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Weis, V.M., Reynolds, W.S., deBoer, M.D. and Krupp, D.A. (2001) *Coral Reefs* 20: 301-308.

Hawaii – Quantification of infected *Fungia scutaria* larvae

Materials/Reagents

1x PBS

Cell counter

Slides/coverslips

Light microscope

Putty – molding clay

10X PBS

Make up in 800 ml	800 ml
0.02M NaH ₂ PO ₄ (monobasic) 120 g/m	1.92
0.077M Na ₂ HPO ₄ (dibasic) 142 g/m	8.8
1.4 M NaCl 58 g/m	69.6
add DH ₂ O to 800 ml	
Autoclave	

1X PBS

100 ml 10X PBS
900 ml DH₂O water

Procedure

- A. Rinse fixed *Fungia scutaria* larvae
 1. Spin larvae 1min, ~2,000xg
 2. Remove sup (toxic – paraformaldehyde)
 3. Rinse w/ 1x PBS 3x
 4. Re-suspend in 50ml 1x PBS

- B. Quantification of algae
 1. Place ~20ml larvae onto slide
 2. Scrap putty onto 4 corners of coverslip
 3. Gently depress coverslip, putty side down, onto larvae until no air is visible between slide and coverslip
 4. **ALTERNATIVE:** do not add putty to coverslip, gently drop onto larvae
Use a Kimwipe to remove excess water until larvae are depressed enough to see all algae
 5. Count 100-150 larvae – record number of larvae with algae (% infection)
 6. Count number of algae in 100 infected larvae (density of algae per larvae)

Comments: