SUPPLEMENT 2

Processing images in imageJ to get x,y coordinates
website manual for MTrackJ Plugin :
http://www.imagescience.org/meijering/software/mtrackj/manual/

1. import folder of files to imageJ as a Virtual Stack (FILE → IMPORT → IMAGE SEQUENCE → Select the folder containing the transformed images, and tick 'USE VIRTUAL STACK' in options dialogue box)
2. calibrate image scale
   1. If first image has quadrat visible :
      1. select line tool
      2. trace a line that marks one black bar on photo quadrat
      3. In Menu, ANALYZE → SET SCALE
      4. 'KNOWN DISTANCE' = '5'
      5. 'UNITS' = 'cm'
      6. tick 'GLOBAL' box
      7. Note the scale ratio (#pixels/cm) on the Tracking Record sheet beside the sequence name
      8. click on 'OK'
   2. If first image has no quadrat visible :
      1. Open a single image from the original sequence that shows the quadrat
      2. select line tool
      3. trace a line that marks one black bar on photo quadrat
      4. In Menu, ANALYZE → SET SCALE
      5. 'KNOWN DISTANCE' = '5'
      6. 'UNITS' = 'cm'
      7. tick 'GLOBAL' box
      8. Note the scale ratio (#pixels/cm) on the Tracking Record sheet beside the sequence name
      9. click on 'OK'
3. Set the time interval to 60 sec per slice : IMAGE → PROPERTIES and time interval = 60 sec
4. Slice one of the virtual stack shows image 1 with 20 random points overlaid (image 000.jpg in the sequence folder). Zoom in as necessary to locate the urchin (diameter >20mm) closest to each point.
   1. Select the 'POINT OR MULTI-POINT' tool from the ImageJ Toolbar.
   2. Select the 'MULTI-POINT' option by right clicking on the icon.
   3. On image, click once on the center of each urchin closest to the red points in order to select a total of 20 urchins. You can click and drag points to relocate.
5. Overlay the urchin selections on the stack
   1. IMAGE → OVERLAY → ADD SELECTION
   2. Save this overlay selection of urchins
      1. IMAGE → OVERLAY → TO ROI MANAGER
      2. Rename the Overlay (RENAME) as 'Sequence.20Urchins'
      3. MORE → SAVE...
      4. Be sure to save the overlay (.roi) in the Results folder with name 'Sequence.20Urchins.roi' Ex. 'GWNik2.20Urchins.roi'
   3. In future sessions, after opening the Virtual Stack, open the .roi file, add it as a selection (IMAGE → OVERLAY → ADD SELECTION) and then use the roi manager to manipulate (IMAGE → OVERLAY → TO ROI MANAGER).

