**Supplemental information and 2016 monitoring on the Buffer Validation Study for the use of imazamox to control Japanese eelgrass in Willapa Bay.**

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**Introduction**: A Buffer Validation Study was done in 2014 and 2015 in Willapa Bay to assess off-site impacts of imazamox on *Z. marina.* Results of this study are provided in reports by UW (Grue 2015) and Confluence (2015). While these reports addressed the regulatory needs of the Buffer Validation Study they did not address the potential long-term impact of imazamox on off-site *Z. marina* that was initially damaged by the herbicide. The objective of this study was to determine if the off-site *Z. marina* that was affected by imazamox in 2014 recovered in the years following treatment.

**Methods:**

*Treatment application:* Treatments were applied 5/18/14. Three ~ 5 ac plots were sprayed using an ATV with a 5 nozzle boom, using 11 oz of imazamox/ac. All three beds were treated in the same day. This was during the end of the low tide series. Treatments were delayed by 5 days from the ideal tide timing in order to accommodate the UW team in collecting *Z. marina* data prior to treatment. Unfortunately, this made for less than ideal spray conditions. There was not enough time for the site to go dry before treatment. Spraying had to start before the beds were completely drained off; at that time there was still a steady shallow 'sheet' flow of water moving off-site to the low end of the plots. The narrow aspect of these plots (~1000' long by 200' wide) amplified the potential for off-site impacts by focusing all the runoff from the 5 acres across and over an ~ 200' band at the lower end of the plots. We finished spraying the bottom end of each plot with less than 20 minutes before tidal inundation. Although unintentional, these treatment conditions represent ideal conditions to maximize the off-site runoff potential for imazamox to impact *Z. marina*. They do not represent what would occur under normal spray conditions as would be typically used by growers to treat their beds.

*Data collection:* The raw data collected by UW and Confluence in 2014 and 2015 respectively was pooled and sorted to determine which quadrats were most affected. Nineteen quadrats out of 135 total quadrats collected on the seaward edge were selected to be the most severely impacted, based on both stem density and percent cover. They averaged 45% and 27% reduction in percent cover and stem density, respectively. There were 5, 8, and 6 quadrats from plots 1, 2, and 3, respectively. Stem density and percent cover were assessed for these quadrats on May 18, 2016. The 2016 quadrats matched the exact 2014 locations. However, the 2015 quadrats were close (within 0.01 to 0.3m), but not always in the exact location of the 2014 and 2016 quadrats. In situations where they were > 0.3 m away, the data were not used in the analysis.

*Data analysis:* The null hypothesis is that affected plants recover, and there will be no long-term treatment effect. To test this null hypothesis, comparisons in percent cover and stem density were made over time with the 19 quadrats (See data in Table A2). Because we selected the mostly highly affected quadrats, the null hypothesis would be expected to be rejected for the first assessment (one month post-treatment). If plants recovered after that, then the null hypothesis would not be rejected in one and two years after treatment. Data were analyzed by ANOVA, with means of quadrats for each plot pooled, to provide for 3 replications per sample time. Comparisons were made between May 2014 vs. June 2014, May 2015 and May 2016. To account for a potential effect of the years, data were collected on the seaward side for the control plots (see data in Table A3) and analyzed similarly to the treated plots. Data are presented graphically for treated and untreated sites by box-whisker graphs.

An additional ANOVA analysis was done comparing only 2014 and 2015 data for just the bottom quadrats of the treated sites, which had > 20% reduction in percent cover one month after treatment (See data in Table A1). There were 44 quadrats that fit in this category.

