Supporting Information

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Intrapopulation variability in the timing of ontogenetic habitat shifts in sea turtles revealed using δ^{15}N values from bone growth rings

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Appendix S1: Methods

Sample collection

All research activities and permits were authorized by the Mexican government through SEMARNAP and SEMARNAT permits 150496-213-03, 280597-213-03, 190698-213-03, 280499-213-03, 280700-213-03, SGPA/DGVS/002 4661, SGPA/DGVS/10358, SGPA/DGVS/03501/06, SGPA/DGVS/03406/07, SGPA/DGVS/03481/09, SGPA/DGVS/04990/10, and SGPA/DGVS/04568/11.

Two groups of bones were collected and sampled for different purposes, one to construct the regional isotope characterization, the second to track the location of individual turtles over multiple years. The first group of bones, collected only from the foraging hotspot in the eastern Pacific Ocean near the Baja California Peninsula (BCP; Fig. 1), was used to investigate stable N (δ^{15}N) isotope patterns of individual turtles by sequentially sampling distinct bone growth layers.

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A second group of samples was used to create a regional isotope characterization by using the $\delta^{15}N$ values of turtle bone at two specific locations, the neritic BCP and the oceanic central North Pacific (CNP). These samples will be referred to as "known-location" samples, and had multiple growth layers homogenized for analysis.

The humerus bones (n=47) collected from loggerheads to characterize the $\delta^{15}N$ values of the nearshore BCP habitat were collected from shoreline surveys of Playa San Lázaro (Fig. 1), Baja California Sur, Mexico, adjacent to the Gulf of Ulloa. This foraging area utilized by loggerheads in the eastern Pacific, near the BCP, is a productive upwelling region within the California Current Large Marine Ecosystem known for its high biodiversity (Etnoyer et al., 2006, Wingfield et al. 2011). To isotopically characterize the oceanic CNP habitat, humerus bones were collected from juvenile North Pacific loggerhead turtles captured as bycatch in the CNP. These bones were previously used in skeletochronology studies by Zug et al. (1995) and Turner Tomaszewicz et al. (2015a; see Zug et al. 1995 and Wetherall et al. 1993 for collection details).

Turtle body size (cm) was measured from the nuchal notch to the posterior marginal tip as either curved carapace length (CCL) or straightline carapace length (SCL; Wyneken 2001) which was converted to CCL. When we were unable to obtain a direct measure of CCL, we estimated it based on an animal's humerus diameter (see Turner Tomaszewicz et al. 2015a).

Skeletochronology

We used skeletochronology to estimate age at stranding for the 45 loggerhead turtles whose bones were collected from the BCP and used for sequential SIA in the current study. The skeletochronology portion (aging) for these 45 bones had been conducted in a previous study (Turner Tomaszewicz et al. 2015a). These humerus bones were cross-sectioned and two 3-mm sections removed, one for sequential SI sampling and one for skeletochronology processing, following the procedures described in Turner Tomaszewicz et al. (2015b, 2016). Skeletochronology was applied to identify and measure annual growth layers separated by distinct lines of arrested growth (LAGs; Goshe et al. 2009, Avens and Snover 2013). Date (year), body size (CCL, cm), and age estimates were assigned to each growth layer following Snover et al. (2007) and Avens et al. (2013). Date and body size estimates were based on year of- and size at- stranding that corresponded to the outermost bone annual growth layer, and each subsequent inner layer was assigned a year and size estimate accordingly. We back-calculated body size estimates at each LAG as described in Snover et al. (2007) by applying the body proportional hypothesis (BPH)-corrected allometric equation (see below). To make the estimates more specific to North Pacific loggerheads, we used the minimum hatchling carapace length (4 cm) from Nishimura (1967, presented in Dodd 1988) for North Pacific loggerheads. There are no reliable data on minimum hatchling humerus diameter for North Pacific loggerheads, so we used the minimum hatchling humerus diameter data (1.5mm; min HD) from Northwestern Atlantic loggerheads (Zug et al. 1983, Zug 1986) for our estimates. The expected variation of this hatchling humerus diameter is minimal.