6. Advance to slice #2 (Add the STACK TOOLS option to your toolbar by clicking on the double red arrow on the right of the toolbar and selecting STACK TOOLS). The overlay with the 20 selected urchins should be visible, with the points numbered 1-20 (but not the random red circles).
7. Measure diameters of 20 urchins
    1. select line tool
    2. trace a line that marks the diameter of urchin number 1
    3. In Menu, ANALYZE → MEASURE (or CTRL → M)=
    4. Repeat for each urchin, being sure to follow the numbers (the number in your Results window should correspond to the number beside the overlay point that marks the urchin you're measuring). CHECK TO BE SURE THAT ALL URCHINS SELECTED ARE >20mm DIAMETER.
    5. Export these results (FILE → SAVE AS) with name 'Sequence.Diameters' (Ex. 'AHPtax1.Diameters'
    6. Clear the results window (Click on the Results window, then RESULTS → CLEAR RESULTS)
8. open MTrackJ plugin (PLUGINS → MTRACKJ)
9. set the stack properties by going to IMAGE → PROPERTIES. Change the 'UNIT of LENGTH' to be 'cm' and the 'FRAME INTERVAL' to be '60 sec'
10. Trace tracks of 20 selected urchins, starting with urchin #1
    1. In MTrackJ → 'ADD' (when selected, the text appears in red). The active track appears as white points on the image stack.
    2. Hide the tracking points/lines. Click on MTrackJ → DISPLAYING and untick the box 'Display reference' and 'Display active track'.
    3. Click on urchin #1 (identified by overlay point labelled '1') in **slice #2** (first slice without red dots). Click on the center of this urchins body in each successive slice until the end of the sequence or until the urchin leaves the frame.
        1. Zoom in on image as necessary to locate the madrapoerite/center of the body using the +/- keys. The zoom will happen with your cursor as the center of the field of view, so position your cursor over the urchin of interest, DON'T CLICK, and press the + key to zoom in on that urchin.
        2. Scroll across the image by holding down the spacebar (will change your imageJ icon for the hand for the period that the spacebar is held). You can then click and drag the image to change your view. Releasing the spacebar will return you to the multipoint tool where you can continue to manipulate the tracks.
    4. Verify the track by watching the sequence ('play' the virtual stack). Individual points can be moved/adjusted if need be. Since every track can have only point per frame, selecting a track (Make sure ADD is selected, hold Ctrl down and move cursor over the track you wish to select and then click once on the track - the active track will be white, so if you have correctly selected the track, it should become white), moving to a frame, and reclicking will automatically replace the previously selected point with the new selection.)
    5. Click on MTrackJ → 'ADD' once more to end the track (the 'ADD' button should go from red text back to plain black text).
    6. Click on MTrackJ → SAVE (name as 'Sequence.mdf', ex 'LHPtax3.mdf', saved in results folder)
    7. Repeat for urchin #2, urchin #3, and so on, adding a new track for each urchin, up to urchin #20, and saving after every track (under the same name – you will end up with one .mdf file with all 20 tracks combined).
    8. Notes for the process of adding tracks
        1. If you click too fast, MTrackJ will finish one track and start another. Watch out for this! If it happens, delete the inadvertant point (Click on MTrackJ → DELETE,

which will appear in red, then move the mouse over the point you want to delete, which will highlight in white, and click to delete) or track (Click on MTrackJ → DELETE, which will appear in red, then hold ctrl down and move the mouse over the track you want to delete, which will highlight in white, and click to delete). Then, to continue working on the track you were in the middle of, click on MTrackJ → ADD, hold the ctrl key down, move the mouse over the track you want to work on until it highlights white, and click on the track. The sequence will automatically advance to the last frame you have a point for. You can then continue adding points as normal.

   2. You can hide certain tracks or hide all but one track to observe or if you have trouble selecting a point you want to delete. Click on MTrackJ → HIDE (it should be in red). Move your mouse over one track until it highlights in white. If you click on this track, only this track will be hidden. If you hold ctrl down and then click on this track, every track BUT this one will be hidden. To unhide all tracks, hold down the ctrl key and click on MTrackJ → HIDE. A dialog will open asking if you want to unhide all tracks; click YES.
   3. You can also remove the finished tracks from view, should they be too overlapped. Click on MTrackJ → DISPLAYING and untick 'DISPLAY FINISHED TRACKS'

11. Record the measurements and positions of the tracks
    1. Check that time interval is set to 60 sec : IMAGE → PROPERTIES and time interval = 60 sec
    2. MTrackJ → 'MEASURE'. This will open two new windows titled 'MTrackJ : Tracks' and 'MTrackJ : Points'
12. Save the results for these tracks
    1. Select 'MTrackJ :Tracks' window
    2. FILE → SAVE AS
    3. Name of file as 'Sequence.Tracks' Ex. 'AHPtax1.Tracks'
    4. Save in 'Results' folder
    5. Repeat for 'MTrackJ:Points' window, saving as 'Sequence.Points' Ex. 'AHPtax1.Points'
13. Quit MTrackJ Plugin (be sure to have saved the tracks (SAVE button in MTrackJ box), and the results (both points and tracks) before quitting).
14. Record and save the coordinates and area of the central weight or piece of kelp
    1. Choose the second photo in the sequence (slice 2 without red points) if the kelp doesn't move after that. If the kelp changes positions in the first few frames, select the first photo where the kelp is holding the position that it then occupies for the rest of the series.
    2. Select the multipoint tool in the ImageJ toolbar
    3. Click on the four (or more) 'corners' of the piece of kelp to create four (or more) points
    4. Click ANALYZE → MEASURE to record the x and y coordinates of these points in the results table. If you have other measurements already showing in the results table, clear them first (ANALYZE → CLEAR RESULTS).
    5. Select the polygon tool in the ImageJ toolbar and outline the piece of kelp or central marker by clicking on successive points around its edge until you have outlined the piece of kelp with a continuous yellow line.
    6. Click ANALYZE → MEASURE to record the area of this polygon.
    7. Export these results and save them
    8. With the Results table selected, click on FILE → SAVE AS
    9. save the file as 'Sequence.Kelp' or 'Sequence.Marker' Ex. 'LHPtax3.Kelp' or 'LHPtax3.Marker' in the Results folder
15. Create a 50cm by 50cm Quadrat in overlay
    1. Select the rectangle tool in the ImageJ toolbar

