SABBATICAL LEAVE REPORT

1992-93 ACADEMIC YEAR

First Year of Ph. D. Preparation and Preliminary Dissertation Research Report

University of California at Riverside

William L. Waggener

Department of Biology

September 9, 1993

Table of Contents

1992-93 SABBATICAL LEAVE PROPOSAL

The purpose of the Sabbatical Leave Proposal is two-fold: (1) to do further graduate study in anatomy and physiology, which are the emphases of both my personal interests and my teaching schedule, and (2) to begin work towards a Ph. D. in the biological sciences with an emphasis in physiology. To these ends, I have already begun an approved time-banking plan to secure and additional semester of full-time study to be taken a year and one-half after my return from this proposed sabbatical leave. I have also spoken (either in person or by telephone) with three members of the Department of Biology at the University of California at Riverside (cited below) regarding this long-term project, and I will be taking the Graduate Record Exam in mid-December as part of the admission requirements for UCR. I currently serve as Course Coordinator and principal instructor for Anatomy 10A/10B (Introductory Human Anatomy and Physiology) [which is a mandatory component of both the Radiation Technology and Respiratory Therapy Programs at MSAC], and I am also deeply involved in teaching the Anatomy 35/36 sequence (Human Anatomy/Human Physiology) [which is a prerequisite for entry into the MSAC RN program].

The following course proposal was developed with the advice of Dr. Prudence Talbot, Dr. Vaughan Shoemaker, and Dr. Roald Roverude, all of whom are advisors of Ph. D. candidates at UCR:

Course Number	Course Title and Description	Quarter Units
Bio 175	**Comparative Animal Physiology** (Nutrition and energy metabolism, gas exchange, circulation, and regulation of body fluid composition.)	3
Bio 175L	**Comparative Animal Physiology Laboratory** (Laboratory exercises in animal physiology.)	2
Bio 297	**Directed Research** (Experimental studies on specifically selected topics in biology under the direction of a faculty member.)	6
Bio 283	**Seminar in Organismal Physiology and Physiological Ecology** (Lectures, discussions, and demonstrations by students, faculty, and invited scholars concerned with the principles of organismal physiology and physiological ecology.)	6
Bio 200A-B	**Cellular and Developmental Biology** (The interrelationship between structural and functional elements of the living cell; genetic physiology and gene expression at the cellular and molecular level.)	10
Bio 252	**Department Colloquium** (A Credit/No Credit departmental seminar in which enrollment is required in each quarter of residence.)	3

Course Number	Course Title and Description	Quarter Units
Biomed 205	**Human Histology** (In-depth study of the microscopic anatomy of normal human tissues and organs. The course will emphasize the morphological basis of physiology.)	5
Biomed 220	**Neuroscience** (This course will emphasize the inter-relationships between the anatomy, physiology, and biochemistry of the nervous system as a basis for understanding its function in health and disease.)	5
Bio 177	**Neurobiology** (The relationships between the neuroanatomy and the behavior of vertebrate organisms.)	4
	Total Quarter Units for this proposal:	44

Full-time enrollment at UCR is considered to be 12 units per quarter, with a total of 36 units per academic year; inasmuch as my proposal exceeds the normal full-time load by some 22%, it is quite possible that I may be advised (if not required) to postpone some of this work in order to avoid an academic overload. Furthermore, as all three members of the UCR faculty were quick to points out, this list of proposed courses may require last-minute revisions for at least two reasons: (1) the UCR 1992-93 schedule of classes has not yet been established, and, therefore, there is a remote possibility that scheduling conflicts may necessitate the substitution of appropriate alternate courses, and (2) if I am formally admitted to the Ph. D. program, my Advisory Committee may demand that I take courses not presently on this list.

Should either of these circumstances occur, I would anticipate that any substituted courses will also be directly related to my fundamental objectives: to enhance my academic preparation in anatomy and physiology so that I may further refine and upgrade the integrity of the MSAC courses for which I am responsible and to prepare myself for the successful completion of a doctoral program in physiology.

wlw
11/21/91

Relevance of Coursework to MSAC Teaching

As supported by the attached documents, my year of full-time graduate study at the University of California at Riverside was most profitable - both personally and professionally.