Stable isotope analysis

The 12 CNP and 47 BCP known-location bones used to create the regional isotope characterization were processed in a different method from the bones processed for sequential sampling. First, multiple outer (recent) growth layers were homogenized for stable isotope analysis. Second, the sequentially sampled bones were not processed at all prior to SI preparation and analysis, whereas the processing for the other bones for SIA included chemical treatments that separate organic and inorganic ^{13}C (Newsome et al. 2006, Post et al. 2007). These samples were demineralized with 0.5 M HCl for ~12-15 hours, rinsed with deionized-water to neutrality, then lipid extracted in a 2:1 chloroform/methanol mixture, then lyophilized. Approximately 1-mg of the demineralized, lipid extracted, and dried samples were weighed for stable isotope analysis. These preparation methods can affect carbon stable isotope values ($\delta^{13}C$) but not the $\delta^{15}N$ values (Post et al. 2007, Schlacher and Connolly 2014, Medeiros et al. 2015, Turner Tomaszewicz et al. 2015b). Therefore, all analysis in the current study used the $\delta^{15}N$ values from samples that were not pretreated.

Bone samples were analyzed for their $\delta^{15}N$ values by combustion in a Carlo Erba NA 1500 CNS elemental analyzer interfaced via a ConFlow II device to a Thermo Electron DeltaV Advantage isotope ratio mass spectrometer in the Stable Isotope Geochemistry Lab at the University of Florida, Gainesville. A conventional delta (δ) notation in parts per thousand or permil (‰) was used to express the stable isotope ratios of the samples relative to the isotope standards:

$$\delta X = ([R_{sample}/R_{standard}] - 1),$$

where the corresponding ratios of heavy to light isotopes ($^{15}N/^{14}N$) in the sample and standard are represented by R_{sample} and $R_{standard}$, respectively. In this analysis $R_{standard}$ for $\delta^{15}N$ was atmospheric N_2, and laboratory reference materials (USGS40) were calibrated at regular intervals against the standards. The average precision for these data was determined using the standard deviations around the means for the internal laboratory standards run at set intervals and was 0.08 ‰ for $\delta^{15}N$.

Back-calculating size estimates

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Carapace lengths at each LAG were estimated using the methods presented by Snover et al. (2007) Eqn 7: $L_i = [L_{op} + 1.96\ (D_i - D_{op})^{1.10}](L_{final})[L_{op} + 1.96\ (D_{final} - D_{op})^{1.10}]^{-1}$

where L_i is carapace length (cm) of the turtle at LAG i, D_i is the diameter (mm) of LAG i, L_{op} is the minimum carapace length (cm) of North Pacific loggerhead turtles at hatching, D_{op} is the minimum diameter (mm) of Northwestern Atlantic loggerhead humeri at hatching, L_{final} is the final carapace length (cm), and D_{final} is the final humerus diameter (mm). Based on the analysis of n=157 samples in Turner Tomaszewicz et al. (2015a), we applied the size back calculation protocol with slope b_1 = 1.956 and proportionality coefficient c_1 = 1.096. Assumptions specific to North Pacific loggerheads were minimum hatchling length (L_{op}) = 4 cm (Dodd 1988, from Nishimura 1967), and, from Northwestern Atlantic loggerheads, minimum hatchling HD (D_{op}) = 1.5 mm (Zug et al. 1983, Zug 1986).

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Fig. S1 – Stable nitrogen isotope values (δ^{15}N, ‰) from all 273 SIA sampled growth layers from 45 different loggerhead bones, aligned with corresponding estimated body sizes (curved carapace length, CCL, cm). Regression line with 95% confidence intervals (grey shading), shows significant positive correlation ($p<0.0001$, $F_{256} = 152.1$). Calculations conducted in R, using ggplot, geom_smooth(method=lm).