2. Draw a rectangle on the stack of images
3. Click on EDIT → SELECTION → SPECIFY...
4. Tick the 'SCALED UNITS (CM)' box
5. Specify both WIDTH and HEIGHT to be 50
6. Specify the X COORDINATE and Y COORDINATE such that the Quadrat is centered on the piece of kelp/center marker
7. Add the rectangle to the overlay (IMAGE → OVERLAY → ADD SELECTION) and open it in the roi manager (IMAGE → OVERLAY → TO ROI MANAGER)
8. Save the rectangle.
    1. Select the rectangle in your roi manager list.
    2. Rename the overlay (RENAME) as 'Sequence.Q' Ex. 'LHPtax2.Q'
    3. Click on MORE → SAVE...
    4. Save as 'Sequence.Q' Ex. 'LHPtax3.Q' in the Results folder

Counting urchins in first and last frame – Difference in Density

1. Open the first image from the sequence
    1. FILE → OPEN
    2. Select image 001.jpg (or the image that you used to create and place the Q if the kelp moved after the first image) from the appropriate sequence folder in the ModSeq folder.
2. Open the Quadrat overlay
    1. FILE → OPEN
    2. Select the roi file that corresponds to this sequence's quadrat ('Sequence.Q.roi' Ex. 'LHPtax3.Q.roi')
    3. Add to Overlay (IMAGE → OVERLAY → ADD SELECTION)
3. Open the CellCounter Plugin (PLUGINS → ANALYZE → CELL COUNTER)
4. Click 'REMOVE' on Cell Counter window until only one type of counter remains ('Type 1' with 0 beside it)
5. Click 'INITIALIZE'. This will open your image with Quadrat in another window (the 'Counter Window – slice#')
6. Select 'TYPE 1' in 'COUNTERS'
7. Click on the urchins visible inside the yellow quadrat (urchin must have center of body inside yellow line to be considered inside the quadrat).
8. Click on 'RESULTS'. This will show the number of selections of Type 1 in the results window. Transfer this number manually to the spreadsheet 'DensityBegEndAllSequences' which is located in the results folder. Make sure to record the Sequence name, the image name/number, the roi file where the quadrat used is located, and whether that image is the beginning (beg) or end (end) of the sequence.

Repeat with the last image in the sequence, selecting the image # that corresponds to the end of the sequence (the last image in the sequence folder).

