**One Tree Island December 2020 - Larval cultures for Coral Larval Restoration Project**

| **Activity** | **Culture 1** (5x5m square pool #4)  Spawned Friday 4 December 2020  Suspect mostly *Acropora* | **Culture 2** (5x5m square pool #2)  Spawned Sat 5 December 2020  Suspect mostly *Porites* | **Culture 3** (Octagon) |
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| Larval collection | Larval slick found in First Lagoon channel while aboard *RV Orcinus* at 12:00 on Sat 5 December.  Slick was collected by leaning overboard with 20 L buckets, then pouring into Nally bins.  Five Nally bins collected, these were all allowed to rest for 10 minutes until embryos collected on surface, then decanted into fresh sea water twice to wash out detritus. Cleaned slick was concentrated into one Nally bin, but we did not sample for density (forgot to bring Falcon tubes on the boat). By eye, it looked like about 3-4 million. Larvae were poured into the rearing pool at ~13:00. Most embryos were pink and looked like Acropora, with Trichodesmium in the mix too. | Coral spawn detected by airborne scent along edge of The Gutter from ~20:00 on Sat 5 Dec.  Spawn collected by careful surface skimming at water’s edge with plastic tumblers, then decanting into 20L buckets, then consolidating into 2 Nally bins.  Initially eggs and bundles were very small and reddish brown, then between 20:30-21:00 some large bright orange bundles appeared (Dex later identified as sea stars), as well as some medium-large, dark green bundles/eggs.  When as much shore slick was collected as possible, Nally bins were carefully carried around to main research station beach and left to rest for a few minutes, then spawn skimmed from surface, decanted into fresh seawater twice, between 21:20-21:40.  5 x 50mL samples were collected for density counts, then Nally bin transferred to rearing pool and poured in at 22:00.  Estimate ~6.5 million embryos (see next row) | Total larvae added to Octagon:  7/12 Bucket A.1 = 1,113,867  7/12 Bucket P.1 = 224,600  8/12 Bucket A.2 = 46,000  8/12 Bucket P.2 = 127,600  10/12 42 L nally = 432,880  Total input = 1,944,667 |
| Larval count  Sat 5 Dec | Did not count | 5 x 50mL samples, from 40 L in Nally bin  Counted 22:30-23:55.  Could only count 3 samples, as not all bundles had broken up when sampled, so samples had excess sperm and were liquefying by 23:30   |  |  | | --- | --- | | S1 = 8,522  S2 = 8,828  S3 = 8,449 | Total = 25,799  /3  = 8,600/50 mL  = ~6.5 million larvae | |  |  | | n/a |
| Larval count  Sun 6 Dec | Niskin bottle into 3 x 500mL sample jars  Collected 15:00   |  |  | | --- | --- | | S1 = 151  S2 = 91  S3 = 129 | Total = 371  /3  = 124 larvae/500 mL | | Did not sample or disturb, larvae < 24 hours old. | n/a |
| Larval count Mon 7 Dec | Niskin bottle into 3 x 500mL sample jars  Collected 10:00   |  |  | | --- | --- | | S1 = 61  S2 = 76  S3 = 108 | Total = 245  /3  = 82 larvae/500 mL | |  |  |   After sampling, Bucket A.1 of larvae extracted with sieving to transfer to octagon. | Niskin bottle into 3 x 500mL sample jars  Collected 10:00   |  |  | | --- | --- | | S1 = 247  S2 = 213  S3 = 264 | Total = 724  /3  = 241 larvae/500 mL |   After sampling, Bucket P.1 of larvae extracted with sieving to transfer to octagon.  Ratio of larvae: 95% Porites, 4.5% Acropora + other, 0.5% sea stars | 3 x 50mL Falcon tubes  Bucket A.1 (20L Acropora mix)   |  |  | | --- | --- | | S1 = 2,537  S2 = 2,839  S3 = 2,978 | Total = 8,354  /3  = 2,785/50 mL  = ~1.14 m larvae |   Bucket P.1 (10L Porites mix)   |  |  | | --- | --- | | S1 = 1,226  S2 = 1,003  S3 = 1,140 | Total = 3,369  /3  = 1,123/50 mL  = 224,600 larvae | |