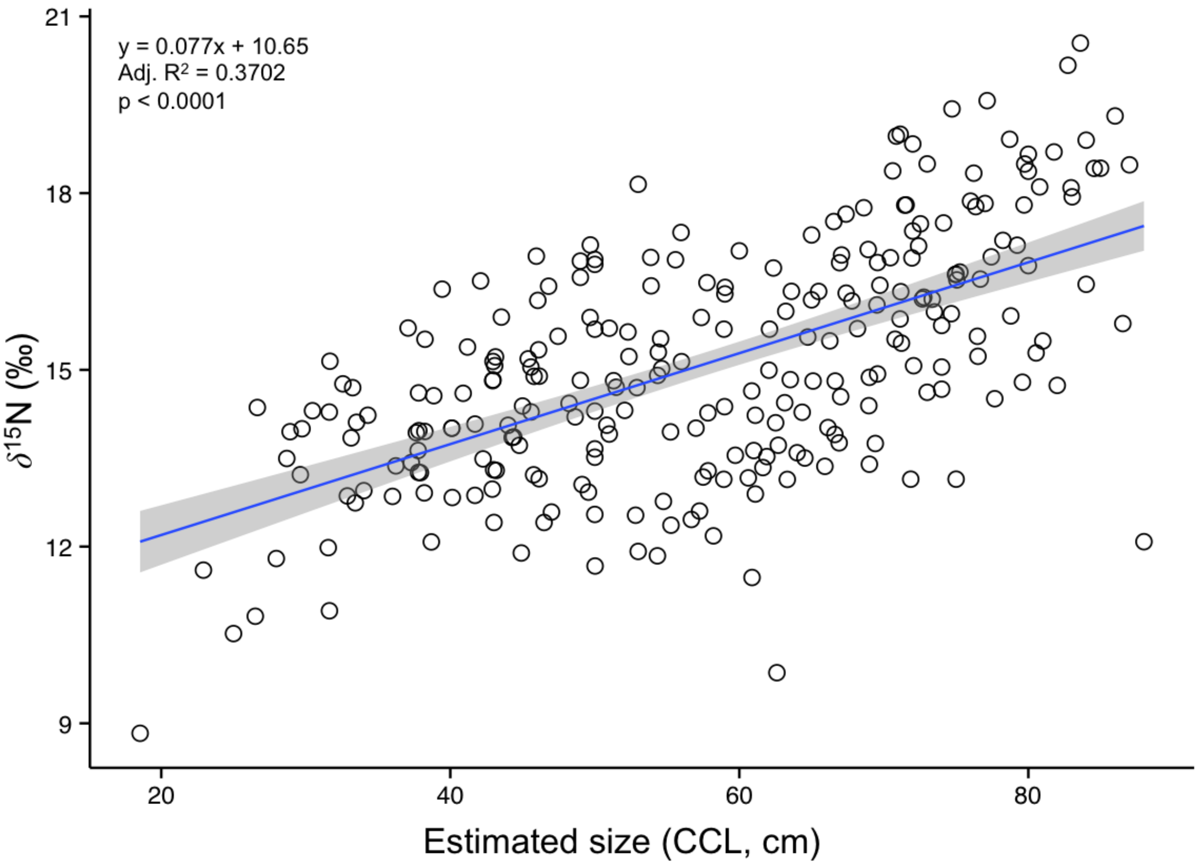

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Fig 2S –The stable nitrogen isotope ($\delta^{15}N$) values (‰), with corresponding estimated body size (curved carapace length, CCL, cm) of each annual growth layer sampled (n=45 turtles). Gray shading indicates the 33 turtles for which the transition year was identified based on the $\delta^{15}N_{HS}$ value shown by the black horizontal line (see text and Fig. 1c for details).