SUPPLEMENT 3

Procedure to extract solid/sand map from substrate info

QGIS

1. New project
2. Open photo number 10 (010 from the 'ModSeq' folder) for the appropriate sequence
    a. LAYER → ADD LAYER → ADD RASTER LAYER
    b. Select photo #10 from appropriate folder and click 'ADD'
3. Right click on layer in 'Layers' pane → SET CRS → SET LAYER CRS
    a. Select: WGS 84 / UTM zone 19N (EPSG 32619)
4. Check properties of layer
    "CRS : EPSG:32619 - WGS 84 / UTM zone 19N - Projected
    Extent: 0.0000000000000000,-2848.0000000000000000 :
    4288.0000000000000000,0.0000000000000000
    Unit: meters
    Width: 4288
    Height: 2848"
    a. Shows the CRS, the extent of the image (which is in pixels from our image), gives the unit 'meters' because of the CRS, and notes the width and height of the image. These values should max the pixel width and height (can check in ImageJ) for the image. The origin (0,0) is in the upper lefthand corner of the image (like in ImageJ).
    b. Setting the coordinate reference system like this will allow combination of urchin track x,y coordinates extracted from images using MTrackJ in ImageJ, with the substrate 'map' we can make here.
5. Right click on layer in 'Layers' pane → SET CRS → SET PROJECT CRS FROM LAYER
6. Import a csv file containing the urchin tracks extracted from MTrackJ/ImageJ plugin
    ************* Emacs/R **************
    a. Open the 'xxx.Points.xls' file corresponding the correct sequence using TextEdit
    b. Create a copy (FILE → DUPLICATE) and save this file as 'xxx.Points.csv' in the appropriate sequence folder for QGIS analysis (e.g., 'Substrate_classification_in_sequences/AHNik2')
    c. Import this csv file into Emacs/R
    d. Use the pixel/cm conversion ratio extracted from ImageJ tracking process (data in excel workbook: 'TrackingData_KNK_sequences.xlsx' for the appropriate sequence) to convert the x and y coordinates from MTrackJ (which are in cm) to pixels measures (and make the y coordinates negative, since from MTrackJ, they are not). Create two new columns (x.pix and y.pix) that contain these positions in pixels.
    e. Add a column with a sequence identifier
    f. Export a copy of this file (only the relevant columns) to the appropriate sequence folder for QGIS analysis (e.g., 'Substrate_classification_in_sequences/AHNik2') with a filename "seq_Points2.csv", e.g., AHNik1_Points2.csv
7. Import urchin tracks into project
    a. LAYER → ADD LAYER → ADD DELIMITED TEXT LAYER
    b. Choose the Seq_Points2.csv file and, if they aren't automatically identified, choose the x.pix and y.pix columns as the x and y for plotting. Use the WGS 84 / UTM 19N as the CRS (should be automatically chosen). Rename this layer as 'Tracks'
8. **EXTENT:** Create a new polygon layer, and save it as 'Extent' (plus change the name in the layer pane)
    a. Toggle into 'editing' mode and then click to add a polygon (green pond icon)

   b. Add a polygon that matches the exact extent of the base photo (010) imported before.
9. **ROCK:** Add a layer for rocky bits of substrate
   a. Add a new layer to map substrate
      i. LAYER → CREATE LAYER → NEW SHAPEFILE LAYER
         1. Choose 'POLYGON' for layer type
         2. Save in appropriate sequence folder (e.g., 'Substrate_classification_in_sequences/AHNik2') with the filename: 'Rocks'
      ii. Rename this layer 'Rocks' in the layers panel
      iii. Toggle editing for this layer on
         1. Select the layer 'Rocks'
         2. Click on the small pencil icon (editing)
      iv. Select the 'ADD POLYGON FEATURE' (small green blob/lake)
      v. Trace all the rocks visible in the photo by creating a sequence of points that follow the outline of the rock. End a polygon by right-clicking (or holding ctrl and clicking). A dialogue box appears with attribute table info. No need to enter anything; just hit 'ok' (or hit enter).
   b. Open the attribute table for the 'Rocks' layer and add properties
      i. Open the attribute table (right click on the layer name → OPEN ATTRIBUTE TABLE
      ii. Toggle editing mode on
      iii. The only column that exists at the moment is the empty 'id' column
      iv. Fill in the id column with a numerical identifier
         1. Select 'id' in the dropdown box at upper left
         2. Use expression builder (click on Epsilon)
         3. $id+1
         4. The +1 just means we start at 1 and not at zero. Make sure you don't have any layers toggled in editing mode or the id will count the rows in those layers as well and the numbering will be off.
      v. Add columns ('New Field' icon) for: area, type, x, y, and perimeter
      vi. Area
         1. NEW FIELD, then fill in the name ('area') and type (integer – whole numbers – because the number of pixels is already super high and the accuracy of tons of decimals here just makes our tables messy afterwards), and create
         2. Then use the Expression manager to fill this column
         3. $area → UPDATE ALL
         4. This will calculate an area (in $pixels^2$, since pixels are the units of the photo) for every row (so every rock we traced)
      vii. Type
         1. Identify these rows as corresponding to rocks
         2. NEW FIELD, then fill in the name ('type') and type (text) and create
         3. Then use the Expression manager to fill this column
         4. title('rock') → UPDATE ALL
         5. This will fill all rows with 'Rock'
      viii. Perimeter
         1. NEW FIELD, then fill in the name ('perimeter') and type (integer – whole numbers – because the number of pixels is already super high and the accuracy of tons of decimals here just makes our tables messy afterwards), and create

