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TITLE OF PROPOSED PROJECT CNIC: WHAT DOES IT TAKE TO BE AN HERBIVOROUS LIZARD? REVEALING THE DIGESTIVE ANATOMY AND PHYSIOLOGY OF MANIPULATED POPULATIONS OF Podarcis sicula						
REQUESTED AMOUNT \$ 46,902	PROPOSED DURATION (1-60 MONTHS) 12 months		REQUESTED STARTING DATE 08/01/13		SHOW RELATED PRELIMINARY PROPOSAL NO. IF APPLICABLE	
CHECK APPROPRIATE BOX(ES) IF THIS PROPOSAL INCLUDES ANY OF THE ITEMS LISTED BELOW						
<input checked="" type="checkbox"/> BEGINNING INVESTIGATOR (GPG I.G.2) <input type="checkbox"/> HUMAN SUBJECTS (GPG II.D.7) Human Subjects Assurance Number _____ Exemption Subsection _____ or IRB App. Date _____						
<input type="checkbox"/> DISCLOSURE OF LOBBYING ACTIVITIES (GPG II.C.1.e) <input checked="" type="checkbox"/> INTERNATIONAL COOPERATIVE ACTIVITIES: COUNTRY/COUNTRIES INVOLVED (GPG II.C.2.j) HR FR						
<input type="checkbox"/> PROPRIETARY & PRIVILEGED INFORMATION (GPG I.D, II.C.1.d) <input type="checkbox"/> HIGH RESOLUTION GRAPHICS/OTHER GRAPHICS WHERE EXACT COLOR REPRESENTATION IS REQUIRED FOR PROPER INTERPRETATION (GPG I.G.1)						
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<input type="checkbox"/> EAGER* (GPG II.D.2) <input type="checkbox"/> RAPID** (GPG II.D.1) <input type="checkbox"/> HIGH RESOLUTION GRAPHICS/OTHER GRAPHICS WHERE EXACT COLOR REPRESENTATION IS REQUIRED FOR PROPER INTERPRETATION (GPG I.G.1)						
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CERTIFICATION PAGE

Certification for Authorized Organizational Representative or Individual Applicant:

By signing and submitting this proposal, the Authorized Organizational Representative or Individual Applicant is: (1) certifying that statements made herein are true and complete to the best of his/her knowledge; and (2) agreeing to accept the obligation to comply with NSF award terms and conditions if an award is made as a result of this application. Further, the applicant is hereby providing certifications regarding debarment and suspension, drug-free workplace, lobbying activities (see below), responsible conduct of research, nondiscrimination, and flood hazard insurance (when applicable) as set forth in the NSF Proposal & Award Policies & Procedures Guide, Part I: the Grant Proposal Guide (GPG) (NSF 11-1). Willful provision of false information in this application and its supporting documents or in reports required under an ensuing award is a criminal offense (U. S. Code, Title 18, Section 1001).

Conflict of Interest Certification

In addition, if the applicant institution employs more than fifty persons, by electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative of the applicant institution is certifying that the institution has implemented a written and enforced conflict of interest policy that is consistent with the provisions of the NSF Proposal & Award Policies & Procedures Guide, Part II, Award & Administration Guide (AAG) Chapter IV.A; that to the best of his/her knowledge, all financial disclosures required by that conflict of interest policy have been made; and that all identified conflicts of interest will have been satisfactorily managed, reduced or eliminated prior to the institution's expenditure of any funds under the award, in accordance with the institution's conflict of interest policy. Conflicts which cannot be satisfactorily managed, reduced or eliminated must be disclosed to NSF.

Drug Free Work Place Certification

By electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative or Individual Applicant is providing the Drug Free Work Place Certification contained in Exhibit II-3 of the Grant Proposal Guide.

Debarment and Suspension Certification

(If answer "yes", please provide explanation.)

Is the organization or its principals presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency?

Yes

No

By electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative or Individual Applicant is providing the Debarment and Suspension Certification contained in Exhibit II-4 of the Grant Proposal Guide.

Certification Regarding Lobbying

The following certification is required for an award of a Federal contract, grant, or cooperative agreement exceeding \$100,000 and for an award of a Federal loan or a commitment providing for the United States to insure or guarantee a loan exceeding \$150,000.

Certification for Contracts, Grants, Loans and Cooperative Agreements

The undersigned certifies, to the best of his or her knowledge and belief, that:

- (1) No federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.
- (2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, "Disclosure of Lobbying Activities," in accordance with its instructions.
- (3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by section 1352, Title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than \$10,000 and not more than \$100,000 for each such failure.

Certification Regarding Nondiscrimination

By electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative is providing the Certification Regarding Nondiscrimination contained in Exhibit II-6 of the Grant Proposal Guide.

Certification Regarding Flood Hazard Insurance

Two sections of the National Flood Insurance Act of 1968 (42 USC §4012a and §4106) bar Federal agencies from giving financial assistance for acquisition or construction purposes in any area identified by the Federal Emergency Management Agency (FEMA) as having special flood hazards unless the:

- (1) community in which that area is located participates in the national flood insurance program; and
- (2) building (and any related equipment) is covered by adequate flood insurance.

By electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative or Individual Applicant located in FEMA-designated special flood hazard areas is certifying that adequate flood insurance has been or will be obtained in the following situations:

- (1) for NSF grants for the construction of a building or facility, regardless of the dollar amount of the grant; and
- (2) for other NSF Grants when more than \$25,000 has been budgeted in the proposal for repair, alteration or improvement (construction) of a building or facility.

Certification Regarding Responsible Conduct of Research (RCR)

(This certification is not applicable to proposals for conferences, symposia, and workshops.)

By electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative of the applicant institution is certifying that, in accordance with the NSF Proposal & Award Policies & Procedures Guide, Part II, Award & Administration Guide (AAG) Chapter IV.B., the institution has a plan in place to provide appropriate training and oversight in the responsible and ethical conduct of research to undergraduates, graduate students and postdoctoral researchers who will be supported by NSF to conduct research.

The undersigned shall require that the language of this certification be included in any award documents for all subawards at all tiers.

AUTHORIZED ORGANIZATIONAL REPRESENTATIVE		SIGNATURE		DATE	
NAME					
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* EAGER - EARly-concept Grants for Exploratory Research

** RAPID - Grants for Rapid Response Research

PROJECT SUMMARY

CNIC: WHAT DOES IT TAKE TO BE AN HERBIVOROUS LIZARD? REVEALING THE DIGESTIVE ANATOMY AND PHYSIOLOGY OF MANIPULATED POPULATIONS OF *Podarcis sicula*

As the supply organ of nutrients to an animal, the digestive tract is a dynamic physiological system that is the focus of the growing field of nutritional physiology. At the core of this research is the Adaptive Modulation Hypothesis (AMH), which suggests that an animal's digestive tract must be tightly regulated in relation to food intake and quality because the gut is expensive to maintain. Indeed, the AMH has been supported by empirical studies in a range of animal taxa, and provides the "ultimate" reasons for why specialization of the digestive tract towards diet arises. However, experimental evolution provides potential to study the "proximate" mechanisms of how the digestive tract adapts to different diets, and the resulting consequences for animal performance. In this study for the CNIC program, researchers from three countries with complementary skillsets will investigate the nutritional physiology of *Podarcis sicula*, a lizard species that has shown rapid evolution of feeding and digestive tract morphology.

Podarcis sicula inhabits islets off the coast of Croatia, and a manipulative study transferred breeding pairs of *P. sicula* from the islet of Pod Kopište to the islet of Pod Mrčaru, which previously lacked this species. After 30 generations on Pod Mrčaru (~36 years), *P. sicula* became more herbivorous, which resulted in marked differences in head size, bite force and gut morphology in comparison to the insectivorous parent population on Pod Kopište. Perhaps most striking was that the Pod Mrčaru lizards had developed a hindgut chamber, including cecal valves, which are only currently known from herbivorous lizards, and have not been observed previously in *P. sicula*. The hindgut chambers in herbivores are a specialization of intestinal morphology, which house endosymbiotic microbes that aid the lizard in the digestion of plant material. Beyond differences in gut morphology, little is known about the digestive physiology of *P. sicula*, or the consequences of this dietary specialization.

This project for the CNIC program will have two main parts comparing lizards from the two populations: **Part 1**—*P. sicula* will be captured from Pod Mrčaru and Pod Kopište for study of their diet (using gut content and stable isotopic analyses), gut morphology (gross, histological and ultrastructural analyses), digestive enzyme activities, levels of gastrointestinal fermentation, and microbial diversity. **Part 2**—*P. sicula* from the two islets will be brought into the laboratory at University of Zagreb and used in common garden experiments in which the digestive efficiency of plant and animal diets will be evaluated, as well as resting, post-prandial, and post-exercise metabolic rates of the lizards.

INTELLECTUAL MERIT: Rarely are we afforded the opportunity to examine evolution in action in vertebrates in a natural setting. The manipulated populations of *P. sicula* provide an opportunity to test how a vertebrate animal modulates its digestive tract in response to dietary changes, and to show how gut function can be matched to gut structure (i.e., AMH). Other studies have examined the acute changes of dietary shifts on gut structure and function in animals, but adaptations of the gut (e.g., hindgut chambers, cecal valves), are currently thought to have evolved over geological timescales. **This project for the CNIC will test the consequences of specialization, which evolved over ecological timescales.** Overall, this project will be one of the first to evaluate the potential metabolic costs of dietary specialization in lizards, and will lead to more detailed investigations of how dietary specializations arise in animals.

BROADER IMPACTS: This project will catalyze an international collaboration between the PI (UC Irvine), Dr. Anthony Herrel (Muséum National d'Histoire Naturelle, Paris, France) and Dr. Zoran Tadić (University of Zagreb, Croatia). The PI is a nutritional physiologist who has studied digestion and metabolism in different animal systems, including amphibians and reptiles. Dr. Herrel is an ecologist who has studied *Podarcis sicula* for over a decade and collaborates with Dr. Zoran Tadić, a physiologist at University of Zagreb. This CNIC grant will allow the PI and his graduate student (Beck Wehrle) to travel to Croatia in the summer of 2013 to collect *P. sicula* and perform the proposed project, which will form the foundation of Mr. Wehrle's dissertation research. Reciprocal training among the collaborators will have an immediate impact for everyone involved, including graduate and undergraduate students from all three institutions. For outreach, the entire group will travel to high schools in Zagreb, Croatia, to share the work with the public and engender interest in the STEM disciplines. Further, outreach at a high school in the US will share this research locally. Finally, findings will be published in scientific journals.

TABLE OF CONTENTS

For font size and page formatting specifications, see GPG section II.B.2.

	Total No. of Pages	Page No.* (Optional)*
Cover Sheet for Proposal to the National Science Foundation		
Project Summary (not to exceed 1 page)	1	_____
Table of Contents	1	_____
Project Description (Including Results from Prior NSF Support) (not to exceed 15 pages) (Exceed only if allowed by a specific program announcement/solicitation or if approved in advance by the appropriate NSF Assistant Director or designee)	8	_____
References Cited	4	_____
Biographical Sketches (Not to exceed 2 pages each)	8	_____
Budget (Plus up to 3 pages of budget justification)	4	_____
Current and Pending Support	1	_____
Facilities, Equipment and Other Resources	1	_____
Special Information/Supplementary Documents (Data Management Plan, Mentoring Plan and Other Supplementary Documents)	12	_____
Appendix (List below.) (Include only if allowed by a specific program announcement/ solicitation or if approved in advance by the appropriate NSF Assistant Director or designee)	_____	_____
Appendix Items:		

*Proposers may select any numbering mechanism for the proposal. The entire proposal however, must be paginated. Complete both columns only if the proposal is numbered consecutively.

CNIC: WHAT DOES IT TAKE TO BE AN HERBIVOROUS LIZARD? REVEALING THE DIGESTIVE ANATOMY AND PHYSIOLOGY OF MANIPULATED POPULATIONS OF *Podarcis sicula*

Project Description

Intellectual merits and objectives

An animal's diet and digestive strategy directly affect its resource acquisition, behavior, and trophic interactions¹. However, beyond feeding observations and gut content analyses, the nutritional physiology of animals is not pursued as often as might be warranted¹⁻⁵. This becomes important when one considers that there is a difference between what an animal *eats* and what it *digests*. For instance, wood-eating catfishes (genus *Panaque*) assimilate little nutriment directly from the wood they consume in copious amounts⁶⁻⁸, and the lizard *Tupinambis merianae*, which appears to be omnivorous based on gut content analyses, does not efficiently digest complex carbohydrates from plants⁴. Studying animal nutritional physiology requires a multi-faceted approach that considers not only the animal's diet, but also its digestive tract structure and function. Central to the field of nutritional physiology is the Adaptive Modulation Hypothesis (AMH^{9,10}), which, grounded in Optimal Foraging (e.g., Pyke et al¹¹), and Symmorphosis¹² theories, takes an economics approach to understand gut function. The AMH posits that because the digestive tract is expensive to maintain¹³, gut structure and function should match with what is consumed in terms of quantity and biochemical composition. In support of the AMH, patterns of gut size and function (from digestive enzyme activities to nutrient transport rates) appear to fit with diet in many vertebrate taxa¹⁴, making the AMH one of the most fruitful hypotheses in comparative physiology.

Within the constructs of the AMH, dietary specialization should lead to specialization of the gut towards digesting a specific diet, as has been confirmed in experimental^{15,16} and phylogenetic¹⁷⁻¹⁹ contexts. Thus, the AMH provides the "ultimate" reasons for why specialization of the gut should arise. Experimental evolution, on the other hand, provides opportunities to test the "proximate" mechanisms by which specialization arises, and the consequences of specialization for animal performance. **Therefore, we will investigate the nutritional physiology of *Podarcis sicula*, a lizard species that has shown rapid evolution of feeding and digestive tract morphology in manipulated populations.**

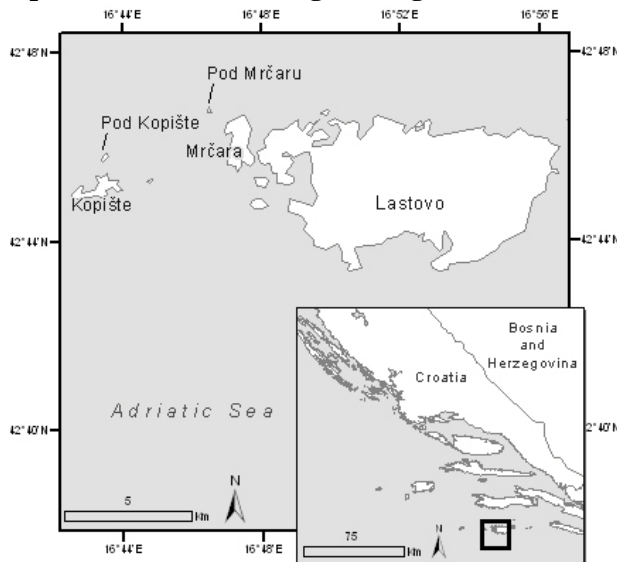


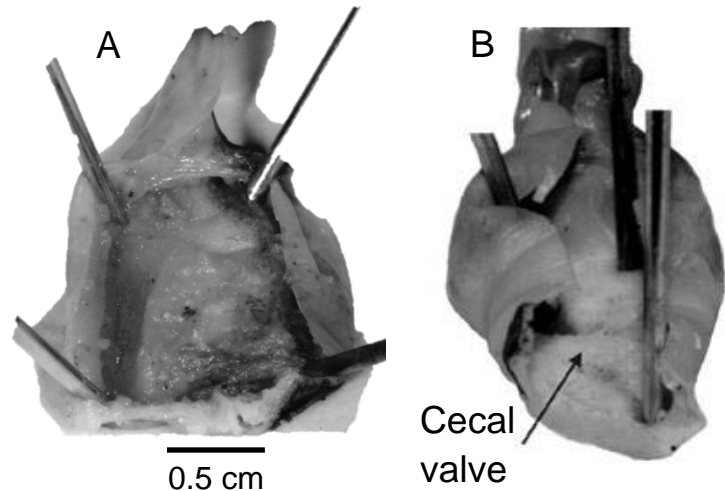
Figure 1. Collection sites of *Podarcis sicula*. The box in the bottom map shows the area of the inset.