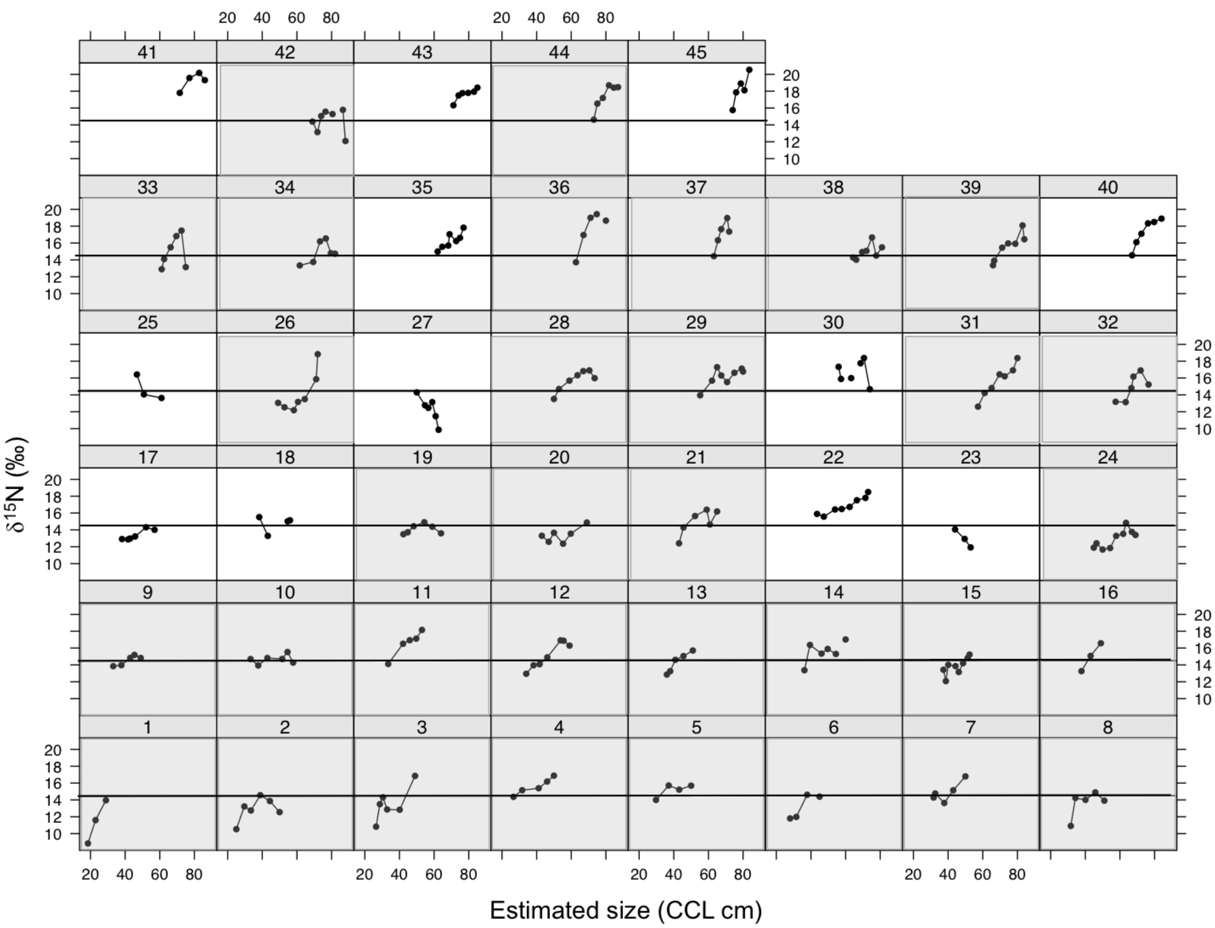

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Fig. S3 – Mean annual growth in carapace length (cm), based on differences measured between annual growth layer increments, as described in Snover et al. (2010), in relation to timing of recruitment to the BCP for 33 individual turtles. Standard error noted in bars, and sample size of increment growth shown for each year as the value above error bars. Recruitment year, noted as positive values on the x-axis, was determined by the $\delta^{15}N_{HS}$ value described in text, and 0 and negative values indicate years spent in the CNP and pelagic habitats, prior to recruitment to the BCP.

