BIOGRAPHICAL SKETCH

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NAME: Ram, Jeffrey L.

eRA COMMONS USER NAME (credential, e.g., agency login): AA2234

POSITION TITLE: Professor, Department of Physiology; Associate member, Dept. of Microbiology, Immunology & Biochemistry and the Center for Molecular Medicine and Genetics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Pennsylvania, Philadelphia, PA	B.A.	1967	Physics (Honors)
Cambridge University, Cambridge, England, UK	Affil. student	1967-68	Hist. Phil. Sci.
California Institute of Technology, Pasadena, CA	Ph.D.	1974	Biochem./Biophysics
University of California, Santa Cruz, CA	Postdoctoral	1973-77	Biology

A. Personal Statement

I have a broad-based biological perspective that underlies my interest in fecal-associated organisms (hence our immediate interest in the presence of SARS-CoV-2 in wastewater), skill in the techniques needed for this project, and on-going collaborations to provide additional expertise to assure successful execution of the proposed experiments. I have an outstanding record of successfully directing federal and state-funded research projects, experience at supervising research personnel (including research assistants, post-docs, and students), and a history of employing and collaborating with a diverse and multidisciplinary group of students, post-docs, and faculty.

My interest in diverse organisms derives in part from my training at Caltech and UC Santa Cruz, in which I analyzed the diversity of proteins in the nervous systems of a model organism (*Aplysia californica*) and its near (and sometimes distant) wild relatives, to understand their function in reproductive and feeding behaviors. More recently, I have used PCR- and sequence-based technologies to analyze the diversity and relative amounts of organisms in aquatic environments (beaches, sewers, lagoons, and rivers) and in the human and oral microbiome. Our characterization of beach bacteria and their fecal sources is directed at protecting people from exposure to pathogenic organisms. Our work with Dr. Ikuko Kato, who is a co-investigator on the current project, on human and oral microbiomes is directly relevant to microorganisms that affect human health.

I am a hands-on research scientist. In addition to training assistants, post-docs, and students in cell culture (Muniyappa et al., 2000; Speyer et al., 2002), animal surgery to obtain cells for primary culture and other *in vitro* techniques (Song et al., 1996), quantitative PCR (Kato et al., 2014; Nechvatal et al., 2008), primer design and other bioinformatics (Ram et al., 1996; Ram et al., 2011; Fujimoto et al., 2014; Farley et al., 2018), biostatistics, and Western blots (Muniyappa et al., 2000), I am in the lab doing many of these activities myself as well. The papers cited here are listed in section C (Contributions to Science) and are selected from >120 peer-reviewed papers, including publications in Proc. Nat. Acad. Sci., FASEB J, American J. Physiol., J. Clin. Investigation, Diabetes, J. Pharm. Exp. Ther., Appl. Env. Microbiol., and Water Res.

In addition to my expertise with laboratory techniques, I have also recruited expert collaborators in order to achieve the goals of this project, including Judy Westrick, Director of the Lumigen chemical analysis facility, Ikuko Kato, who provides epidemiological expertise; Philip Pellett, virologist and Chair of Microbiology, Immunology, and Biochemistry; Carrie Turner, Senior Project Engineer at LimnoTech, with whom we collaborated on a previous wastewater-based project (Ram et al., 2007); and Anthony Lagina,M.D., who brings a clinical perspective and oversight of the university's BioBank to the project.

My editorial work has promoted communication in science and with practitioners, including editing the most recent edition of *Conn's Handbook of Models for Human Aging*, 80 chapters, 1200 pages, published by Elsevier (2018). The fact that the aged are so much more at risk from COVID-19 infection than others suggests one of the themes to be developed if we do a third edition of this compendium. I produced a recent award-winning video to communicate the value of a STEM project that I lead with a local aquarium (Ram, 2020). I also recently collaborated with two law professors to write a paper on legal and ethical issues associated with COVID-19 surveillance using SARS-CoV-2 measurements in wastewater (Gable et al., 2020).