2). Hindgut chambers are observed in other herbivorous lizards (e.g., Agamids, Iguanids), and are densely populated by endosymbiotic microorganisms (including nematodes) that facilitate plant digestion in the lizard host²²⁻²⁵. Indeed, Herrel and co-authors²⁰ noted high densities of nematodes in the hindgut chambers of Pod Mrčaru lizards. In just 30 generations, the Pod Mrčaru *P. sicula* apparently adopted a mostly herbivorous lifestyle from the perspective of food intake and gut structure. However, little is

Among vertebrates, few cases of rapid specialization to diet are more remarkable than that observed in the lacertid lizard *Podarcis sicula*²⁰. In 1970, five breeding pairs of *P. sicula* were transferred from the islet of Pod Kopište to the islet of Pod Mrčaru, Croatia²¹ (Fig. 1). The source *P. sicula* population on Pod Kopište was insectivorous. Herrel and colleagues²⁰ returned to these islets 36 years later (representing about 30 *P. sicula* generations) and discovered that the transplanted *P. sicula* on Pod Mrčaru had become mostly herbivorous. This dietary shift resulted in changes in head size and bite force among the populations on the two islands. Perhaps most striking, however, were the changes that occurred in the lizards guts. The herbivorous Pod Mrčaru lizards had developed an elaborate hindgut chamber with cecal valves, which had not been seen in any other population of *P. sicula*²⁰ (Fig.

known about the function of the digestive tract in *P. sicula* from the two populations, whether any changes in gut function have occurred, nor whether there have been any performance consequences for the apparent dietary specialization in the Pod Mrčaru lizards. **Thus, the focus of this CNIC proposal will be to return to the islets of Pod Kopište and Pod Mrčaru to investigate potential specializations of gut function and performance consequences in the Pod Mrčaru lizards consuming an herbivorous diet.** The data gathered in this study will further test the AMH, showing that changes in gut function match changes in gut structure. These data will be used to apply for a follow-on grant to study how this specialization has arisen on multiple levels, from molecular, to whole animal performance.

Figure 2. The large intestine of *Podarcis sicula* from Pod Kopište (A) and from Pod Mrčaru (B). Note the thicker gut wall and presence of cecal valves in the Pod Mrčaru individual. The large intestine in the Pod Kopište lizard is artificially enlarged by the probes, as these animals do not show a hindgut chamber as seen in (B). Photos by Anthony Herrel.



Consistent with the AMH, the primary hypothesis in this study is that because there have been significant changes in the gut structure of *P. sicula* from Pod Mrčaru, there have been concomitant changes in gut function in these lizards towards the digestion of an herbivorous diet. **Therefore, the Pod Mrčaru *P. sicula* should possess a suite of digestive tract features observed in other herbivorous vertebrates allowing them to subsist on low-quality plant foods.** This project for the NSF CNIC program is envisioned in two main parts using lizards from the two islets: (1) an analysis of diet, gut structure and function; and (2) an analysis of animal performance. This project will be the most detailed study of the digestive tract in a wild-caught lizard species to date, and will be one of the first to evaluate the potential metabolic costs of dietary specialization in lizards. Perhaps most importantly, this study will lead to more detailed investigations of how dietary specializations arise in animals.

Part 1: Diet, gut structure and gut function of lizards from the two islets

Initial capture and care of lizards

Adult male *Podarcis sicula* will be captured by hand from the islets of Pod Kopište and Pod Mrčaru, located just west of the island of Lastovo, Croatia (Fig. 1), during the summer of 2013 (i.e., during the non-breeding season). Upon capture, 13 lizards from each island will be euthanized by injection of sodium pentobarbital. These lizards will immediately be measured, weighed, and dissected. The digestive tracts of seven of these specimens will be prepared for gut content, digestive enzyme activity and fermentation analyses (see detailed sections below). The gut contents will be collected from different regions of the intestine (small and large intestine) of three lizards, and frozen in liquid nitrogen for molecular analyses of the gut microbiome (Hong et al.²⁶). The digestive tracts of the three remaining lizards will be fixed and used for histological and gut ultrastructural analyses. Fifteen additional lizards will be captured and held in large plastic containers (L x W x H; 150 cm x 50 cm x 50 cm). At the end of each day during the collection period (~14 days), these lizards will be transferred to large mesh terraria (1000-L) located on the island of Lastovo, which will contain sand and cover, thus allowing the lizards to thermoregulate. During this period, captive lizards from two the islets, which will be housed separately, will be given access to water, meal worms dusted with vitamin premix²⁷, and plants. Upon completion of collection activities, lizards will be transported in the large plastic containers described above to the

University of Zagreb, where they will be housed at 30°C in groups of four in 76-L terraria equipped with cover and heat lamps, and under a 14:10 (Light:Dark) lighting regime, consistent with the summer photoperiod in Croatia. Lizards will be given unfettered access to meal worms and plant material for one week to allow them to acclimate to the laboratory environment before beginning experiments.

Diet and gut structure

Hypothesis 1: The *P. sicula* on Pod Mrčaru will have greater amounts of fibrous plant material in their stomach contents than the Pod Kopište lizards, and the former will show carbon and nitrogen stable isotopic signatures indicative of long-term plant consumption.

Although the diets of *P. sicula* on the two islets are known^{20,27}, it is necessary to confirm the diets of the lizards used in the current study. The guts of seven individuals will be dissected out as described by Herrel et al.²⁰, and placed on a chilled cutting board⁷. Each gut will then be measured (to the nearest 0.1 mm^{28,29}), and cut into three distinct regions: stomach, small intestine, and large intestine. The stomach contents will be separated from the stomach tissue using sterile forceps, and the contents and stomach tissue will be weighed individually. The stomach contents and stomach tissue will then be individually placed in centrifuge vials and frozen in liquid nitrogen. The stomach contents will be used to evaluate diet using gravimetric analyses²⁰ at University of Zagreb within one month of collection. The contents and tissues from the small and large intestine will be collected in a similar manner, but will be used for analyses of digestive enzyme activities and levels of gastrointestinal fermentation (see detailed section below). The guts of three additional lizards will be fixed in Trump's fixative (4% formaldehyde, 1% glutaraldehyde, in 10 mM monobasic sodium phosphate and 6.75 mM sodium hydroxide³⁰) for histological and TEM analyses following the methods described by German⁷. The expectation is that changes in gross gut morphology often mirror changes on the histological and ultrastructural levels^{31,32}. Thus, in addition to the hindgut chamber, we expect to find that the herbivorous Pod Mrčaru lizards have greater intestinal (histology) and microvilli (ultrastructure) surface areas than the Pod Kopište lizards due to the increased intake of a low-quality diet in the former^{31,32}. Increased surface area should increase absorptive capacity, which will be further tested by measuring digestive enzyme activities along the gut.

Although gut content analysis provides a snap shot of an animal's diet, stable isotopic analysis has emerged as a powerful tool that integrates dietary information over time³³⁻³⁵. The dietary carbon (C) and nitrogen (N) assimilated into an animal's tissues can be used to trace dietary diversity within species, as well as changes in trophic level with dietary shifts^{5,6,8,35}. To further examine differences in diet among the two populations, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ will be measured in potential dietary items collected from each islet, and in several tissues (blood, including plasma and red blood cells (RBCs) analyzed separately, liver, and muscle) from the lizards. Blood plasma and liver show rapid rates of dietary isotopic incorporation (days to weeks) in ectotherms, whereas muscle and RBCs show slower turnover rates (weeks to months^{6,36}). Thus, these tissues integrate dietary signals on different time scales and can allow for the examination of diet ($\delta^{13}\text{C}$) and trophic standing ($\delta^{15}\text{N}$) in the short and long term. We expect that the Pod Mrčaru lizards will show lower $\delta^{15}\text{N}$ in their tissues than the Pod Kopište lizards, and $\delta^{13}\text{C}$ consistent with cellulose assimilation⁸, thus confirming the long-term herbivory of Pod Mrčaru *P. sicula*.

Digestive enzyme activities, gastrointestinal fermentation, and microbial diversity

Hypothesis 2: The *P. sicula* on Pod Mrčaru will have elevated cellulase activities, higher concentrations of short chain fatty acids, and a different microbial community in their large intestines than the Pod Kopište *P. sicula*.

Because lizards do not masticate, trituration and digestive enzymes provide the physical and chemical means, respectively, through which these animals reduce ingesta particle size and access nutrients contained therein^{1,37,38}. Surprisingly few studies have examined digestive enzyme activities in lizards in relation to diet⁵, nor how patterns of digestive enzyme activity vary along the digestive tract³⁹. Most studies of digestive enzymes in reptiles have focused on enzymes in venom (e.g., Aminetzach et al.⁴⁰), or

the guts of snakes as they ramp up digestive function following consumption of a carnivorous meal (e.g., Cox and Secor⁴¹). One study compared digestive enzyme activities among populations of *Liolaemus pictus* with slight dietary variation, finding differences only in aminopeptidase (a protease) activity⁵. Thus, we know little about the digestive biochemistry of omnivorous and herbivorous reptiles.

In other vertebrates, herbivores rely on endogenous digestive enzymes in the stomach and small intestine to digest soluble nutrients in their food, whereas exogenously produced (microbial) enzymes aid in the digestion of insoluble dietary components (e.g., cellulose) in the region of the gut where fermentative digestion is active. In non-ruminants, including *Iguana iguana*, this is in the hindgut chamber^{25, 38, 42, 43}. A typical pattern of digestive enzyme activity in an animal with hindgut fermentation is elevated protease and soluble polysaccharide-degrading enzyme (e.g., amylase) activities in the small intestine, and elevated refractory polysaccharide-degrading enzyme (e.g., cellulase) activities in the hindgut¹. Based on the observations of Herrel et al.²⁰, we expect to find different patterns of activity in the Pod Mrčaru *P. sicula* in comparison to the *P. sicula* on Pod Kopište (Table 1).

Following the methodology of German and Bittong⁴⁴, we will examine endogenous and exogenous enzyme activities in the small and large intestines of the lizards. We will do this by collecting three components from the small and large intestines: gut wall tissue (endogenous enzymes), gut fluid (enzymes secreted either by the lizard or microorganisms), and microbial extract (exogenous enzymes). If, similar to other herbivorous animals, the Pod Mrčaru *P. sicula* are relying on endosymbionts in their hindgut to digest cellulose in their plant diet, we would expect cellulase activities to be highest in the microbial extract of the hindgut (Table 1). Because the digestion of cellulose requires three enzymes to degrade the polysaccharide to monomeric glucose, we will measure the activities of cellulase, cellobiohydrolase, and β -glucosidase.

In addition to cellulases, we will measure the activities of eight enzymes that digest carbohydrates, proteins, and lipids (Table 1). Based on the findings that amylase and α -glucosidase activities are elevated in herbivorous vertebrates in comparison to carnivores^{17, 18}, we expect these enzyme activities to be higher in Pod Mrčaru lizards, whereas we expect the insectivorous Pod Kopište *P. sicula* to have elevated chitin-degrading enzyme^{17, 45} and trehalase¹⁹ activities. No clear patterns of activity to diet have emerged for proteases and lipases, probably because all animals need protein and essential fatty acids^{1, 2, 16, 18}. Thus, we expect no differences for these enzymes. Overall, these data will be the most comprehensive study to date of lizard digestive enzyme activities in relation to diet.

Table 1. Digestive enzymes to be assayed in this study of *Podarcis sicula*.

Enzyme	Substrate	Dietary source	Expected region ^a	>Component ^b	Population ^c
Amylase	Starches	Plants, Seeds, Glycogen	Small Int	Fluid	Mrčaru
α -glucosidase	α -glucosides	Plants, Seeds, Glycogen	Small Int	Gut wall	Mrčaru
Cellulase	Cellulose	Plant cell walls	Large Int	Contents	Mrčaru
Cellobiohydrolase	Cellulose	Plant cell walls	Large Int	Contents	Mrčaru
β -glucosidase	β -glucosides	Plant cell walls	Large Int	Contents	Mrčaru
Chitinase	Chitin	Insect exoskeletons	Stomach	Gut wall	Kopište
N-acetyl- β -D-glucos ^d	NAG	Insect exoskeletons	Small Int	Gut wall	Kopište
Trehalase	Trehalose	Insects	Small Int	Gut Wall	Kopište
Trypsin	Protein	Plants, Seeds, Insects	Small Int	Fluid	Equivocal
Aminopeptidase	Dipeptides	Plants, Seeds, Insects	Small Int	Gut wall	Equivocal
Lipase	Lipid	Plants, Seeds, Insects	Small Int	Fluid	Equivocal

^a The expected patterns of activity between the small and large intestine (Int). e.g., “Large Int” means that the activity of this enzyme should be higher in the large intestine. ^b Predictions of which assayed components will have higher activity of a particular enzyme. ^c Predictions regarding what population of *P. sicula* will have higher activities for a particular enzyme. Equivocal means there is no expected difference for that enzyme. ^d Complete name of the enzyme is N-acetyl- β -D-glucosaminidase, and the substrate is N-acetyl- β -D-glucosaminide (NAG).

When endosymbiotic microorganisms digest refractory organic matter in animal guts, they do so under anaerobic conditions. The enteric microbial community is thus forced to use fermentation to recycle electron acceptors and maintain the functionality of metabolic pathways^{1,46}. The waste products of these fermentative pathways include short chain fatty acids (SCFAs), which can be absorbed and used by the vertebrate host in its own metabolism⁴⁷. The SCFAs Acetate, Propionate, and Butyrate are utilized efficiently to produce ATP by vertebrates and represent the key mechanism by which ruminant and hindgut-fermenting mammals make a living^{46,48,49}. Indeed, *Iguana iguana*^{25,42} and *Uromastix aegyptius*⁴⁷ have elevated SCFA concentrations in their hindguts that rival those seen in hindgut fermenting mammals and herbivorous fishes⁵⁰. Therefore, following the methodology of Pryor et al.⁵¹, we will measure the concentrations of SCFAs in the fluid of the small and large intestines of *P. sicula* from the two islets, with the expectation of higher SCFA concentrations in the Pod Mrčaru lizards.

Beyond the functional characteristics of digestive physiology, the diversity of the enteric microbial community provides insight into what microorganisms aid the vertebrate host in their digestive strategy^{26,52-54}. Clear differences are apparent in the intestinal microbial diversity among species with different diets^{26,55}, and in a single species in response to gut fullness⁵⁶, geographic location⁵², and dietary changes⁵⁷. Following Hong et al.²⁶, we will use pyrosequencing to generate 16s rRNA-based sequences of the microbial communities in the small and large intestines of *P. sicula* from the two islets. Previous investigations have shown shifts in the operational taxonomic units of microbial communities in the guts of animals consuming different diets, and differences in the community among different regions of the gut⁵⁵. We, therefore, expect that microbial diversity will be higher in Pod Mrčaru *P. sicula* than in the Pod Kopište lizards, and higher in the large intestine than the small intestine of lizards from both populations. This information will then be used to infer function of the community based on functional groupings for known taxa.

Part 2: Animal performance

Digestibility of herbivorous, omnivorous, and insectivorous diets

Hypothesis 3: The *P. sicula* on Pod Mrčaru will have longer transit times of food through the gut, and higher digestibility of plant and animal foods than the Pod Kopište *P. sicula*.