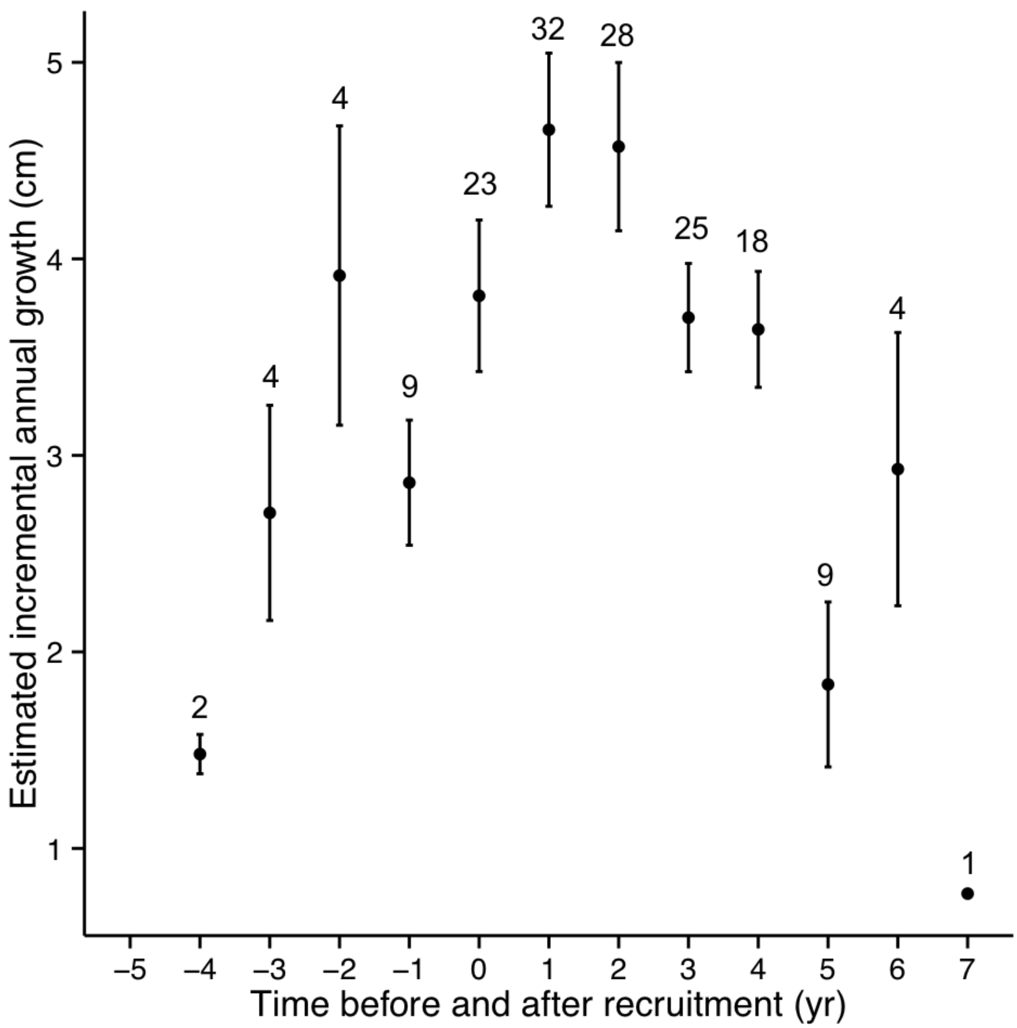

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Table S1 – Stable nitrogen isotope values ($\delta^{15}N$, ‰), percent N (%N), and estimated body size (CCL, cm) and estimated age for all 273 growth layers sampled from all 45 turtles.

(See attached).

Turtle Number	LAG ID	d15N	%N	Estimated CCL (cm)	Estimated Age
1	LAG_A	9	4.16	18.5	0
1	LAG_B	12	4.27	22.9	1
1	LAG_C	14	7.26	28.9	2
1	LAG_D	NA	NA	30.0	3
2	LAG_A	11	4.42	25.0	4
2	LAG_B	13	4.28	29.6	5
2	LAG_C	13	4.43	33.4	6
2	LAG_D	15	4.58	38.9	7
2	LAG_E	14	5.26	44.4	8
2	LAG_F	13	6.50	50.0	9
3	LAG_A	11	4.60	26.5	4
3	LAG_B	13	4.33	28.7	5
3	LAG_C	14	4.18	30.5	6
3	LAG_D	13	4.38	32.9	7
3	LAG_E	13	4.81	40.1	8
3	LAG_F	17	7.64	49.0	9
4	LAG_A	14	4.46	26.6	4
4	LAG_B	15	4.31	31.7	5
4	LAG_C	15	4.41	41.2	6
4	LAG_D	16	4.43	46.1	7
4	LAG_E	17	5.14	50.0	8
5	LAG_A	NA	NA	27.5	3
5	LAG_B	14	4.12	29.7	4
5	LAG_C	16	4.31	37.1	5
5	LAG_D	15	4.70	43.1	6
5	LAG_E	16	5.83	50.0	7
6	LAG_A	12	4.43	28.0	4
6	LAG_B	12	4.68	31.6	5
6	LAG_C	15	4.74	37.8	6
6	LAG_D	14	5.57	45.0	7
7	LAG_A	14	4.36	31.6	5
7	LAG_B	15	3.99	32.6	6
7	LAG_C	14	4.63	37.8	7
7	LAG_D	15	4.80	43.0	8
7	LAG_E	17	4.88	50.0	9
8	LAG_A	11	4.11	31.6	4
8	LAG_B	14	4.32	34.3	5
8	LAG_C	14	4.18	40.1	6
8	LAG_D	15	4.56	45.8	7
8	LAG_E	14	5.32	51.0	8
9	LAG_A	14	4.21	33.1	6
9	LAG_B	14	4.15	37.8	7