**Results:** Two data sets were compared, one using the most seriously affected quadrats (19 out of 135) based on stem density, and the other using all quadrats (44 out of 135) with >20% reduction in percent cover. For the most seriously affected quadrats, based on stem density, there was no difference in percent cover or stem density one or two years after treatment compared to the initial quadrat data in May 2014 (Table 1). Average percent cover of the 19 most affected quadrats was 61% in May 2014. Following treatment it was reduced to 39% in June 2014, and then increased to 60% cover in May 2015 and 81% cover in May 2016. In contrast, stem density in these 19 quadrats declined from an initial density of 33 stems/0.25 $m^2$ before treatment to 23 stems/0.25 $m^2$ following treatment. There was no change in subsequent years. There was, however, no statistical difference for these quadrats between pre-treatment densities and densities 1 and 2 years after treatment. There was no difference in percent cover or stem density over time in the control sites, although percent cover was numerically higher in 2016 than in previous years (Table 2). These results are displayed graphically in Figure 1.

For all quadrats that showed >20% decline in percent cover between May and June of 2014, the percent cover completely recovered by 2015. Stem density data did not track with percent cover, and showed no initial decline after treatment (Table 3).

Table 1. Differences between pre-treatment and post-treatment percent cover and stem density in 2014, 2015 and 2016 on the quadrats with a mean decrease in stem density of 25% one month post-treatment.

| Date of assessment | *Z. marina* (% cover and density) with significance of change compared to May 2014 (P=)* | |
|---|---|---|
| | Percent cover | Stem density (#/0.25$m^2$) |
| May 2014 | 61 | 30.3 |
| June 2014 | 39 (P=0.12) | 22.8 (P= 0.005) |
| May 2015 | 60 (P=0.9) | 23.3 (P=0.48) |
| May 2016 | 81 (P=0.12) | 23.5 (P=0.28) |

*P= Probability of significant F test based on ANOVA from pooled quadrats for within each replication (N=3)

Table 2. Differences between the control plots in percent cover and stem density in 2014, 2015 and 2016 on the quadrats with a mean decrease in stem density of 25% one month post-treatment.

| Date of assessment | *Z. marina* (% cover and density) with significance of change compared to May 2014 (P=)* | |
|---|---|---|
| | Percent cover | Stem density (#/0.25 $m^2$) |
| May 2014 | 44 | 21.7 |
| June 2014 | 46 (P=0.65) | 19.6 (P= 0.12) |
| May 2015 | 60 (P=0.55) | 218.0 (P=0.33) |
| May 2016 | 80 (P=0.09) | 18.7 (P=0.16) |

*P= Probability of significant F test based on ANOVA from pooled quadrats within each replication (N=3)

| Table 3. Differences between pre-treatment and post-treatment percent cover and stem density in 2014 and 2015 on the quadrats with a mean decrease in percent cover one month post-treatment. | | |
|---|---|---|
| Date of assessment | *Z. marina* (% cover and density) with significance of change compared to May 2014(P=)* | |
| | Percent cover | Stem density (#/0.25 $m^2$) |
| May 2014 | 58 | 25.4 |
| June 2014 | 36 (P=0.001) | 23.4 (P= 0.2) |
| May 2015 | 59 (P=0.89) | 26.7 (P=0.14) |
| *P= Probability of significant F test based on ANOVA from pooled quadrats for within each replication (N=3) | | |