2. Then use the Expression manager to fill this column
3. $perimeter → UPDATE ALL
4. This will calculate a perimeter (in pixels, since pixels are the units of the photo) for every row (so every rock we traced)

ix. X

1. Add x coordinates of the centroid of each polygon
2. NEW FIELD, then fill in the name ('x') and type (integer – whole numbers – because the number of pixels is already super high and the accuracy of tons of decimals here just makes our tables messy afterwards), and create
3. Then use the Expression manager to fill this column
4. x($geometry) → UPDATE ALL
5. This will give us an x coordinate (remember that the origin is in the upper lefthand corner) for every polygon that corresponds to the centroid

x. y

1. Add y coordinates of the centroid of each polygon
2. NEW FIELD, then fill in the name ('y') and type (integer – whole numbers – because the number of pixels is already super high and the accuracy of tons of decimals here just makes our tables messy afterwards), and create
3. Then use the Expression manager to fill this column
4. y($geometry) → UPDATE ALL
5. This will give us a y coordinate (remember that the origin is in the upper lefthand corner) for every polygon that corresponds to the centroid

10. **SHELLS:** Add another layer as above in point 6, but rename it 'Shells' and trace all the bits of shell, etc.
11. **TURF:** Create a new polygon layer for turf algae
12. **MARKER/KELP:** Add a third and fourth layer as above but trace the center marker on one and the piece of kelp on the other.
13. **COMBINED SOLID BITS:** Solid substrate bits – extract centroid positions for these three types (if all three types exist in this photo background)

a. Combine these three layers into a single layer with all 'solid' portions of the area

i. VECTOR → DATA MANAGEMENT TOOLS → MERGE VECTOR LAYERS…
ii. Select all three polygon layers as input layers and then click RUN
iii. This creates a temporary layer called 'Merged'
iv. Click on the scratch icon beside this layer's name (or right click on this temporary layer → EXPORT → SAVE FEATURES AS or right click → MAKE PERMANENT) and save this layer as an ESRI shapefile to the appropriate sequence folder (e.g., 'Substrate_classification_in_sequences / AHNik2') with the filename 'Rocks_Shells_Marker"

b. Create points for the centroids of all these polygons (Solid areas in the sequence)

i. VECTOR → GEOMETRY TOOLS → CENTROIDS
ii. Select the input layer ('Rocks_Shells_Marker')
iii. RUN
iv. This creates a temporary point layer called 'Centroids'
v. Click on the scratch icon beside this layer's name (or right click on this temporary layer → EXPORT → SAVE FEATURES AS or right click → MAKE PERMANENT) and save this layer as an ESRI shapefile to the appropriate sequence folder (e.g., 'Substrate_classification_in_sequences / AHNik2') with the filename 'Rocks_Shells_Marker_Centroids'
vi. Change the layer name to be 'Rocks_Shells_Marker_Centroids' also

   c. Add a unique ID to the rows of this new combined points polygon layer to uniquely identify each solid thing (because as it is, the ids are replicated; there's a 1 rock, a 1 shell and a 1 marker)
      i. Open the attribute table
      ii. Toggle editing
      iii. Add a column with the name 'total_id'
      iv. Populate this column using the expression editor ($id + 1)

14. **BUFFER SOLID AREAS**
   a. VECTOR → GEOPROCESSING TOOLS → BUFFER
   b. Input layer: Choose the combined solid bits layer that was created in step 11
   c. Distance
      i. Use the pixel/cm conversion extracted from ImageJ tracking process (data in excel workbook: 'TrackingData_KNK_sequences.xlsx' for the appropriate sequence)
      ii. Calculate a buffer distance that corresponds to 2cm (2*conversion factor) in pixels
      iii. Use this distance in pixels as the 'Distance' for the buffer
   d. Click RUN
   e. This creates a temporary file called 'Buffer'
   f. Save this temporary file as an ESRI shapefile with the filename: 'Rocks_Shells_Marker_buffered' and change the layer name.
   g. These buffers extend beyond the outer limits of the photo, though. So clip this layer by the extent of the image.
   h. VECTOR → GEOPROCESSING TOOLS → CLIP
      i. Input layer: Rocks_Shells_Marker_Buffered
      ii. Overlay layer: Extent
      iii. Click RUN
      iv. This will create a temporary file called 'clipped'
      v. Save this temporary file as an ESRI shapefile with the filename: 'Rocks_Shells_Marker_Buffered_Clipped' and change the layer name.

