# Lab and field evaluation of tagging methods for the use of acoustic telemetry to observe sea urchin movement behaviour at ecologically relevant spatio-temporal scales

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## Additonal file

### Details of tagging methods:

The two fishing line methods (FL1 and FL2) consisted of threading a length of fishing line directly through the test to form a loop (*sensu* Ebert 1965). Two types of fishing line were tested: Dyneema® nylon fishing line (0.18 mm diameter) and a more durable but less flexible nickel titanium fishing line (0.254 mm diameter). The urchin's test was pierced with a 0.69 mm diameter hypodermic needle, first on the aboral side about 1 cm from the mouth in an interambulacral plate to enter the test and then on the oral side to exit the test, again through an interambulacral plate. The fishing line was maintained in place as the needle was withdrawn, the two ends of line knotted together, and the tag fixed to the resulting loop using a combination of electrical tape and glue (Lepage UltraGel super glue). The nickel titanium fishing line was evaluated because of concerns that urchins might graze on the dyneema fishing line, thereby detaching tags over longer temporal scales and at higher urchin densities (Ebert, 1965).

The third method (TB) used commercially available plastic T-bar tags (*sensu* Clemente et al. 2007). A tagging gun for clothing was used to pierce the test and insert a T-bar tag on the aboral side of the urchin about 1 cm from the anus in an interambulacral plate and the printed tag was attached to the stalk of the plastic tag using electrical tape and glue. The fourth method (NS) used a nylon screw cut to a length of 6mm (diameter: 3mm; *sensu* Duggan & Miller 2001). A hole slightly smaller in diameter than the gauge of the screw was made on the aboral side of the urchin using a dissection needle and the nylon screw carefully rotated into the hole. The tag was then glued on the head of the screw.

Clemente, S., Hernández, J. C., & Brito, A. (2007). An external tagging technique for the long-spined sea urchin *Diadema* aff. *antillarum*. *Journal of the Marine Biological Association of the United Kingdom*, *87*(3), 777–779. doi:10.1017/S0025315407053349

Duggan, R. E., & Miller, R. J. (2001). External and internal tags for the green sea urchin. *Journal of Experimental Marine Biology and Ecology*, *258*, 115–122.

Ebert, T. A. (1965). A technique for the individual marking of sea urchins. *Ecology*, *46*(1–2), 193–194.

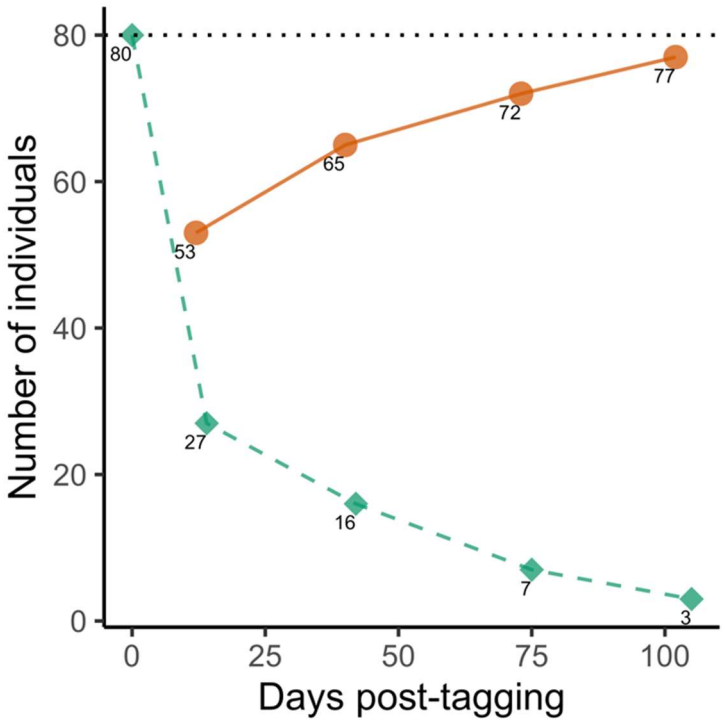



**Figure S1.** Number of nylon screw tagged urchins (green dashed line) and number of hole urchins (orange solid line) through time (days post-tagging), showing that hole urchins are almost entirely composed of nylon screw tagged urchins that have lost their tags (sum of the two groups is always equal or close to the 80 individuals initially tagged with nylon screws).