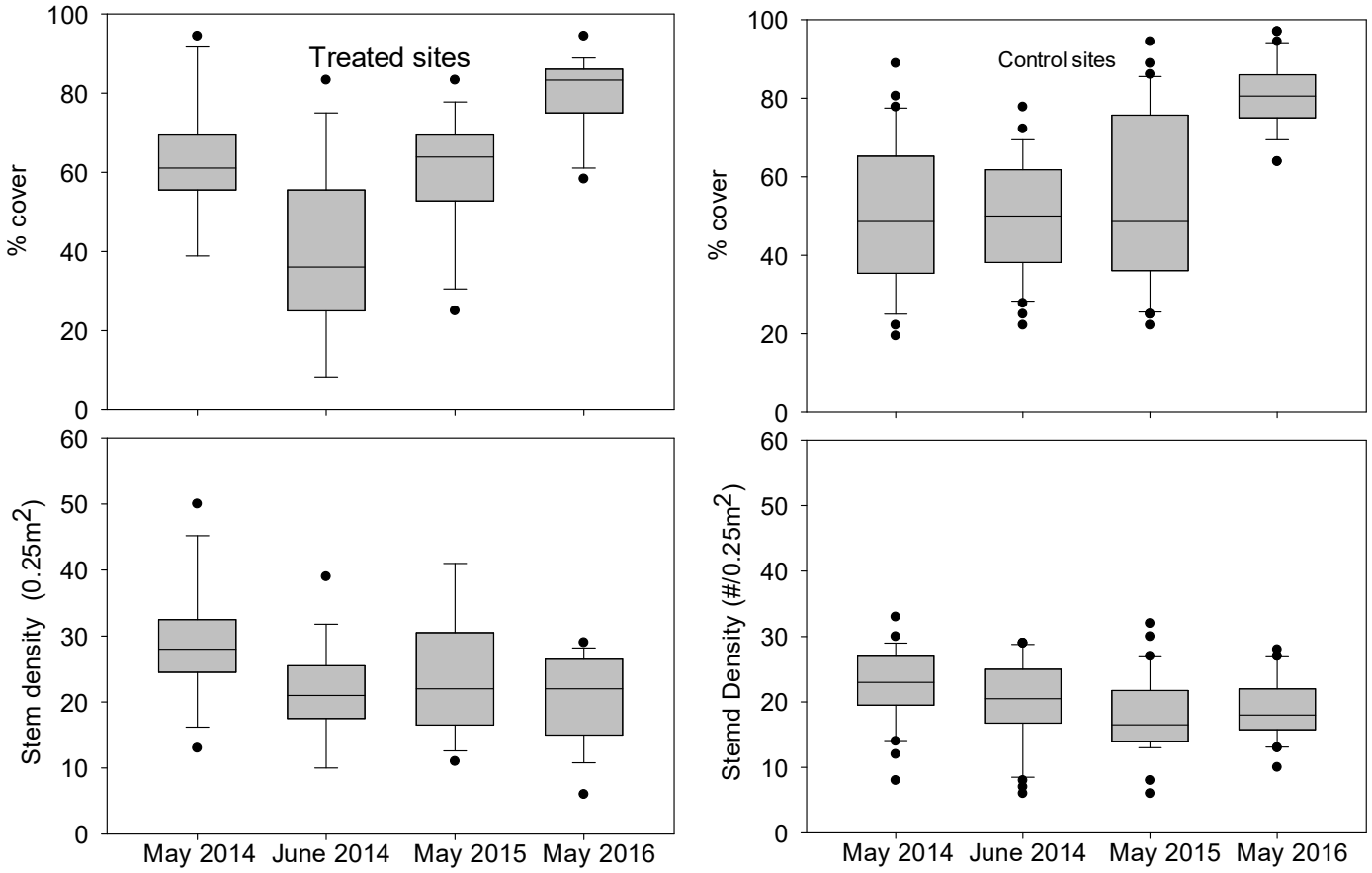


Figure 1. Box whisker graph of stem density and percent cover over time of the most affected *Z. marina* quadrats (top 14% based on stem density) in the treated site and in the control site.

**Discussion:**

The objective of this study was to assess if *Z. marina* that was affected by imazamox recovered following treatment. Only quadrats with the most severely affected eelgrass were selected to make this assessment. Based on the quadrats assessed in 2016, there were no difference in *Z. marina* percent cover or stem density between pre-treatment and one or two years post-treatment. Reduction in stem density one-month post-treatment did not translate to plant death of the affected plants, as suggested might occur by Grue (2015). Therefore it is unlikely that there would be any long-term impacts of imazamox on off-site *Z. ma*rina populations near treated sites in Willapa Bay.