a. Gable, L., Ram, N., and Ram, J.L. (published on-line, 24 June 2020; in press, 2020). Legal and Ethical Implications of Wastewater SARS-CoV-2 Monitoring for COVID-19 Surveillance. https://academic.oup.com/jlb/article/doi/10.1093/jlb/lsaa039/5861905. 11 pages, plus a supplement
b. Ram JL, Thompson B, Turner C, Nechvatal J, Sheehan H, Bobrin J (2007) Identification of pets and raccoons as sources of bacterial contamination of urban storm sewers using a sequence-based bacterial source tracking method. Water Research 41: 3605-3614.
c. Ram, J. L. Creating STEM Pathways in Detroit. 2020 STEM for ALL Video Showcase, at https://stemforall2020.videohall.com/presentations/1753. Facilitators' Choice (award) for video on NSF project 1614187 with the Detroit Public Schools Community District, 3 min (2020).
d. Ram, J.L. and Conn, P.M. (editors). *Conn's Handbook of Models for Human Aging, 2nd Edition* (2018) Published May 2018. London: Elsevier, Academic Press. 80 chapters, 1218 pages in length.

B. Positions and Honors
Positions and Employment
Summer, 1975	Marine Biological Laboratory. Independent Investigator (recipient of MBL-STIP grant)
1977-1982	Assistant professor of Physiology, Wayne State University
1982-1993	Associate professor of Physiology, Wayne State University
1984-1985	Visiting Professor (sabbatical), Biophysics, Technion, Haifa, Israel
1999-	Associate, Center for Molecular Medicine & Genetics, Wayne State University
2003-	Associate, Department of Immunology & Microbiology (now the Department of Microbiology, Immunology, & Biochemistry), Wayne State University
1993-	Professor of Physiology, Wayne State University

Honors and Awards
1963-1967	Benjamin Franklin Scholar, U. of Penn.
1966	Phi Beta Kappa
1967-68	Thouron Scholar at Cambridge University
1968-1971	NSF Predoctoral Fellow at Caltech
1972	Elected to Sigma Xi
1975-1977	NIH Post-doctoral Fellow (NRSA) at University of California, Santa Cruz
1984	Elected Fellow, Amer. Assoc. Adv. Sci.
1984-1985	Lady Davis Fellow, Technion, Haifa, Israel
1996-1997	Picchione Scholar, Dalhousie Univ.
2001	Wayne State Exceptional Service Award
2003	President's Award for Excellence in Teaching
2005-2011	Honorary Professor, Hull University, UK

C. Contributions to Science
1. Gastrointestinal microbiome and human behavior
Our established credentials in microbiological DNA analysis (see contribution #5, below) led to collaborations with Dr. Ikuko Kato on epidemiological studies of the association of the fecal microbiome of humans with smoking and other human behaviors. We established reliable techniques (including a patented collection device) for preserving and extracting bacterial DNA and RNA and human markers from stool samples, and then applied these technologies to analysis of human characteristics, such as smoking and diet, to determine associations with various bacteria of the fecal microbiome. Also, recent studies relating systemic health to oral microbiome.

a. Nechvatal JM, **Ram JL**, Basson MD, Namprachan P, Niec SR, Badsha KZ, Matherly LH, Majumdar AP, Kato I. Fecal collection, ambient preservation, and DNA extraction for PCR amplification of bacterial

and human markers from human feces. J Microbiol Methods. 2008 Feb;72(2):124-32. PubMed PMID: 18162191.

b. Kato I, Startup J, **Ram JL**. Fecal biomarkers for research on dietary and lifestyle risk factors in colorectal cancer etiology. Current colorectal cancer reports. 2014 March; 10(1):114-131.

c. Kato I, Vasquez AA, Moyerbrailean G, Land S, Sun J, Lin, H-S, **Ram JL** (2016). Oral microbiome and history of smoking and colorectal cancer. Journal of Epidemiological Research. Vol 2, No 2, at http://www.sciedu.ca/journal/index.php/jer/article/view/9047 ISSN 2377-9306(Print) ISSN 2377-9330(Online)

d. Vasquez AA*, **Ram JL***, Qazazi MS, Sun J, Kato I. 2018. Oral microbiome; Potential link to systemic disease and oral cancer. Book chapter. In J. Sun, P.K. Dudeja (eds.), *Mechanisms Underlying Host-Microbiome Interactions in Pathophysiology of Human Diseases, Physiology in Health and Disease,* Amer Physiol Soc. https://doi.org/10.1007/978-1-4939-7534-1_9 *Both authors contributed equally to this work.

