**Releasing Kelp Spores**

**Materials:** (note, this protocol is written for sporing 5 samples of kelp soros, but can be modified for any number of samples)

* Prepared Soros tissue samples
* Submersible thermometer
* 1000mL (1 L) beakers
* Chilled and filtered seawater, 50oF/10oC • Culture nutrients: PES, Vitamins, GeO2 • Graduated cylinder
* Pipettes
* Spatula
* Paper towels
* Exam gloves
* Clorox bleach
* 70% isopropyl alcohol

**Protocol:**

1. Remove spools from the freezer and let defrost. It is important to have the settling tubes filled with chilled, filtered seawater 50 F. Culture nutrients can be added to the settling tubes later, after the release solution is added to the tubes.
2. Prepare (5) 1000 mL beakers with 1000 mL filtered sea water cooled to 50 F.
3. Label 2 beakers “Alaria" and 3 beakers “Sugar Kelp.”
4. Add 9 mL Culture Nutrients to each beaker.
5. Collect the prepared sorus tissue from the refrigerator. Remove pieces of sorus tissue from the paper towels. If there is a brownish residue left on the paper towel, this is evidence of prior spore release and is a good sign that the soros tissue will release zoospores. Cut the sorus tissue into smaller pieces if necessary to fit into the beaker. About 10–15 pieces of sorus per beaker were frequently used in the OA nursery.
6. Record release data on the Release of Zoospores Worksheet, including: species, when and where collected, starting temperature, and time.
7. Occasionally stir sori(plural version of soros) gently in the beakers (every couple of minutes) with a sterile pipette. A cloudy plume(cloud) emerging from the sori signals the start of a spore release.
8. It is important to monitor the beaker temperature because temperatures rising over 60oF/16oC can damage the newly released spores. Write down the time, temperature, and release activity notes on the worksheet every few minutes to keep track of the progression.
9. Pour out 1000 mL of sea water from each settling tube - pour into orange bucket.
10. Place a wrapped kelp line spool into each settling tube.
11. Pour solution from Release Beaker into each settling tube.
12. Add chilled sea water to each in order to cover the wrapped spool.
13. Cover the tubes with the plexi-glass and let the tubes sit in the chilled tank for 24 hours.
14. Remove spools from settling tubes.
15. Remove settling tubes from the nursery tank.
16. Carefully stand spools in the nursery tank.