To complement the studies described above of digestive physiology in *P. sicula*, it is also important to move beyond such inferential data and directly evaluate animal digestive performance in response to diet. The best way to examine specialization towards diet is with common garden experiments, where, under the same conditions, true differences in function can be quantified²⁷. In this experiment, we will quantify digestibilities of animal and plant foods, and transit times of food through the lizards' guts. Thus, we will transfer 15 individuals of *P. sicula* from each of the islets back to the laboratory of Zoran Tadić at the University of Zagreb for use in feeding trials. After an initial holding period of one week to acclimate the lizards to the laboratory environment, lizards will be housed in individual 15-L terraria equipped with heating lamps and cover (bark and stones⁵⁸), and maintained at 30°C under a 14:10 Light:Dark cycle. Lizards from both islets will be offered unfettered access to meal worms (dusted with vitamin premix), plant material (from each islet), and water for an additional week under these conditions to confirm that they are viable and at least maintaining body mass⁵⁸. Intake of each dietary item will be monitored to assess dietary preferences of lizards from the islets, and to confirm voluntary intake of each individual lizard. Lizards that do not eat voluntarily and lose weight will be removed from the experiment. At the conclusion of the two week acclimation, the lizards will be deprived of food for 3-4 days, until defecation of previous digesta ceases. At this time, the lizards will be randomly assigned to one of three treatments: plant only diet (herbivorous), plant and meal worm diet (omnivorous), and a meal worm only (insectivorous) diet. Because it is possible that the Pod Kopište lizards will not voluntarily consume plant material in the laboratory, it will be necessary to force feed them the herbivorous diet, and thus, for consistency, to force feed lizards in all treatments⁵⁹. The herbivorous diet will be composed of fresh plant material (e.g., leaves, seeds, flowers) commonly consumed by *P. sicula* from Pod Mrčaru. The

omnivorous diet will be 50% Pod Mrčaru plant material and 50% meal worms. The insectivorous diet will consist solely of meal worms. All dietary items will be dried to a constant weight at 60°C, and ground in a coffee grinder to pass through a 1-mm screen. Just before feeding, the ground food will be mixed with water (50:50, food:water) and placed in small gelatin capsules (Erawat Pharma, Ltd., Madhya Pradesh, India). The leading end of the capsules will be lubricated with a small amount (<0.01 g) of vegetable oil, the capsules placed in the lizards mouths, and gently pushed into the esophagus with a glass stir rod⁶⁰. Each lizard will be weighed prior to feeding, and the appropriate amount of food fed to each animal as is necessary to maintain their body mass (~10% of body mass⁵⁹). The lizards will be fed daily in this manner until enough fecal material is produced for analyses of assimilation efficiencies of various constituents of the diet (2-3 weeks). Because we will be feeding the lizards known quantities of food, and we will collect all feces and urates, we will use the “total collection method” of measuring digestive efficiency, as opposed to using an inert marker (e.g., ash⁶¹⁻⁶³). The digestibility of protein, lipid, and total sugars will be performed following Pafilis et al.⁵⁸, whereas the digestibility of neutral detergent and acid detergent fiber (NDF and ADF, respectively), will be determined using modified protocols of Goering and Van Soest⁶⁴ as described by German⁷. We will then directly compare the digestibilities of plant and animal dietary constituents by the lizards from the two islets. It should be noted that lacertid lizards do readily consume food in captivity^{58, 65}, and thus, if lizards in all treatments do voluntarily consume their food, we will not force feed them, but instead allow the animals to control their own intake on each diet.

Following the digestibility trials, we will analyze gut transit times of food in the lizards’ alimentary canals. On day one of this trial, lizards in all groups will be fed their respective diet (as described above), with the exception that the food will be mixed with carmine dye to stain the food red⁷. The lizards will then be left in their terraria and monitored hourly until the appearance of red feces, which will mark transit time of food through the gut. If this takes longer than one day, as is possible in small lizards^{27, 58}, the animals will be fed unstained food on day two to maintain normal intake of ingesta.

One previous study of digestibility and gut transit of meal worms found longer transit times and higher digestibilities of protein, lipids, and sugars in the Pod Mrčaru lizards than in the Pod Kopač lizards²⁷. However, the study by Vervust and colleagues²⁷ only examined gut transit time of a plastic marker (not marked food itself, which is superior¹), and only examined digestibility of the soluble fractions of an insectivorous diet. Thus, the current study will examine digestibility of herbivorous and omnivorous diets, and most importantly, digestibilities of the fibrous portions of these diets. Moreover, in conjunction with all of the digestive physiological characteristics in different regions of the gut, and stable isotope analyses, this study will provide the mechanistic evidence to understand how the Pod Mrčaru lizards have increased their digestive capacities of plant material.

Standard, post-prandial, and post-exercise metabolic rates of lizards on different diets

Hypothesis 4: Despite their dietary differences, the lizards will not have different standard metabolic rates, but the post prandial response (i.e., specific dynamic action) will be greater in the lizards from both populations fed higher protein diets. Because of the similar sizes of *P. sicula* from both populations, the active metabolic rates will not differ.

A key measure of animal function is the heat generated by the totality of their metabolic processes, referred to as metabolic rate. Commonly measured via oxygen consumption or CO₂ production, metabolic rate can be used as a metric of animal function in response to different environmental variables⁶⁶. In ectotherms, like reptiles, it is routine to determine the metabolic rate of an animal that is at rest, not stressed, and not digesting a meal. This “standard metabolic rate” (SMR) is used as a baseline from which to compare other physiological states in that same animal. SMR scales with temperature and body mass in a very predictable manner⁶⁷, and does not necessarily vary in individual reptiles and amphibians consuming different diets^{59, 68}.

A common observation in animals is an increase in metabolic rate following the consumption of a meal, representing the ramping up of digestion and metabolism involved in nutrient processing. This post-prandial metabolic increase is commonly referred to as “specific dynamic action” (SDA⁶⁹). Among

reptiles, SDA is largest (25X higher than SMR) in sit-and-wait predators, like *Python molurus*⁷⁰, consuming large, proteinaceous meals, and may be lowest (<2X SMR) in herbivores⁷¹. This is primarily due to meal size and composition: proteins require more energy to process than carbohydrates or fats⁷²⁻⁷⁴, and sit-and-wait predators consume single, large meals, and then are inactive during digestion^{69, 75}. In contrast, herbivores consume frequent, smaller meals, constantly experience a small SDA, and are active during digestion⁷⁵. Thus, herbivores and carnivores may have similar metabolic demands over time, and our dataset can be used to evaluate this idea.

Finally, the post-exercise, or active metabolic rate (AMR) is frequently used as a metric of animal performance and correlates with animal size⁷⁶. However, it is unclear how AMR might vary among similarly sized individuals of the same species that have diverged in their dietary preferences. Thus, we will measure the SMR, SDA, and AMR in the *P. sicula* from the two islets that are consuming the different diets in the laboratory. Immediately following our assimilation efficiency trials, we will fast the lizards for 3-4 days to confirm that they have empty guts. Using closed-system respirometry⁷⁷, we will place individual lizards in 1-L glass jars fitted with incurrent and excurrent air ports, each attached to a three-way stopcock, and held at 30°C. We will start each measurement by withdrawing a 20-ml air sample from an excurrent air port, and close both ports to seal the chamber. One-half hour later, a second 20-ml air sample will be withdrawn. Air samples will be injected through an incurrent tube (100 ml min⁻¹), through a column of water absorbent Drierite and CO₂ absorbent soda lime (Ascarite II) and past an O₂ sensor (HighOxyR, Ocean Optics, Dunedin, FL). A second 8-ml sample will be taken and injected directly into an infrared CO₂ analyzer (EGM-4, PP Systems, Amesbury, MA, USA), which includes its own Drierite scrubber. We will calculate whole-animal (ml h⁻¹) rates of O₂ and CO₂ corrected for standard pressure and temperature⁷⁷. We will measure each lizard twice a day, and will take measurements across two days. The lowest value in fasted animals will represent SMR⁶⁸. We will then follow the same procedures using fed animals, and fasted lizards that have run to exhaustion on a treadmill⁷⁸ to calculate SDA and AMR, respectively. Because protein has such an important impact on SDA⁷²⁻⁷⁴, we expect lizards from both islets fed the insectivorous and omnivorous diets to exhibit the largest SDA values. However, with little insight on potential differences for AMR, we will assume a null hypothesis for these measurements in the lizards from the two populations. Nevertheless, inherent physiological differences between the divergent populations of *P. sicula* may result in differences in AMR in lizards from the two islets. At the end of the experiments, all animals will be euthanized and tissues harvested to observe any changes in gut structure and function in response to the experimental diets. All bodies will be tissue and deposited in the CNRS in Paris, France (Dr. Herrel's institution).

Significance, broader impacts, and appropriateness for the CNIC program

The *Podarcis sicula* system provides an unprecedented opportunity to study specialization of the digestive tract to a recent change in diet. Many studies have investigated the plasticity of the digestive tract towards acute dietary shifts in animals, and a few have examined these patterns in a phylogenetic context (summarized in Karasov and Martínez del Río¹). Rarely, however, are we afforded an opportunity to examine evolution in action in a vertebrate species living under natural conditions, and to investigate microevolutionary changes that have occurred over *ecological* time scales that resemble macroevolutionary changes that occur over *geological* time scales. The experiments outlined in this project will allow us to directly test whether the apparent rapid evolution in *P. sicula* has achieved the functional differences that are currently only assumed to have taken place. Moreover, this study will provide a baseline for understanding the role of *P. sicula* in their environments in terms of trophic position and potential contributions to ecological fluxes^{1, 8}. In terms of application, relevant nutritional physiological data can lead to better management decisions regarding conservation of that species or their habitat³. Thus, the data gathered in this study will not only be useful for understanding the evolution of dietary specialization, but can provide a model for future nutritional physiological studies.

This project for the CNIC will initiate a productive international collaboration among researchers in the USA, France, and Croatia. The PI is a nutritional physiologist who has studied digestion and metabolism in many different animal systems, including amphibians and reptiles. Dr. Anthony

Herrel is an ecologist who has been studying *Podarcis sicula* for over a decade (e.g., Herrel et al.²⁰). Dr. Herrel has also established a collaboration with Dr. Zoran Tadić, a physiologist at University of Zagreb, on other ecological projects⁷⁹. The project described here, therefore, uniquely brings together scientists from three different countries whose skillsets complement each other's in a synergistic fashion. Furthermore, a true hurdle to studying *P. sicula* in captivity in the past has been transporting these animals out of Croatia and maintaining their health²⁷. This project overcomes this problem by involving a Croatian scientist, and using his laboratory for experiments, thereby minimizing animal transport.

From a training perspective, this project will form the basis of the dissertation research of the PI's graduate student, Beck Wehrle. Mr. Wehrle is a budding herpetologist who did his Masters of Science degree under the guidance of herpetologist Dr. Robert E. Espinoza at California State University, Northridge. The PI, Mr. Wehrle, and an undergraduate assistant (Abraham Sosa) will spend three months at the University of Zagreb, which is ample time for reciprocal training for all researchers and students involved. Moreover, Mr. Sosa is part of the Minority Science Program at UC Irvine, and thus, adds to the diversity of the personnel included in the project. This study will produce data for at least two manuscripts to be submitted to international scientific journals: Part 1 (Diet, gut structure and gut function of lizards from the two islets) will be submitted to *Functional Ecology*, and Part 2 (Animal performance and fitness) will be submitted to *Journal of Experimental Biology*.

The PI has an active outreach program with Valencia High School (with a 78% minority population) in Placentia, CA, and is engaged with other diversity programs in the community (see support letter from Dr. Luis Mota-Bravo). Dr. Tadić has established connections at two local schools in Zagreb, Croatia (the 4th and 5th Gymnasiums, which are equivalent to high schools in the US; see support letters from the principals of both schools). During the research period (Summer 2013), the US scientists (German, Wehrle, and Sosa), along with Dr. Herrel and Dr. Tadić, will travel to the schools to engage the students in discussions about our research program and the importance of the STEM disciplines in society. Moreover, students from both schools will be invited to Dr. Tadić's laboratory at University of Zagreb to observe how our research is performed. Thus, we have outreach in the US and Croatia. Finally, Dr. Herrel has applied for funding through the European Research Council to examine the genetic underpinnings of the cecal valves. That research and funding will complement the current study.

Schedule of activities

August 2013: German, Wehrle, and Sosa travel to Zagreb, Croatia.

Weeks 1-2: With Dr. Tadić and his students, prepare laboratory for experiments (including reagents).

Weeks 3-4: Collect lizards on Pod Mrčaru and Pod Kopašće with collaborators. Prepare tissues.

September-November 2013: Laboratory phase.

Weeks 5-10: Conduct all feeding and metabolic rate trials at University of Zagreb. Measure activity levels of digestive enzymes, and concentrations of SCFA in lizard guts. Send guts and lizard tissues to US for microbial community and stable isotopic analyses, respectively. Begin data analysis and drafting of manuscripts.

Weeks 11-12: Complete all analyses on live animals. Tear down laboratory and return to US.

November 2013: Continue with data analysis and drafting of manuscripts for publication.

December 2013: Submit the first manuscript (Diet, gut structure and function) for publication.

January 2014: With foundational data from this project, submit a preproposal to NSF Division of Integrative Organismal Systems for a larger follow-on grant to further understand the evolution of digestive specialization in *Podarcis sicula*. Submit manuscript on animal performance for publication.

Investigator roles (all will participate in animal collection and writing of manuscripts)

Dr. German: Dissection, tissue processing, enzyme assays, gas chromatography, histology, TEM, SIA.

Dr. Herrel: Animal husbandry, metabolic rate measurements, digestibility analyses, enzyme assays.

Dr. Tadić: Animal husbandry, metabolic rate measurements, digestibility analyses, enzyme assays.

Mr. Wehrle: Dissection, tissue processing, enzyme assays, molecular techniques, microbial diversity.

Mr. Sosa: Dissection, tissue processing, enzyme assays, digestibility analyses, histology.