The content of the my coursework that is relevant to my teaching assignment in the Biology Department is reviewed below:

Bio 111 **Cell Biology**

Structure and function of subcellular components with emphasis on the investigative techniques employed to resolve questions at the cellular level.

Bio 114 **Cell Biology Laboratory**

Practical experience in laboratory techniques in cellular physiology with emphasis on SDS-PAGE electrophoresis and membrane electrophysiology.

Bio 128 **Immunology**

Lecture and discussion on humoral and cellular immunology including lymphoid systems, cells, antigens, antibodies, antibody formation, cellular immunity, and tumor and transplantation immunology.

Bio 175 **Animal Physiology**

A thorough review of vertebrate systemic physiology with primary emphasis on mammalian function.

Bio 200A/B **Cell, Molecular, and Developmental Biology**

Current techniques in electron and light microscopy; molecular motors; sperm-egg interaction at fertilization; lipid membrane dynamics.

Bio 252 **Colloquium in Biology**

Presentation of current research interests and techniques by UCR faculty and outside guests.

Bio 262 **Colloquium in Cell Biology**

Lectures and discussion by graduate students and faculty on current research in cell biology, including evolution, cancer-related issues blocks to polyspermy, and relevant issues.

Bio 281 **Seminar in Fertilization**

Lectures and discussion by students and faculty on mechanisms of fertilization with particular emphasis on current journal publications related to the specific research interests of the seminar participants.

Bio 281 **Seminar in Cell Signalling**

Lectures and discussion by students and faculty on mechanisms of intercell communication at the membrane surface.

Bio 283 **Seminar in Evolutionary and Ecological Physiology**

Lectures and discussion by students and faculty on physiological adaptations to environmental forces with particular emphasis on energy flow.

Bio 287 **Colloquium in Neurophysiology**

Weekly guest lecturers speaking on their current research in neurophysiology.

Bio 291 **Individual Study**

Assigned review readings in physiology in preparation for taking the Written Qualifying Exam in Physiology.

Bio 297 **Directed Research**

Individual research experience under the guidance of a dissertation advisor in membrane potential measurement of anuran oocytes in four stages of development: (1) preovulation; (2) coelomic; (3) post-oviducal; and (4) post-fertilization.

Statement of Purpose

As presented in the **1992-93 SABBATICAL LEAVE PROPOSAL**, the purpose of my sabbatical leave was to "... (1) to do further graduate study in anatomy and physiology, which are the emphases of both my personal interests and my teaching schedule, and (2) to begin work towards a Ph. D. in the biological sciences with an emphasis in physiology." Those objectives provided the focus for my work during the 1992-93 academic year at the University of California at Riverside and continue to do so as I proceed with my Ph. D. program at UCR.

Preliminary Dissertation Research Report

The Oocytes of *Lepidobatrachus laevis* Have A Measurable Pre-Fertilization Membrane Potential

Abstract. Membrane potentials of *Lepidobatrachus laevis* oocytes in three different stages of development were measured. The three oocyte stages were *ovarian* (i. e., follicular oocytes), *coelomic* (i. e., ovulated but not passed through the oviduct) and *oviducal* (i. e., ovulated and released with a full jelly coat). Membrane potentials were measured with the oocytes bathed in Ringer's solution in order to maintain an environment isoosmotic and isoionic with the female reproductive tract. The mean *ovarian* oocyte membrane potential was 40.0 ± 2.8 mv (n = 2), the mean *coelomic* oocyte membrane potential was 36.5 ± 5.8 mv (n = 4),and the mean *oviducal* oocyte membrane potential was 12.0 ± 1.6 mv (n = 4). Implications of the rise in membrane potential with increasing oocyte maturity are discussed.