The two previous reports by Grue (2015) and Confluence (2015) addressed statistical inferences about the validity of the buffer zone. Irrespective of the inferences from these two independent studies, this report would indicate minimal permanent impact of imazamox in zones where imazamox did traverse into the buffer zone. These "zones" of affected *Z. marina* were in the small drainage swales that flowed off-site through the buffer zone. In this particular study, applications were made under non-ideal conditions and this accentuated that level of imazamox moving off-site in the drainage swales. Hence, inferences made from this study or either of the buffer validation studies could be considered conservative. Another approach to validating the buffer zone is to trace the perimeters of beds sprayed by growers and compare the area treated to the area affected. This was done for 32 grower-treated sites in 2014 and 2015 (Patten and Norelius, 2016). The total area of off-site affected ground, based on impacted *Z. marina* or *Z. japonica*, was <0.6% of the total treated ground. In conclusion, the results of this study and our monitoring of grower-treated beds would suggest that buffers set in place by the NPDES are conservative enough to minimize off-site long-term impacts.

**Literature cited:**

Confluence Environmental Company. 2015. Impacts of Imazamox on Native Eelgrass Following Application to Control Exotic Eelgrass in Willapa Bay, Washington: An Evaluation of Buffer Width One Year Post-application. Prepared for: Plauché & Carr LLP, by N. Grant.

Grue, C. & L. Conquest. 2015. Impacts of native eelgrass following application to control exotic eelgrass in Willapa Bay Washington: an evaluation of buffer width. Univ. of WA.

Patten, K. and Norelius. 2016. 2015-2016. Progress report to Washington Department of Fish and Wildlife: Japanese Eelgrass Research in Willapa Bay. Washingon State University

## Appendix – data used in analysis

Table A1. 2014 and 2015 quadrat raw data with >20% reduction in percent cover.