References Cited

1. Karasov, W.H. and C. Martínez del Rio. 2007 *Physiological ecology: how animals process energy, nutrients, and toxins* Princeton, NJ USA: Princeton University Press. 741.
2. Clements, K.D. and D. Raubenheimer. 2006. Feeding and nutrition, in *The physiology of fishes*, D.H. Evans, Editor CRC Press: Boca Raton, FL. p. 47-82.
3. Tracy, C.R., et al. 2006. The importance of physiological ecology in conservation biology. *Int Comp Biol.* 46: 1191-1205.
4. Vega Parry, H., et al. 2009. Nutritional performance of *Tupinambis meriana* lizards fed with corn starch as source of energy. *Acta Herpetol.* 4: 29-36.
5. Vidal, M.A. and P. Sabat. 2010. Stable isotopes document mainland–island divergence in resource use without concomitant physiological changes in the lizard *Liolaemus pictus*. *Comp Biochem Physiol B.* 156: 61-67.
6. German, D.P. and R.D. Miles. 2010. Stable carbon and nitrogen incorporation in blood and fin tissue of the catfish *Pterygoplichthys disjunctivus* (Siluriformes, Loricariidae). *Environ Biol Fish.* 89: 117-133.
7. German, D.P. 2009. Inside the guts of wood-eating catfishes: can they digest wood? *J Comp Physiol B.* 179: 1011-1023.
8. Lujan, N.K., D.P. German, and K.O. Winemiller. 2011. Do wood grazing fishes partition their niche? Morphological and isotopic evidence for trophic segregation in Neotropical Loricariidae. *Funct Ecol.* 25: 1327-1338.
9. Ferraris, R.P. and J. Diamond. 1989. Specific regulation of intestinal nutrient transporters by their dietary substrates. *Ann Rev Physiol.* 51: 125-141.
10. Karasov, W.H. and J. Diamond. 1983. Adaptive regulation of sugar and amino acid transport by vertebrate intestine. *Am J Physiol.* 245: G443-G462.
11. Pyke, G., H. Pulliam, and E. Charnov. 1977. Optimal foraging: a selective review of theory and tests. *Quart Rev Biol.* 52: 137-154.
12. Weibel, E., C. Taylor, and H. Hoppeler. 1991. The concept of symmorphosis: A testable hypothesis of structure-function relationship. *PNAS.* 88: 10357-10361.
13. Cant, J.P., B.W. McBride, and W.J. Croom. 1996. The regulation of intestinal metabolism and its impact on whole animal energetics. *J Anim Sci.* 74: 2541-2553.
14. Karasov, W.H., C. Martínez del Rio, and E. Caviedes-Vidal. 2011. Ecological physiology of diet and digestive systems. *Ann Rev Physiol.* 73: 69-93.
15. Buddington, R.K., J.W. Chen, and J. Diamond. 1987. Genetic and phenotypic adaptation of intestinal nutrient transport to diet in fish. *J Physiol.* 393: 261-281.
16. German, D.P., M.H. Horn, and A. Gawlicka. 2004. Digestive enzyme activities in herbivorous and carnivorous pricklyback fishes (Teleostei: Stichaeidae): ontogenetic, dietary, and phylogenetic effects. *Physiol Biochem Zool.* 77: 789-804.
17. German, D.P., et al. 2010. Evolution of herbivory in a carnivorous clade of minnows (Teleostei: Cyprinidae): effects on gut size and digestive physiology. *Physiol Biochem Zool.* 83: 1-18.
18. Kohl, K.D., et al. 2011. Pancreatic and intestinal carbohydrases are matched to dietary starch level in wild passerine birds. *Physiol Biochem Zool.* 84: 195-203.
19. Schondube, J., L. Herrera, and C. Martinez Del Rio. 2001. Diet and the evolution of digestion and renal function in phyllostomid bats. *Zool.* 104: 59-73.
20. Herrel, A., et al. 2008. Rapid large-scale evolutionary divergence in morphology and performance associated with exploitation of a different dietary resource. *PNAS.* 105: 4792-4795.
21. Nevo, E., et al. 1972. Competitive exclusion between insular *Lacerta* species (Sauria, Lacertidae). *Oecologia.* 10: 183-190.
22. Bjorndal, K. 1997. Fermentation in reptiles and amphibians, in *Gastrointestinal Microbiology, volume 1: Gastrointestinal ecosystems and fermentations*, R. Mackie and B. White, Editors. Chapman and Hall: New York, NY. p. 199-230.

23. O'Grady, S.P. and M.D. Dearing. 2006. Isotopic Insight into host-endosymbiont relationships in liolaemid lizards. *Oecologia*. 150: 355-361.
24. Pryor, G.S. and K.A. Bjorndal. 2005. Effects of the nematode *Gyrinicola batrachiensis* on development, gut morphology, and fermentation in bullfrog tadpoles (*Rana catesbeiana*): a novel mutualism. *J Exp Zool*. 303A: 704-712.
25. Troyer, K. 1984. Structure and function of the digestive tract of a herbivorous lizard *Iguana iguana*. *Physiol Zool*. 57: 1-8.
26. Hong, P.Y., et al. 2011. Phylogenetic analysis of the fecal microbial community in herbivorous land and marine iguanas of the Galapagos Islands using 16S rRNA-based pyrosequencing. *ISME J*. 5: 1461-1470.
27. Vervust, B., et al. 2010. Anatomical and physiological changes associated with a recent dietary shift in the lizard *Podarcis sicula*. *Physiol Biochem Zool*. 83: 632-642.
28. Herrel, A., B. Vanhooydonck, and R. Van Damme. 2004. Omnivory in lacertid lizards: adaptive evolution or constraint? *J Evol Biol*. 17: 974-984.
29. O'Grady, S.P., et al. 2005. Correlating diet and digestive tract specialization: Examples from the lizard family Liolaemidae. *Zool*. 108: 201-210.
30. McDowell, E. and B. Trump. 1976. Histological fixatives for diagnostic light and electron microscopy. *Arch Pathol Lab Med*. 100: 405-414.
31. German, D.P., et al. 2010. Feast to famine: the effects of dietary quality and quantity on the gut structure and function of a detritivorous catfish (Teleostei: Loricariidae). *Comp Biochem Physiol A*. 155: 281-293.
32. Karasov, W.H. and I.D. Hume. 1997. Vertebrate Gastrointestinal System, in *Handbook of Comparative Physiology*, W.H. Dantzler, Editor American Physiological Society: Bethesda, MD, USA. p. 407-480.
33. DeNiro, M.J. and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta*. 42: 495-506.
34. DeNiro, M.J. and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim Cosmochim Acta*. 45: 341-351.
35. Fry, B. 2007 *Stable Isotope Ecology* New York, NY: Springer. 308.
36. Warne, R.W., C.A. Gilman, and B.O. Wolf. 2010. Tissue-carbon incorporation rates in lizards: implications for ecological studies using stable isotopes in terrestrial ectotherms. *Physiol Biochem Zool*. 83: 608-617.
37. Vonk, H.J. and J.R.H. Western. 1984 *Comparative Biochemistry and Physiology of Enzymatic Digestion* London: Academic Press. 501.
38. Stevens, C.E. and I.D. Hume. 1995 *Comparative physiology of the vertebrate digestive system*. Melbourne, Australia: Press Syndicate of the University of Cambridge. 400.
39. Iglesias, S., et al. 2009. Seasonal effects on intestinal enzyme activity in the Australian agamid lizard, *Lophognathus temporalis*. *Comp Biochem Physiol B*. 153: 89-94.
40. Aminetzach, Y.T., et al. 2009. Convergent evolution of novel protein function in shrew and lizard venom. *Curr Biol*. 19: 1925-1931.
41. Cox, C.L. and S.M. Secor. 2008. Matched regulation of gastrointestinal performance in the Burmese python, *Python molurus*. *J Exp Biol*. 211: 1131-1140.
42. McBee, R.H. and V.H. McBee. 1982. The hindgut fermentation in the green iguana, *Iguana iguana*, in *Iguanas of the world*, G.M. Burghardt and A.S. Rand, Editors. Noyes Publications: Park Ridge, NJ. p. 77-83.
43. Nagy, K.A. 1977. Cellulose digestion and nutrient assimilation in *Sauromalus obesus*, a plant-eating lizard. *Copeia*. 1977: 355-362.
44. German, D.P. and R.A. Bittong. 2009. Digestive enzyme activities and gastrointestinal fermentation in wood-eating catfishes. *J Comp Physiol B*. 179: 1025-1042.
45. Marsh, R.S., et al. 2001. Characterization of gastrointestinal chitinase in the lizard *Sceloporus undulatus garmani* (Reptilia: Phrynosomatidae). *Comp Biochem Physiol B*. 128: 675-682.

46. Bergman, E. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol Rev.* 70: 567-590.
47. Foley, W.J., et al. 1992. Microbial digestion in the herbivorous lizard *Uromastix aegyptius* (Agamidae). *J Zool.* 226: 387-398.
48. Stevens, C.E. and I.D. Hume. 1998. Contributions of Microbes in Vertebrate Gastrointestinal Tract to Production and Conservation of Nutrients. *Physiol Rev.* 78: 393-427.
49. Van Soest, P. 1994 *Nutritional Ecology of the Ruminant* Ithica, NY: Cornell University Press. 476.
50. Pryor, G.S. and K. Bjorndal. 2005. Symbiotic fermentation, digesta passage, and gastrointestinal morphology in bullfrog tadpoles (*Rana catesbeiana*). *Physiol Biochem Zool.* 78: 201-215.
51. Pryor, G.S., D.P. German, and K. Bjorndal. 2006. Gastrointestinal fermentation in greater sirens (*Siren lacertina*). *J Herpetol.* 40: 112-117.
52. Lankau, E.W., P.Y. Hong, and R.I. Mackie. 2012. Ecological drift and local exposures drive enteric bacterial community differences within species of Galápagos iguanas. *Mol Ecol.* 21: 1779-1788.
53. Semova, I., et al. 2012. Microbiota regulate intestinal absorption and metabolism of fatty acids in the zebrafish. *Cell Host & Microb.* 12: 277-288.
54. Sullam, K.E., et al. 2012. Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. *Mol Ecol.* 21: 3363-3378.
55. Ley, R.E., et al. 2008. Evolution of mammals and their gut microbes. *Science.* 320: 1647-1651.
56. Costello, E.K., et al. 2010. Postprandial remodeling of the gut microbiota in Burmese pythons. *ISME J.* 4: 1375-1385.
57. Middelbos, I.S., et al. 2010. Phylogenetic characterization of fecal microbial communities of dogs fed diets with or without supplemental dietary fiber using 454 pyrosequencing. *PLoS ONE.* 5: e9768.
58. Pafilis, P., et al. 2007. Digestive performance in five Mediterranean lizard species: effects of temperature and insularity. *J Comp Physiol B.* 177: 49-60.
59. Nussear, K.E., et al. 1998. Diet quality does not affect resting metabolic rate or body temperatures selected by an herbivorous lizard. *J Comp Physiol B.* 168: 183-189.
60. Horn, M.H., et al. 1985. Assimilation of carbon from dietary and nondietary macroalgae by a temperate-zone intertidal fish, *Cebidichthys violaceus* (Girard) (Teleostei: Stichaeidae). *J Expl Mar Biol Ecol.* 86: 241-253.
61. Bjorndal, K. 1985. Use of ash as an indigestible dietary marker. *Bull Mar Sci.* 36: 224-230.
62. Galetto, M.J. and D.R. Bellwood. 1994. Digestion of algae by *Stegastes nigricans* and *Amphiprion akindynos* (Pisces: Pomacentridae) with an evaluation of methods used in digestibility studies. *J Fish Biol.* 44: 415-428.
63. German, D.P. 2011. Digestive Efficiency, in *Encyclopedia of Fish Physiology: From Genome to Environment*, A.P. Farrel, Editor Academic Press: San Diego. p. 1596-1607.
64. Goering, H.K. and P. Van Soest. 1970 *Forage fiber analyses (apparatus reagents, procedures and some applications)*. Vol. Number 379. Washington, D.C.: United States Department of Agriculture.
65. Huyghe, K., et al. 2010. Effects of testosterone on morphology, performance and muscle mass in a lizard. *J Exp Zool A.* 313A: 9-16.
66. McNab, B.K. 2002 *The physiological ecology of vertebrates* Ithica, N.Y.: Cornell University Press.
67. Gillooly, J.F., et al. 2001. Effects of size and temperature on metabolic rate. *Science.* 293: 2248-2251.
68. Secor, S.M., J.A. Wooten, and C.L. Cox. 2007. Effects of meal size, meal type, and body temperature on the specific dynamic action of anurans. *J Comp Physiol B.* 177: 165-182.
69. Secor, S.M. 2009. Specific dynamic action: a review of the postprandial metabolic response. *J Comp Physiol B.* 179: 1-56.

70. Secor, S.M. 1995. Digestive response to the first meal in hatchling Burmese pythons (*Python molurus*). *Copeia*. 1995: 947-954.
71. Clarke, B.C. and S.W. Nicolson. 1994. Water, energy, and electrolyte balance in captive Namib sand-dune lizards (*Angolosaurus skoogi*). *Copeia*. 1994: 962-974.
72. Secor, S.M. and J. Diamond. 1997. Effects of meal size on postprandial responses in juvenile Burmese pythons (*Python molurus*). *Am J Physiol*. 272: R902-R912.
73. Secor, S.M. and J. Diamond. 1997. Determinants of the postfeeding metabolic response of Burmese pythons, *Python molurus*. *Physiol Zool*. 70: 202-212.
74. Secor, S.M., E.D. Stein, and J. Diamond. 1994. Rapid upregulation of snake intestine in response to feeding: a new model of intestinal adaptation. *Am J Physiol*. 266: G695-G705.
75. Fu, S.J., et al. 2009. The behavioural, digestive and metabolic characteristics of fishes with different foraging strategies. *J Exp Biol*. 212: 2296-2302.
76. Hicks, J.W. and A.F. Bennett. 2004. Eat and run: prioritization of oxygen delivery during elevated metabolic states. *Resp Physiol Neurobiol*. 144: 215-224.
77. Vleck, D. 1987. Measurement of O₂ consumption, CO₂ production, and water vapor production in a closed system. *J App Physiol*. 62: 2103-2106.
78. Vanhooydonck, B., R. Van Damme, and P. Aerts. 2001. Speed and stamina trade-off in lacertid lizards. *Evol*. 55: 1040-1048.
79. Lisičić, D., et al. 2012. Effect of competition on habitat utilization in two temperate climate gecko species. *Ecol Res*. 27: 551-560.

Donovan P. German

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PROFESSIONAL PREPARATION

University of San Diego	Marine Science	B.A.	1999
California State Univ., Fullerton	Biology	M.S.	2003
University of Florida	Zoology	Ph.D.	2008
University of California, Irvine	Ecology & Evolutionary Biology	Postdoc	2009-2011

APPOINTMENTS

- 2009-2010 **Postdoctoral scholar**, Department of Ecology & Evolutionary Biology, University of California, Irvine
- 2010-2011 **University of California President's Postdoctoral Fellow**, Department of Ecology & Evolutionary Biology, University of California, Irvine
- 2011-present **Assistant Professor**, Department of Ecology & Evolutionary Biology, University of California, Irvine

PUBLICATIONS

Five publications most closely related to the proposed project

1. **German, D.P.**, D.T. Neuberger, M.N. Callahan, N.R. Lizardo, and D.H. Evans (2010) Feast to famine: the effects of food quality and quantity on the gut structure and function of a detritivorous catfish (Teleostei: Loricariidae). *Comparative Biochemistry and Physiology A* 155: 281-293
2. **German, D.P.**, B.C. Nagle, J.M. Villeda, A.M. Ruiz, A.W. Thomson, S. Contreras-Balderas, and D.H. Evans (2010) Evolution of herbivory in a carnivorous clade of minnows (Teleostei: Cyprinidae): effects on gut size and digestive physiology. *Physiological and Biochemical Zoology* 83: 1-18
3. **German, D.P.** (2009) Inside the guts of wood-eating catfishes: can they digest wood? *Journal of Comparative Physiology B* 179: 1011-1023
4. **German, D.P.**, and R.A. Bittong (2009) Digestive enzyme activities and gastrointestinal fermentation in wood-eating catfishes. *Journal of Comparative Physiology B* 179: 1025-1042).
5. **German, D.P.**, M.H. Horn and A. Gawlicka (2004). Digestive enzyme activities in herbivorous and carnivorous pricklyback fishes (Teleostei: Stichaeidae): ontogenetic, dietary, and phylogenetic effects. *Physiological and Biochemical Zoology* 77: 789-804

Other significant publications

1. **German, D.P.**, K.R.B. Marcelo, M.M. Stone, and S.D. Allison. (2012) The Michaelis-Menten kinetics of soil extracellular enzymes in response to temperature: a cross-latitudinal study. *Global Change Biology* 18: 1468-1479
2. **German, D.P.**, S. Chacon, and S.D. Allison. (2011) Substrate concentration and enzyme allocation can affect rates of microbial decomposition. *Ecology* 92: 1471-1480

3. Lujan, N.K., **D.P. German**, and K.O. Winemiller. (2011) Do wood grazing fishes partition their niche? Morphological and isotopic evidence for trophic segregation in Neotropical Loricariidae. *Functional Ecology* 25: 1327-1338
4. **German, D.P.**, and R.D. Miles (2010) Stable carbon and nitrogen incorporation in blood and fin tissue of the catfish *Pterygoplichthys disjunctivus* (Siluriformes, Loricariidae). *Environmental Biology of Fishes* 89: 117-133
5. **German, D.P.** (2009) Do herbivorous minnows have "plug-flow reactor" guts? Evidence from digestive enzyme activities, gastrointestinal fermentation, and luminal nutrient concentrations. *Journal of Comparative Physiology B* 179: 759-771

SYNERGISTIC ACTIVITIES

K-12 Outreach: As a NSF GK-12 Fellow (University of Florida, 2004-2006), the PI engaged under-resourced middle school students in inquiry-based science activities. The PI also involved two middle school students in his research as a graduate student, propelling both students into science majors when they entered community college. The PI has a newly established outreach program between his laboratory at UC Irvine and Valencia High School (Placentia, CA), which is a high school with a large population (78%) of underrepresented groups (see collaboration letter from Valencia High School). This will be further enhanced through interactions with UCI-FOCUS and NSF-NOYCE, which brings middle and high school teachers to UCI for interactions with UCI scientists (see letter of collaboration from Debra Mauzy-Meltiz).