Introduction

For several decades, the existence of a prefertilization membrane potential and a transient postfertilization membrane depolarization in anuran eggs has been well documented (Grey, *et al.*, 1982; Elinson, 1986; Kline and Nuccitelli, 1985). In those anurans that have been investigated, the depolarization at fertilization has been shown to serve as an immediate but temporary block to

receptivity to subsequent spermatozoa (the "fast block") which is followed by the cortical granular reaction which generates the fertilization envelope (the "slow block"). The membrane depolarization has been termed the *activation potential* (Maéno, 1959) when stimulated by artificial means (e. g., needle stick, electric shock, or Ca^{++} ionophore A23187) and the *fertilization potential* when triggered by fertilization, *per se* (Cross and Elinson, 1980). As a consequence of this sequential pair of mechanisms, the monospermic fertilization of anuran eggs is virtually assured, and polyspermy, which produces a nonviable zygote in anurans, is rare.

In *Xenopus*, the prefertilization potential has been measured to be about -19 mV and the postfertilization potential to be about +8 mV (Grey, *et al.*, 1982). Egg membrane potentials in several other anurans have been found to be of the same order of magnitude (Iwao, 1989; Iwao and Jaffe, 1989). The immediate questions addressed in this paper are: do the eggs of *L. laevis* exhibit a prefertilization membrane potential that is typical of anurans, and if so, what is that potential?

Methods and Materials

Oocyte Collection

Female *Lepidobatrachus laevis* were injected with Luteinizing Hormone Releasing Hormone (LH-RH) at a concentration of approximately 0.375 mg/kg body weight eight to twelve hours prior to oocyte collection. A lobe of the ovary approximately 1.0 cc. in volume was removed surgically, and the oocytes were released from their follicles by microdissection and/or digestion with collagenase

in Ringer's solution. Coelomic oocytes were collected by flushing the coelomic cavity of injected females with sterile Ringer's solution. Oviducal oocytes were collected by allowing injected females to release directly into Ringer's solution. Oocytes were stored in Ringer's solution at approximately 4° C. Anesthesia for all surgical procedures consisted of 100 ml 0.5% (W/V) solution of MS222 in which the animal was immersed until righting activity ceased. Additional anesthesia was administered topically via saturated Kimwipes as necessary. Incisions were dusted twice daily with nitrofurazone powder for two to three days prior returning the animals to their routine aquatic environment.

Solution Preparation

The composition of the Ringer's solution used in these procedures was 113 mM NaCl, 1.4 mM $CaCl_2$, 2.0 mM KCl, 3.6 mM $NaHCO_3$, pH 7.2-7.4 (Hedrick and Nishihara, 1991).

Membrane Potential Measurements

Microelectrodes were drawn from glass capillary tubing with a conductive resin filament core and backfilled with 3M KCl. Electrode resistance values were on the order of 8 to 12 megohms. Oocytes were placed in a 10 mm (W) x 20 mm (L) 5 mm (D) well within a cast block of silicon rubber. A cage of 4 minuten nadlen within the well held each oocyte in place during measurement procedures. Membrane voltages were measured with a bioamplifier and monitored via digital LED display.

Results

Measurements of Ovarian Oocyte Membrane Potentials

Membrane potentials of 4 ovarian oocytes showed a mean of 26.8 ± 15.5 mv (n = 4) (Table 1.). However, the morphology of 2 of those oocytes was clearly abnormal inasmuch as their internal pressure was reduced sufficiently to cause major surface crenation. Their membrane potentials were also well above those of oocytes with normal appearance. When the data from the abnormal oocytes are rejected, the mean membrane potential is 40.0 ± 2.8 mv (n = 2).

Date	Egg #	MP(mv)	Notes/Morphology
3/3/93	1	**-38**	**normal**
3/3/93	2	**-42**	**normal**
3/3/93	3	-16	crenated; floated
3/3/93	4	-11	crenated; floated

Mean for all oocytes = 26.8 ± 15.5 mv (n = 4).
Mean for **normal oocytes** = **40.0 ± 2.8 mv** (n = 2).

Table 1. Ovarian Oocyte Membrane Potentials. All measured values are shown. Data from eggs with normal appearance are shown in **boldface**.

Measurements of Coelomic Oocyte Membrane Potentials

Membrane potentials of 13 coelomic oocytes showed a mean of 22.2 ± 12.1 mv (n = 13) (Table 2.). However, the morphology and/or collection circumstances of 8 of those oocytes were judged to be abnormal inasmuch as they also showed major surface crenation or had a substantially elevated membrane potential. When the data from the abnormal oocytes are rejected, the mean membrane

potential is 36.5 ± 5.8 mv (n = 4).