| | | | | Percent cover | | | Stem density | | |
|---|---|---|---|---|---|---|---|---|---|
| Plot | Location | Transect | Quad # | May 2014 | June 2014 | June 2015 | May 2014 | June 2014 | June 2015 |
| 1 | Bottom | A | 1 | 24 | 19 | 23 | 27 | 26 | 24 |
| 1 | Bottom | A | 4 | 21 | 15 | 11 | 15 | 29 | 31 |
| 1 | Bottom | A | 8 | 16 | 8 | 28 | 29 | 25 | 25 |
| 1 | Bottom | A | 9 | 14 | 10 | 12 | 23 | 22 | 27 |
| 1 | Bottom | A | 13 | 15 | 8 | 17 | 24 | 20 | 19 |
| 1 | Bottom | A | 14 | 10 | 6 | 21 | 28 | 38 | 28 |
| 1 | Bottom | B | 17 | 13 | 10 | 9 | 19 | 17 | 21 |
| 1 | Bottom | B | 19 | 7 | 5 | 15 | 18 | 8 | 8 |
| 1 | Bottom | B | 20 | 9 | 6 | 20 | 28 | 19 | 15 |
| 1 | Bottom | B | 21 | 19 | 15 | 32 | 31 | 34 | 38 |
| 1 | Bottom | B | 24 | 20 | 11 | 21 | 26 | 31 | 35 |
| 1 | Bottom | B | 25 | 18 | 9 | 22 | 25 | 21 | 27 |
| 1 | Bottom | B | 26 | 13 | 9 | 21 | 27 | 18 | 21 |
| 1 | Bottom | B | 29 | 20 | 13 | 19 | 28 | 30 | 44 |
| 1 | Bottom | C | 33 | 23 | 18 | 24 | 28 | 39 | 50 |
| 1 | Bottom | C | 34 | 15 | 11 | 12 | 16 | 26 | 31 |
| 2 | Bottom | B | 25 | 26 | 20 | 23 | 18 | 24 | 31 |
| 2 | Bottom | B | 26 | 27 | 20 | 14 | 15 | 30 | 42 |
| 2 | Bottom | C | 38 | 24 | 15 | 32 | 27 | 27 | 34 |
| 2 | Bottom | C | 39 | 31 | 20 | 26 | 30 | 32 | 23 |
| 2 | Bottom | C | 40 | 31 | 23 | 22 | 32 | 27 | 31 |
| 2 | Bottom | C | 42 | 21 | 13 | 31 | 34 | 18 | 22 |
| 2 | Bottom | C | 43 | 24 | 18 | 21 | 27 | 25 | 23 |
| 3 | Bottom | A | 1 | 24 | 12 | 23 | 36 | 21 | 30 |
| 3 | Bottom | A | 2 | 25 | 10 | 27 | 29 | 25 | 30 |
| 3 | Bottom | A | 3 | 25 | 0 | 20 | 26 | 20 | 25 |
| 3 | Bottom | A | 4 | 26 | 3 | 19 | 22 | 22 | 27 |
| 3 | Bottom | A | 5 | 21 | 4 | 24 | 28 | 18 | 21 |
| 3 | Bottom | A | 6 | 20 | 3 | 11 | 17 | 10 | 17 |
| 3 | Bottom | A | 7 | 26 | 8 | 28 | 23 | 22 | 27 |
| 3 | Bottom | A | 8 | 14 | 4 | 24 | 21 | 20 | 23 |
| 3 | Bottom | A | 9 | 20 | 6 | 25 | 31 | 14 | 18 |
| 3 | Bottom | A | 10 | 22 | 3 | 33 | 33 | 20 | 30 |
| 3 | Bottom | A | 11 | 14 | 4 | 21 | 22 | 10 | 13 |
| 3 | Bottom | A | 12 | 25 | 13 | 18 | 23 | 18 | 16 |
| 3 | Bottom | A | 13 | 27 | 7 | 11 | 14 | 19 | 25 |
| 3 | Bottom | A | 14 | 18 | 11 | 19 | 32 | 21 | 28 |
| 3 | Bottom | A | 15 | 23 | 13 | 19 | 33 | 18 | 24 |
| 3 | Bottom | B | 18 | 26 | 19 | 36 | 35 | 17 | 16 |
| 3 | Bottom | B | 23 | 19 | 15 | 22 | 31 | 26 | 22 |
| 3 | Bottom | C | 33 | 16 | 11 | 17 | 30 | 18 | 20 |
| 3 | Bottom | C | 40 | 9 | 7 | 9 | 20 | 13 | 15 |
| 3 | Bottom | C | 43 | 15 | 12 | 7 | 12 | 22 | 26 |
| 3 | Bottom | C | 44 | 20 | 16 | 11 | 22 | 13 | 13 |
| 3 | Bottom | C | 45 | 28 | 22 | 18 | 25 | 34 | 37 |

Table A 2. 2014 and 2016 quadrat raw data for the control plots.