Mentoring of Minorities in Science: With support from the UCI Biological Sciences Outreach, Research Training and Minority Science Program, the PI has advised two minority undergraduate researchers since 2009 (see collaboration letter from Luís Mota-Bravo), one as a postdoctoral fellow (Stephany Chacon, who became a co-author on an article published in the journal *Ecology*), and one who is currently working in the PI's laboratory (Abraham Sosa). The PI also has a minority postdoctoral researcher, who is a UC President's Postdoctoral Fellow.

COLLABORATORS & OTHER AFFILIATIONS

Collaborators

Kendall Clements	University of Auckland, New Zealand
Andres Lopez	University of Alaska, Fairbanks
Nathan Lujan	University of Toronto
Ian Tibbetts	University of Queensland, Australia
Anthony Herrel	Muséum National d'Histoire Naturelle, Paris, France
Zoran Tadić	University of Zagreb, Croatia

Graduate Advisors

Michael H. Horn	California State University, Fullerton
David H. Evans	University of Florida

Postdoctoral Mentor

Steven D. Allison	University of California, Irvine
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Graduate students and postdocs

Kwasi Connor	UC President's Postdoctoral Fellow (2012-present)
Beck Wehrle	PhD student (2012-present)

Biographical sketch

Anthony Herrel

Address : UMR 7179 C.N.R.S/M.N.H.N., Département d'Ecologie et de Gestion de la Biodiversité, 57 rue Cuvier, Case postale 55, 75231, Paris Cedex 5, France; e-mail: anthony.herrel@mnhn.fr; phone: ++33-140798120 - fax: ++33-140793773.

a. professional preparation

University of Antwerp, Master (Zoology), 1992

University of Antwerp, Ph.D. (Sciences), 1998

University of Antwerp, postdoctoral research FWO-Vlaanderen (advisor: Frits De Vree), 1998-2007

Northern Arizona University, postdoctoral research (advisor: Kiisa Nishikawa), 02/1999-02/2000 (FWO-VI mobility grant)

Tulane University, postdoctoral research (advisor: Duncan Irschick), 09/2001-09/2002 (FWO-VI mobility grant)

Harvard University, postdoctoral research (advisor: Jonathan Losos), 2008

Habilitation à Diriger des Recherches (HDR): Université Paris-Sud 11, Orsay, 23/02/2011.

b. appointments

01/2009-present, Permanent researcher, CNRS, France (CR1).

07/2009 - present, Associate of the Museum of Comparative Zoology, Harvard University.

02/2008 - present, Research associate, University of Antwerp.

c. publications

(a) 5 publications most closely related to this project

Verwajen, D., R. Van Damme and **A. Herrel** (2002) Relationships between head size, bite force, prey handling efficiency and diet in two sympatric lacertid lizards. *Funct Ecol.* 16: 842-850.

Herrel, A., K. Huyghe, B. Vanhooydonck, T. Backeljau, K. Breugelmans, I. Grbac, R. Van Damme and D.J. Irschick (2008) Rapid large scale evolutionary divergence in morphology and performance associated with exploitation of a different dietary resource. *Proc. Natl. Acad. Sci.* 105: 4792-4795.

Herrel, A., G.J. Measey, B. Vanhooydonck and K.A. Tolley (2011) Functional consequences of morphological differentiation between populations of the Cape Dwarf Chameleon (*Bradypodion pumilum*). *Biol. J. Linn. Soc.* 104: 692-700.

Mallarino R., P.R. Grant, B.R. Grant, **A. Herrel**, W. Kuo and A. Abzhanov (2011) Two developmental modules establish 3-D beak shape variation in Darwin's finches. *Proc. Natl. Acad. Sci. USA.* 108: 4057-4062.

Cornette, R., **A. Herrel**, J-F. Cosson, F. Poitevin and M. Baylac (in press) Rapid morpho-functional changes among insular populations of the greater white-toothed shrew. *Biol. J. Linn. Soc.*

(b) 5 other significant publications

Kohlsdorf, T., M.B. Grizante, C.A. Navas and **A. Herrel** (2008) Head shape evolution in Tropicodurinae lizards: does locomotion constrain diet? *J. Evol. Biol.* 21: 781-790.

Herrel, A., S.E. Vincent, M.E. Alfaro, S. Van Wassenbergh, B. Vanhooydonck and D.J. Irschick (2008) Morphological convergence as a consequence of extreme functional demands: examples from the feeding system of natricine snakes. *J. Evol. Biol.* 21: 1438-1448.

Van Wassenbergh, S., J. Brecko, P. Aerts, I. Stouten, G. Vanheusden, A. Camps, R. Van Damme and **A. Herrel** (2010) Hydrodynamic constraints on prey-capture performance in forward-striking snakes. *J. R. Soc. Interface* 7: 773-785.

Herrel, A. and C. Bonneaud (2012) Trade-offs between burst performance and maximal exertion capacity in a wild amphibian, *Xenopus tropicalis*. *J. Exp. Biol.* 215: 3106-3111.

Sustaita, D., E. Pouydebat, A. Manzano, V. Abdala, F. Hertel and **A. Herrel** (in press) Getting a grip on tetrapod grasping: form, function, and evolution. *Biol. Rev.*

d. Synergistic activities

1. dissemination of research to public, including articles in *Andira*, *Celso*, *Jara y Sadal*; collaboration with natural history programs (e.g. *BBC*, *Discovery Chanel*); collaboration with Pearson Educational Measurement to develop student assessment tests. My research has been covered in American and international magazines (e.g. *Discovery*, *Geo*, *Highlights for Children*, *Quark*, *National Geographic*, *Nature Australia*, *New Scientist*, *Sciences et Avenir*, *Science et Vie*, *Scientific American*, *Yes magazine*, *Wild magazine*) and newspapers (e.g. *De Morgen*, *De Standaard*, *Der Standard*, *New York Times*).

2. undergraduate and graduate training including 19 undergraduates several of which are currently in graduate school and one is currently a postdoctoral researcher at the FWO-Vlaanderen.

3. Associate editor for *Functional Ecology*, *The Herpetological Journal*; Editorial board member for the *Journal of Zoology*.

e. Collaborators and other affiliations

(1) graduate students (total 4)

Sam Van Wassenbergh (University of Antwerp), Shawn Vincent (Tulane University), Vicky Schaerlaeken (University of Antwerp), Raphael Cornette (Museum National d'Histoire Naturelle).

(2) graduate and postdoctoral advisors

Frits De Vree (University of Antwerp), Peter Aerts (University of Antwerp), Kiisa Nishikawa (Northern Arizona University), Duncan Irschick (University of Massachusetts, Amherst), Jonathan Losos (Harvard University).

(3) other collaborators (recent – last 48 months only)

Virginia Abdala (Instituto Miguel Lillo, Tucuman), Arhat Abzhanov (Harvard University); Dominique Adriaens (Ghent University), Peter Aerts (University of Antwerp), Denis Andrade (Universidade Estadual Sao Paulo), Vincent Bels (MNHN); Renaud Boistel (Université de Poitiers); Michel Baguette (MNHN, Paris), Camille Bonneaud and Jean Clobert (Station d'Ecologie Experimentale du CNRS à Moulis), Otger Campas (Harvard University); Aurora Castilla (CSIC, Spain); Felix Cruz (Universidad Nacional del Comahue, Bariloche), Gheyleen Daghfous (Université de Montreal); Joris Dirckx (University of Antwerp), Betsy Dumont (University of Massachusetts, Amherst), Donovan P. German (University of California, Irvine), Andrew Hendry (McGill University); Katleen Huyghe (University of Antwerp), Nate Kley (Stony Brook University), Tiana Kohlsdorf (Universidade de Sao Paulo – Ribeira Preto), Nicolai Konow (Brown University), Marta Lopez-Darias (CSIC, Spain); Luke Mahler (Harvard University); Ricardo Mallarino (Harvard University); Adriana Manzano (CONICET-UADER, Entre Rios), John Measey (SANBI, Cape Town), Carlos Navas (Universidade de Sao Paulo), Nicola Nelson (Victoria University, Wellington), Jeff Podos (University of Massachusetts, Amherst), Zbynek Rocek (Charles University, Prague), Callum Ross (University of Chicago), Zoran Tadic (University of Zagreb), Omar Torres (Pontificia Universidad Catolica de Quito); Krystal Tolley (SANBI, Cape Town), Arie van der Meijden (CIBIO, Porto); Sam Van Wassenbergh (University of Antwerp); Bieke Vanhooydonck (University of Antwerp).

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PROFESSIONAL PREPARATION

University of Zagreb	Biothecnology	B.Sc. 1986
University of Zagreb	Biotechnology	Ph.D. 1992

APPOINTMENTS

1992-1993 **Postdoctoral associate**, Department of Physiology & Biophysics, Indiana University School of Medicine, Indiana University, Indianapolis, IN, USA

1993-1996 **Postdoctoral fellow**, Department of Animal Physiology, Faculty of Science, University of Zagreb, Zagreb, Croatia

1996-1999 **Senior teaching & research assistant**, Department of Animal Physiology, Faculty of Science, University of Zagreb, Zagreb, Croatia

1999-present **Assistant Professor**, Department of Animal Physiology, Faculty of Science, University of Zagreb, Zagreb, Croatia

PUBLICATIONS

Five publications most closely related to the proposed project

1. Huyghe K., Vanhooydonck B., Herrel A., **Tadić Z.**, Van Damme R. (2007) Morphology, performance, behavior and ecology of three color morphs in males of the lizard *Podarcis melisellensis*. *Integr. Comp. Biol.* **47**: 211 - 220
2. Huyghe K., Husak J. F., Herrel A., **Tadić Z.**, Moore I. T., Van Damme R., Vanhooydonck B. (2009) Relationships between hormones, physiological performance and immunocompetence in a color-polymorphic species, *Podarcis melisellensis*. *Horm. Behav.* **55**: 488 –
3. Strunjak-Perović I., Lisičić D., Čož-Rakovac R., Topić-Popović N., Jadan M., Benković V., **Tadić Z.** (2010) Evaluation of micronucleus and erythrocytic nuclear abnormalities in Balkan whip snake, *Hierophis gemonensis*. *Ecotoxicology* **19**: 1460 – 1465
4. Čož-Rakovac R., Lisičić D., Šmuc T., Topić Popović N., Strunjak-Perović I., Jadan M., **Tadić Z.**, Jug Dujaković J. (2011) Classification modelling of physiological stages in captive Balkan whip snakes using blood biochemistry parameters. *J. Herpetol.* **45**: 525 – 529
5. Lisičić D., Drakulić S., Herrel A., Đikić D., Benković V. **Tadić Z.** (2012) Effect of competition on habitat utilization in two temperate climate gecko species. *Ecol. Res.* **27**: 551 - 560

Other significant publications

1. Heulin B., Guillaume C. P., Vogrin N., Surget - Groba Y., **Tadić Z.** (2000) Further evidence of the existence of oviparous populations of *Lacerta (Zootoca) vivipara* in the NW of the Balkan Peninsula. *C. R. Biol.* **323**: 461 - 468

- Huyghe K., Breugelmans K., Small M., **Tadić Z.**, Van Damme R., Vanhooydonck B., Backeljau T. (2009) Characterization of polymorphic microsatellite markers in the Dalmatian wall lizard *Podarcis melisellensis* (Squamata: Lacertidae). *Mol. Ecol. Resources* **9**: 299 - 301
- Huyghe K., Herrel A., Adriaens D., **Tadić Z.**, Van Damme R. (2009) It is all in the head: Morphological basis for differences in bite force among colour morphs of the Dalmatian wall lizard. *Biol. J. Linn. Soc.* **96**: 13 – 22
- Huyghe K., Van Oystaeyen A., Pasmans F., **Tadić Z.**, Vanhooydonck B., Van Damme R. (2010) Seasonal changes in parasite load and a cellular immune response in a colour polymorphic lizard. *Oecologia* **163**: 867 – 874
- Herrel A., Huyghe K., Oković P., Lisičić D., **Tadić Z.** (2011) Fast and furious: Effects of body size on strike performance in an arboreal viper *Trimeresurus (Cryptelytrops) albolabris*. *J. Exp. Zool. A* **315**: 22 – 29
- Huyghe K., Vanhooydonck B., Herrel A., **Tadić Z.**, Van Damme R. (2012) Female lizards ignore the sweet scent of success: male characteristics implicated in female mate preference. *Zoology* **115**: 217 - 222

SYNERGISTIC ACTIVITIES

Several popular talks in high schools and in public for the public understanding of science (in croatian):

- “What’s the use of these snakes anyway?”, 2001
- “Animal senses: How do animals perceive the world around them?”, 2001
- “The importance of being a snake”, 2004 (in english)
- “Why is snake necessary in every household?”, 2004
- “Disappear in fall, return in spring: The mechanisms of orientation in migrating birds”, 2005
- “Sex is joyfull, sex is fun, sex is thrilling one on one. . .but what for?: The biology and evolution of sex”, 2005
- “The introduction to the tasty morsels of anatomy, physiology, ecology and behavior of reptiles for complete idiots”, 2010
- “Why should gastroenterologists love snakes, cardiologists crocodiles, molecular biologists ecologists, ecologists physiologists, and physiologists animal behavior and physicians?: Multidisciplinary and integrative approach to the complex problems and challenges in biology and biomedicine.”, 2012

Appearing in the documentary series on animals filmed by the Croatian Broadcasting Company (HRT):

- “Birds & People” (appeared in the first two episodes (“The Pigeons”, “The Birds of Prey”), HRT, 2002
- “The Secret Life of Reptiles” (wrote the story and appeared in the both episodes: “Lizards” and “Snakes”), HRT, 2005

COLLABORATORS

Anthony Herrel	CNRS, France	anthony.herrel@mnhn.fr
Donovan P. German	University of California, Irvine	dgerman@uci.edu
Gordon Burghardt	University of Tennessee, Knoxville	gburghar@utk.edu
Anna Wilkinson	University of Lincoln, UK	awilkinson@lincoln.ac.uk
Daniel Simberloff (2002-09)	University of Tennessee, Knoxville	dsimberloff@utk.edu

GRADUATE STUDENTS

Ivna Tomašković	MSc student	2008-2002
Duje Lisičić	MSc student	2000-2006
Duje Lisičić	PhD student	2007-2013

UNDERGRADUATE STUDENTS AND SUPERVISIONS

Up till now, I have co-supervised 14 and supervised 46 undergraduate students.

