CUSTOMER SPOTLIGHT

Novel Approaches for Unconventional Research Subjects Yield Unexpected Results

The "Crab Lab" at Colorado State University relies on unique procedures to make a positive impact on marine-based food supplies.

The natural world provides life-sustaining food and medicines, and fundamental research is essential to unlocking unknown resources. However, the research can be challenging, particularly with life forms that haven't been studied very much. For the team at the Mykles Lab at Colorado State University, informally known as the "Crab Lab," meeting those challenges means developing new protocols and procedures.

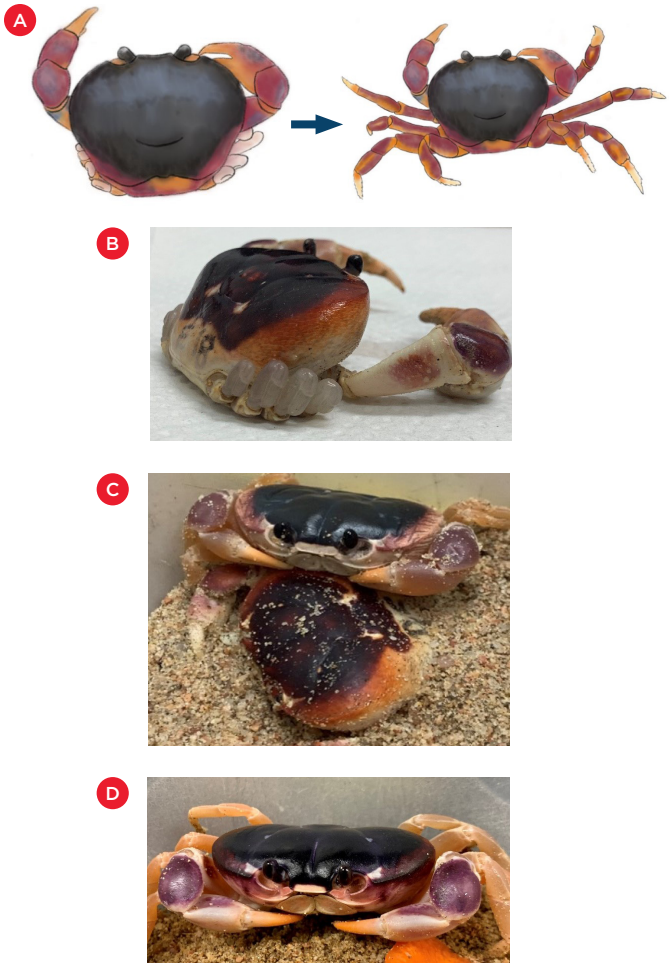
The Mykles Crab Lab at Colorado State University. Top left to right: Ashlynn Madril, Olivia Garvin, Avery Hunter, Kendal Berasley, Mihika Kozma Ph.D. (Postdoc). Bottom left to right: Kyle Raney, Talia Head (Ph.D. candidate), Julia Newcomb, Laura Antizzo, Sydney Collins, An-Ping Yu, Vanessa Bentley (Ph.D. candidate), Jorge Perez-Moreno Ph.D. (Postdoc), Donald Mykles Ph.D. (PI). Not pictured: New Crab Lab members, Levi Friss, Avery Kruger, Madison Pelletier, Jay Peralta.

To learn more about this approach, we asked Jorge L. Perez-Moreno, a postdoctoral fellow, to tell us more.

The team is focused on the cellular and molecular mechanisms that control molting, muscle atrophy, and limb regeneration in crabs, using the blackback land crab (*Gecarcinus lateralis*) as a model system. A combination of cellular, molecular, and genomic/transcriptomic methods are used to explore numerous aspects of the crustacean physiology.

Understanding the important physiological pathways and mechanisms that are part of such a complex biological transformation can have far reaching implications. Molting is essential for crustacean growth, development, reproduction, and regeneration. So a better understanding of the process can reveal environmental pressures that can influence sustainable practices in aquaculture and the fisheries industry, improving a crucial worldwide food source.

Characterizing the complexity of the processes will necessarily include details about the diversity of the peptides and metabolites involved at every stage. These in turn may offer potential biomedical and biotechnological uses. But the tissues of crustaceans can be quite tricky to work with due to the presence of lipids, mucopolysaccharides, chitin, and other compounds. These complicate isolating pure high-molecular-weight (HMW) nucleic acids, among other compounds.



Crab limb-regeneration & molting. (A) Crabs have the ability to voluntarily autotomize their limbs, as a mechanism to survive both encounters with predators and fights with other crabs. They are also able to regenerate previously lost limbs, which become fully functional once the animal successfully molts. **(B)** Shortly after limb autotomy, limb regeneration is evident by the appearance of "limb-buds". **(C)** The crab will then molt and shed its exoskeleton. Fully regenerated limbs are now visible. **(D)** Once the crab's pneumo-hydrostatic pressure is restored and the new exoskeleton has hardened, the new limbs become fully functional.

*Blackback land crab illustrations and photographs by Crab Lab's undergraduate student An-Ping Yu. For more details on crabs' ability to regenerate via molting, please visit **https://www.youtube.com/watch?v=KBMUhG0aEFg**

As a result, the team has invested a significant amount of time and effort into developing and optimizing custom protocols and reagents to achieve research goals. Perez-Moreno is working in conjunction with another postdoctoral fellow, Mihika Kozma, to develop the bioinformatics, pipelines, and databases, and standardize procedures that will enable the lab to efficiently use their large RNAseq datasets. These efforts will rely on an existing Crab Lab process created to yield very good quality RNA and HMW DNA.

The most noteworthy aspects of the DNA isolation are custom digestion buffers to deal with excess mucopolysaccharides from tissues, the separation of nuclei from other cellular components, and the use of Beckman Coulter Life Sciences SPRIselect paramagnetic bead-based chemistry to separate, purify, concentrate, and size-select the nucleic acid isolations. The beads proved extremely useful in the lab setting for numerous tasks, which streamlined a way of obtaining clean and HMW DNA.

As the lead for the lab's genome sequencing project identifying the molting pathway and the mechanisms that regulate the model species, *Gecarcinus lateralis*, Perez-Moreno will rely on Beckman Coulter Life Sciences SPRIselect to isolate HMW DNA for long-read sequencing. DNA/RNA isolation, purification, and the preparation of sequencing libraries will all benefit from the efficiency of the beads.

Perez-Moreno recognizes that the initial stages of such non-traditional model systems can seem daunting. So he encouraged researchers to take a long-term approach.

"This effort is compensated by the breadth of research questions that can be answered, the satisfaction of developing new successful methods and protocols, and the ability to obtain novel insights that perhaps might not have been evident in other systems," he said.

Dr. Donald Mykles, primary investigator, and postdocs from the lab – including Dr. Perez-Moreno and Dr. Kozma– shared some methods for obtaining insights from genomic/transcriptomic data that are often overlooked, particularly by researchers working with non-traditional model organisms. The workshop for the Society for Integrative and Comparative Biology was presented in January 2023 thanks to funding by a National Science Foundation Research Coordination Network grant.