Date	Egg #	MP(mv)	Notes/Morphology
2/12/93	1	-18	had been in HOH w/Hb
3/12/93	1	-19	somewhat crenated
3/12/93	2	-16	somewhat crenated
3/12/93	3	**-35**	**normal**
3/12/93	4	-10	deeply crenated
3/12/93	5	-14	slightly crenated
3/12/93	6	0	dead
3/12/93	7	**-44**	**normal**
3/12/93	8	-11	cytoplasmic bleb
3/12/93	9	0	dead
3/12/93	10	-10	normal (?)
3/12/93	11	**-30**	**normal**
3/12/93	12	**-37**	**normal**

Mean for all oocytes = 22.2 ± 12.1 mv (n = 13).
Mean for **normal oocytes = 36.5 ± 5.8 mv** (n = 4).

Table 2. Coelomic Oocyte Membrane Potentials. All measured values are shown. Data from eggs with normal appearance are shown in **boldface**.

Measurements of Oviducal Oocyte Membrane Potentials

Membrane potentials of 4 oviducal oocytes showed a mean of 12.0 ± 1.6 mv (n = 4) (Table 3.). Inasmuch as all of these oocytes possessed both normal morphologies and very similar membrane potentials, none of their measurements were rejected.

Date	Egg #	MP(mv)	Notes/Morphology
2/26/93	1	**-14**	**normal**
2/26/93	2	**-12**	**normal**
2/26/93	3	**-12**	**normal**
2/26/93	4	**-10**	**normal**

Mean for all oocytes = 12.0 ± 1.6 mv (n = 4).

Table 3. Oviducal Oocyte Membrane Potentials. All measured values are shown. Data from eggs with normal appearance are shown in **boldface**.

Discussion

The initial question of this investigation has been answered: that the oocytes of *Lepidobatrachus laevis* do possess a prefertilization membrane potential. However, the value of that membrane potential appears to be dependent on the degree of maturation of the oocyte. Whereas there is no significant difference between the coelomic and ovarian membrane potentials (Table 4.), there is a statistically significant difference between both the coelomic and ovarian membrane potentials with the oviducal membrane potentials. Although the limited data presented here can be considered only to be suggestive, the apparent difference in membrane potential between eggs with and without the jelly coat may have some implications with regard to their maturation. Inasmuch as the presumed function of any anuran prefertilization oocyte membrane potential is to generate the voltage-dependent fast block to polyspermy (Charbonneau, *et al.*, 1983), a rise in membrane potential in *L. laevis* oocytes during maturation to a level closer to the depolarization threshold (Figure 1.) would be consistent with preparation for eminent fertilization and depolarization (Schlichter and Elinson, 1981). Hyperpolarization prior to the addition of the jelly coat, then, may ensure that premature depolarization does not occur within either the ovarian follicle or

the coelomic cavity.