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Ph.D. Student
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PROFESSIONAL PREPARATION

University of California, Davis	Biological Science	B.S.	2008
California State Univ., Northridge	Biology	M.S.	2013 (expected)
University of California, Irvine	Ecol. & Evol. Biol.	Ph.D.	2018 (expected)

PROFESSIONAL EXPERIENCE

2012 **Teaching Assistant:** Human Physiology Labs, Department of Ecology & Evolutionary Biology, University of California, Irvine

2009-2011 **Teaching Associate:** Human Anatomy Labs, Department of Biology, California State University, Northridge

2009, 2011 **Graduate Assistant:** Vertebrate Biology, Department of Biology, California State University, Northridge

2010 **Graduate Assistant:** Tropical Ecology (field course in Ecuador), Department of Biology, California State University, Northridge

2009 **Junior Specialist** (Post Graduate Research Assistant), Gail L. Patricelli Lab, Department of Evolution and Ecology, University of California, Davis

2008-2009 **Undergraduate Research Assistant**, Gail L. Patricelli Lab, Department of Evolution and Ecology, University of California, Davis

2006-2009 **Field Assistant** to Christopher A. Searcy, Ph.D. candidate, Department of Population Biology, University of California, Davis

2005-2009 **Student Assistant II**, H. Bradley Shaffer Lab, Department of Evolution and Ecology, University of California, Davis

2006-2008 **Field Assistant** to Maureen E. Ryan, Ph.D. candidate, Department of Evolution and Ecology, University of California, Davis

PUBLICATIONS

- 1.) **Wehrle, B. A.** (*in prep.*) Variation in the gut microbial communities of an herbivorous lizard, *Iguana iguana* (Green Iguana). M.S. thesis, California State University, Northridge
- 2.) **Wehrle, B. A.**, and J. A. Guzman (2012) *Iguana iguana* (Green Iguana) Predation. *Herpetological Review* 43: 134

SELECTED RELEVANT PROFESSIONAL PRESENTATIONS

Wehrle, B. A. and R.E. Espinoza. 2012. Why do lizards lounge? The role of sociality in exchanging microbial communities among hatchling Iguana iguana. World Congress of Herpetology 7, University of British Columbia, Vancouver, BC, Canada.

Wehrle, B. A. 2011. Eat poop and thrive: testing the role of the lizard lounge for transferring digestive microbes. Joint Meeting of Ichthyologists and Herpetologists, Minneapolis, MN.

Wehrle, B. A. 2011. Lizard herbivores, digestive microbes, and social aggregations. Capybara Seminar, Smithsonian Tropical Research Institute, Barro Colorado Island, Panamá

Wehrle, B. A. 2010. Iguanas and microbes. Capybara Seminar, Smithsonian Tropical Research Institute, Barro Colorado Island, Panamá

PROFESSIONAL SERVICE

- 2011- Current Research Section Editor, *Herpetological Review*, Society for the Study of Amphibians and Reptiles
- 2012 Judge, *West Contra Costa Unified School District Annual Science Fair*
- 2009-2012 Vice President, *Behavior, Ecology, and Evolution Research Club*, California State University, Northridge

MENTORING AND MINORITY OUTREACH

He worked for the Gay Lesbian Straight Education Network as a student educator for two years and has been a part of LGBT task forces at multiple campuses and was part of the *Strategies for Ecology Education, Development and Sustainability (SEEDS)* program and *Biology Undergraduate Scholars Program* as an undergraduate, both aimed at increasing minority involvement in science. In the Patricelli Lab at UC Davis, he oversaw/mentored a minority undergraduate researcher (Claudia Lopez). As an M.S. student, he mentored four field assistants (Lauren W. Steely, Navasha Singh, Max W. Dresow, and Jorge A. Guzman), three of whom belong to minority groups. Three of the field assistants have since been accepted to graduate programs in biology. As a Ph.D. student at UC Irvine, he is a mentor in the campus LGBT Mentor Program.

GRADUATE ADVISORS

Robert E. Espinoza California State University, Northridge
Donovan P. German University of California, Irvine

SUMMARY PROPOSAL BUDGET

YEAR 1

ORGANIZATION University of California-Irvine				FOR NSF USE ONLY			
				PROPOSAL NO.	DURATION (months)		
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR Donovan German				AWARD NO.	Proposed	Granted	
				A. SENIOR PERSONNEL: PI/PI, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)			
				CAL	ACAD	SUMR	
1.				0.00	0.00	0.00	
2.							
3.							
4.							
5.							
6.	(0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)			0.00	0.00	0.00	0
7.	(1) TOTAL SENIOR PERSONNEL (1 - 6)			0.00	0.00	0.00	0
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)							
1.	(0) POST DOCTORAL SCHOLARS			0.00	0.00	0.00	0
2.	(0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)			0.00	0.00	0.00	0
3.	(0) GRADUATE STUDENTS						0
4.	(0) UNDERGRADUATE STUDENTS						0
5.	(0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)						0
6.	(0) OTHER						0
TOTAL SALARIES AND WAGES (A + B)							0
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)							0
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)							0
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING \$5,000.)							
TOTAL EQUIPMENT							0
E. TRAVEL							0
1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)							0
2. FOREIGN							26,224
F. PARTICIPANT SUPPORT COSTS							
1.	STIPENDS \$ _____						0
2.	TRAVEL _____						0
3.	SUBSISTENCE _____						0
4.	OTHER _____						0
TOTAL NUMBER OF PARTICIPANTS (0)							
TOTAL PARTICIPANT COSTS							0
G. OTHER DIRECT COSTS							
1. MATERIALS AND SUPPLIES							11,000
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION							0
3. CONSULTANT SERVICES							0
4. COMPUTER SERVICES							0
5. SUBAWARDS							0
6. OTHER							0
TOTAL OTHER DIRECT COSTS							11,000
H. TOTAL DIRECT COSTS (A THROUGH G)							37,224
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) Modified total direct cost (Rate: 26.0000, Base: 37224)							
TOTAL INDIRECT COSTS (F&A)							9,678
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)							46,902
K. RESIDUAL FUNDS							0
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)							46,902
M. COST SHARING PROPOSED LEVEL \$ 0				AGREED LEVEL IF DIFFERENT \$			
PI/PI NAME Donovan German				FOR NSF USE ONLY			
ORG. REP. NAME*				INDIRECT COST RATE VERIFICATION			
		Date Checked		Date Of Rate Sheet		Initials - ORG	

SUMMARY PROPOSAL BUDGET Cumulative

ORGANIZATION University of California-Irvine				FOR NSF USE ONLY				
				PROPOSAL NO.	DURATION (months)			
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR Donovan German				AWARD NO.				
				Proposed	Granted			
A. SENIOR PERSONNEL: PI/PI, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)				NSF Funded Person-months				
				CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1.				0.00	0.00	0.00		
2.								
3.								
4.								
5.								
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)				0.00	0.00	0.00	0	
7. (0) TOTAL SENIOR PERSONNEL (1 - 6)				0.00	0.00	0.00	0	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)								
1. (0) POST DOCTORAL SCHOLARS				0.00	0.00	0.00	0	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)				0.00	0.00	0.00	0	
3. (0) GRADUATE STUDENTS							0	
4. (0) UNDERGRADUATE STUDENTS							0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)							0	
6. (0) OTHER							0	
TOTAL SALARIES AND WAGES (A + B)							0	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)							0	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)							0	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING \$5,000.)								
TOTAL EQUIPMENT							0	
E. TRAVEL							0	
1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)							0	
2. FOREIGN							26,224	
F. PARTICIPANT SUPPORT COSTS								
1. STIPENDS \$ _____				0				
2. TRAVEL _____				0				
3. SUBSISTENCE _____				0				
4. OTHER _____				0				
TOTAL NUMBER OF PARTICIPANTS (0)								
TOTAL PARTICIPANT COSTS							0	
G. OTHER DIRECT COSTS								
1. MATERIALS AND SUPPLIES							11,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION							0	
3. CONSULTANT SERVICES							0	
4. COMPUTER SERVICES							0	
5. SUBAWARDS							0	
6. OTHER							0	
TOTAL OTHER DIRECT COSTS							11,000	
H. TOTAL DIRECT COSTS (A THROUGH G)							37,224	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE)								
TOTAL INDIRECT COSTS (F&A)							9,678	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)							46,902	
K. RESIDUAL FUNDS							0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)							46,902	
M. COST SHARING PROPOSED LEVEL \$				0	AGREED LEVEL IF DIFFERENT \$			
PI/PI NAME Donovan German				FOR NSF USE ONLY				
ORG. REP. NAME*				INDIRECT COST RATE VERIFICATION				
		Date Checked		Date Of Rate Sheet		Initials - ORG		

C *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

CNIC: WHAT DOES IT TAKE TO BE AN HERBIVOROUS LIZARD? REVEALING THE DIGESTIVE ANATOMY AND PHYSIOLOGY OF MANIPULATED POPULATIONS OF *Podarcis sicula*

BUDGET JUSTIFICATION

Travel Costs

The travel costs are to allow the PI, his graduate student (Mr. Beck Wehrle), and an undergraduate assistant (Mr. Abraham Sosa) to travel to Zagreb, Croatia, for 90 days to engage in a collaborative research project with Dr. Anthony Herrel and Dr. Zoran Tadić. Croatia allows stays for United States citizens for up to 90 days with just a passport (no visa required). The PI and his students will travel together to Croatia in August 2013 and return to the US in November 2013. This length of time will be necessary to optimize the laboratory portion of experiments. We will need several weeks of preparation before we even head into the field to collect lizards, and once we have lizards in the laboratory, we will need time to optimize the conditions so that the lizards will be stable under our experimental design. Moreover, many of the tasks (enzyme activity, gas chromatography analyses) will be performed at University of Zagreb, allowing for reciprocal training among the collaborating groups.

The cost of roundtrip airfare is approximately \$1700 per person (using Delta Airlines, with a single stopover in Amsterdam). We will be taking a fair amount of equipment to the University of Zagreb for our experiments. Thus, excess baggage fees are added at \$100 per person, per direction. Thus, total airfare costs are estimated at \$5100 for the plane fare, plus \$600 in baggage fees, and a total of \$5700. Once in Croatia, the primary cost of staying in Zagreb is housing. There are no dormitories or on-campus housing for visiting scientists at University of Zagreb (see letter from Zoran Tadić). Thus, we will rent a two bedroom suite with a kitchenette at the Hotel Ilica in Zagreb, Croatia (<http://www.hotel-ilica.hr/>), which is just adjacent to the University of Zagreb. Because this suite (at \$129/night) will house all three travelers from the US, the cost per person per night is \$43. We will stay in this hotel for 76 nights (total cost is \$9804). During field collections, we will rent a flat on the island of Lastovo. This flat will again house all three US travelers at a cost of \$100 (\$33.33 per person per night; and \$1400 for the two weeks). The cost of food during our stay is \$30/day (\$8100 total for all three travelers for 90 days).

It will be necessary to travel to Lastovo from the Croatian town of Split by boat. Furthermore, all excursions to Pod Kopač and Pod Mrčaru will be by boat originating from the island of Lastovo. Dr. Tadić has made arrangements with a boat owner to use a 7.5 m rigid-hull inflatable boat (RHIB), equipped with a Mercury 350HP engine and propeller. The only costs associated with its use are fuel and incidental repair. We estimate that we will need two weeks to collect enough lizards from Pod Kopač and Pod Mrčaru with which to perform our study. We estimate that we will travel about 30 km per day on the water during this period. The boat engine burns 1 L of fuel per km, and the cost of fuel is currently about \$2.60 per L in Croatia. Thus, we estimate \$1100 for boat fuel, which is significantly cheaper (by nearly an order of magnitude) than renting a boat and hiring a driver. We will also incur the costs of driving the 250 mile trip from Zagreb to Split by car. Dr. Tadić has a vehicle that we will all share, but we will need to share the fuel costs, which we estimate at \$120 per direction (we will pay for one direction). Thus, with the boat and car fuel costs, we estimate \$1220.

Research Costs

We will need to purchase many supplies in Croatia for our work there. For field work, we will need to purchase PVC and mesh with which to build field terraria on Lastovo (estimated at \$200), as well as veterinary drugs (sodium pentobarbital; \$285) for animal euthanasia, and ethanol (\$300) for sterilization and preservation. For our laboratory work, we will need to purchase substrates, microplates, chemicals, and vials for enzyme assays (\$5000) and gas chromatography (\$1000) to be performed at the University of Zagreb. We will need to purchase terraria and heat lamps (\$1000). The PI will bring important pieces of equipment (e.g., liquid nitrogen dewar, tissue homogenizer, O₂ analyzer, flow meter) for the work, but it will be necessary to purchase liquid nitrogen (\$500), tubing (\$20), syringes (\$50), and

other consumables (\$500) for the work. We wish to add \$1500 for any incidental costs and unforeseen expenses of the research. We will also ship frozen (for determination of microbial diversity) and dried (for stable isotopic analyses) samples back to UC Irvine during our stay in Zagreb so that PI's postdoctoral researcher can get those samples prepped for analyses. Overnight shipping (which would include dry ice) from Zagreb to Irvine is approximately \$500 (including USDA import fees). Overall, we estimate that we will need approximately \$11,000 for supplies and services to properly perform the research.

The total requested amount (including research expenditures) is **\$37,224**, which is \$138 per person per day. The allowable per diem amount for Zagreb, Croatia, for our length of stay is \$288 per person per day for the first 30 days, and \$144 per person per day thereafter (http://aoprals.state.gov/web920/per_diem_action.asp?MenuHide=1&CountryCode=1358). At those rates, the maximal allowable per diem amount would be \$51,840. Thus, we are economical in our approach for a stay of this length, and given the amount of work to be performed. The Facilities and Administrative Costs for this type of grant with work performed off site from UC Irvine are 26% ($0.26 \times 37224 = \$9678.24$). Thus, the total requested amount for this proposal is **\$46902**.

Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.				
Investigator: Donovan P. German	Other agencies (including NSF) to which this proposal has been/will be submit-			
Support: <input checked="" type="checkbox"/> Current <input type="checkbox"/> Pending <input type="checkbox"/> Submission Planned in Near Future <input type="checkbox"/> *Transfer of Support				
Project/Proposal Title: University of California Laboratory Start-up Funds				
Source of Support: University of California				
Total Award Amount: \$475,000		Total Award Period Covered: 7/1/2011 - 6/30/2014		
Location of Project: UC Irvine				
Person-Months Per Year Committed to the Project.	0.0	Cal: 0.0	Acad: 0.0	Sumr: 2.0
Support: <input type="checkbox"/> Current <input checked="" type="checkbox"/> Pending <input type="checkbox"/> Submission Planned in Near Future <input type="checkbox"/> *Transfer of Support				
Project/Proposal Title: CNIC: What does it take to be an herbivorous lizard? Revealing the digestive anatomy and physiology of manipulated populations of <i>Podarcis sicula</i>				
Source of Support: NSF				
Total Award Amount: \$46,902		Total Award Period Covered: 8/1/2013 – 7/31/2014		
Location of Project: Croatia				
Person-Months Per Year Committed to the Project.	0.0	Cal: 0.0	Acad: 0.0	Sumr: 0.0
Support: <input type="checkbox"/> Current <input type="checkbox"/> Pending <input type="checkbox"/> Submission Planned in Near Future <input type="checkbox"/> *Transfer of Support				
Project/Proposal Title:				
Source of Support:				
Total Award Amount: \$		Total Award Period Covered:		
Location of Project:				
Person-Months Per Year Committed to the Project.		Cal:	Acad:	Sumr:
Support: <input type="checkbox"/> Current <input type="checkbox"/> Pending <input type="checkbox"/> Submission Planned in Near Future <input type="checkbox"/> *Transfer of Support				
Project/Proposal Title:				
Source of Support:				
Total Award Amount: \$		Total Award Period Covered:		
Location of Project:				
Person-Months Per Year Committed to the Project.		Cal:	Acad:	Sumr:
Support: <input type="checkbox"/> Current <input type="checkbox"/> Pending <input type="checkbox"/> Submission Planned in Near Future <input type="checkbox"/> *Transfer of Support				
Project/Proposal Title:				
Source of Support:				
Total Award Amount: \$		Total Award Period Covered:		
Location of Project:				
Person-Months Per Year Committed to the Project.		Cal:	Acad:	Sumr:

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.