| Plot | Location | Tran-sect | Quad # | Percent cover | | | Stem density | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | May 2014 | June 2014 | May 2016 | May 2014 | June 2014 | May 2016 |
| 1 | Bottom | A | 3 | 16 | 27 | 29 | 18 | 22 | 20 |
| 1 | Bottom | A | 4 | 10 | 28 | 28 | 15 | 16 | 18 |
| 1 | Bottom | A | 5 | 9 | 24 | 23 | 21 | 21 | 10 |
| 1 | Bottom | A | 6 | 15 | 29 | 28 | 23 | 25 | 17 |
| 1 | Bottom | A | 7 | 11 | 28 | 25 | 27 | 20 | 18 |
| 1 | Bottom | A | 8 | 16 | 34 | 29 | 33 | 29 | 22 |
| 1 | Bottom | A | 9 | 13 | 31 | 30 | 22 | 24 | 24 |
| 1 | Bottom | A | 10 | 8 | 32 | 29 | 12 | 13 | 25 |
| 2 | Bottom | B | 16 | 25 | 17 | 30 | 24 | 23 | 20 |
| 2 | Bottom | B | 17 | 19 | 16 | 33 | 14 | 8 | 13 |
| 2 | Bottom | B | 18 | 19 | 14 | 31 | 29 | 18 | 26 |
| 2 | Bottom | B | 19 | 23 | 13 | 25 | 21 | 18 | 28 |
| 2 | Bottom | B | 20 | 32 | 13 | 34 | 26 | 21 | 21 |
| 2 | Bottom | B | 21 | 22 | 9 | 27 | 29 | 27 | 14 |
| 2 | Bottom | B | 22 | 25 | 15 | 33 | 27 | 23 | 27 |
| 2 | Bottom | B | 23 | 26 | 17 | 32 | 23 | 19 | 27 |
| 2 | Bottom | B | 24 | 29 | 20 | 27 | 26 | 25 | 21 |
| 2 | Bottom | B | 25 | 21 | 11 | 28 | 26 | 20 | 18 |
| 2 | Bottom | B | 26 | 22 | 9 | 29 | 28 | 29 | 13 |
| 2 | Bottom | B | 27 | 27 | 11 | 28 | 27 | 27 | 16 |
| 2 | Bottom | B | 28 | 23 | 14 | 29 | 30 | 29 | 17 |
| 2 | Bottom | B | 29 | 28 | 13 | 23 | 29 | 25 | 22 |
| 2 | Bottom | B | 30 | 22 | 16 | 26 | 21 | 16 | 14 |
| 3 | Bottom | A | 1 | 14 | 24 | 35 | 15 | 6 | 16 |
| 3 | Bottom | A | 2 | 12 | 25 | 31 | 26 | 17 | 15 |
| 3 | Bottom | A | 3 | 7 | 24 | 31 | 21 | 20 | 15 |
| 3 | Bottom | A | 4 | 9 | 18 | 27 | 8 | 7 | 18 |
| 3 | Bottom | A | 5 | 14 | 26 | 29 | 20 | 20 | 19 |
| 3 | Bottom | A | 6 | 13 | 8 | 35 | 15 | 14 | 21 |
| 3 | Bottom | A | 7 | 13 | 29 | 27 | 21 | 25 | 16 |
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| Table A 2. 2014 and 2016 quadrat raw data for the control plots. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Percent cover | | | Stem density | | |
| Plot | Location | Tran-sect | Quad # | May 2014 | June 2014 | May 2016 | May 2014 | June 2014 | May 2016 |
| 1 | Bottom | A | 3 | 16 | 27 | 29 | 18 | 22 | 20 |
| 1 | Bottom | A | 4 | 10 | 28 | 28 | 15 | 16 | 18 |
| 1 | Bottom | A | 5 | 9 | 24 | 23 | 21 | 21 | 10 |
| 1 | Bottom | A | 6 | 15 | 29 | 28 | 23 | 25 | 17 |
| 1 | Bottom | A | 7 | 11 | 28 | 25 | 27 | 20 | 18 |
| 1 | Bottom | A | 8 | 16 | 34 | 29 | 33 | 29 | 22 |
| 1 | Bottom | A | 9 | 13 | 31 | 30 | 22 | 24 | 24 |
| 1 | Bottom | A | 10 | 8 | 32 | 29 | 12 | 13 | 25 |
| 2 | Bottom | B | 16 | 25 | 17 | 30 | 24 | 23 | 20 |
| 2 | Bottom | B | 17 | 19 | 16 | 33 | 14 | 8 | 13 |
| 2 | Bottom | B | 18 | 19 | 14 | 31 | 29 | 18 | 26 |
| 2 | Bottom | B | 19 | 23 | 13 | 25 | 21 | 18 | 28 |
| 2 | Bottom | B | 20 | 32 | 13 | 34 | 26 | 21 | 21 |
| 2 | Bottom | B | 21 | 22 | 9 | 27 | 29 | 27 | 14 |
| 2 | Bottom | B | 22 | 25 | 15 | 33 | 27 | 23 | 27 |
| 2 | Bottom | B | 23 | 26 | 17 | 32 | 23 | 19 | 27 |
| 2 | Bottom | B | 24 | 29 | 20 | 27 | 26 | 25 | 21 |
| 2 | Bottom | B | 25 | 21 | 11 | 28 | 26 | 20 | 18 |
| 2 | Bottom | B | 26 | 22 | 9 | 29 | 28 | 29 | 13 |
| 2 | Bottom | B | 27 | 27 | 11 | 28 | 27 | 27 | 16 |
| 2 | Bottom | B | 28 | 23 | 14 | 29 | 30 | 29 | 17 |
| 2 | Bottom | B | 29 | 28 | 13 | 23 | 29 | 25 | 22 |
| 2 | Bottom | B | 30 | 22 | 16 | 26 | 21 | 16 | 14 |
| 3 | Bottom | A | 1 | 14 | 24 | 35 | 15 | 6 | 16 |
| 3 | Bottom | A | 2 | 12 | 25 | 31 | 26 | 17 | 15 |
| 3 | Bottom | A | 3 | 7 | 24 | 31 | 21 | 20 | 15 |
| 3 | Bottom | A | 4 | 9 | 18 | 27 | 8 | 7 | 18 |
| 3 | Bottom | A | 5 | 14 | 26 | 29 | 20 | 20 | 19 |
| 3 | Bottom | A | 6 | 13 | 8 | 35 | 15 | 14 | 21 |
| 3 | Bottom | A | 7 | 13 | 29 | 27 | 21 | 25 | 16 |