9	LAG_C	15	4.10	43.0	8
9	LAG_D	15	4.85	45.4	9
9	LAG_E	15	7.83	49.0	10
10	LAG_A	15	4.23	33.2	5
10	LAG_B	14	4.34	37.6	6
10	LAG_C	15	4.51	42.9	7
10	LAG_D	15	4.75	51.5	8
10	LAG_E	16	4.89	54.6	9
10	LAG_F	14	5.44	57.8	10
10	LAG_G	NA	NA	58.0	11
11	LAG_A	14	4.00	33.5	4
11	LAG_B	17	4.04	42.1	5
11	LAG_C	17	4.23	46.0	6
11	LAG_D	17	4.63	49.7	7
11	LAG_E	18	5.87	53.0	8
12	LAG_A	13	4.13	34.0	6
12	LAG_B	14	4.02	38.2	7
12	LAG_C	14	4.19	41.7	8
12	LAG_D	15	4.28	46.2	9
12	LAG_E	17	4.54	53.9	10
12	LAG_F	17	4.40	55.6	11
12	LAG_G	16	4.82	59.0	12
13	LAG_A	13	4.04	36.0	6
13	LAG_B	13	4.16	37.9	7
13	LAG_C	15	4.31	40.9	8
13	LAG_D	15	4.61	45.6	9
13	LAG_E	16	5.15	51.0	10
14	LAG_A	13	3.91	36.2	5
14	LAG_B	16	4.02	39.4	6
14	LAG_C	15	4.05	46.1	7
14	LAG_D	16	4.23	49.7	8
14	LAG_E	15	4.48	54.4	9
14	LAG_F	NA	NA	55.2	10
14	LAG_G	17	6.91	60.0	11
15	LAG_A	13	3.94	37.3	7
15	LAG_B	12	4.37	38.7	8
15	LAG_C	14	4.08	40.1	9
15	LAG_D	14	4.22	44.3	10
15	LAG_E	13	4.34	46.1	11
15	LAG_F	14	4.49	48.6	12
15	LAG_G	15	5.01	51.3	13
15	LAG_H	15	6.03	52.4	14
15	LAG_I	NA	NA	53.0	15

15	LAG_J	NA	NA	53.0	16
16	LAG_A	13	4.48	37.8	5
16	LAG_B	15	4.50	43.1	6
16	LAG_C	17	4.83	49.0	7
17	LAG_A	13	4.26	38.2	7
17	LAG_B	13	4.53	41.7	8
17	LAG_C	13	4.53	42.9	9
17	LAG_D	13	4.60	45.8	10
17	LAG_E	14	4.61	52.1	11
17	LAG_F	14	5.98	57.0	12
18	LAG_A	16	4.12	38.2	7
18	LAG_B	13	4.55	43.2	8
18	LAG_C	NA	4.61	50.8	9
18	LAG_D	15	6.05	54.6	10
18	LAG_E	15	9.15	56.0	11
19	LAG_A	13	4.08	42.3	7
19	LAG_B	14	3.99	44.8	8
19	LAG_C	14	4.56	48.2	9
19	LAG_D	15	4.23	54.3	10
19	LAG_E	14	4.86	59.0	11
19	LAG_F	14	5.27	64.0	12
20	LAG_A	13	4.14	43.0	10
20	LAG_B	13	4.22	47.0	11
20	LAG_C	14	4.10	50.0	12
20	LAG_D	12	4.71	55.3	13
20	LAG_E	14	4.35	59.7	14
20	LAG_F	15	4.82	69.0	15
21	LAG_A	12	4.55	43.0	8
21	LAG_B	14	4.21	45.6	9
21	LAG_C	16	4.28	52.3	10
21	LAG_D	16	4.26	59.0	11
21	LAG_E	15	4.78	60.9	12
21	LAG_F	16	4.96	65.0	13
22	LAG_A	16	3.88	43.5	8
22	LAG_B	16	3.87	47.5	9
22	LAG_C	16	3.74	53.9	10
22	LAG_D	16	3.77	57.8	11
22	LAG_E	17	3.91	62.4	12
22	LAG_F	18	4.00	66.5	13
22	LAG_G	18	4.27	71.4	14
22	LAG_H	18	4.58	73.0	15
23	LAG_A	14	4.36	44.0	10
23	LAG_B	13	4.87	49.6	11

