The proteomic response in the crustacean molting gland of land crab *Gecarcinus lateralis* in response to artificially induced molting throughout its molting cycle.

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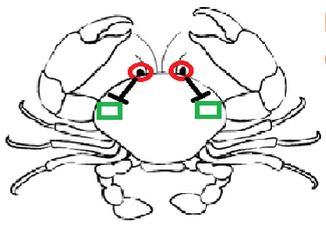
Background

- Y-organ (YO) is located in the cephalothorax and produces ecdysteroid which induces molting.
- X-organ (XO) is located in the eyestalks and produces molt-inhibiting hormone (MIH) into the hemolymph, which, through a GPCR cell signaling pathway, down regulates ecdysteroid function in the YO. It also has other, less well defined, endocrine functions
- MIH has a direct inverse relationship with the production of ecdysteroids and indirectly inhibits molting.



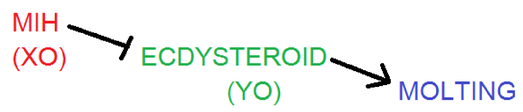


Figure 1. Depicts location of the XO in the eyestalks, producing MIH (red), which has an inhibitory relationship with production of ecdysteroids. The YO in the cephalothorax is also shown which synthesizes ecdysteroids (green), inducing molting in the land crab *G. lat.*

- Progression in the molt cycle is determined by the R-index which is the ratio of the length of the carapace to the length of their limb buds. When this value reaches >16 the crab is committed to molt.

	Intermolt	Early Premolt	Mid Premolt	Late Premolt	Postmolt
YO state:	Basal	Activated	Committed	Repressed	Basal
Sensitivity to MIH:	High	High	Low	Low	High
R-index: (limb regeneration)	0-10	11-15	16-23	23	

Figure 2. The YO state, sensitivity to MIH and R-index based on molting state. Yellow highlighting delineates the point at which the crab becomes committed to molt and the R-index threshold where this occurs. Figure adapted from Mykles and Chang (2011)

- In nature, environmental cues induce molting which causes behavioral changes in *G. lat.* such that they burrow in solidarity, away from predators, as their exoskeleton softens and they are able to grow and experience an influx of metabolic activity.
- Consumption of the old exoskeleton provides them with calcium and they peristaltically wiggle out of the old exoskeleton while synthesizing a new one.

Table 1. Description of the two ways to induce molting in *G. lat.* in the laboratory

Treatments to induce molting in the lab:	What happens?	How does it induce molting?	Benefits	Drawbacks
Eye-stalk ablation (ESA)	Removes the XO and MIH source	YO is not suppressed by MIH allowing ecdysteroids to increase	Fast; don't regrow eyes	XO makes other hormones which may have other physiological functions including actions on YO
Multiple leg autotomy (MLA)	Removes 5 or more walking legs	Growth of limb buds is restricted to premolt where upon ecdysis they become functional appendages	No endocrine organs or function in legs, replicates what occurs in nature	Slow; must fully regrow limbs before moving on

Primary goal: Determine if ESA mimics the normal physiology of a molting crab accurately by comparing the proteome of their YOs to those of the MLA treated crabs.

Relevance: Being able to artificially induce molting has broad economic impacts for crustacean fisheries as industry demands increase

Experimental Design

ANIMAL COLLECTION

Dominican Republic

HOUSING & ACCLIMATION

Colorado State University

MOLT STAGE DETERMINATION

R-index and confirmed using ELISA to determine ecdysteroid hemolymph titer

SAMPLE POOLING

Molt Stage	Sample Size	YO Pairs per Biological Replicate	Biological Replicates
Intermolt	12	3	4
Early Premolt	12	3	4
Mid Premolt	12	3	4
Late Premolt	12	3	4
Postmolt	12	3	4
0 Days Post-ESA	6	2	3
1 Day Post-ESA	6	2	3
3 Days Post-ESA	6	2	3
7 Days Post-ESA	6	2	3
14 Days Post-ESA	6	2	3

YOs were dissected out and pooled in groups of 2-3 pairs per biological replicate based on similar ecdysteroid titers because a single pair doesn't produce enough protein concentration

SAMPLE COLLECTION

Proteomics Workflow

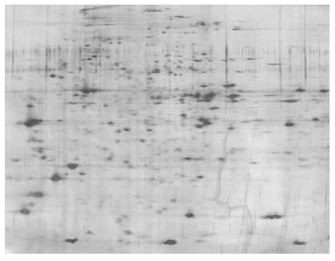
SAMPLE PREPARATION

Tissue homogenized in protein denaturant solution, then precipitated in TCA/acetone and solubilized in rehydration buffer

ISOELECTRIC FOCUSING

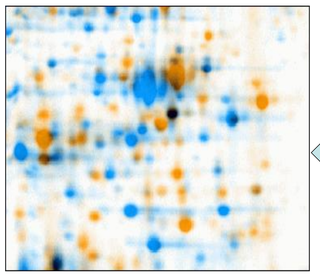
1st dimension: proteins migrate along a charge gradient that separates based on isoelectric (pI) point within pH 3-10 range

SDS-PAGE



2nd dimension: proteins are separated further by molecular mass (kDa), polyacrylamide gel contains SDS-an anionic detergent that denatures proteins

ANALYSIS: Delta2D



Gel images are superimposed to detect significant changes in expression patterns and protein abundance levels based on different treatments

