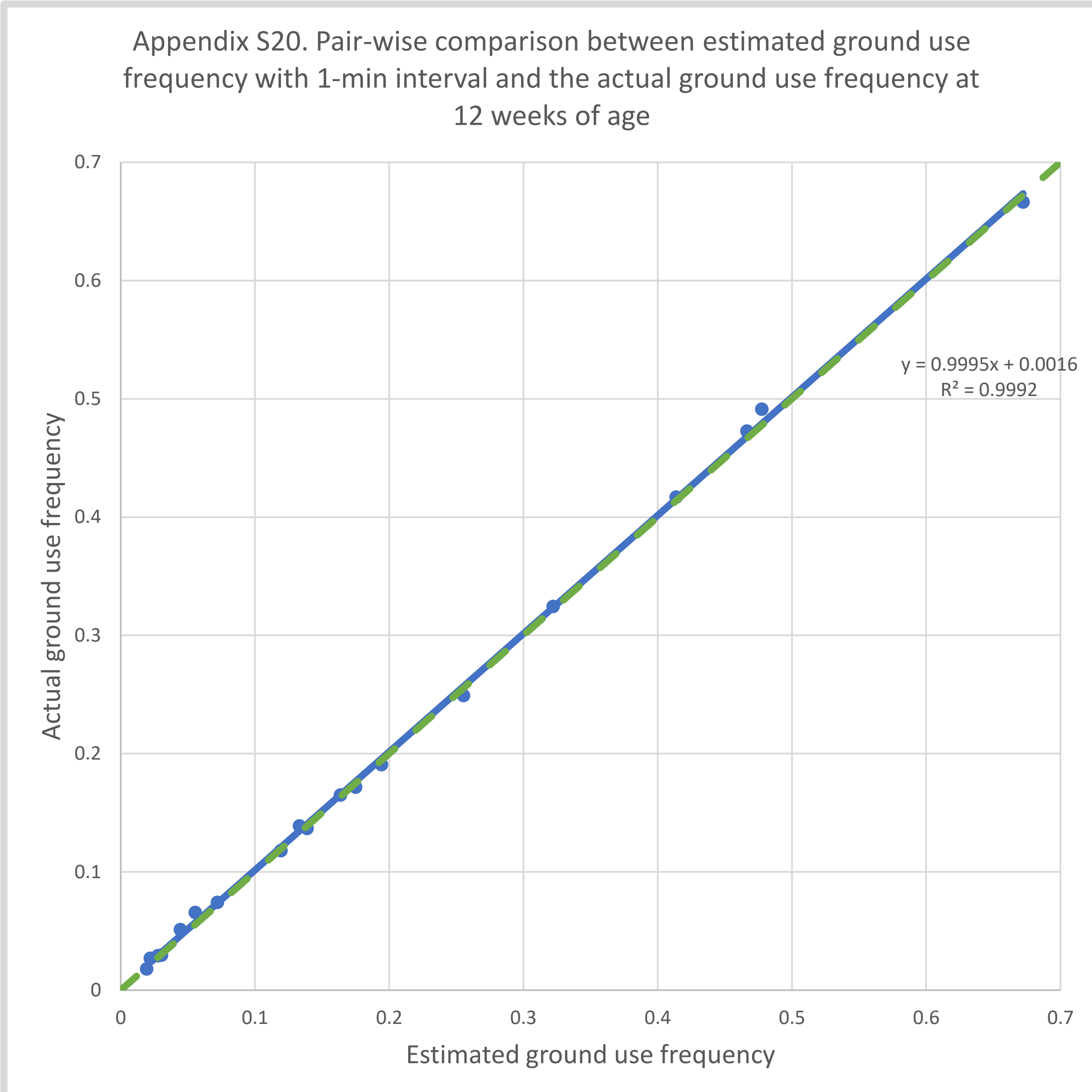
Appendix S17. Pair-wise comparison between estimated platform use frequency with 5-min interval and the actual platform use frequency at 12 weeks of age

