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Bioassay directed isolation and characterization of a natural sea lamprey deterrent phase-II

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ABSTRACT:
The sea lamprey (*Petromyzon marinus*) is a semelparous ectoparasitic fish that relies extensively on olfaction to complete its terminal spawning migration from the open waters of oceans or large lakes into streams. Sea lamprey tissue, in particular the skin, contains naturally aversive compounds that constitute a conspecific alarm cue. Application of the alarm cue to stream waters has proven effective as a repellent that can be used to guide migrants toward traps and other control measures. We pursued isolation and identification of the sea lamprey alarm cue components through behaviorally guided fractionation. The goal of this project was to identify and characterize previously unidentified (Phase-I study, project ID: 2016-NAI-54051) potential repellent compounds and to test their repellent activity in laboratory raceways. To isolate and spectroscopically characterize the remaining potential alarm cue constituents from Phase-I project, we modified the fractionation of the active water-soluble fraction of the aqueous ethanolic (80:20 *v/v*) extract of sea lamprey and afforded six distinct sub-fractions. Based on HPLC analyses, four of these fractions contained only previously reported nitrogenous compounds (Dissanayake et al. PLOS ONE, 2019, 14(5):e0217417) (appendix 1 and 2). MPLC fractionation and repeated HPLC purifications of the remaining two fractions yielded 12 pure compounds. Spectroscopic (NMR and MS) experiments were used to chemically identify these pure isolates. Among these pure isolates, one of the compounds was a novel substituted uracil analogue and we named it as petromyzonacil. Three amino acids (proline, aspartic acid and serine), two organic acids (3-phenyllactic acid and β-hydroxybutyric

acid), four keto acids (α-ketobutyric acid, α-ketoisovaleric acid, α-ketovaleric acid and pyruvic acid) and two aliphatic amines (putrescine and spermine) were the rest of the 11 compounds. Both water-soluble and chloroform-soluble fractions elicited 75% of the avoidance response observed from crude skin extract. Recombining the two fractions restored full reactivity, suggesting alarm cue components may include or require lipids in addition to water-soluble compounds. We further screened 13 individual compounds or pure isolates and 6 sub-fractions from the water-soluble fraction and found one individual compound of the pure isolates, isoleucine, evoked an avoidance response on its own, but not consistently when found in other mixtures. In a third experiment, we observed no behavioral response after recombining 32 compounds isolated and identified from the water-soluble fraction. These results confirm other suggestions that the process of elucidating alarm cue constituents are will prove challenging. We also evaluated the behavioral response of invasive sea lamprey to putrescine, a decay molecule that many prey organisms avoid. Putrescine is found in tissue extracts that contain the sea lamprey alarm cue, and in human saliva, two mixtures known to elicit flight and avoidance responses in migratory sea lamprey. We used two behavioral assays to evaluate metrics of repellency: behavioral preference (space use) and change in activity rates and found context-dependent results. In a smaller assay with individual fish, we found that putrescine had no effect on sea lamprey activity but did increase repellency. In a larger assay with multianimals, we found no repellent behavior to any putrescine treatment. However, our results also showed consistent changes in activity and avoidance behavior in sea lamprey exposed to alarm cue in the smaller assay, concluding that this design could prove useful as a high-throughput screening tool. We also investigated a novel odor identified in sea lamprey skin, petromyzonacil and found no behavioral effects to this odor on its own or in synergy with putrescine. Our results show limited evidence that putrescine acts as robust repellent for sea lamprey and highlight the importance of environmental context in anti-predator behaviors