

In situ tissue analysis of metabolites, lipids, and proteins in the gill glands of Bloodfin Tetra by LAESI Mass Spectrometry

Bindesh Shrestha, Robert Javonillo, John Burns, and Akos Vertes, *The George Washington University, Washington, DC 20052*

Introduction

- Adult males of the Glandulocaudine subfamily of fish, such as the Bloodfin Tetra (*Aphyocharax anisitsi*), have modified caudal-fin scales associated with hypertrophied glandular tissue.^[1]
- No such glands are present in females or immature males and the functions of these glands are not established.
- *In situ* analysis of the exposed gill glands for metabolites, lipids and proteins could provide further insight into their functions.
- Laser ablation electrospray ionization (LAESI) mass spectrometry (MS)^[2,3] is employed to simultaneously analyze small metabolites, lipids and some proteins at ambient conditions from *A. anisitsi* gill glands.

Material and Methods

- *A. anisitsi* was euthanized by submersion in buffered tricaine methanesulfonate for 10 minutes and the gill glands were surgically exposed.
- Gill glands were ablated by mid-infrared radiation at 2940 nm produced by a Nd:YAG laser driven optical parametric oscillator (OPO).
- Electro spray intercepted and ionized the neutrals produced by the ablation and the generated ions were analyzed by a mass spectrometer (MS) (see Fig. 1).
- Alignment of the laser beam on the sample was aided by visualization using a CCD camera fitted with a macro lens.

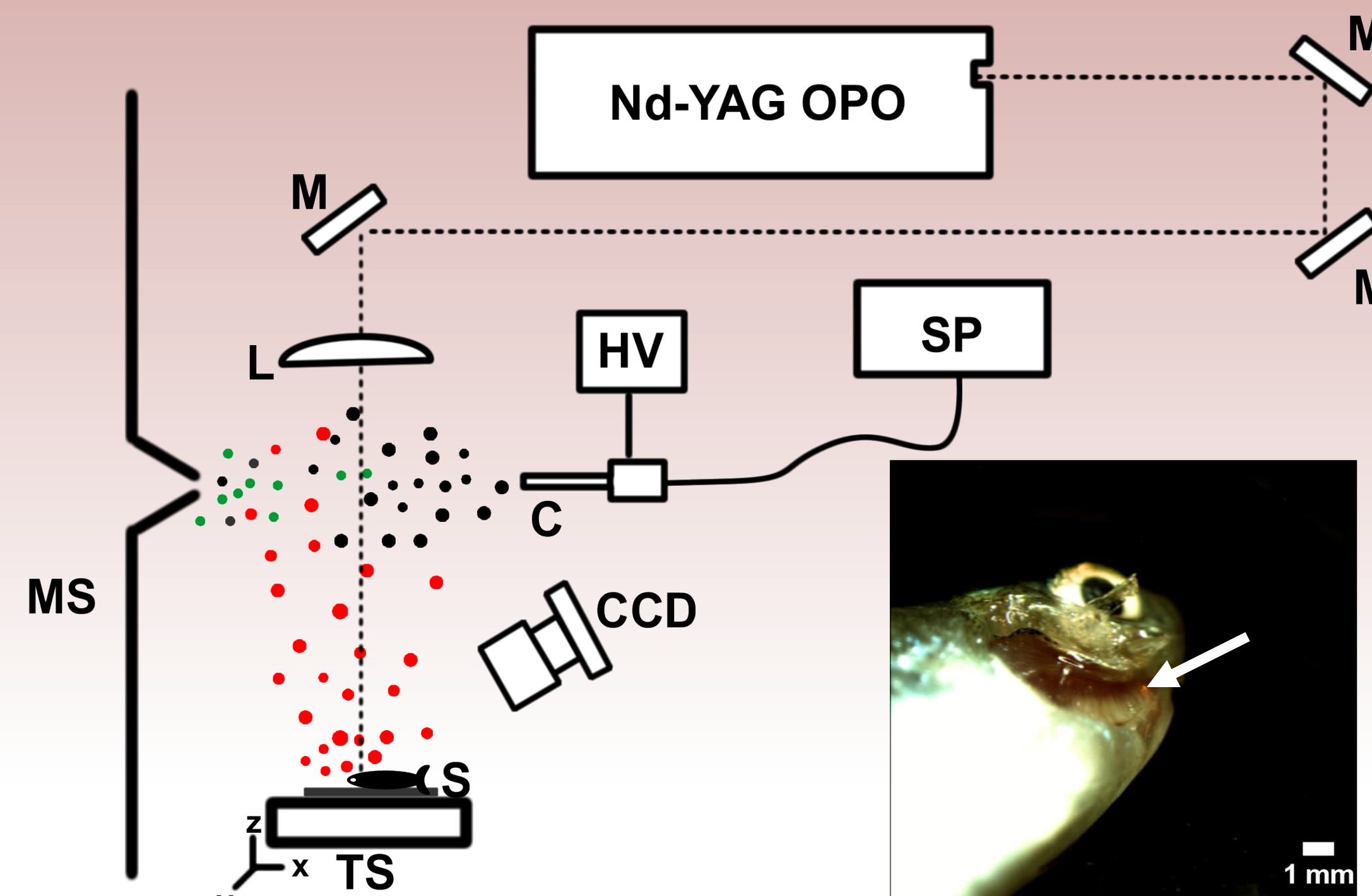


Fig. 1. Schematic of LAESI-MS for analysis of *A. anisitsi* gill glands. Capillary (C), CCD camera with a macro lens (CCD), high-voltage power supply (HV), CaF₂ lens (L), Au-coated mirrors (M), mass spectrometer (MS), exposed gill glands sample (S), syringe pump (SP), translation stage (TS)