15. N**ON-SAND SUBSTRATE**: create two layers, one with non-sand objects (solid bits and turf and kelp) and one with buffered solid bits + kelp + turf
   a. Non-sand objects
      i. VECTOR → GEOPROCESSING TOOLS → UNION
      ii. Input layer: Rocks_Shells_Marker
      iii. Overlay layer: Turf (and/or kelp)
      iv. Click RUN
      v. This creates a temporary file called 'Union'
      vi. Save this temporary file as an ESRI shapefile with the filename: 'Rocks_Shells_Marker_Turf' and change the layer name.
   b. Repeat but use the Rocks_Shells_Marker_Buffered_Clipped file as the input layer and save the output file as 'Rocks_Shells_Marker_Buffered_Clipped_Turf'

16. **SAND:** Calculate the difference between the extent (total area covered by the photos) and the solid bits we mapped previously.
   a. **Sand around solid bits and turf/kelp**
      i. VECTOR → GEOPROCESSING TOOLS → DIFFERENCE
      ii. Input layer: Extent
      iii. Overlay layer: Rocks_Shells_Marker_Turf
      iv. Click RUN
      v. This will create a temporary layer called DIFFERENCE
      vi. Save this temporary layer: Click on the scratch icon beside this layer's name (or right click on this temporary layer → EXPORT → SAVE FEATURES AS or right

click → MAKE PERMANENT) and save this layer as an ESRI shapefile to the appropriate sequence folder (e.g., 'Substrate_classification_in_sequences / AHNik2') with the filename 'Sand'

vii. Change the layer name to be 'Sand' also

viii. Convert this one polygon layer (attribute table with a single row) to a layer with one row per each separate polygon

1. VECTOR → GEOMETRY TOOLS → MULTIPART TO SINGLEPARTS
2. This creates a temporary file called 'Singleparts'
3. Save this temporary layer: Click on the scratch icon beside this layer's name (or right click on this temporary layer → EXPORT → SAVE FEATURES AS or right click → MAKE PERMANENT) and save this layer as an ESRI shapefile to the appropriate sequence folder (e.g., 'Substrate_classification_in_sequences / AHNik2') with the filename 'Sand_Singleparts'

b. **Sand around <u>buffered</u> solid bits and turf/kelp**

i. VECTOR → GEOPROCESSING TOOLS → DIFFERENCE

ii. Input layer: Extent

iii. Overlay layer: Rocks_Shells_Marker_Buffered_Clipped_Turf

iv. Click RUN

v. This will create a temporary layer called DIFFERENCE

vi. Save this temporary layer: Click on the scratch icon beside this layer's name (or right click on this temporary layer → EXPORT → SAVE FEATURES AS or right click → MAKE PERMANENT) and save this layer as an ESRI shapefile to the appropriate sequence folder (e.g., 'Substrate_classification_in_sequences / AHNik2') with the filename 'Sand_minus_buffer'

vii. Change the layer name to be 'Sand_minus_buffer' also

viii. Convert this one polygon layer (attribute table with a single row) to a layer with one row per each separate polygon

1. VECTOR → GEOMETRY TOOLS → MULTIPART TO SINGLEPARTS
2. This creates a temporary file called 'Singleparts'
3. Save this temporary layer: Click on the scratch icon beside this layer's name (or right click on this temporary layer → EXPORT → SAVE FEATURES AS or right click → MAKE PERMANENT) and save this layer as an ESRI shapefile to the appropriate sequence folder (e.g., 'Substrate_classification_in_sequences / AHNik2') with the filename 'Sand_minus_buffer_Singleparts'