2. Regulation of invertebrate reproduction: model system studies
Background: Understanding the mechanisms by which peptides regulate biophysical mechanisms was difficult to study in the pre-patch-clamping era. The neuropeptide egg-laying hormone system of *Aplysia* was an excellent model system to study these mechanisms, and, as it turns out also an excellent model system for studying the regulation of reproduction in diverse gastropods (snails, related to *Aplysia*) used for food. We demonstrated the diversity of neuropeptide hormones that activate egg-laying behavior in *Aplysia* and applied what we learned to large snails used for food in New England and Chile. In *Aplysia*, we identified specific neurons that are depolarized by the *Aplysia* egg-laying hormone and serotonin that are hypothesized to mediate changes in feeding behavior that accompany reproduction and analyzed mechanisms mediating the responses, which included the *first ever publication* of the effect of the adenylate cyclase activating agent forskolin on neuronal biophysics. Other laboratories followed up on our findings (not always crediting the original source). Publications include:

a. **Ram, Jeffrey L.** Hormonal control of reproduction in *Busycon*: Laying of egg capsules caused by nervous system extracts. Biol. Bull., 152: 221-232, 1977.

b. **Ram, Jeffrey L.** Neuropeptide activation of an identifiable buccal ganglion motoneuron in *Aplysia*. Brain Res., 288: 177-186, 1983.

c. **Ram, Jeffrey L.** Forskolin activation of an identified peptide-sensitive motoneuron in Aplysia. Brit. J. Pharm., 79: 631-633, 1983.

d. **Ram, J.L.**, Gallardo, C., Ram, M.L., Merino, R. and Navarro, J. Neural extract induction of egg-laying and subsequent embryological development in hard and soft egg capsules of the prosobranch snail, Chorus giganteus. Journal of Shellfish Research 19: 905-911, 2000.

3. Smooth muscle and other vascular cell physiology
Background: Having initiated studies of the biophysical, second messenger, and contractile responses to hormones and neurotransmitters on *Aplysia* smooth muscle, we subsequently began mammalian medically related studies of biophysical and second messenger mechanisms in vascular cells (endothelial cells, smooth muscle cells, and pericytes) associated with diabetes, diabetes and cancer drugs, sepsis, and gender. In relation to sepsis, we discovered non-nitric oxide-mediated mechanisms that relaxed pericytes. Publications include:

a. Standley, P.R., Zhang, F., **Ram, J.L.**, Zemel, M.B., and Sowers, J.R. Insulin attenuates vasopressin-induced calcium transients and a voltage-dependent calcium response in rat vascular smooth muscle. J. Clin. Inv. 88: 1230-1236, 1991.

b. Song, J., Standley, P.R., Zhang, F., Joshi, D., Gappy, S., Sowers, J.R., and **Ram, J.L**. Tamoxifen (estrogen antagonist) inhibits voltage-gated calcium current and contractility in vascular smooth muscle from rats. J. Pharm. Exp. Ther. 277: 1444-1453, 1996.

c. Muniyappa, R., Xu, R., **Ram, J.L.**, and Sowers, J.R. Inhibition of Rho protein stimulates iNOS expression in rat vascular smooth muscle cells. American Journal of Physiology 278: H1762-H1768, 2000.

d. Speyer, C.L., Steffes, C.P., Tyburski, J.G., Homan, R., and **Ram, J.L.** Lipopolysaccharide-induced secretory phospholipase A2 activity in pericytes: A possible mechanism for mediating relaxation. Microvascular Research 63: 239-242, 2002.