Summary and References

- Detection of diverse of molecular classes by direct analysis of unprocessed tissue using LAESI-MS demonstrates its potential for the simultaneous detection of small metabolites, lipids and peptides/proteins from biological samples.
 - A unique protein that was found only in the mature male gill glands using rapid screening by LAESI-MS can be related to the function of this modified organ.
- [1] Burns, J. R.; Weitzman, S. H., "Novel Gill-Derived Gland in the Male Swordtail Characin, *Corynopoma riisei*" *Copeia*, **1996**, 3, 627.
 [2] Nemes, P.; Vertes, A., "Laser Ablation Electrospray Ionization for Atmospheric Pressure, in Vivo, and Imaging Mass Spectrometry," *Analytical Chemistry*, **2007**, 79, 8098.
 [3] Shrestha, B.; Vertes, A., "In Situ Metabolic Profiling of Single Cells by Laser Ablation Electrospray Ionization Mass Spectrometry" *Analytical Chemistry*, **2009**, 81, 8265.

The authors acknowledge the financial support from the W. M. Keck Foundation (041904), the Department of Energy (DEFG02-01ER15129), and the George Washington University Research Enhancement Fund for this work.

LAESI-MS of Gill Gland

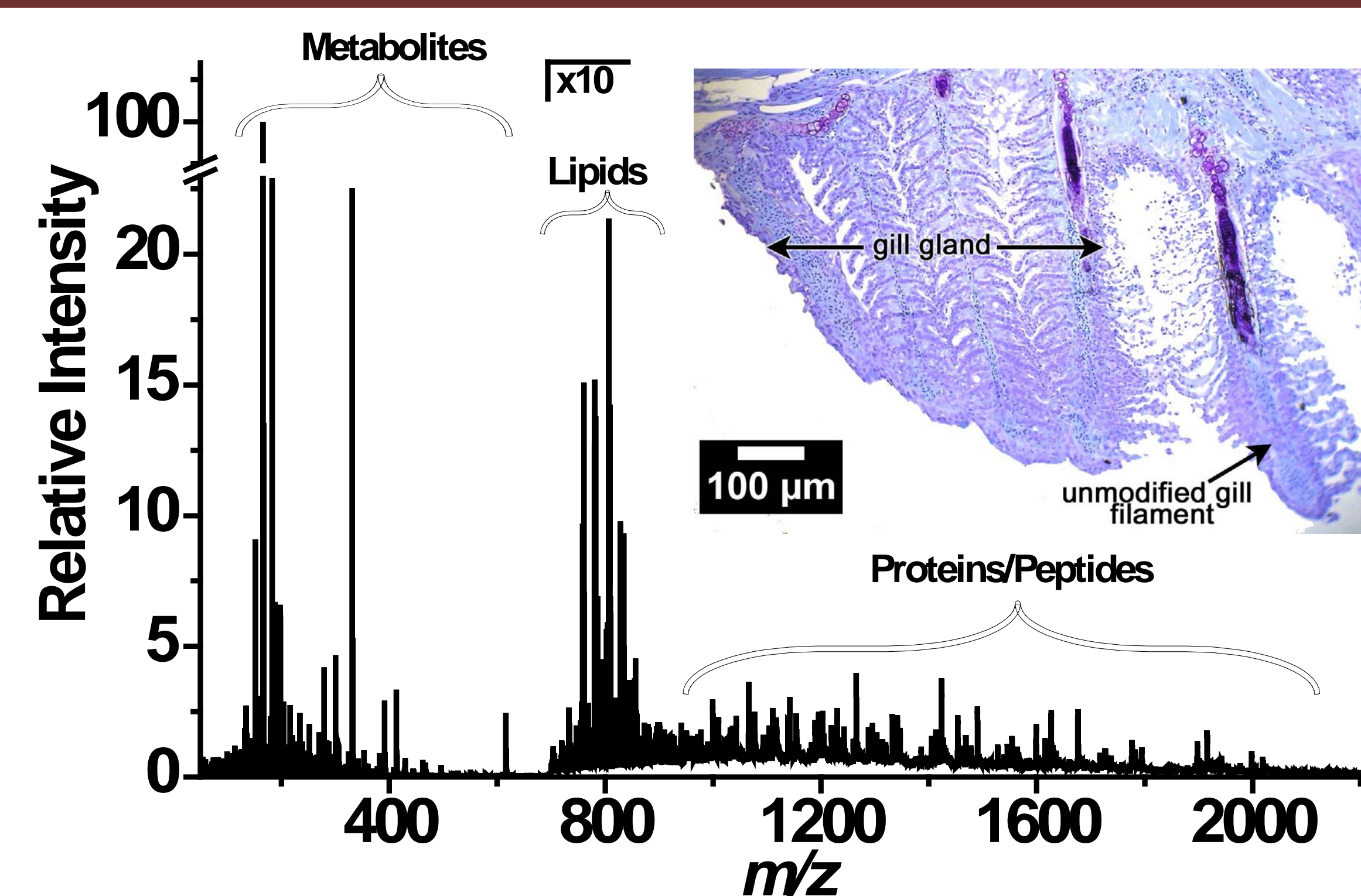


Fig. 2. LAESI mass spectrum from the gill glands of the adult male *A. anisitsi* contains approximately two hundred different ions corresponding to metabolites, lipids and proteins/peptides.

Metabolites and Lipids