17. **Export .csv files**

a. for the **Sand** polygon layer (singleparts)

i. Right click on the layer name (e.g., Sand_singleparts)

ii. EXPORT → SAVE FEATURES AS

iii. Select ".csv file" in Format box

iv. Select a location (csv_outputs) folder in the appropriate sequence folder (e.g., 'Substrate_classification_in_sequences / AHNik2') and name the file 'Sand'.

v. In Layer Options, change String_Quoting to 'IF_NEEDED'

vi. Click OK

b. for the **Sand_minus_buffer** polygon layer (singleparts)

i. Right click on the layer name (e.g., Sand_minus_buffer_singleparts)

ii. EXPORT → SAVE FEATURES AS

iii. Select ".csv file" in Format box

    iv. Select a location (csv_outputs) folder in the appropriate sequence folder (e.g., 'Substrate_classification_in_sequences / AHNik2') and name the file 'Sand_minus_buffer_singleparts'
    v. In Layer Options, change String_Quoting to 'IF_NEEDED'
    vi. Click OK
  c. Repeat for **Non-sand bits** (e.g, Rocks_Shells_Marker, or Rocks_Marker_Turf) polygon layer
  d. Repeat for the **Solid (rocks, shells and marker) buffered** area (Rocks_Marker_Buffered_Clipped_Dissolved) layer

18. Calculate a **summary distance matrix** for the solid bits in this area
  a. VECTOR → ANALYSIS TOOLS → DISTANCE MATRIX…
  b. Input point layer: choose the 'Rocks_Shells_Marker_Centroids' points layer
  c. Input unique ID field: total_id
  d. Target point point layer: 'Rocks_Shells_Marker_Centroids'
  e. Target unique ID field: total_id
  f. Output matrix type: 'Summary distance matrix (mean, std. dev., min, max)
  g. Click RUN
  h. This creates a temporary file called 'Distance'
  i. Save this temporary layer: Click on the scratch icon beside this layer's name (or right click on this temporary layer → EXPORT → SAVE FEATURES AS or right click → MAKE PERMANENT) and save this layer as and ESRI shapefile to the appropriate sequence folder (e.g., 'Substrate_classification_in_sequences / AHNik2') with the filename 'Distance_matrix'
  j. Save this layer as a csv file, also (to the appropriate sequence folder - e.g., 'Substrate_classification_in_sequences / AHNik2' - with the filename 'Distance_matrix'

19. Create a **Delauney Triangulation** for the dataset of centroid positions for these solid bits
  a. VECTOR → GEOMETRY TOOLS → DELAUNEY TRIANGULATION …
  b. Select Input layer: Rocks_Shells_Marker_Centroids
  c. Click RUN
  d. This creates a temporary file called 'Delauney Triangulation'
  e. Save this temporary layer: Click on the scratch icon beside this layer's name (or right click on this temporary layer → EXPORT → SAVE FEATURES AS or right click → MAKE PERMANENT) and save this layer as an ESRI shapefile to the appropriate sequence folder (e.g., 'Substrate_classification_in_sequences / AHNik2') with the filename 'Delauney_Triangulation'
  f. Export this layer as a csv file as well
    i. Right click on this temporary layer → EXPORT → SAVE FEATURES AS or right click → MAKE PERMANENT) and save this layer as an ESRI shapefile to the appropriate sequence folder (e.g., 'Substrate_classification_in_sequences / AHNik2/csv_outputs') with the filename 'Delauney_triangulation

20. **Nearest Neighbour** analysis
  a. Use the NNJoin plugin to run a nearest neighbour analysis
  b. Input vector layer: e.g., Rocks_Marker (layer that contains solid bits only, but no Turf, or kelp or algal stuff included, and no buffering)
  c. Join vector layer: same layer
  d. Add 'NNJoin' to the input layer for Export layer name (e.g., 'Rocks_Marker_NNJoin')
  e. Click RUN
  f. This creates a temporary file
  g. Save this temporary layer: Click on the scratch icon beside this layer's name (or right click on this temporary layer → EXPORT → SAVE FEATURES AS or right click → MAKE

PERMANENT) and save this layer as an ESRI shapefile to the appropriate sequence folder (e.g., 'Substrate_classification_in_sequences / AHNik2') with the filename 'Rocks_Marker_NNJoin'

h. Save this layer as a csv file, also (to the appropriate sequence folder - e.g., 'Substrate_classification_in_sequences / AHNik2/csv_outputs' - with the filename 'Rocks_Marker_NNJoin'

