

Squid Ink as a Chemical Defense against Predatory Sea Catfish

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Introduction: In a previous study, ink from the squid *Doryteuthis pealeii* was tested to determine its palatability on a predatory fish, the flounder *Paralichthys dentatus*. It was concluded that the ink acted as a chemical defense. Melanin, a key component that causes the inks black color, may serve as a chemical cue. Determining if melanin is essential for the ink will be useful in finding the compounds responsible for the chemical defense. We hypothesize that: 1) squid ink will work as a chemical defense on the predatory sea catfish *Ariopsis felis*, and 2) when fractionated, the melanin-containing ink pellet will have a higher overall rejection rate from the sea catfish than the supernatant fraction, which contains no melanin.

Methods: To test our hypothesis, shrimp were soaked in water, drained, and then freeze dried. Treatments were then added to pieces of freeze dried shrimp in 0.5 mL amounts. This experiment included a negative control (artificial salt water-ASW) and a positive control (10 mM of quinine). The other treatments included 1:5 diluted squid ink (un-separated), the ink pellet, and the ink supernatant. An ASW- treatment- ASW feeding test method was conducted every other day to prevent false results. Data was collected from sea catfish that accepted both pieces of ASW shrimp. The shrimp pieces were presented to the sea catfish by touching the fish's barbels. Data was categorized into accept (ate), intra-oral reject (take into mouth and spit out), or extra-oral reject (reject before entering mouth).

Results: Over 70% of the catfish rejected (either intra- or extra-orally) the ink. Less than 35% of the fish rejected the ink supernatant, but 75% rejected the resuspended ink pellet containing melanin. All of the fish accepted the negative control and extra-orally rejected the positive control.

Conclusion: Our findings supported our hypothesis that ink from the squid *Doryteuthis pealeii* works as a chemical defense against predatory sea catfish *Ariopsis felis*. The significant differences between the overall rejection rates for the fractionated ink portions confirmed that there is a component within the melanin-containing pellet fraction that is more unpalatable to the catfish than the supernatant fraction.