Over 120 small metabolites ($m/z < 600$ range) and lipid-related species ($600 < m/z < 900$) were found in a typical LAESI mass spectrum (Fig. 2). Examples are:

choline, hydroxybutyric acid, erythritol ketobutyric acid, methylhistamine, creatine, adenine, urea, hypoxanthine, proline, histidinal, spermidine, lysine, ciliatine, ornithine, histidine, phenylpyruvate, phenylalanine, glutamic acid or acetyl serine, diethylthiophosphate, acetylspermidine,

PC(32:0), PC(34:2), PC(34:1), PC(34:0), PE(38:4), PC(36:5), PC(36:4), PC(36:3), PC(36:2), PC(36:1), PS(36:0), PC(38:8), PC(38:7), PC(38:6), PC(38:5), PC(38:4), PC(38:3), PC(36:4), PC(36:1), PC(40:9), PC(38:6), PC(40:8), PS(40:2), PC(42:10) and PC(40:6).

PC = Glycerophosphocholines, PE = Phosphatidylethanolamines

Peptides and Proteins in *A. anisitsi* Glands

Mass spectrum of the gill glands of sexually matured male showed the presence of multiply charged ions in the +6 to +10 charge states corresponding to a protein with a molecular weight of 11.38 kDa (denoted by red) (see inset in Fig. 3b), which is absent in the unmodified areas of the male specimen (see Fig. 3a) and in the corresponding area of the female specimen (see Fig. 3c).

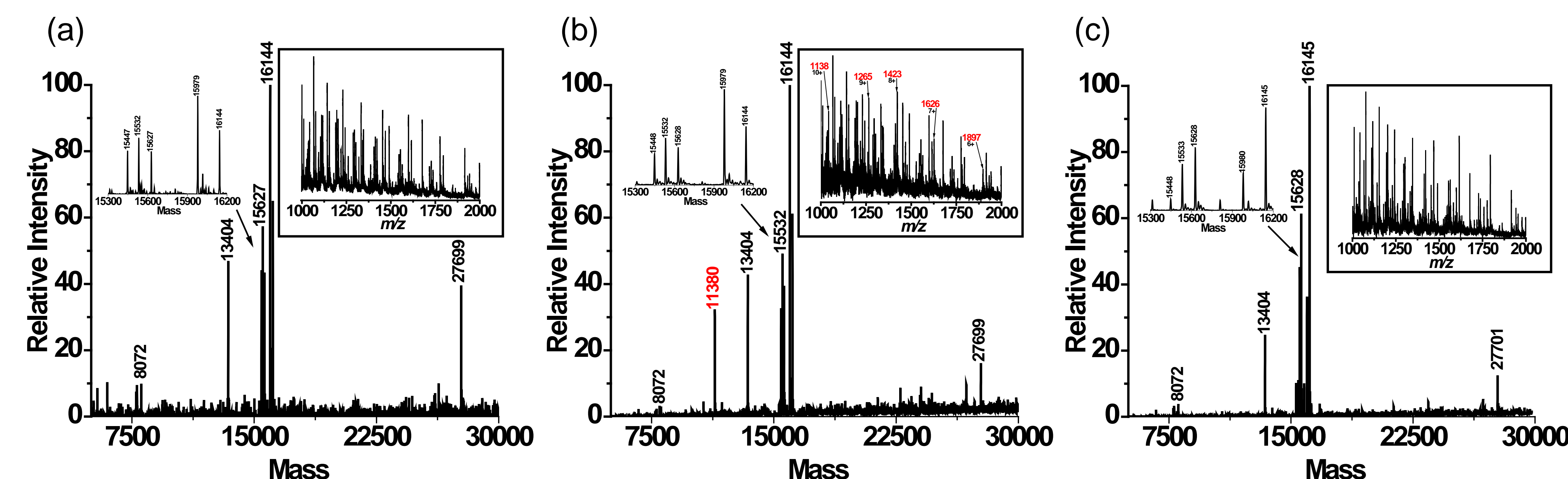


Fig. 3. Deconvoluted high-mass section of LAESI mass spectrum revealed the presence of peptides and proteins in (a) unmodified male gland, (b) modified mature male gland and (c) female gland.