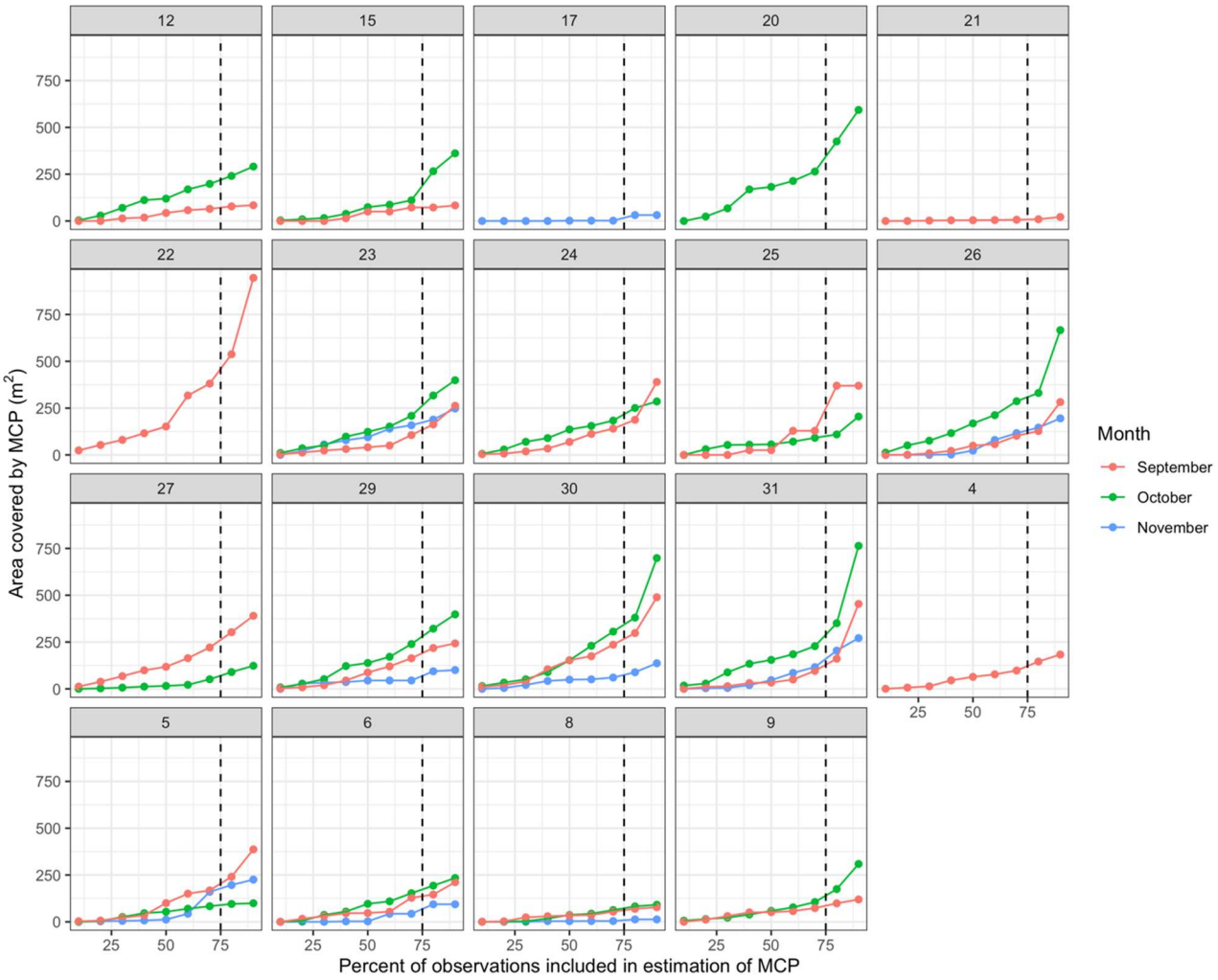



**Figure S2.** The effect of including more points (increasing the percentage included) on Minimum Convex Polygon area. Estimated areas for 10% to 90% of points are shown, coloured by month of observation. Vertical dashed line indicates the chosen cut-off point (75%) where the small percentage of points very far from the majority cause a steep increase in estimated area. This should ensure that large-error points are excluded from these estimates, while retaining points which represent actual movement and space use by individual urchins.