21. Link **Urchin track** information to **substrate** information and calculate distances to central marker and/or kelp for track info
    a. **Rocks:** Use the Intersection tool to classify every position of urchins as being on rock
        i. VECTOR → GEOPROCESSING TOOLS → INTERSECTION
        ii. Input Layer: Tracks
        iii. Overlay Layer: Rocks
        iv. This will create a temporary layer (Intersection) that will need to be saved (Tracks_on_rock) and the layer name changed
    b. Repeat this process for other substrate types: **Sand, Turf, Marker, Shells, Kelp**
    c. Repeat for the set of buffered substrates (**Solid with buffer** – use the Rocks_Marker_Buffered_Clipped_Dissolved layer – and **Sand minus buffered** solid area)
    d. Export all these files as csv files (to csv_outputs/Track_Info folder)
22. Calculate a **distance** for each point in the urchin track **info to the center marker or piece of kelp** in the sequence
    a. Distance to the centroid
        i. Create a centroid layer with 1 point for the centroid of the marker/kelp
            1. VECTOR → GEOMETRY TOOLS → CENTROIDS
            2. Input Layer: Marker
            3. This creates a temporary layer; save it and change the name of the new layer (Marker_centroid)
        ii. VECTOR → NNJOIN → NNJOIN
        iii. Input Layer: Tracks
        iv. Join vector layer: Marker_centroid
        v. Save and rename the temporary file created after running this (Tracks_with_distance_to_Marker_centroid)
    b. Distance to the outside of the central marker
        i. Create a line layer that traces the outside of the central Marker
            1. VECTOR → GEOMETRY TOOLS → POLYGONS TO LINES
            2. Input Layer: Marker
            3. This creates a temporary layer; save it and change the name of the new layer (Marker_Line)
        ii. VECTOR → NNJOIN → NNJOIN
        iii. Input Layer: Tracks
        iv. Join vector layer: Marker_Line
        v. Save and rename the temporary file created after running this (Tracks_with_distance_to_outside_Marker_Line)
    c. Export these two distance layers to csv files (csv_outputs/Track_info folder)
23. **Repairing invalid geometries** (if polygons are overlapping when created for rocks, for example)
    a. Verify validity of a layer using the 'Check Validity' function
        i. PROCESSING → TOOLBOX
        ii. CHECK VALIDITY
        iii. Choose the layer under question as the Input Layer
        iv. Click RUN
        v. This will create three output files (temporary) showing valid and invalid areas

        vi. If there are invalid areas, proceed to repair stage
    b. Use GRASS to buffer at a very small distance (1 pixel) to recreate a layer that repairs the geometry of this layer
        i. Open GRASS plugin : PLUGINS → GRASS → OPEN MAPSET (choose the Saint Lawrence, Katie mapset already created)
        ii. PLUGINS → GRASS → OPEN GRASS TOOLS
        iii. First import the layer we want to repair into grass (v.in.ogr.qgis), e.g., Rocks
        iv. Then use the function v.buffer
            1. Choose the layer to repair as the Input Vector Layer (e.g., Rocks)
            2. Buffer distance in map units: 1
            3. Click RUN
            4. Click VIEW OUTPUT
            5. Save this temporary layer: Click on the scratch icon beside this layer's name (or right click on this temporary layer → EXPORT → SAVE FEATURES AS or right click → MAKE PERMANENT) and save this layer as an ESRI shapefile to the appropriate sequence folder (e.g., 'Substrate_classification_in_sequences / AHNik2') with the filename 'Rocks_clean'

24. ************** Emacs/R **************
    a. Import the csv files (Sand and Rocks_Shells_Marker)
    b. Use the pixel/cm conversion ratio extracted from ImageJ tracking process (data in excel workbook: 'TrackingData_KNK_sequences.xlsx' for every sequence to convert the distances and areas from QGIS (which are in pixels) to cm measures.
    c. Calculate total area, proportion of area, distribution of patches in the photo etc. (see Excel data sheet)
    d. Import substrate and distance track info and combine with track info per sequence from MTrackJ