Facilities, Equipment and Other Resources

Laboratory: Dr. Zoran Tadić has a 1100 square foot laboratory in which he has a large vivarium at University of Zagreb. The facility houses around 200 specimens of snakes. This facility is equipped with timed lighting (for controlling photoperiod), temperature controlled rooms, and hundreds of square feet of bench space on which to set up experiments (see support letter from Dr. Tadić). This will be where all feeding trials and metabolic rate measurements are carried out. The Department of Biology at University of Zagreb (UZ) has common use -80°C freezers, and several laboratories have microplate spectrophotometers for use in digestive enzyme activity assays. There is a gas chromatograph in the Medical School at UZ, which we will be able to use for measuring short chain fatty acid concentrations in the lizard's guts. Dr. German has extensive gas chromatography experience, so he will work closely with technicians in the UZ medical school to ensure that the machine is functioning properly. Stable isotope samples will be prepared and sent to the UC Davis Stable Isotope Facility for mass spectroscopy analysis. Histological analysis will be performed at UC Irvine using an Olympus BX60 bright-field light microscope equipped with a Hitachi KP-D50 digital camera. Ultrastructural analyses will be performed using a Phillips CM-10 transmission electron microscope in the medical school at UC Irvine. Pyrosequencing for gut microbial diversity will be performed at the UC Irvine Institute of Genomics and Bioinformatics.

At UC Irvine, Dr. German has a 1300 square foot laboratory equipped with microscopes, ovens, reagents, electrophoresis rigs, and PCR machine for molecular analyses.

Clinical: N/A

Animal: See above for "Laboratory". Dr. German will have IACUC approval from his institution (UCI) before any animals are collected or experiments performed.

Computer: Dr. German, Mr. Wehrle, and Mr. Sosa will each bring laptop computers for use in data collection. See data management plan for data storage.

Office: At UC Irvine, Dr. German has a 150 square foot office with a desktop and laptop computer for data analysis and writing of manuscripts. We will work in Dr. Tadić's laboratory and our hotel room while in Zagreb.

MAJOR EQUIPMENT

Coming from UCI to Croatia: Tissue homogenizer (Kinematica Polytron 10-35), O₂ analyzer (HIOXY-R fiberoptic O₂ analyzer, Ocean Optics, Inc.), CO₂ analyzer (EGM-4, PP Systems).

At University of Zagreb: Microplate spectrophotometer (BioTek Synergy H1), Gas chromatograph (Shimadzu GC-9AM).

OTHER RESOURCES

For all purchases, Dr. German will utilize his support staff at UC Irvine. There are dedicated personnel for all financial and ordering interests.

DATA MANAGEMENT PLAN

Types of Data

The proposed work will generate numerical data in the forms of O₂ and CO₂ concentrations (direct from instruments), enzyme activity levels (from spectrophotometry), short chain fatty acid (SCFA) concentrations (from gas chromatography), stable isotope signatures (from mass spectroscopy), micrograph images (TEM and light microscope), and DNA sequence data generated for microbial diversity.

Data Standards

All numerical data will be transferred to a spreadsheet for management. The O₂ and CO₂ concentration data will be transferred directly from the instruments to the recording computer. Enzyme activity data will start as raw absorbance or fluorescence values, which will be exported from the spectrophotometer software to a spreadsheet for use in activity calculations. The gas chromatography data will be gathered from retention times (indicated by area under specific curves) from the chromatographer software. Traces of flame ionization detector intensity per unit time will be saved as raw data, with area under the curve at specific retention times used to determine concentrations of different SCFAs. Mass spectroscopy data will be delivered as “delta” values (in a spreadsheet) directly from the laboratory that conducts the analyses. Gene sequence data will be analyzed using Quantitative Insights into Microbial Ecology (QIIME; <http://qiime.org/>). Finally, surface area measurements taken from TEM and light micrographs will be compiled in a spreadsheet as other forms of data described above. Thus, for most data types, we will retain raw and processed data, thereby allowing for the reprocessing of data in the event reanalysis is necessary. The lab has begun using a standardized format for all files that follows the scheme: *species_date_type of experiment_last name of primary researcher*. A sample file name might read: *PsMrc1_12-7-2012_amylase_German*.

Data Sharing Plan

All data will be made publicly available after publication. If the results are published in a journal that offers the opportunity to include electronic supplemental material (ESM), data tables relating to the manuscript will be made available as ESM. In fact many journals (e.g., *Functional Ecology*) now ask the authors to include raw data files as part of the final submission. If a journal does not have the option of the submitting the ESM, then the data will be made available through the PI's website (<http://german.bio.uci.edu>) and linked to a specific paper under the publications page. Similarly, tissue samples collected during the study will be made available to the scientific community by special request.

Plans for Archiving Data

The German lab utilizes the software program Crashplan (<http://www.crashplan.com/>), which is used for immediate backup of all files on lab computers (including laptops) to the Academic data center at the University of California, Irvine. This facility is available to all PIs and includes raised flooring, uninterrupted power supplies, and generator power backup. All data gathered in Croatia will also be backed up on three Lacie 1-Terabyte external hard drives per researcher, ensuring that data will remain in Croatia, but also will be safely transferred to France and the US. Extra tissue samples from histological and ultrastructural analyses will be kept in 70% ethanol, whereas dried tissue samples from stable isotopic analyses will be kept in a drying chamber (including drierite) at room temperature. Any remaining gut tissue homogenates and gut contents will be maintained in a -80°C freezer operating with a backup power supply.



Mécanismes Adaptatifs : des organismes aux Communautés
UMR 7179 C.N.R.S./M.N.H.N

Département d'Ecologie et de Gestion de la Biodiversité
57, rue Cuvier – Case postale 55 – 75231 Paris Cedex 5
anthony.herrel@mnhn.fr



Paris, 03-12-2012

To: Nancy Sung, Program Director
NSF Catalyzing New International Collaborations Program
4201 Wilson Boulevard
Arlington, VA 22230
USA

Re: Letter of support for the CNIC project of Dr. Donovan German

This is a letter of support for the NSF-CNIC proposal by Dr. Donovan German entitled 'What does it take to be an herbivorous lizard? Revealing the digestive anatomy and physiology of manipulated populations of *Podarcis sicula*'. This is a highly innovative and important project that will provide significant insights into the evolution of herbivory in lizards and other vertebrates. The experimental introduction of *P. sicula* on tiny islets in the S-Adriatic offers a unique window on evolutionary processes in action, and as such ties in micro-evolutionary processes and mechanisms with macro-evolutionary changes observed on geological time-scales. Although my previous work has documented that lizards changed diets and morphology on ecological time-scales (30 years), the mechanisms allowing these lizards to eat mostly plant matter remain undocumented. The present proposal addresses this issue by proposing to investigate the anatomical and physiological mechanisms underlying the digestion of plant matter under controlled laboratory conditions.

I am very excited to embark upon this novel collaboration with Dr. German to increase our understanding of the evolution of herbivory in lizards. This project has the potential to provide important insights and will provide base-line data needed to submit a subsequent collaborative grant to further explore this system. I have applied for a European ERC advanced researcher grant to investigate the genetic basis of the cecal valves in lizards from Pod Mrčaru and to quantify selection on performance traits. If successful this grant will provide an additional impetus and important co-financing for the current project. My family, nor I have any significant financial interests related to the work to be conducted as part of the above-referenced project. Moreover the CNRS has institutional laws in place to ensure full disclosure and no conflicts of interest.

I will provide my expertise and knowledge on the system and through my existing collaborations with Dr. Z. Tadić, can assure the feasibility of this project. In summary, I am very pleased to embark upon this new collaboration and am confident that this project will result in significant publications in internationally renowned journals. Feel free to contact me for any further information you might need.

Sincerely,

Anthony Herrel



University of Zagreb
Faculty of Science
Department of Animal Physiology
Rooseveltov trg 6
HR-10000 Zagreb
Croatia
tel +385 1 4877747 ♦ fax +385 1 4826260

Nancy Sung, program Director
NSF – CNIC
4201 Wilson Boulevard
Arlington, VA 22230
USA

December 9, 2012

Ref: *A letter of support for the NSF-CNIC project of Prof. Donovan P. German*

I am strongly supporting the research proposal “What does it take to be a herbivorous lizard? Revealing the digestive anatomy and physiology of manipulated populations of *Podarcis sicula*“ by prof. Donovan German of the University of California at Irvine. This project is truly integrative in its approach, and will provide important data in the anatomy, physiology and the evolution of herbivory in lizards.

More than 30 years ago, *P. sicula* from the island Pod Kopište were transferred to the island Pod Mrčaru. Both islands are located in the vicinity of the island of Lastovo in the eastern Adriatic and belong to Croatia. Recent research done by one of the collaborators on this project (Dr. Anthony Herrel) has shown that the descendants of these lizards have changed their morphology and have become mostly herbivorous, consequently changing their gut anatomy and physiology. However, the exact mechanism of digestion and utilization of an herbivorous diet remains unknown. The lizards on the eastern Adriatic islands provide a unique model to study coupling micro- and macroevolutionary processes and the link between various aspects of physiology, from molecules to the organ system level and beyond, and to link physiology to behavior. The proposed project presents a great advancement in the integrative biology of the digestive system and its link to the fitness consequences of herbivory.

Therefore, I am very excited to collaborate with Prof. German. The collaboration on this project with him and Dr. Anthony Herrel of the CNRS, will present the unique opportunity to gather important data to use in follow-on proposals to the NSF and other funding agencies. More importantly, the complementary skillsets of the principal investigator and collaborators will assure various levels of the study will be accomplished thoroughly.

The Department of Animal Physiology at the University of Zagreb is equipped with microplate spectrophotometers and a gas chromatograph. These will be used to study digestive enzyme activities and measure levels of fermentation end products (called Short Chain Fatty Acids) found in the lizards digestive tracts. Further, I have a state-of-the-art facility for reptile husbandry in my laboratory. For instance, I currently manage a large (178 individuals representing 24 species) academic snake colony and house various exotic and native lizards for research purposes. Thus, I have the facilities in which to carry out the experiments outlined in the proposal. Rooms can be maintained at set temperatures and photoperiods, and we have terraria with thermal gradients on the bottom, which reptiles prefer in captivity. The equipment that Dr. German will be bringing with him (e.g., tissue homogenizer, O₂ sensor, CO₂ analyzer) complements what we have at University of Zagreb, and will allow us to complete the proposed

research in a timely manner.

I will provide my facilities and my expertise in lizard husbandry and biochemical analyses of digestive enzymes. The joint expertise of all three faculty participants and Mr. Beck Wehrle (the PI's graduate student) assures the feasibility of the project. It is also of importance that Dr. Anthony Herrel is currently applying for the European Research Council Grant assessing the genetics of cecal valves in Pod Mrčaru lizards which complements nicely to Dr. German's proposal. Dr. Herrel's proposal will quantitatively measure selection on performance traits and will give momentum to the PI's project, both scientifically and financially.

It is a matter of regret University does not have a guesthouse/dormitory/cabins for a longer accommodation of the guests. However, a nearby hotel "Ilica" has a small suite with all the necessary facilities (e.g. kitchenette, laundry machine). We always get good discounts in this hotel which is within the walking distance (approx. 400 meters) of the biology main building.

Again, I am very eager to participate in this project because of its integrative nature and the possibility to produce important data and insights into the digestive physiology of herbivory and its evolution. I am especially excited that the whole study will be based on lizards that inhabit the Adriatic islands – a true evolutionary pearl.

Neither my family nor I have any significant financial interests related to the above-referenced project. The University of Zagreb abides to the Croatian laws to ensure full disclosure and no conflict of interest.

If you might need any further information, please contact me on my E-mail.

Sincerely yours

A handwritten signature in black ink, appearing to read "Zoran Tadić". The signature is fluid and cursive, with a large initial 'Z' and a long, sweeping tail.

Prof. Zoran Tadić
ztadic@biol.pmf.hr



IV. GIMNAZIJA UNESCO ASP - pridružena škola

10010 ZAGREB, Av. Dubrovnik 36, tel: +385 1 6677-188, fax: +385 1 6677-228
e-mail: 4.gimnazija-zg@zg.htnet.hr, www.cetvrtagimnazija.hr
MB: 3773159, OIB: 24771132599, ŽR: 2360000-1101449187

Klasa: 602-03/12-09/844
Urbroj: 251-282-03-12
Zagreb, 27. 11. 2012.

A letter of support for the NSF grant of Prof. Donovan P. German, Department of Ecology and Evolutionary Biology and Center for Comparative and Evolutionary Physiology, University of California

To whom it may concern:

Dear Sir/Madam,

Recently I learned from Prof. Zoran Tadić of the University of Zagreb (cocollaborator from the University of Zagreb) on the proposed NSF project) about the possibility to include the students of the 4th Grammar School (a type of secondary school comparable to the college-preparatory high schools in the US), in the outreach activities proposed in the NSF grant of Prof. Donovan P. German of the University of California at Irvine.


Although our school is primarily a language school, we pride ourselves in having achieved and achieving excellent results in teaching subjects belonging to the natural sciences, especially biology. One of our students won first place at the national competition in biology last year. This collaboration would also be very useful from the linguistic point of view because our students would have the opportunity to use and practice English in a natural environment.

I therefore eagerly support the outreach of the US University to our school. During this process the students would take part in modern research done at the US university, and would get an invaluable hands-on experience in the interactions with foreign scientists. They would also see and feel the modern research techniques in animal and comparative physiology. This may be very important in their future education particularly at the university level.

For further questions, you can reach me on my e-mail.

Yours sincerely,




Boris Štimac
Principal of the 4th Grammar School
boris.stimac3@gmail.com

A letter of support for the NSF grant of prof. Donovan P. German, Department of Ecology and Evolutionary Biology and Center for Comparative and Evolutionary Physiology, University of California at Irvine

To whom it may concern:

Dear Sir/Madam,

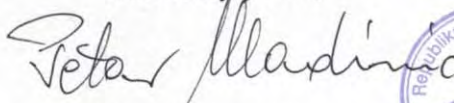
Prof. Zoran Tadić from the Faculty of Science, University of Zagreb has informed me that there is a possibility of cooperation of The 5th Gymnasium (the college-preparatory high school) in the outreach activities in the NSF grant of prof. Donovan P. German of the University of California at Irvine.

Our school is one of the best, if not the best secondary school in Croatia. It is a gymnasium (high school) with a strong natural sciences programme, but just as strong in the humanities, languages, special projects section, including excellent results in national and international competitions in many fields. Our school has a long history of excellence in teaching subjects such as biology, chemistry, physics and mathematics. This year there are students preparing for the international Olympiads in science, while many students participated in 15 state competitions winning numerous first, second...etc. awards. Out of 800 students in our school, over 100 students participate in competitions at national state level, from mathematics, physics, chemistry, biology, to geography, Croatian, Latin, English, Philosophy, Logic etc.

Therefore, I am strongly supporting the outreach of the US university to our school. During this project the students will get in touch with modern research and will get a hands-on experience of science in interaction with scientists from the US. This may be very important for their future education.

If you have any further questions, you can reach me on my E-mail.