| Table A 3. 2014, 2015 and 2016 quadrat raw data with >25% reduction in percent stem density. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Percent cover | | | | Stem density | | | |
| Plot | Location | Tran-sect | Quad # | May 2014 | June 2014 | May 2015 | May 2016 | May 2014 | June 2014 | May 2015 | May 2016 |
| 1 | Bottom | A | 3 | 21 | 25 | 30 | 31 | 35 | 30 | 22 | 35 |
| 1 | Bottom | A | 9 | 14 | 10 | 27 | 28 | 27 | 22 | 13 | 31 |
| 1 | Bottom | B | 10 | 18 | 9 | 28 | 25 | 27 | 21 | 29 | 15 |
| 1 | Bottom | B | 14 | 20 | 13 | 15 | 30 | 44 | 30 | 18 | 24 |
| 1 | Bottom | C | 3 | 23 | 18 | 24 | 34 | 50 | 39 | 28 | 29 |
| 2 | Bottom | A | 8 | 33 | 27 | 13 | 30 | 39 | 35 | 18 | 17 |
| 2 | Bottom | A | 13 | 34 | 30 | 20 | 21 | 25 | 17 | 19 | 18 |
| 2 | Bottom | B | 10 | 26 | 20 | 22 | 22 | 31 | 24 | 22 | 23 |
| 2 | Bottom | B | 11 | 27 | 20 | 23 | 29 | 42 | 30 | 18 | 21 |
| 2 | Bottom | B | 13 | 20 | 15 | 19 | 27 | 31 | 23 | 13 | 12 |
| 2 | Bottom | B | 30 | 25 | 24 | 9 | 29 | 33 | 23 | 15 | 28 |
| 2 | Bottom | C | 6 | 16 | 13 | 25 | 27 | 25 | 19 | 16 | 18 |
| 2 | Bottom | C | 8 | 24 | 15 | 24 | 32 | 34 | 27 | 11 | 24 |
| 3 | Bottom | A | 1 | 24 | 12 | 23 | 32 | 30 | 21 | 36 | 27 |
| 3 | Bottom | A | 6 | 20 | 3 | 11 | 30 | 17 | 10 | 17 | 13 |
| 3 | Bottom | A | 9 | 20 | 6 | 25 | 31 | 18 | 14 | 41 | 27 |
| 3 | Bottom | A | 10 | 22 | 3 | 25 | 29 | 30 | 20 | 41 | 22 |
| 3 | Bottom | A | 11 | 14 | 4 | 21 | 30 | 13 | 10 | 22 | 26 |
| 3 | Bottom | A | 15 | 23 | 13 | 19 | 32 | 24 | 18 | 32 | 28 |