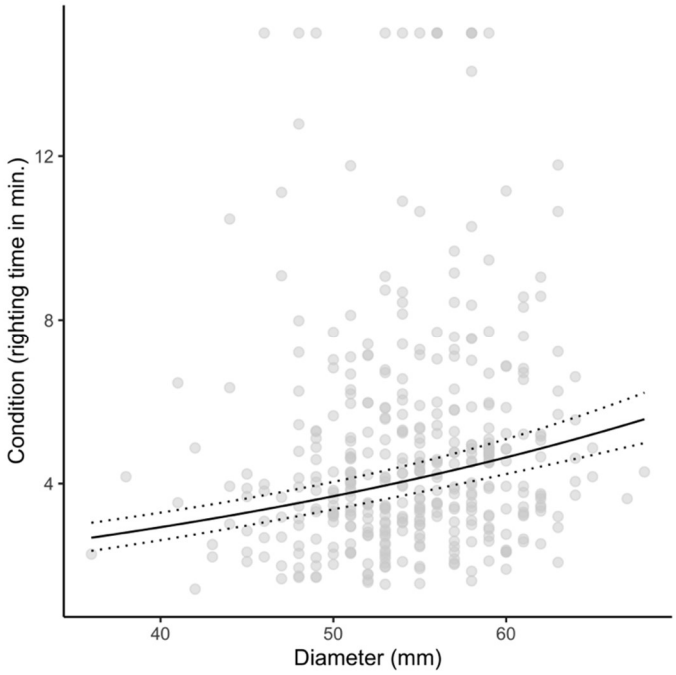



**Figure S3.** Larger urchins take more time to right themselves than smaller urchins (significant effect of diameter), although there is significant variability between individuals and measures; points and predictions are from Control urchins only.

**Table S1.** Parameter estimates (beta coefficients) from models, with 95% confidence intervals.

| | **beta** | **lower-95** | **upper-95** | **random effect (SD)** |
|---|---|---|---|---|
| **log(Condition)[1]** | | | | |
| (Intercept) | 4.26 | 3.98 | 4.54 | - |
| Urchin ID | - | - | - | 0.25 |
| Tank ID | - | - | - | 0.07 |
| Tag | | | | |
| Fishing line (1) | 0.07 | -0.04 | 0.19 | - |
| Fishing line (2) | 0.10 | -0.03 | 0.23 | - |
| T-bar tag | -0.01 | -0.12 | 0.11 | - |
| Nylon screw | -0.12 | -0.24 | 0.0075 | - |
| Hole | -0.10 | -0.24 | 0.0483 | - |
| Days after tagging | 8e-04 | -5e-04 | 0.0021 | - |
| Diameter | 0.02 | 0.02 | 0.03 | - |
| Tag : Days after tagging | | | | |
| Fishing line (1) : Days | 8e-04 | -9e-04 | 0.0024 | - |
| Fishing line (2) : Days | 4e-04 | -0.0015 | 0.0023 | - |
| T-bar tag : Days | 0.0039 | 0.002 | 0.0059 | - |
| Nylon screw : Days | 0.0023 | -0.0012 | 0.0059 | - |
| Hole : Days | 1e-04 | -0.0021 | 0.0023 | - |

| | | | | |
|---|---|---|---|---|
| **Diameter** | | | | |
| (Intercept) | 54.6376 | 53.8283 | 55.4469 | - |
| Urchin ID | - | - | - | 5.093 |
| Tank ID | - | - | - | 1.491 |
| Tag | | | | |
| Fishing line (1) | -1.0567 | -2.4573 | 0.344 | - |
| Fishing line (2) | 2.7052 | 1.0294 | 4.3809 | - |
| T-bar tag | -0.3543 | -1.7505 | 1.042 | - |
| Nylon screw | -0.1628 | -1.5714 | 1.2457 | - |
| Hole | -0.272 | -1.8277 | 1.2836 | - |
| Days after tagging | -0.0047 | -0.0184 | 0.009 | - |
| Tag : Days after tagging | | | | |
| Fishing line (1) : Days | 0.0067 | -0.0075 | 0.021 | - |
| Fishing line (2) : Days | 8e-04 | -0.0138 | 0.0153 | - |
| T-bar tag : Days | 0.0116 | -0.0031 | 0.0263 | - |
| Nylon screw : Days | 0.0079 | -0.0114 | 0.0272 | - |
| Hole : Days | 0.002 | -0.0215 | 0.0254 | - |
| **Difference in weight (final – initial)** | | | | |
| (Intercept) | -0.3974 | -0.8528 | 0.0567 | - |
| Tank ID | - | - | - | 0.0729 |
| Tag | | | | |
| Fishing line (2) | -0.3579 | -1.096 | 0.3789 | - |
| T-bar tag | -1.7642 | -2.6949 | -0.8288 | - |
| **log(Gonad weight)** | | | | |
| (Intercept) | -1.5731 | -2.11 | -1.0363 | - |
| Tag | | | | |
| Fishing line (1) | -0.1086 | -0.2566 | 0.0393 | - |
| Fishing line (2) | -0.0605 | -0.2352 | 0.1141 | - |
| T-bar tag | -0.1491 | -0.3707 | 0.0724 | - |
| Hole | 0.015 | -0.1309 | 0.161 | - |
| Diameter | 0.0699 | 0.0601 | 0.0797 | - |
| **Injury severity**[2] | | | | |
| Tag | | | | |
| Fishing line (2) | -0.1827 | -1.4585 | 0.9849 | - |
| T-bar tag | 0.9382 | 0.1358 | 1.79 | - |
| Nylon screw | 3.1666 | 2.4085 | 3.9877 | - |
| Days after tagging | 0.0444 | 0.0365 | 0.0532 | |
| Tag : Days after tagging | | | | |
| Fishing line (2) : Days | -0.0063 | -0.0203 | 0.0085 | - |
| T-bar tag : Days | 0.0233 | 0.0121 | 0.0343 | - |
| Nylon screw : Days | 0.0462 | 0.0305 | 0.0626 | - |