Preliminary Results

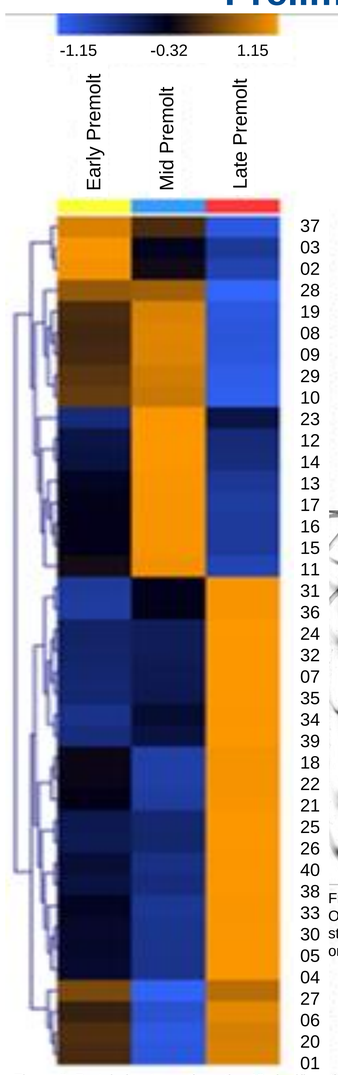


Figure 3. Protein heat map from the 40 significantly different proteins throughout early, mid, late premolt. Orange signifies up regulation of a given protein and blue signifies down regulation of a given protein.

Significant genes
# of Significant Genes: 40
% of Genes that are significant: 14%

Non-significant genes
# of non-significant Genes: 242
% of Genes that are not significant: 86%

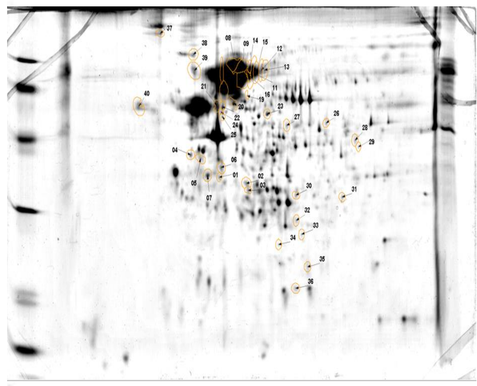


Figure 4. Fused gel image from early, mid, and late premolt showing YOs proteins with significantly different abundances between the each stage (compared to early premolt). Significant spots are designated by orange circles.

Future Analysis

- Analysis of relative protein abundance and regulation of those proteins throughout the crustacean molting cycle.
- Determine the mechanism in which the YO transitions from the activated to the committed state in terms of the signaling pathway involved in the negative control of ecdysteroidogenesis.
- Changes in the protein expression profile in the YOs between ESA treated crabs and natural molting crabs will determine the viability of ESA as a molting manipulation technique in a laboratory setting.
- Identification of proteins of interest between ESA and MLA treated crabs using MALDI-TOF mass spectrometry.

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References

Abuhagr, A.M., K.S. MacLea, E.S. Chang, D.L. Mykles. 2014. Mechanistic target of rapamycin (mTOR) signaling genes in decapod crustaceans: Cloning and tissue expression of mTOR, Akt, Rheb, and p70 S6 kinase in the green crab , Carcinus maenas, and blackback land crab, Gecarcinus lateralis. Comp. Biochem. Physiol. 168:25-39. Chang, E.S., and D.L. Mykles. 2011. Regulation of crustacean molting: A review and our perspectives. Gen. Comp. Endocr. 172:323-330. Covi, J.A., E.S. Chang, and D.L. Mykles. 2009. Conserved role of cyclic nucleotides in the regulation of ecdysteroidogenesis by the crustacean molting gland. Comp. Biochem. Physiol. 152:470-477. Garland, M., Stillman, J.H., Tomanek, L. 2015. The proteomic response of cheeped myofibril tissue in the eurythermal porcelain crab Petrolisthes cinctures the heat shock following acclimation to daily temperature fluctuations. J. Exp. Biol. 218:388-403. Hamer, M. 2015. The proteomic response of the Carcinus maenas Y-organ over the course of the molt cycle. Masters Thesis , California Polytechnic State University. Lee, S.G., B.D. Bader, E.S. Chang, and D.L. Mykles. 2007. Effects of elevated ecdysteroid on tissue expression of three guanylyl cyclades in the tropical land crab Gecarcinus lateralis: possible roles of neuropeptide signaling in the molting gland. J. Exp. Biol. 210:3245-3254. Lee, S.G., and D.L. Mykles. 2006. Proteomics and signal transduction in the crustacean molting gland. Integr. Comp. Biol. 46(6):965-977. McDonald, A.A., E.S. Chang, and D.L. Mykles. 2011. Cloning of a nitric oxide synthase from green shore crab, Carcinus maenas: A comparative study of the effects of eyestalk ablation on expression in the molting glands (Y-organs) of C. maenas, and blackback land crab, Gecarcinus lateralis. Comp. Biochem. Physiol. 158:150-162. Mykles, D.L. 2011. Ecdysteroid metabolism in crustaceans. J. Steroid Biochem. 127:196-203. Stentiford, from the tropical land crab, Gecarcinus lateralis, that inhibits Y-organ ecdysteroidogenesis in vitro. Mol. Biol. Rep. 36:1231-1237. G.D., D.M. Niel, E.J. Peeler, J.D. Shields, H.J. Small, T.W. Flegel, J.M. Vlak, B. Jones, F. Morado, S. Moss, J. Lotz, L. Bartholomay, D.C. Behringer, C. Hauton, and D.V. Lightner. 2012. Disease will limit future food supply from the global crustacean fishery and aquaculture sectors. J. Invert. Pathol. 110: 141-157. Zarubin, T.P., E.S. Chang, and D.L. Mykles. 2009. Expression of recombinant eyestalk crustacean hyperglycemic hormone