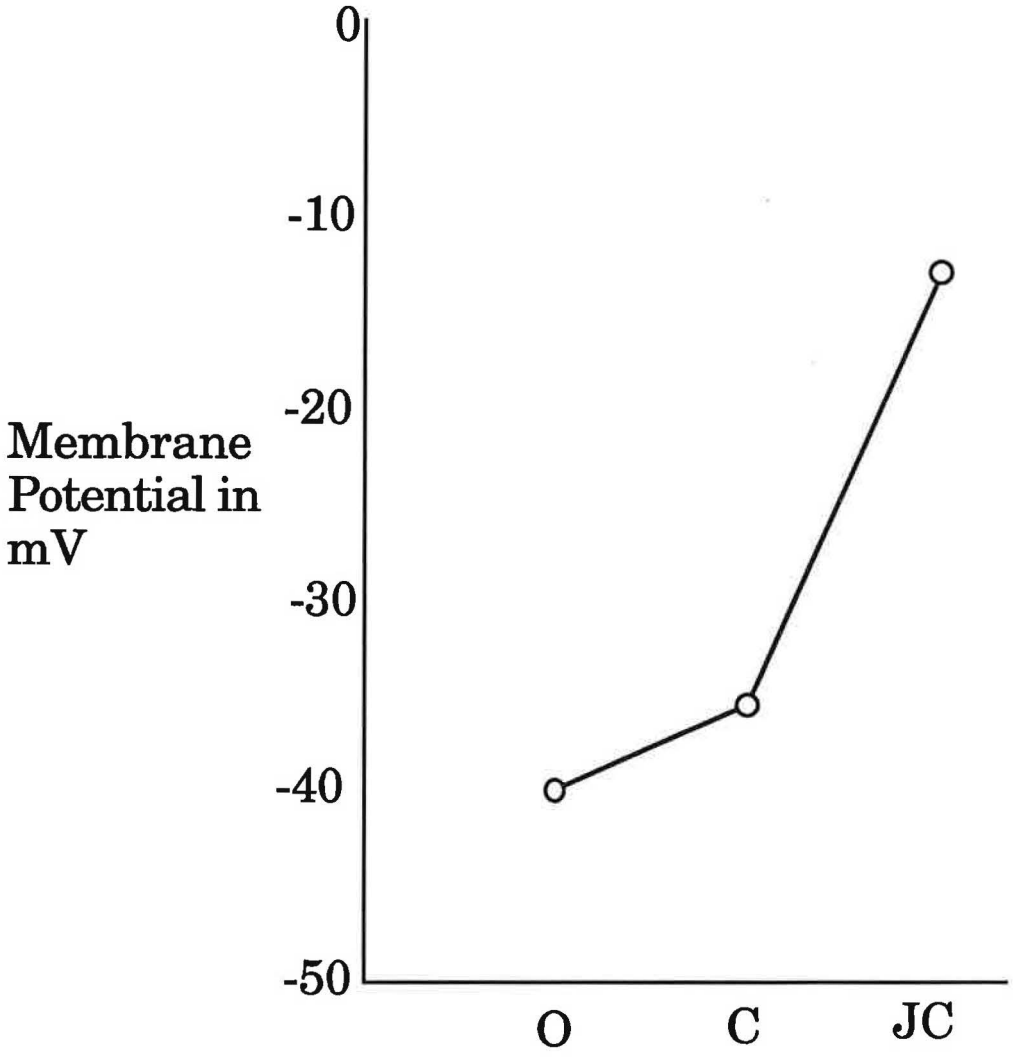

Figure 1. Membrane Potentials Rise With Maturation. **O**, ovarian oocytes; **C**, coelomic oocytes; **JC**, oviducal oocytes with jelly coat.

Coelomic vs.Ovarian	t = 0.77 *(t = 2.78 @ df = 4)*	NSD
Coelomic vs. Oviducal	t = 6.25 *(t = 2.45 @ df = 6)*	**SD**
Ovarian vs.Oviducal	t = 6.15 *(t = 2.78 @ df = 4)*	**SD**

Table 4. Comparison of Membrane Potentials. Means were compared using Student's **t**-Test. Tabular **t** values for significant difference at the 95% confidence levels are shown in italics. Significant difference between membrane potentials is shown in **boldface**.

Literature Cited

Charbonneau, M., Moreau, M., Picheral, B., Vilain, J. P., and Guerrier, P. (1983). Fertilization of Amphibian Eggs: A Comparison of Electrical Responses between Anurans and Urodeles. *Developmental Biology* **98**:304-318.

Cross, N. L., and Elinson, R. P. (1980). A Fast Block to Polyspermy In Frogs Mediated By Changes In The Membrane Potential. *Developmental Biology* **75**: 187-198.

Elinson, Richard P. (1986). Fertilization in Amphibians: The Ancestry of the block to Polyspermy. *International Review of Cytology,* **101**: 59-100.

Grey, Robert D., Bastiani, Michael J., Webb, Dennis J., and Shertel, Eric R. (1982). An Electrical Block Is Required to Prevent Polyspermy in Eggs Fertilized by Natural Mating of *Xenopus laevis*. *Developmental Biology* **89**: 475-484.

Hedrick, Jerry L., and Nishihara, Tatsuro (1991). Structure and Function of the Extracellular matrix of Anuran Eggs. *Journal of Electron Microscopy Technique* **17**:319-335.