[1] Interpretation of log-transformed response variable: Exponentiate the coefficient, subtract one from this number, and multiply by 100. This gives the percent increase (or decrease) in the response for every one-unit increase in the independent variable. Example: the coefficient is 0.198. (exp(0.198) – 1) * 100 = 21.9. For every one-unit increase in the independent variable, our dependent variable increases by about 22%.

[2] Tagging methods are compared to Control. Every tagging method is by far and away more likely to cause injury than the control

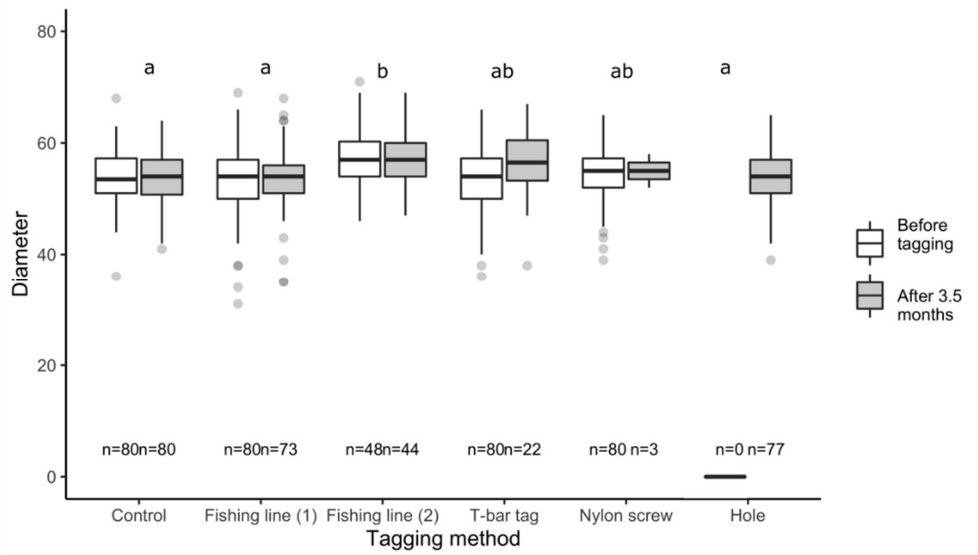



**Figure S4.** Test diameter (mm) of urchins before tagging and at the end of the experiment after 3.5 months. Because of mortality and tag loss, the number of individuals in each group after 3.5 months is less than at the beginning, except for the Hole treatment. Significant differences between tagging treatments from post-hoc comparisons are shown with different letters.