4. Physiology of invasive mussels and other aquatic invertebrates

Background: Aquatic nuisance species and diseases have been introduced into the Great Lakes by the frequent discharge of ballast water of foreign ships that have entered the Great Lakes via the St. Lawrence Seaway since the 1950's. Huge impacts occurred most recently due to the introduction of foreign mussels (zebra mussels and quagga mussels), which filter microorganisms from the water, damage structures in power plants and drinking water facilities, and impact humans through accumulations on beaches and impacts on aquatic disease organisms. Our laboratory applied our knowledge of molluscan reproduction to demonstrate, for the first time, the regulation of zebra mussel reproduction by serotonin (now the most commonly used method in studies of their reproduction and development). We also developed PCR-based detection techniques (eDNA analysis) to detect the presence and density of the early reproductive stages in plankton. Regulation of reproduction by a glutathionergic pheromone was also demonstrated in *Nereis succinea*. With regard to human health, we collaborated with civil engineers to apply zebra mussel filtering capabilities to source tracking of *E. coli* contamination of environmental waters, subsequently investigated with eDNA techniques (see contribution #5).

Publications include:

   a. **Ram, J.L.**, Crawford, G.W., Walker, J.U., Mojares, J.J., Patel, N., Fong, P.P., and Kyozuka, K. Spawning in the zebra mussel (*Dreissena polymorpha*): Activation by internal or external application of serotonin. J. Exp. Zool. 265: 587-598, 1993.
   b. **Jeffrey L. Ram**, Aos S. Karim, Payel Archarya, Pranav Jagtap, Sonal Purohit, and Donna R. Kashian (2011) Reproduction and Genetic Detection of Veligers from Changing Dreissena Populations in the Great Lakes. Ecosphere 2(1):art3. 16 pages. doi:10.1890/ES10-00118.1 (on-line journal)
   c. **Ram, J.L.**, Muller, C.T., Beckmann, M., and Hardege, J.D. The spawning pheromone cysteine-glutathione disulfide ("Nereithione") arouses a multicomponent nuptial behavior and electrophysiological activity in *Nereis succinea* males. FASEB Journal 13: 945-952, 1999.
   d. Selegean, J.P.W., Kusserow, R., Patel, R., Heidtke, T.M., and **Ram, J.L.** Using zebra mussels to monitor *Escherichia coli* in environmental waters. Journal of Environmental Quality. 30: 171-179, 2001.

5. DNA analyses for biodiversity, forensic, and biodetection applications

Background: The convenience of PCR and the decreasing cost of DNA sequence analysis made it possible to apply bioinformatic methods to the detection and diversity analysis of organisms in the environment, including health-related organisms such as waterborne *E. coli*. Starting with a forensic study of fish (our frequently cited forensic study of labeling of imported tuna was one of the first tests of this application), we applied these techniques to analyze the origin of invasive fish and the sources of *E. coli* at the beach. This work included identification of novel strains (some might even say, given the genetic distances involved, new species) of *Escherichia*. Applied to environmental samples, we detected changes in the reproduction of invasive mussels in the Great Lakes and the microbiome of ballast water undergoing treatment to prevent new invasions of pathogens and microeukaryotes.

Publications include:

