

Water Acclimation Experiment

Brine Shrimp Kinetics Study

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1. Remove ~100 adult, age-matched artemia from main culture tank and rinse in fresh media in a 200-ml beaker.
2. Add 1 L of 100-g/L GSL media to each of three 1-L tripour beakers.
3. Carefully transfer 30 artemia to each beaker with a plastic transfer pipette, minimizing the amount of liquid transferred with each artemia.
4. Gently aerate each beaker with capillary tubing to ensure even mixing and full air saturation.
5. Spike one beaker with an appropriate volume of Se-75 labeled Se stock (of known specific activity) to achieve 2 $\mu\text{g Se/L}$ in the media. Spike another beaker with the same volume of unlabelled Se stock. The third beaker receives no addition of Se.
6. Take 3-mL initial water samples (in duplicate) from the Se-75 beaker and measure radioactivity on the gamma counter to verify exposure concentration.
7. Take duplicate 3-mL water samples daily from the Se-75 beaker, measure radioactivity, and spike with additional Se-75 labeled Se stock (or dilute with GSL media) to maintain 2 $\mu\text{g Se/L}$. Mirror the Se spikes and/or dilutions in the second beaker with unlabeled Se stock.

8. Feed each beaker daily with equal amounts of *Dunaliella viridis*. Feeding should be done 3-4 hours before water sampling and Se spiking to minimize uptake of Se by the algae cells.
9. Maintain waterborne exposures in the beakers for 2 weeks.
10. Perform waterborne uptake and depuration experiment with artemia exposed to 2 $\mu\text{g/L}$ unlabeled Se (from second beaker) and artemia not exposed to Se in the media (from third beaker) according to **Standard Procedures for Se-75 Experiments - Uptake from the Water**. (Note: artemia from beaker containing Se-75 are not used in uptake and depuration experiment but simply serve to monitor exposure concentrations).