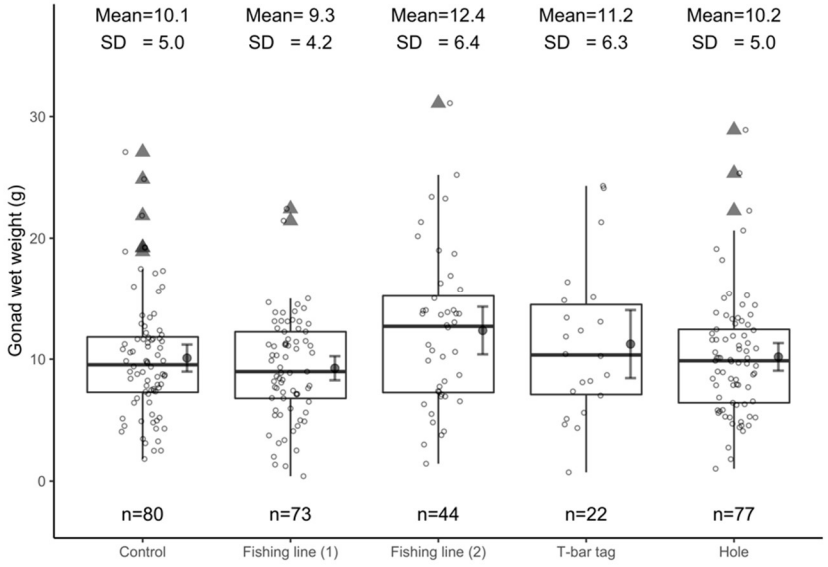



**Figure S5.** Gonad weight as a function of tagging treatment. There were no significant differences between tagging treatments.

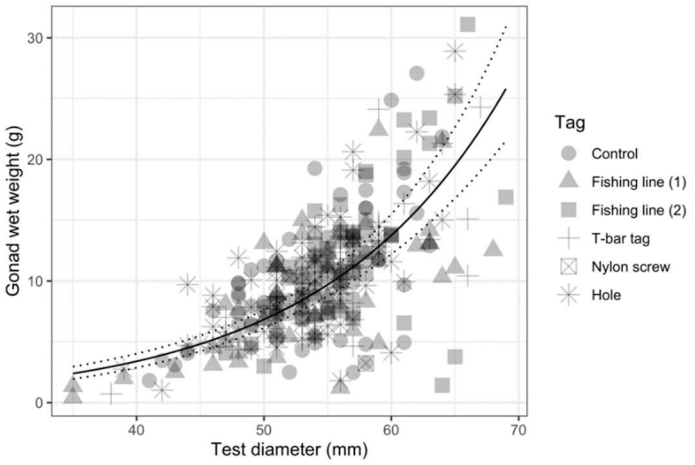



**Figure S6.** Relationship between gonad wet weight and test diameter for all tagging treatments. Model results (predicted values and 95% Confidence Intervals) are shown.

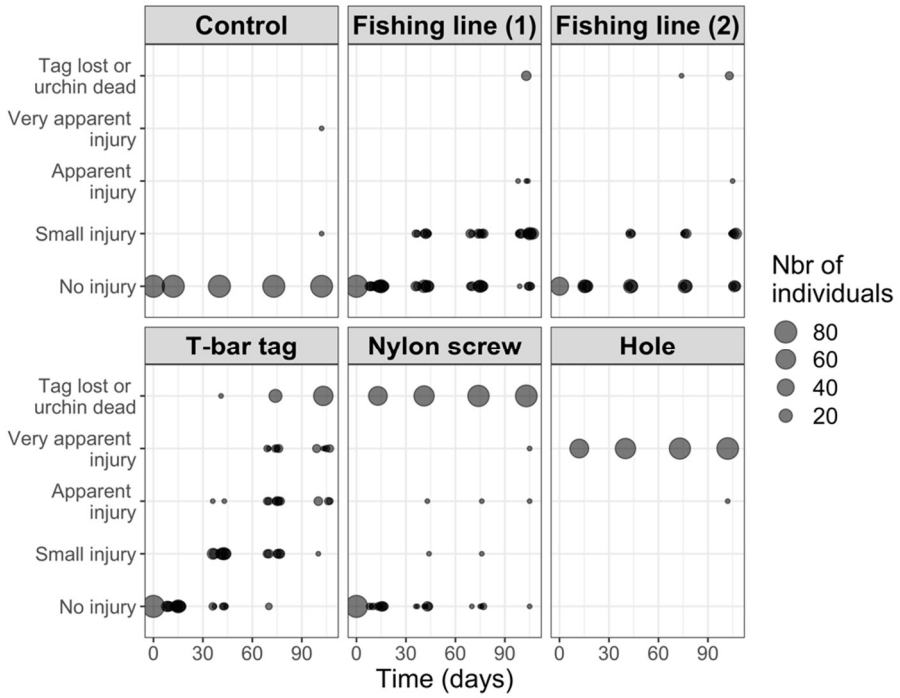



**Figure S7.** Injuries observed through time as a function of tagging method. No mortality and only two injuries were observed in control urchins. Because injuries could only be followed and definitively assigned to a tagging method as long as the tag was attached, the number of urchins removed from these observations at each time period is shown as the category "Tag lost or urchin dead". For example, the small number of injuries assigned to the Nylon screw tagging method is a function of the high mortality and high tag loss seen in this treatment; these urchins are also shown in the Hole tag treatment here, as urchins with Very apparent injuries.