Iwao, Yashiro (1989). An Electrically Mediated Block to Polyspermy in the Primitive Urodele *Hynobius nebulosus* and Phylogenetic Comparison with Other Amphibians. *Developmental Biology* **134**: 438-445.

Iwao, Yashiro, and Jaffe, Laurinda A. (1989). Evidence That the Voltage-Dependent Component in the Fertilization Process Is Contributed by The Sperm. *Developmental Biology* **134**: 446-451.

Kline, Douglas, and Nuccitelli, Richard (1985). The Wave of Activation Current in *Xenopus* Egg. *Developmental Biology* **111**:471-487.

Maéno, T. (1959). Electrical Characteristics and Activation Potential of *Bufo* Eggs. *Journal of General Physiology* **43**: 139-157.

Schlichter, Lyanne C., and Elinson, Richard P. (1981). Electrical Responses of Immature and Mature *Rana pipiens* Oocytes to Sperm and Other Activating Stimuli. *Developmental Biology* **83**:33-41.

Conclusions

Inasmuch as my teaching responsibilities are exclusively in anatomy and physiology, virtually every formal course in which I participated will be applied to upgrading the content of my courses here at MSAC. The dissertation research and directed reading component of my work has given me fresh insights of current trends in the biological sciences which also will be reflected in my teaching - most particularly in the laboratory exercises which I supervise. Over a span of five days at the beginning of June, 1993, I took the Ph. D. Written Qualifying Exam in Physiology. I am delighted to report that the exam was passed on my first attempt and that I am now in the process of writing my dissertation research proposal, which is the next step prior to sitting for the Oral Qualifying Exam.

A tangential but nonetheless significant observation that struck me repeatedly during my year at UCR is that, overall, the faculty regard for and interest in the quality of undergraduate teaching at the university level is substantially below that at MSAC. Although the major intellectual mission of most university faculty clearly lies in research (*and* the journal publications that result from that research), it strikes me as tragic that although the graduate students who serve as Teaching Assistants receive on-going assistance and encouragement in developing their teaching abilities, professorial skill in instructional technique (or lack thereof) is largely ignored. My reluctant conclusion from this is that MSAC seems to be doing a far superior job of preparing its transfer students for success at the upper division level than the

UCs - which I view as a most distressing indictment of many of the UC's undergraduate programs.

A parallel observation is a reflection upon the dividends that active participation in research *does* provide to teaching faculty. I return to MSAC with a far more current and vastly greater knowledge of the field of physiology than when I left. An enormous portion of that understanding is derived directly from my experiences in the research laboratory setting. Although the major intellectual mission of most community college faculty clearly lies in teaching, it also strikes me as tragic that there seems to be little formal encouragement and support for community college faculty as a whole to develop (or even maintain) their research interests and, therefore, to be able to pass on to their students the tremendous excitement and stimulation that come from such endeavors. My reluctant conclusion from this is that many of our MSAC faculty tend to lag behind the current level of knowledge in their fields - even to the point of stagnation. Indeed, I must include myself in this group - prior to this sabbatical leave. Unfortunately, in both the long term and the short term, it is our students who may ultimately suffer from hoary scholarship!

I am not blind to the obvious financial constraints that preclude wider faculty participation in the sabbatical leave or similar programs. However, I also have to believe that some creative (and inexpensive) program could and must be devised to encourage our faculty to set aside the time to participate in relevant long-term activities that *will* upgrade and maintain their academic currency.

Regrettably, most short-term and/or one-time presentations are ineffective in this regard; afterwards, they simply vanish into the busy schedules that most of us follow. [Furthermore, most such activities that I have attended recently have been directed more to teaching strategies and tactics rather than to contemporary scholarly development within the disciplines - which is the concern I am addressing here.]

Given the present rate with which advances both in technology and in human knowledge are taking place, it seems imperative to me that the faculty be motivated in some significant way to move forward concurrently. No less than our integrity as an academic institution may be at stake. Any suggestions?

Appendix A: Letters from Dr. Vaughan Shoemaker

July 7, 1993

To: William Waggener

From: Vaughan Shoemaker VAS

I am pleased to inform you that you have passed the written Ph.D. qualifying exam in Physiology with flying colors. Attached is a summary of the faculty evaluations of your answers. If you wish you may solicit additional feedback from the faculty involved.