   a. **Ram JL**, Ram ML, Baidoun F. Authentication of canned tuna and bonito by sequence and restriction site analysis of polymerase chain reaction (PCR) products of mitochondrial DNA. Journal of agricultural and food chemistry. 1996; 44:2460-2467.
   b. Walk ST, Alm EW, Gordon DM**, Ram JL**, Toranzos GA, Tiedje JM, Whittam TS. Cryptic lineages of the genus Escherichia. Appl Environ Microbiol. 2009 Oct;75(20):6534-44. PubMed PMID: 19700542; PubMed Central PMCID: PMC2765150.
   c. Fujimoto M, Moyerbrailean GA, Noman S, Gizicki JP, Ram ML, Green PA, **Ram JL**. Application of ion torrent sequencing to the assessment of the effect of alkali ballast water treatment on microbial community diversity. PLoS One. 2014;9(9):e107534. PubMed PMID: 25222021; PubMed Central PMCID: PMC4164647.
   d. Nicole J. Farley, Adrian A. Vasquez, Richard Kik IV, Solomon R. David, Arjun S. Katailiha, Xavier N. Walker, **Jeffrey L. Ram** (2018). Primer designs for identification and eDNA detection of gars (Lepisosteidae). Transactions of the American Fisheries Society. 147: 687-695. https://onlinelibrary.wiley.com/doi/abs/10.1002/tafs.10043

Complete List of Published Work

The Pubmed URL for "Ram JL" (http://www.ncbi.nlm.nih.gov/pubmed/?term=Ram+JL) lists 96 publications

The following publications URL by Web of Science, which has a broader general science coverage than PubMed, shows 158 publications, in response to an author search for "Ram JL":
http://apps.webofknowledge.com.proxy.lib.wayne.edu/Search.do?product=WOS&SID=7AcDEupuRLYtnLA7AtZ&search_mode=GeneralSearch&prID=1b5c6d9f-8113-4c45-9d2b-dd35acba92a4
- o Also we have published numerous sequences to GenBank (find by searching Nucleotide database for "Ram JL")

D. Additional Information: Research Support and/or Scholastic Performance
Ongoing Research Support
NSF 1614187   Ram (PI)   09/1/2016-08/31/2021
Promoting Student Interest in Science and Science Careers through a Scalable Place-based Environmental Educational Program at a Public Aquarium
The goal of this study is to provide professional development for teachers and educational field trips for their students at the Belle Isle Aquarium, and to do research on the effectiveness of these experiences on teacher self-efficacy and the attitudes and choices of students about science and science careers.
Role: Project Director

NSF 1735038   Kashian, Miller, Guerra-Lopez, Mohamed, Sopory (mPIs)   09/01/17 - 08/31/22
Transformative Research in Urban Sustainability and Training
The purpose of this project is to create a new integrative transdisciplinary model for graduate education that will help create a more sustainable environment
Role: Senior personnel

State of Michigan   Shuster (PI)   10/2020 -12/2020
Dorm-level Wastewater monitoring for SARS CoV-2 material: a process-based approach to inform early-warning and control
Wastewater from five dorms and a sewershed will be monitored by SARS-CoV-2 markers using qPCR and ddPCR in the Ram lab in the med school and will be biobanked in a facility directed by Dr. Anthony Lagina. The Ram lab has received supplies and equipment for the project from the state, while final memoranda of agreement are in process.
Role: Co-PI

Completed projects
Great Lakes Protection Fund, Project #964   Ram (PI)   10/1/2012-1/31/2020
Automated Ballast Treatment Verification Project
The goal of this project was to develop an automated system for differentiating live from dead organisms in ballast water of ships after the water has undergone treatment and before discharge, to protect the Great Lakes from the discharge of potential invasive eukaryotes and pathogens into the environment.
Role: PI

Michigan Invasive Species Grant Program, Project # IS16-4002   Ram(PI)   2/1/2017 – 2/29/2020
Educating Educators and their Students Everywhere to Prevent New Invaders
The goal of this project was to educate educators throughout Michigan about teaching about invasive species in multiple curricula, including biology, math, social studies, and language arts, for the purpose of increasing knowledge of students and their families about invasive species to protect the state from new invaders.
Role: PI

Healthy Urban Waters: HEART Field Station Investigator Research Award   Ram (PI)   3/1/2017- 6/30/2018
Microbial Contamination of Beaches in Huron to Erie Corridor: Investigating Dynamics and Potential Sources
The goal of this project was to use high throughput sequencing to characterize and compare the types and changes of bacteria at Belle Isle Beach and a Canadian beach sampled daily over an entire summer
Role: PI