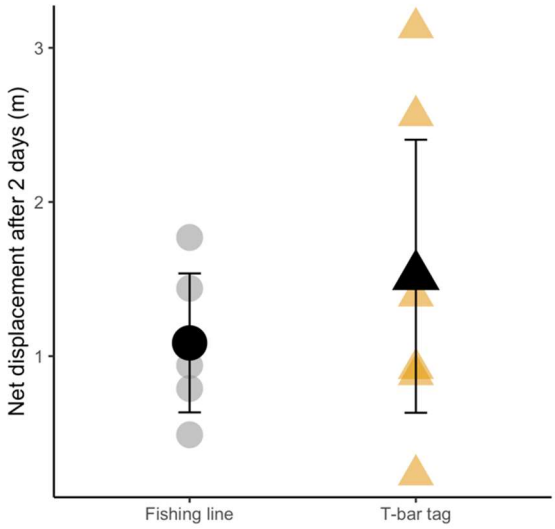



**Figure S8.** Net displacement of tagged individuals after 2 days in the field calculated from diver observations

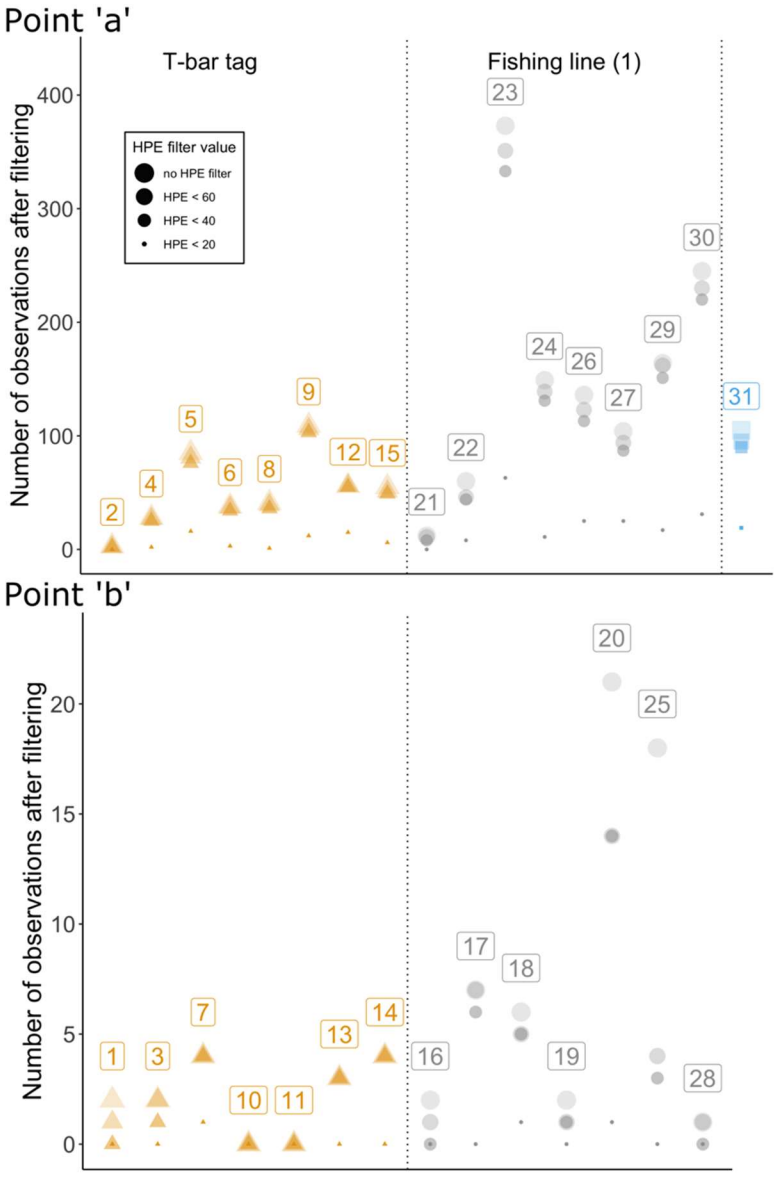
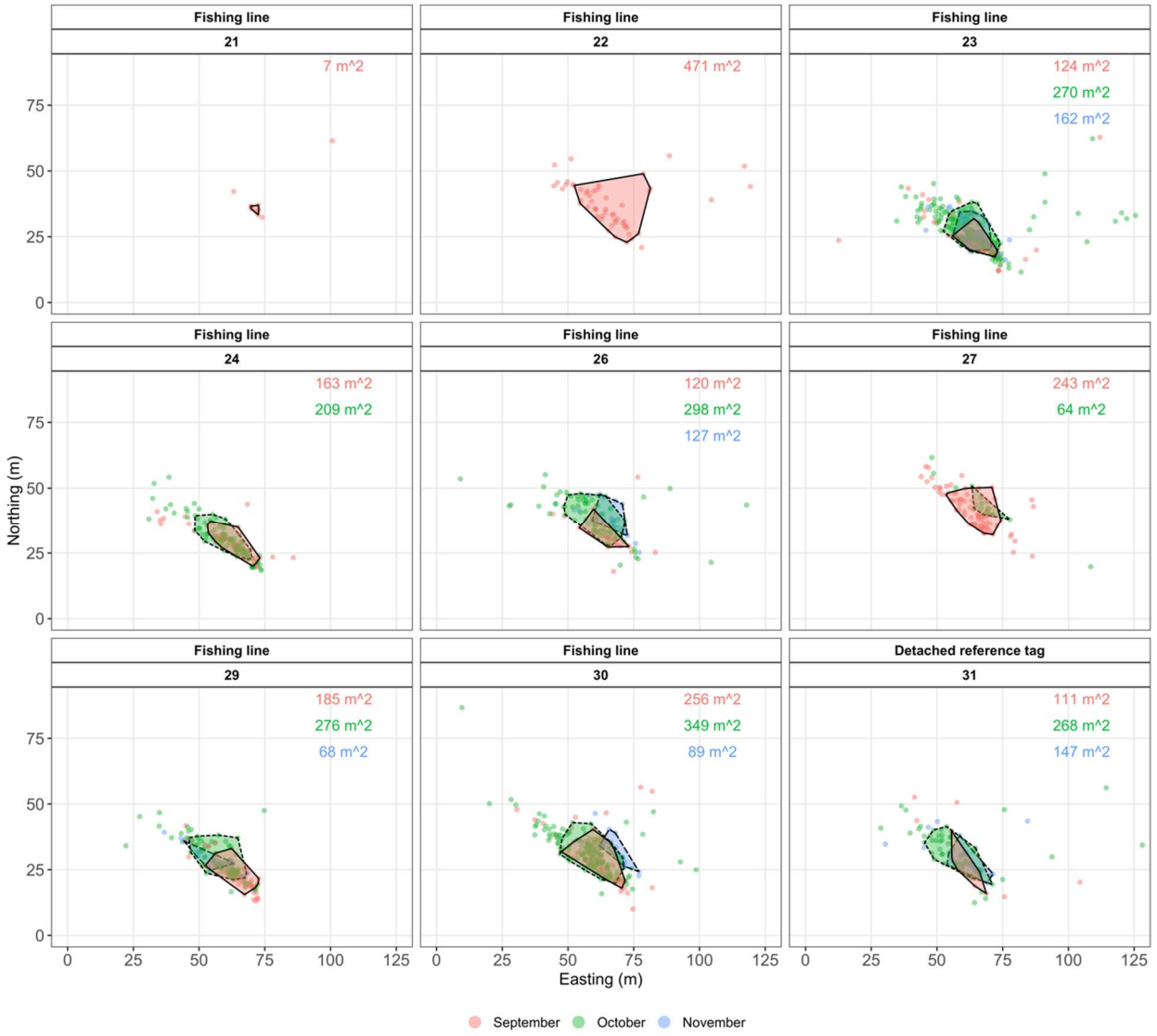



**Figure S9.** Number of observations per tagged individual. The two tagging methods are shown as different shapes and colours, while release point 'a' and 'b' are the top and bottom panels, respectively. Points are shown for the total number of observations filtered by the positioning error (HPE). All urchins have at least one point (not filtered: largest and palest point) although for certain individuals that were never detected by the array (ID 10 and 11) this is zero. Successively darker and smaller points are filtered by lower HPE values. Size of the points is scaled by the HPE filter (points filtered by larger values, are larger). Note the different y-axis scales for the two panels.