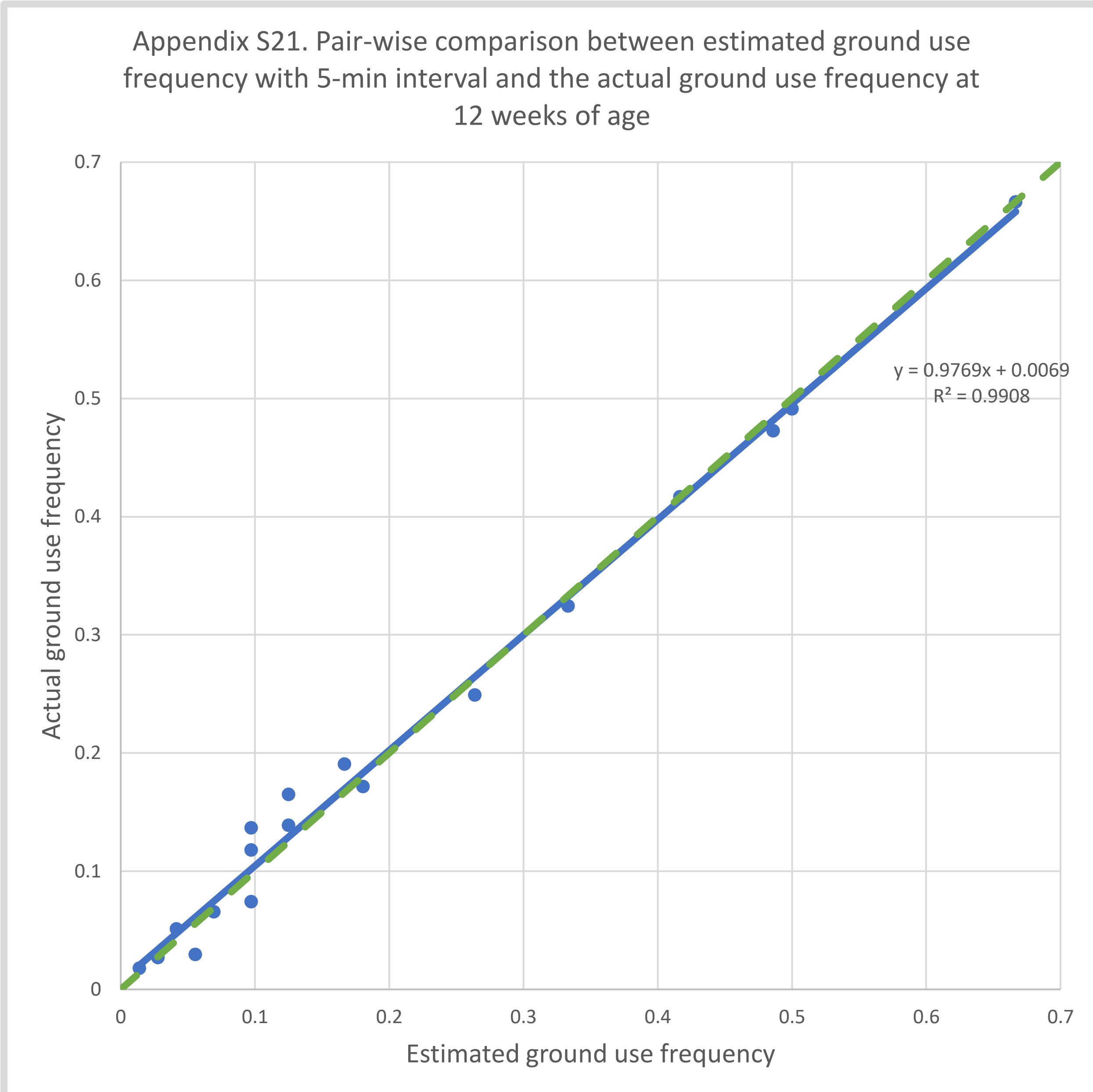


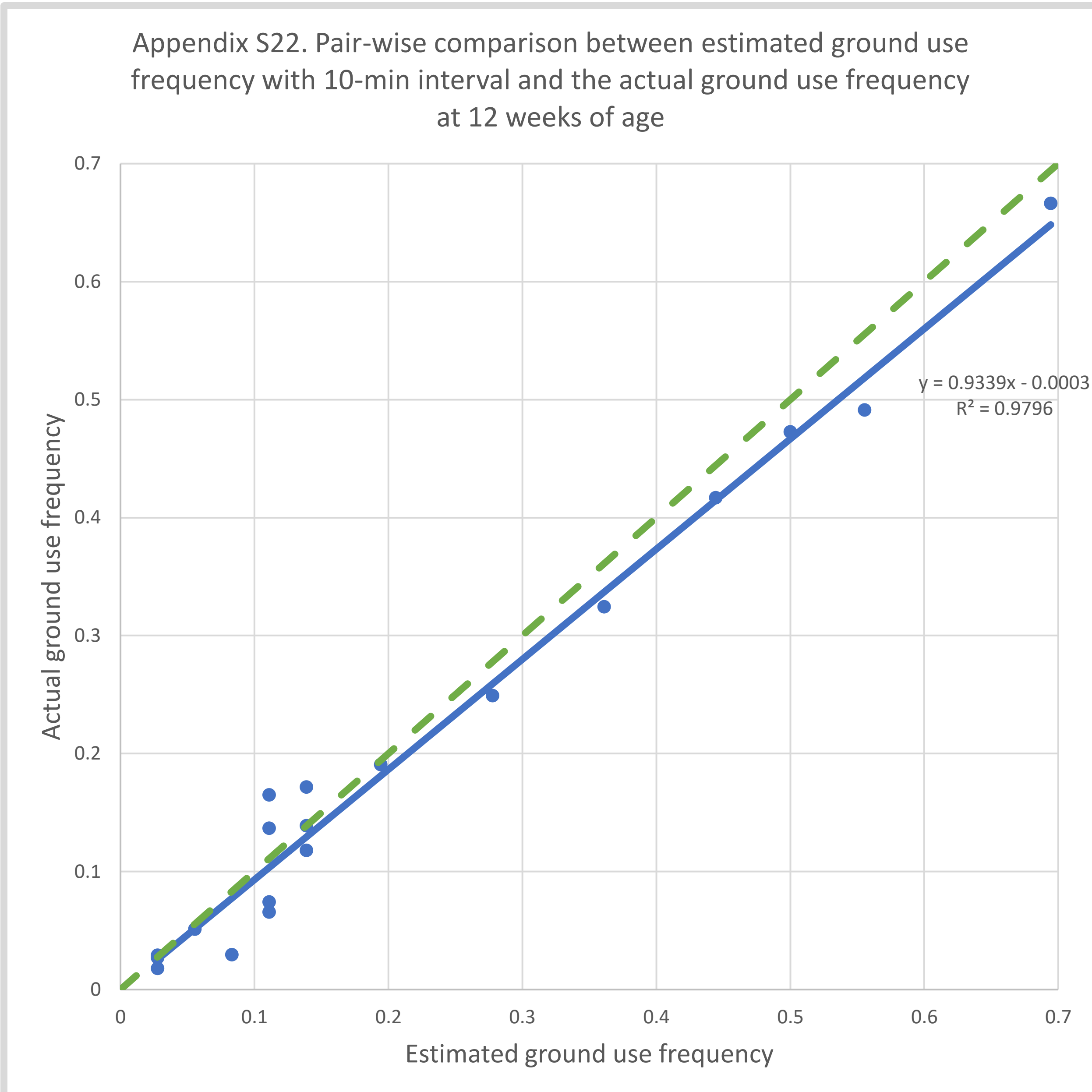



*Appendix S16-19. Pair-wise comparison between estimated platform use frequency using tested intervals (1, 5, 10 and 15 min) and the actual platform use frequency derived from 1-s samples at 12 weeks of age. Green dash line: reference line indicating a 1:1 alignment between data from the 1-s sampling and tested intervals. Blue solid line: actual relationship between the results of 1-s samples and the tested intervals.*


Appendix S19. Pair-wise comparison between estimated platform use frequency with 15-min interval and the actual platform use frequency at 12 weeks of age
Actual platform use frequency
Estimated platform use frequency
y = 0.9213x + 0.0318
R² = 0.9545
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1


Appendix S20. Pair-wise comparison between estimated ground use frequency with 1-min interval and the actual ground use frequency at 12 weeks of age
y = 0.9995x + 0.0016
R² = 0.9992
Actual ground use frequency
Estimated ground use frequency
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7


Appendix S21. Pair-wise comparison between estimated ground use frequency with 5-min interval and the actual ground use frequency at 12 weeks of age
y = 0.9769x + 0.0069
R² = 0.9908
Actual ground use frequency
Estimated ground use frequency
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7




*Appendix S20-22. Pair-wise comparison between estimated ground use frequency using tested intervals (1, 5 and 10 min) and the actual ground use frequency derived from 1-s samples at 12 weeks of age. Green dash line: reference line indicating a 1:1 alignment between data from the 1-s sampling and tested intervals. Blue solid line: actual relationship between the results of 1-s samples and the tested intervals.*