Derek Brady

Research Proposal

28 OCT 2011

FISH 441 - Lab

**Background:**

 The green sea urchin (Strongylocentrotus droebachiensis) is a marine invertebrate osmoconformer (Lange, R., 1964) that exhibits a covering behavior in which tube feet are utilized to bring molluscs shell fragments, rock, and other substances onto the its dorsal surface. The behavior decreases in frequency as the urchin size increases and it is thought to be triggered by multiple stressors such as light, predator proximity, and tidal action (Ciocan, C. M., 2010). While the covering behavior is widely observed within this species the function and mechanism of the behavior is poorly understood. My experiment proposal looks at the behavior from a physiological stand point by analyzing changes in gene expression of Na-K-2Cl ion pumps when exposed to stressful conditions. The stressful conditions examined are direct exposure to a known predator (Pisaster ochraceus) and synergistically exposure to known predator (Pisaster ochraceus) and low salinity conditions.

**Research Objectives:**

 The objective of the experiment is to examine how Na-K-2Cl ion pump expression is affected by predator proximity and synergistically predator and low salinity conditions.

**Methods:**

 Experimental set-up will consist of 3 separate tanks and 20 specimens. Tank 1, the control tank will contain 6 urchins (salinity 30psu) with a wall separating it from the two experimental tanks. Tank 2 will contain 7 urchins and 1 sea star and salinity (30psu). Tank 3 will contain 7 urchins, 1 sea star, and a lowered salinity (20psu). The experiment will run 120 hours with feeding once a day and x2 water changes to remove excess ammonia waste. Daily observations will be made to include temperature, salinity, and number of fragments collected by each urchin. Upon completion 10-15 tube feet per urchin will be collected by placing each into ~1-2in salt water and clipping tube feet (Kabatt-Zinn et al, 1981). Dissection for protein analysis will be completed on x1 urchin per group by cutting horizontally along the urchin’s equator and removing the dorsal portion for gonadal and digestive tract tissue collection. All tissue samples will be directly placed onto dry ice and then transferred to -80C freezer for storage.

 The tissue collected will be converted into cDNA through RNA extraction and isolation procedures to protocol. Tissue collected form the tube feet will be utilized with the Na-K-2Cl co-transporter primer whereas the digestive tract and gonadal tissue will be used in stress related protein extraction by separate members of the experiment. Techniques such as qPCR will be required to examine the differences in gene expression of the Na-K-2Cl co-transporter pump.

Primer (Strongylocentrotus purpuratus)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Name | Gene | Primer Sequence | Ascension # | Bps | Product Bps |
| Sp\_NaK2Cl\_F | Na-K-2Cl co-transporter 1 | TGAGATACGACACGCCACCA | NM\_001113236.1 | 20 | 186 |
| Sp\_NaK2Cl\_R | Na-K-2Cl co-transporter 1 | GTTCTCTTTCGGGGCAGCTT | NM\_001113236.1 | 20 | 186 |

**Timeline:**

|  |  |
| --- | --- |
| Date | Experimental Plan |
| Exp Day 1 – Wednesday, October 19, 2011 | Experimental set-up, organism collection, primer design, begin acclimatization of specimens. Feed 2-3 algal pellets each urchin. |
| Exp Day 2 – Thursday, October 20, 2011 | Begin Experiment 1330. Add predators to respective tanks 2 & 3. Lower tank 3 salinity using DI water to 20psu. Feed 2-3 algal pellets each urchin. |
| Exp Day 3 – Friday, October 21, 2011  | Monitor shell fragments collected, Feed 2-3 algal pellets each urchin. |
| Exp Day 4 – Saturday, October 22, 2011 | Monitor shell fragments collected, change water, check salinity. Feed 2-3 algal pellets each urchin. |
| Exp Day 5 – Sunday, October 23, 2011 | Monitor shell fragments collected, Feed 2-3 algal pellets each urchin. |
| Exp Day 6 – Monday, October 24, 2011 | Monitor shell fragments collected, Feed 2-3 algal pellets each urchin. Water change, check salinity. |
| Exp Day 7 – Tuesday, October 25, 2011 | Collect tissue samples and store in -80C freezer. |
| Lab Week 1 – Tuesday November 1 – November 7 | Begin RNA extraction and Isolation of tube feet tissue. |
| Lab Week 2 – Nov 8th – Nov 14th  | Run PCR procedures |
| Lab Week 3 – Nov 15th – Nov 21st  | Run qPCR procedures |
| Lab Week 4 – Nov 22nd – Nov 30th  | Review data/ write paper/ design presentation |

 **Expected Results:**

1. Shell Behavior-

I expect that urchins within tanks 2 & 3 will have an increased shell covering response due to the stress of predator proximity. Tank 3 should have an even higher covering response due to the synergistic effects of both predator proximity and low salinity conditions.

2. Na-K-2Cl co-transporter pump –

I would expect the co-transporter pump expression to decrease in response to stress due to the required shift in energy devoted toward stress protein production such as HSP70 and movement. This could be problematic when faced with multiple stressors such as predator and low salinity conditions as the urchin may not be able to conform as effectively to the osmotic conditions.

**References:**

Dumont, Clément P., et al. "Multiple Factors Explain the Covering Behaviour in the Green Sea Urchin, Strongylocentrotus Droebachiensis." *Animal Behaviour* 73.6 (2007): 979-86. Web

Kabat-Zinn, J.,Singer, R H., Sea urchin tube feet: unique structures that allow a cytological and molecular

approach to the study of actin and its gene expression.1981/04/01 The Journal of Cell Biology.P.109-114., 10.1083/jcb.89.1.109, Vol. 89

R. Lange, The osmotic adjustment in the echinoderm, Strongylocentrotus droebachiensis, Comparative Biochemistry and Physiology, Volume 13, Issue 3, November 1964, Pages 205-216, ISSN 0010- 406X, 10.1016/0010-406X(64)90117-3.