**a)**


Fishing line
21
7 m^2
Fishing line
22
471 m^2
Fishing line
23
124 m^2
270 m^2
162 m^2
Fishing line
24
163 m^2
209 m^2
Fishing line
26
120 m^2
298 m^2
127 m^2
Fishing line
27
243 m^2
64 m^2
Fishing line
29
185 m^2
276 m^2
68 m^2
Fishing line
30
256 m^2
349 m^2
89 m^2
Detached reference tag
31
111 m^2
268 m^2
147 m^2
Northing (m)
Easting (m)
0
25
50
75
100
125
September
October
November

**b)**

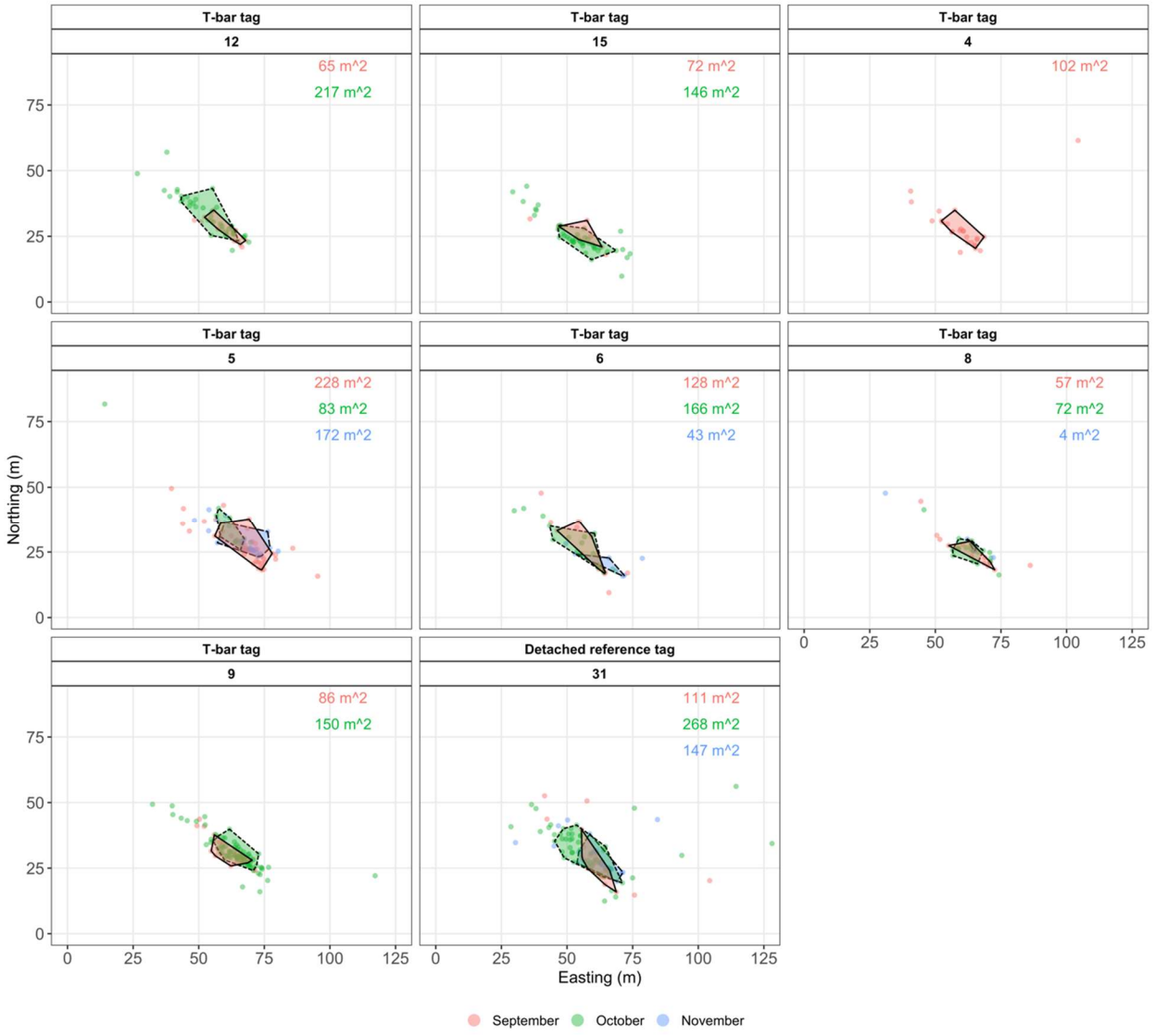



**Figure S10.** Minimum convex polygons (MCPs) per month for each individual. Points are all detections (HPE < 200), polygons are monthly 75% MCPs and numbers in the upper right of each panel are the MCP area per month in $m^2$. a) Fishing line tagged urchins and b) T-bar-tagged urchins. The detached reference tag is shown in both a and b (id #31) for comparison.