23	LAG_C	12	7.63	53.0	12
24	LAG_A	12	4.03	44.9	8
24	LAG_B	12	4.06	46.5	9
24	LAG_C	12	4.13	50.0	10
24	LAG_D	12	4.03	54.3	11
24	LAG_E	13	4.23	57.8	12
24	LAG_F	14	4.25	61.9	13
24	LAG_G	15	4.43	63.5	14
24	LAG_H	14	4.82	66.9	15
24	LAG_I	13	5.54	69.0	16
25	LAG_A	16	4.21	46.8	8
25	LAG_B	14	4.86	50.8	9
25	LAG_C	14	5.44	61.0	10
26	LAG_A	13	4.16	49.1	10
26	LAG_B	13	4.17	52.8	11
26	LAG_C	12	4.20	58.2	12
26	LAG_D	13	4.30	60.6	13
26	LAG_E	14	4.33	64.5	14
26	LAG_F	16	4.42	71.1	15
26	LAG_G	19	4.76	72.0	16
27	LAG_A	14	3.99	50.0	12
27	LAG_B	13	4.51	54.7	13
27	LAG_C	12	4.51	56.7	14
27	LAG_D	13	4.53	58.9	17
27	LAG_E	11	5.15	60.9	18
27	LAG_F	10	6.15	62.6	20
28	LAG_A	14	4.55	50.0	12
28	LAG_B	15	4.20	52.9	13
28	LAG_C	16	4.36	58.9	14
28	LAG_D	16	4.23	63.6	15
28	LAG_E	17	4.36	66.9	16
28	LAG_F	17	4.60	70.5	17
28	LAG_G	16	5.05	73.5	18
29	LAG_A	14	3.91	55.2	12
29	LAG_B	16	3.97	62.1	13
29	LAG_C	17	3.91	65.0	14
29	LAG_D	16	4.22	67.4	15
29	LAG_E	16	4.09	70.8	16
29	LAG_F	17	4.17	75.0	17
29	LAG_G	17	4.79	79.2	18
29	LAG_H	17	8.26	80.0	19
30	LAG_A	17	4.23	56.0	13
30	LAG_B	16	4.25	57.4	14