Congratulations!

July 7, 1993

To: Prue Talbot, Graduate Advisor

From: V. Shoemaker VHS

I am pleased to inform you that Lisa Hazard and William Waggener have completed and passed the written Ph.D. qualifying exam in Physiology. Copies of the exam, answers, and a summary of the faculty evaluations have been placed in the students' folders. Both Bill and Lisa performed at a high level.

xc:
Lisa Hazard
William Waggener

Appendix B: Transcript

UNIVERSITY OF CALIFORNIA, RIVERSIDE
OFFICIAL TRANSCRIPT WITH SEAL AFFIXED

PRINTED: 09/09/93

William Lloyd Waggener
560-58-9450 06-24-41

Official GRADUATE ACADEMIC RECORD

Current Academic Program:
Graduate Division
Doctor of Philosophy
Major: Biology

Previous Degrees:
Master of Science Jun 10, 1977
California State U - Pomona
Major: Biology
Cum GPA: 3.810
Master of Arts Jun 7, 1969
Claremont Graduate School
Major: Music
Bachelor of Arts Jun 9, 1963
Pomona College
Major: Music
Cum GPA: 2.150

----------1992 Fall----------

Admitted Program:
Graduate Division
Doctor of Philosophy
Major: Biology

BIOL-200A	CELL, MOLECULAR & DEVLPMNTL	B+	4.00	13.20
BIOL-252	COLLOQUIUM IN BIOLOGY	S	1.00	
BIOL-281	MOLECULAR ASPCTS:CELL SIGNALING	A	2.00	8.00
BIOL-283	EVOLUTIONARY & ECOLOGICAL ASPCTS	A	2.00	8.00
BIOL-287	COLLOQUIUM IN NEUROSCIENCE	S	1.00	
BIOL-297	DIRECTED RESEARCH	S	2.00	

	AHRS	EHRS	QHRS	QPTS	GPA
Current	12.00	12.00	8.00	29.20	3.650
Cumulative	12.00	12.00	8.00	29.20	3.650

----------No Further Entries This Column----------

----------1993 Winter----------

BIOL-111	CELL BIOLOGY	B	4.00	12.00
BIOL-114	CELL BIOLOGY LABORATORY	A	3.00	12.00
BIOL-175	ANIMAL PHYSIOLOGY	A	3.00	12.00
BIOL-200B	CELL, MOLECULAR & DEVLPMNTL	B+	4.00	13.20
BIOL-252	COLLOQUIUM IN BIOLOGY	S	1.00	

	AHRS	EHRS	QHRS	QPTS	GPA
Current	15.00	15.00	14.00	49.20	3.514
Cumulative	27.00	27.00	22.00	78.40	3.564

----------1993 Spring----------

BIOL-128	IMMUNOLOGY	A-	3.00	11.10
BIOL-252	COLLOQUIUM IN BIOLOGY	S	1.00	
BIOL-262	ADVANCES:CELL, MLCLR & DVLP	S	2.00	
BIOL-281	FERTILIZATION	B+	2.00	6.60
BIOL-291	INDIVIDUAL STUDY:COORD	S	2.00	
BIOL-297	DIRECTED RESEARCH	S	3.00	

	AHRS	EHRS	QHRS	QPTS	GPA
Current	13.00	13.00	5.00	17.70	3.540
Cumulative	40.00	40.00	27.00	96.10	3.559

----------1993 Fall----------

BIOL-250	SPECIAL TOPICS	NR	(2.00)
BIOL-291	INDIVIDUAL STUDY:COORD	NR	(5.00)
BIOL-297	DIRECTED RESEARCH	NR	(5.00)

	AHRS	EHRS	QHRS	QPTS	GPA
Current	12.00	0.00	0.00	0.00	0.000
Cumulative	52.00	40.00	27.00	96.10	3.559

----------End of GRADUATE ACADEMIC RECORD----------
----------No Further Entries This Column----------

William Lloyd Waggener 1 1
1631 N. Oxford Ave.
Claremont CA 91711