Sincerely yours,


Petar Mladinić, prof.
The principal of The 5th Gymnasium

petar.mladinic@zg.t-com.hr



Valencia High School

500 North Bradford Avenue
Placentia, CA 92870
(714) 996-4970
FAX (714) 996-3159



December 11, 2012

Jim Bell, Principal
Rita Phillips, Biology Teacher
Valencia High School
500 N. Bradford Ave.
Placentia, CA 92870

Dear Dr. German,

As the Principal and Biology Teacher at Valencia High School in Placentia, CA, we are writing to offer our support for your NSF grant proposal. Your proposal to do hands-on, inquiry-based science activities with biology students at Valencia High School (VHS), located about 20 minutes from the campus of UCI, is stellar. As you know, 78% of the population of VHS is composed of under-represented groups. Thus, your outreach program is like its own contained GK-12 program operated out of your laboratory at a time when NSF GK-12 programs (like the one at UCI) are being discontinued. We are excited that you, your postdoctoral researchers, and graduate students will come to our campus once a month and engage our students with well-developed, inquiry-based science laboratory activities. We think this is an excellent opportunity because of your history in the NSF GK-12 Program. Hence, you already have designed several modules and lesson plans that are ready to be used at the high school level, and you will work closely with one of us (Ms. Phillips) to develop new lesson plans that suit the strengths of those involved (e.g., the expertise in genetics offered by your postdocs and graduate students). Each lesson plan will be crafted to meet Science Content Standards for California Public Schools and include Common Core Standards for the grade levels involved. In addition to your monthly visits, twice during the school year, our biology students will be bussed to the UCI campus for tours of your laboratory to learn more about your research on genetics and biochemistry. These are invaluable opportunities for our students.

Outreach programs like this one will be crucial in increasing scientific literacy and general interest in science, especially in youth at the high school age, the age at which natural curiosity in nature and science appear to wane. We can't imagine a better way to pique the students' interest in science than by having actual scientists come to their classroom and engage them in exciting activities.

Thank you,

James E. Bell, Principal
Valencia High School

Rita Phillips, Teacher
Valencia High School

Expected Schoolwide Learning Results
Valencia High School will prepare its graduates to be
Academic Achievers, Effective Communicators, Healthy Individuals, Complex Thinkers, Self-directed Learners,
Community Participants, Effective Technologists and Collaborative Workers
Instilling Pride-Tradition-Excellence since 1933



Dr. Luis Mota-Bravo
Director of Outreach, Research Training and Minority Science Programs (MSP)
Office of the Dean
School of Biological Sciences

1106 Biological Sciences III
Irvine, CA 92697-2527
lmota@uci.edu
Fax: (949) 824-6599
<http://port.bio.uci.edu>

December 5, 2012

Dr. Donovan P. German
Department of Ecology & Evolutionary Biology
University of California, Irvine
Irvine, CA 92697-2525

Dear Donovan,

I am writing this letter to follow up on our recent conversation about opportunities for outreach and research training for underrepresented students in your laboratory at UCI. We would be very pleased to have your NSF project contribute to our ongoing efforts.

The Outreach, Research Training and Minority Science Programs (MSP) at the UCI School of Biological Sciences Office of the Dean are a comprehensive effort, from K-12 to graduate studies, to increase the number and academic excellence of underrepresented students pursuing careers in science. MSP efforts are funded by the following NSF grants.

- The NSF-Undergraduate Research and Mentoring in the Biological Sciences (URM) Program is designed to broaden career options in biology for undergraduates from underrepresented groups. The program's objective is to prepare underrepresented undergraduates to excel in PhD programs in areas of biological research supported by the NSF Directorate for Biological Sciences (BIO). During their junior and senior years, students are funded to conduct year-round independent research under the direction of a UCI faculty mentor, and receive preparation for successful entry into graduate programs.
- The GK-12 Program at the UCI School of Biological Sciences is designed to bring innovation to graduate and K-12 education by integrating investigation and experimentation conducted by UCI faculty and graduate students with teaching of Biology/Life Sciences in grades 7-12 at partner schools in Santa Ana and Westside of Newport-Mesa Unified School Districts. GK-12 graduate students have improved communication, teaching and team-building skills as they acted as partners and mentors and incorporate their own research to support and serve as resources for teachers to enrich learning for K-12 students in minority-serving schools in grades 9-12.

In November 2005, MSP received the institutional Presidential Award for Excellence in Science, Mathematics, and Engineering Mentoring (PAESMEM) for developing innovative activities that promote the academic achievement of students beginning in the middle schools and that act as pathways to two-year, four-year college and graduate studies.

Since arriving at UCI, you have been a consistent supporter of, and participant in our MSP programs. First, you served as a research advisor of our MSP undergraduate Stephany Chacon. Thanks to your mentoring, Stephany was admitted and she is currently in her second year in the PhD program in the Department of Crop and Soil Science at Oregon State University. Currently, MSP undergraduate Abraham Sosa is receiving research training in your laboratory. Last quarter, you gave a highly inspirational seminar to our group of MSP undergraduates trainees. This quarter, you gave an excellent seminar to our GK-12 teachers and graduate students. I hope that you are successful with this grant, not just for your science, but because I know that it will allow you to continue your high level of participation in our MSP programs.

There are many ways in which your NSF project will contribute to ongoing efforts of MSP to provide training opportunities for underrepresented students and K-12 teachers. I would welcome the opportunity to place MSP undergraduates in your laboratory, visits to your laboratory by prospective community college underrepresented students, seminars to participants in our programs and your contribution of lessons for teachers.

Sincerely,

A handwritten signature in black ink that reads "Luis Mota-Bravo". The signature is written in a cursive, flowing style.

Luis Mota-Bravo, Ph.D.
Director of Outreach, Research Training and Minority Science Programs (MSP)
Office of the Dean
School of Biological Sciences
1106 Biological Sciences III
University of California, Irvine
Irvine CA, 92697-2527

E-mail: lmota@uci.edu
Ph. (949) 824-5680; FAX (949) 824-6599
<http://port.bio.uci.edu>



December 10, 2012

Dear Dr. German,

I am writing to express my enthusiasm about your engagement in diversity and outreach programs offered here at UC Irvine. In addition to your activities at Valencia High School, you have a history of involvement in our on-campus programs. Your current outreach program will expand on prior activities developed during the NSF-funded UCI FOCUS Math and Science Partnership grant. FOCUS, "Faculty Outreach Collaboratives Uniting Scientists, Students and the Schools," encompassed over 100 schools in three urban school districts, including 30 secondary schools in Compton Unified in Los Angeles and Santa Ana Unified in Orange County, which serve high percentages of African American and Hispanic/Latino students, respectively. I served as the FOCUS Director of Development for Faculty Outreach Collaboration before my appointment as an assistant professor at UC Irvine in 2011. Our work together with K-12 teachers to develop hands on experiences to showcase your research on fish, digestion, and physiology can be improved with the further addition of pre and post activities to the activities already developed. Your research closely augments many of the California Science Standards.

Immersing the teachers in hands-on activities will help them design and conduct experiments appropriate for use in their own classrooms. As a Co-Pi of a UC Irvine NSF NOYCE Internship and Scholarship Program grant, our continued interaction will benefit undergraduate students interested in pursuing a K-12 teaching profession. Your research experiences and my K-12 outreach experience will be beneficial to help NOYCE undergraduate students develop pre and post activities and lessons appropriate to students from high needs schools.

Sincerely,

A handwritten signature in blue ink that reads "Debra Mauzy-Melitz".

Debra Mauzy-Melitz, Ph.D.
Developmental & Cell Biology
School of Biological Sciences
University of California Irvine

CNIC: Evidence of prior communication and consultation

On Oct 18, 2012, at 2:36 PM, "Nancy Sung" <nsung@nsf.gov> wrote:

Dear Donovan-

Based on this exchange with Gary, you may prepare a CNIC proposal. Please be sure to attach the correspondence below to your application.

As I am on travel I cannot access our prior correspondence to see information about what you plan to request in your budget-so will just remind you to read the solicitation carefully so that you include only those things that are covered by the program.

Funding of your proposal will depend upon the results of external review as well as on available funds. Good luck!

Nancy

On Oct 18, 2012, at 3:47 AM, "Donovan P. German" <dgerman@uci.edu> wrote:

Dear Nancy,

Below you will see a response from Gary Gillis, the PO for IOS. He agrees that our proposal for the CNIC program is appropriate for submission through IOS. Thus, that will be where we send our proposal. We are shooting for early November submission. I thought you should be aware of this since it appears to be a requirement for the program and for submission.

Thank you,

Donovan

~~~~~  
Donovan P. German, PhD  
Assistant Professor  
Department of Ecology and Evolutionary Biology  
University of California  
Irvine, CA 92697  
[dgerman@uci.edu](mailto:dgerman@uci.edu)

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**From:** Gillis, Gary [<mailto:ggillis@nsf.gov>]  
**Sent:** Tuesday, October 16, 2012 10:46 AM  
**To:** Donovan P. German  
**Subject:** RE: NSF Electronic Proposal Review Request

**Hey Donovan, just wanted to let you know that I've read through your proposal and you are right, this is a good fit for IOS, and specifically for the PSS (Physiological and Structural Systems) Cluster. Hope this helps.**  
**Gary**

--

Gary Gillis, Ph.D.  
Expert/Program Director  
Physiological and Structural Systems Cluster  
Division of Integrative Organismal Systems  
Directorate for Biological Sciences  
Suite 685, National Science Foundation  
4201 Wilson Blvd.  
Arlington, VA 22230

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**From:** Gillis, Gary [<mailto:ggillis@nsf.gov>]  
**Sent:** Monday, October 01, 2012 7:31 AM  
**To:** Donovan P. German  
**Subject:** RE: NSF Electronic Proposal Review Request

Hi Donovan, please do send me the 1-page summary when you've got it as well as some rationale/motivation for the work (if that's not included in the 1-page document). It's my job to get a sense of program affiliation (i.e., does it look like it belongs in Physiological and Structural Systems, and if so, where).

Thanks  
Gary

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**From:** Donovan P. German [[dgerman@uci.edu](mailto:dgerman@uci.edu)]  
**Sent:** Friday, September 28, 2012 8:12 PM  
**To:** Gillis, Gary  
**Subject:** RE: NSF Electronic Proposal Review Request

Dear Gary,

I just submitted my review.

On another note, I am developing a collaboration with Dr. Anthony Herrel (France) and Dr. Zoran Tadic (Croatia) to study the nutritional physiology of the Italian Wall Lizard (*Podarcis sicula*) in Croatia. We are working on a proposal for the Catalyzing New International Collaboration (**CNIC**) program through the Office of International Science and Engineering. In the call (NSF 12-573), it says to contact the program officer for the respective division through which a proposal will be submitted. I believe that IOS is the correct division, but I want to be sure. Please let me know what you think. I have already reached out to Nancy Sung (the PO for the CNIC program) and Bonnie Thompson (the NSF contact for research in Croatia). I have heard enthusiastically from Bonnie, but I am waiting for Nancy to reply. I thought I would go ahead and reach out to you since I was submitting a review anyway. In the call for the CNIC program, it says that we should submit a 1-page summary of the project to the PO of the correct division. If you would like that, I can furnish that by Tuesday of next week. We hope to submit the full proposal in early November.

Please reply at your convenience.  
Sincerely,  
Donovan

German

CNIC: Evidence of prior communication

**Subject** FW: Award Id : 1318059, PI : German  
**Sender** Donovan German <dgerman@uci.edu>  
**Recipient** Zoran Tadic <ztadic@biol.pmf.hr>  
**Date** 05.09.2013 14:37



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Award Date: August 15,  
2013  
Award No. IIA-1318059  
Proposal No. IIA-1318059

Ms. Nancy Lewis  
Director of Sponsored Projects  
University of California, Irvine  
5171 California Avenue, Ste 150  
IRVINE, CA 92697-7600

Dear Ms. Lewis:

The National Science Foundation hereby awards a grant of \$46,902 to University of California, Irvine for support of the project described in the proposal referenced above .

This project, entitled "CNIC: US-Croatian-French Research on the Digestive Anatomy and Physiology of Herbivorous Populations of the Lizard Species, Podarcis sicula," is under the direction of

Donovan P. German.

This award is effective August 15, 2013 and expires July 31, 2014.

This grant is awarded pursuant to the authority of the National Science Foundation Act of 1950, as amended (42 U.S.C. 1861-75) and is subject to Research Terms and Conditions (RTC, dated June 2011) and the NSF RTC Agency-Specific Requirements (dated January 14, 2013) are available at <http://www.nsf.gov/awards/managing/rtc.jsp>.

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase V Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots. and the following terms and conditions:

This award is subject to the Federal Funding Accountability and Transparency Act (FFATA) award term entitled, Reporting Subawards and Executive Compensation, which has been incorporated into the NSF Terms and Conditions referenced above.

If the awardee has any questions related to the pre-populated data associated with this award in the FFATA Subaward Reporting System, such questions should be submitted to: [FFATAREporting@nsf.gov](mailto:FFATAREporting@nsf.gov) or by phone to: (800) 673-6188.

The provisions of NSF 12-573, Catalyzing New International Collaborations are applicable to this award.

The attached budget indicates the amounts, by categories, on which NSF has based its support.

Please view the project reporting requirements for this award at the following web address [<https://reporting.research.gov/fedAwardId/1318059>].

The cognizant NSF program official for this grant is Bonnie Thompson, (703) 292-8703  
 The cognizant NSF grants official contact is Denise O. Young, (703) 292-4837.

Sincerely,

John C. Robey  
 Grants and Agreements Officer

CFDA No. 47.079  
[awards@research.uci.edu](mailto:awards@research.uci.edu)

IIA-1318059 000

SUMMARY PROPOSAL BUDGET

|                                                |      |      |      | Funds   |
|------------------------------------------------|------|------|------|---------|
|                                                |      |      |      | granted |
|                                                | cal  | acad | sumr | By NSF  |
| A. (0.00) Total Senior personnel               | 0.00 | 0.00 | 0.00 | \$0     |
| B. Other Personnel                             |      |      |      |         |
| 1. (0.00) Post Doctoral associates             | 0.00 | 0.00 | 0.00 | \$0     |
| 2. (0.00) Other professionals                  | 0.00 | 0.00 | 0.00 | \$0     |
| 3. (0.00) Graduate students                    |      |      |      | \$0     |
| 4. (0.00) Secretarial-clerical                 |      |      |      | \$0     |
| 5. (0.00) Undergraduate students               |      |      |      | \$0     |
| 6. (0.00) Other                                |      |      |      | \$0     |
| Total salaries and wages (A+B)                 |      |      |      | \$0     |
| C. Fringe benefits (if charged as direct cost) |      |      |      | \$0     |
| Total salaries wages and fringes (A+B+C)       |      |      |      | \$0     |

|                                                                                       |          |
|---------------------------------------------------------------------------------------|----------|
| D. Total permanent equipment                                                          | \$0      |
| E. Travel                                                                             |          |
| 1. Domestic                                                                           | \$0      |
| 2. Foreign                                                                            | \$26,224 |
| F. Total participant support costs                                                    | \$0      |
| G. Other direct costs                                                                 |          |
| 1. Materials and supplies                                                             | \$11,000 |
| 2. Publication costs/page charges                                                     | \$0      |
| 3. Consultant services                                                                | \$0      |
| 4. Computer (ADPE) services                                                           | \$0      |
| 5. Subcontracts                                                                       | \$0      |
| 6. Other                                                                              | \$0      |
| Total other direct costs                                                              | \$11,000 |
| H. Total direct costs (A through G)                                                   | \$37,224 |
| I. Total indirect costs                                                               | \$9,678  |
| J. Total direct and indirect costs (H+I)                                              | \$46,902 |
| K. Residual funds / Small business fee                                                |          |
| 1. Residual funds (if for further support of<br>current projects AAG I.D.2 and I.D.3) | \$0      |
| 2. Small business fee                                                                 | \$0      |
| L. Amount of this request (J) or (J-K1+K2)                                            | \$46,902 |
| M. Cost sharing                                                                       | \$0      |