| Larval count Tues 8 Dec  Niskin not working, changed to paddle and dunk with sample jars | 3 x 500mL sample jars  Collected 10:00   |  |  | | --- | --- | | S1 = 52  S2 = 49  S3 = 48 | Total = 149  /3  = 50 larvae/500 mL | |  |  |   After sampling, Bucket A.2 of larvae extracted with sieving to transfer to octagon. | 3 x 500mL sample jars  Collected 10:00   |  |  | | --- | --- | | S1 = 133  S2 = 120  S3 = 150 | Total = 403  /3  = 134 larvae/500 mL |   After sampling, Bucket P.2 of larvae extracted with sieving to transfer to octagon. | 3 x 50mL Falcon tubes  Bucket A.2 (20L Acropora mix)   |  |  | | --- | --- | | S1 = 114  S2 = 117  S3 = 113 | Total = 344  /3  = 115/50 mL  = 46,000 larvae |   Bucket P.2 (20L Porites mix)   |  |  | | --- | --- | | S1 = 311  S2 = 321  S3 = 324 | Total = 956  /3  = 319/50 mL  = 127,600 larvae | |
| Larval count Wed 9 Dec  Niskin not working, so stir with paddle then dunk with sample jars  Very windy | 3 x 500mL sample jars  Collected 10:00   |  |  | | --- | --- | | S1 = 170  S2 = 142  S3 = 117 | Total = 429  /3  = 143 larvae/500 mL | |  |  |   No larvae transferred to octagon, too rough to sieve. | 3 x 500mL sample jars  Collected 10:00   |  |  | | --- | --- | | S1 = 137  S2 = 206  S3 = 191 | Total = 534  /3  = 178 larvae/500 mL |   No larvae transferred to octagon, too rough to sieve. | 3 x 500mL sample jars  Collected 10:00   |  |  | | --- | --- | | S1 = 4  S2 = 3  S3 = 4 | Total = 11  /3  = 4 larvae/500 mL | |
| Larval count Thu 10 Dec  Niskin not working, so stir with paddle then dunk with sample jars | 3 x 500mL sample jars  Collected 10:00   |  |  | | --- | --- | | S1 = 26  S2 = 31  S3 = 37 | Total = 94  /3  = 31 larvae/500 mL | |  |  |   In the afternoon (~16:00), Bucket A.3 of larvae extracted with sieving to keep aside for Chris to dye.  Bucket A.3 (20L Acropora mix, 3 x 50mL samples)   |  |  | | --- | --- | | S1 = 155  S2 = 150  S3 = 143 | Total = 448  /3  = 149/50 mL  = 59,600 larvae | | 3 x 500mL sample jars  Collected 10:00   |  |  | | --- | --- | | S1 = 50  S2 = 59  S3 = 63 | Total = 172  /3  = 57 larvae/500 mL | | 3 x 500mL sample jars  Collected 10:00   |  |  | | --- | --- | | S1 = 0  S2 = 2  S3 = 1 | Total = 3  /3  = 1 larvae/500 mL | |
| Larval count **before** removal from pools  Fri 11 Dec | Niskin bottle into 3 x 500mL sample jars  Collected ~10:00   |  |  | | --- | --- | | S1 = 53  S2 = 33  S3 = 26 | Total = 112  /3  = 37 larvae/500 mL | |  |  | | Bucket P.3 of larvae extracted with sieving for Chris to dye, extracted ~09:00  Bucket P.3   |  |  | | --- | --- | | S1 = ?  S2 = ?  S3 = ? | Total = ?  /3  = ?/50 mL  = ? larvae |   Niskin bottle into 3 x 500mL sample jars  Collected ~11:00   |  |  | | --- | --- | | S1 = 57  S2 = 20  S3 = 57 | Total = 134  /3  = 45 larvae/500 mL | | Niskin bottle into 3 x 500mL sample jars  Collected ~15:00   |  |  | | --- | --- | | S1 = 5  S2 = 8  S3 = 4 | Total = 17  /3  = 6 larvae/500 mL | |
|  |  |  |  |
| Larval counts **after** release Fri 11 Dec | 3 x 15mL Falcon tubes, vol in large Nally = 100L   |  |  | | --- | --- | | S1 = 159  S2 = 200  S3 = 221 | Total = 580  /3  = 193.3 larvae/15 mL | |  | = 1,288,867 larvae |   *This pool identification for these counts was transcribed from the whiteboard, but looking at these numbers a month later, I wonder if they were the wrong way round? Over the preceding days, there generally seemed a lot more larvae in Pool #2 (Culture 2) than Pool #4 (Culture 1).* | 3 x 15mL Falcon tubes, vol in large Nally = 78L   |  |  | | --- | --- | | S1 = 97  S2 = 89  S3 = 92 | Total = 278  /3  = 92.67 larvae/15 mL | |  | = 481,884 larvae | | **Combined larvae from 5 x 5 m pools (excluding Octagon).**  Total volume 178 L  5 x 15mL Falcon tubes   |  |  | | --- | --- | | S1 = 127  S2 = 152  S3 = 151  S4 = 174  S5 = 169 | Total = 773  /5  = 154.6 larvae/15 mL | |  | = 1,834,587 total larvae | |