30	LAG_C	NA	4.13	60.2	15
30	LAG_D	16	4.24	63.2	16
30	LAG_E	NA	4.21	65.9	17
30	LAG_F	18	4.26	68.6	18
30	LAG_G	18	4.63	70.6	19
30	LAG_H	15	6.12	74.0	20
31	LAG_A	13	3.94	57.2	12
31	LAG_B	14	4.02	61.1	13
31	LAG_C	15	4.22	65.1	14
31	LAG_D	16	4.23	69.7	16
31	LAG_E	16	4.38	72.7	17
31	LAG_F	17	4.64	77.4	18
31	LAG_G	18	4.86	80.0	19
32	LAG_A	13	3.94	57.5	12
32	LAG_B	13	4.24	63.3	13
32	LAG_C	15	3.86	66.6	14
32	LAG_D	16	4.37	67.8	15
32	LAG_E	17	4.68	71.9	16
32	LAG_F	15	5.36	76.5	17
32	LAG_G	NA	NA	77.0	18
33	LAG_A	13	4.28	61.1	14
33	LAG_B	14	4.05	62.5	15
33	LAG_C	15	4.26	66.3	16
33	LAG_D	17	4.15	69.6	17
33	LAG_E	17	4.41	72.5	19
33	LAG_F	13	6.16	75.0	21
34	LAG_A	13	5.10	61.6	15
34	LAG_B	14	4.54	69.4	16
34	LAG_C	16	4.29	73.4	17
34	LAG_D	17	4.39	76.7	18
34	LAG_E	15	4.99	79.6	19
34	LAG_F	15	6.88	82.0	20
35	LAG_A	15	4.18	62.1	12
35	LAG_B	16	4.12	64.7	13
35	LAG_C	16	4.14	68.2	14
35	LAG_D	17	3.94	68.9	15
35	LAG_E	16	4.25	72.8	17
35	LAG_F	17	6.34	75.0	18
35	LAG_G	18	8.41	77.0	19
36	LAG_A	14	4.28	62.7	13
36	LAG_B	17	4.22	67.1	14
36	LAG_C	19	4.18	71.1	15
36	LAG_D	19	4.43	74.7	16

36	LAG_E	NA	NA	78.2	17
36	LAG_F	19	4.66	80.0	18
37	LAG_A	14	3.98	63.1	16
37	LAG_B	16	4.75	65.5	18
37	LAG_C	18	4.32	67.4	19
37	LAG_D	19	4.20	70.9	20
37	LAG_E	17	4.22	72.0	21
38	LAG_A	14	4.40	64.4	14
38	LAG_B	14	4.61	66.1	15
38	LAG_C	NA	NA	67.3	16
38	LAG_D	15	5.92	69.6	17
38	LAG_E	15	3.86	72.1	18
38	LAG_F	17	4.24	75.3	19
38	LAG_G	15	4.72	77.7	20
38	LAG_H	15	4.34	81.0	21
39	LAG_A	13	4.71	65.9	15
39	LAG_B	14	4.78	66.6	16
39	LAG_C	15	4.62	71.2	17
39	LAG_D	16	4.48	74.7	18
39	LAG_E	16	4.70	78.8	19
39	LAG_F	18	4.74	83.0	21
39	LAG_G	16	5.23	84.0	22
40	LAG_A	15	4.30	67.0	15
40	LAG_B	16	4.21	69.5	16
40	LAG_C	17	4.07	72.4	17
40	LAG_D	18	4.09	76.2	18
40	LAG_E	18	4.26	79.7	19
40	LAG_F	19	4.17	84.0	20
41	LAG_A	NA	NA	67.7	13
41	LAG_B	NA	NA	69.3	14
41	LAG_C	18	3.80	71.5	15
41	LAG_D	20	5.50	77.1	16
41	LAG_E	20	4.20	82.7	17
41	LAG_F	19	6.50	86.0	18
42	LAG_A	14	4.01	69.0	16
42	LAG_B	13	5.07	71.9	17
42	LAG_C	15	4.49	74.0	18
42	LAG_D	16	4.28	76.5	19
42	LAG_E	15	4.62	80.5	20
42	LAG_F	NA	NA	81.0	21
42	LAG_G	16	5.04	86.5	22
42	LAG_H	12	6.17	88.0	23
43	LAG_A	16	4.07	71.2	16

43	LAG_B	17	4.14	74.1	17
43	LAG_C	18	4.44	76.4	18
43	LAG_D	18	4.53	79.7	19
43	LAG_E	18	4.62	83.0	20
43	LAG_F	18	5.07	85.0	21
44	LAG_A	15	4.72	73.0	16
44	LAG_B	17	4.29	75.1	17
44	LAG_C	17	4.15	78.2	18
44	LAG_D	19	4.31	81.8	19
44	LAG_E	18	4.40	84.6	20
44	LAG_F	18	4.72	87.0	21
45	LAG_A	16	4.51	74.0	16
45	LAG_B	18	4.39	76.0	17
45	LAG_C	19	4.35	78.7	18
45	LAG_D	18	4.54	80.8	19
45	LAG_E	21	4.52	83.6	20