Project Summary

Intellectual merit: This research tests effects of a predator on competition between an invasive and a resident species in a community of container mosquitoes. Unlike native *Ochlerotatus triseriatus*, invading *Aedes albopictus* does not modify behavior in response to water-borne cues from the predator *Toxorhynchites rutilus*. *Aedes albopictus* is superior to *O. triseriatus* in resource competition. In the field, there is little evidence of competitive displacement of *O. triseriatus* (Florida). We test the hypothesis that lack of behavioral responses to *T. rutilus* predation leads to greater vulnerability to predation, preventing displacement of *O. triseriatus*. We test whether: 1) interspecific competition is affected by predation risk cues from *T. rutilus*; 2) natural abundances of *O. triseriatus* and *A. albopictus* are positively and negatively correlated, respectively, with abundance of *T. rutilus*; 3) behavioral responses of *A. albopictus* to predation cues differ among populations; and 4) there is potential for evolution of *A. albopictus* behavioral responses if subjected to consistent selection by predation.

Broader impacts: Educational impacts include completion of the Ph.D. by the graduate student and research experiences for undergraduates who will aid in the laboratory research. Practical impacts include increased knowledge of population limitation by predation for vectors of human disease.

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Description of the proposed research

Introduction

Predation has long been recognized as an important factor structuring communities (Glasser 1979, Addicott 1974). In some natural communities predators facilitate the survival of competitively inferior species by preferentially attacking competitively superior species and reducing their numbers. In the absence of such predation, poorer competitors may be excluded by the competitively superior species (Chase et al. 2002). Although the details of the mechanisms involved in these indirect mutualisms vary, one key feature seems to be a tradeoff between low vulnerability to predation and competitive ability (Blaustein et al. 1995, Leibold 1996, Stav et al. 2000, Chase et al. 2002). In addition to their direct effect as a source of mortality for prey, predators can have important indirect effects on prey. Many prey reduce vulnerability to predation via adaptive changes in life history or behavior, but such changes incur costs (McPeek and Peckarsky 1998, Caudill and Peckarsky 2003). Such costs can have significant consequences for prey competitive abilities (Werner and Anholt 1996, Werner 1991). Behavioral adaptations appear to predominate in aquatic systems (Sih 1984). Prey that alter their behavior in response to predation risk have been shown to be less vulnerable to predation (Kats et al. 1988, Buskirk et al. 1997, Relyea 2002b). The costs of these behavioral adaptations is usually reduced movement, reduced foraging, and associated reduced growth rate (Werner and McPeek 1994, Lima and Dill 1990, Van Buskirk 2000). Sometimes the behavioral changes can affect competitive ability of the prey and so they play an important role in determining the tradeoff between invulnerability to predation and competitive ability (Sih 1992, Milbrink and Bengtsson 1991, Relyea 2002a).

Among the subdisciplines within evolutionary ecology, invasion biology is increasingly perceived as vital for conservation because invasion by exotic species is increasing at an unprecedented rate and these species have enormous economic and ecological impacts (Kolar and Lodge 2001). Although there are many examples of impacts of invasion by exotic species, our knowledge of the factors involved in facilitation or prevention of invasion by an organism is limited (Miller et al. 2002). Predators could have an impact on the density and population growth of invaders, and these effects could act as barriers to successful invasion (Lodge 1993). Predators may preferentially feed on invaders, thereby facilitating survival of native prey relative to that attained in the absence of predation when faced with an invader that is superior in resource competition (Garvey et al. 2003). Recent studies have shown that animal behavior can play an important role in animal communities, especially behavior related to predator-prey interactions (Relyea 2000). Consider a system where a native prey alters its behavior to avoid predation from the native predator. The success of an invading prev that is also a competitor of the native prev could depend upon whether it can also show appropriate behavior modifications (or other facultative changes) in response to the novel predators it encounters as it invades a new area. Without such appropriate responses, predation may act as a barrier to invasion. Thus, animal behavior can be one of the important factors that could influence the processes and outcomes of invasion, especially if the invasion involves predator-prey interactions. Despite this, behavioral mechanisms have received relatively little attention in the field of invasion biology, especially with respect to predator-prey interactions (Holway and Suarez 1999).

Study system

The Asian container-dwelling mosquito Aedes albopictus (Skuse) was introduced into the United States in the mid-1980ís (Hawley et al. 1987). Ochlerotatus triseriatus (Say) is a container dwelling mosquito native to the United States. Toxorhynchites rutilus, also a mosquito, is a native predator and feeds on other aquatic insects in tree holes and man made containers, including immatures of A. albopictus and O. triseriatus. T. rutilus primarily use mechanoreceptors to detect their prey and primarily hunt at the bottom of the containers (Steffan and Evenhuis 1981). Therefore, prey that are less active at the surface of the containers are less vulnerable to predation from *T. rutilus* than are prey that are active at the bottom of the containers (Juliano and Reminger 1992). Ochlerotatus triseriatus alters its behavior in response to water-borne cues to predation risk from T. rutilus, becoming less active and moving away from the bottom of the container, apparently reducing risk of predation (Grill and Juliano 1996, Juliano and Gravel 2002). Several studies have shown that A. albopictus are superior competitors relative to other co-occurring mosquitoes like O. triseriatus and Aedes aegypti (Ho et al. 1989, Livdahl and Willey 1991, Novak et al. 1993, Juliano 1998, Teng and Aperson 2000, Aliabadi and Juliano 2002). Aedes albopictus has displaced A. aegypti in many parts of Florida (Lounibos et al. 2001), but despite A. albopictus being the superior competitor to O. triseriatus under laboratory conditions, O. triseriatus does not appear to have suffered population declines since the arrival of A. albopictus in Florida (Lounibos et al. 2001). There have been many studies on competitive interactions of A. albopictus with North American filter-feeding mosquitoes, but relatively few on the vulnerability of A. albopictus to North American predators (Lounibos et al. 2001) or the impact of predation on A. albopictusis interspecific competitive interactions with O. triseriatus.

We propose investigations of the effects *T. rutilus* on competition between *O. triseriatus* and invading *A. albopictus* and the impacts of those effects on the invasive capabilities of *A. albopictus*.

Previous results

i) Interspecific comparison: We have tested the hypothesis that *A. albopictus* shows a behavioral response to predation risk from *T. rutilus* that is similar to that of *O. triseriatus* (Juliano and Gravel 2002). *Aedes albopictus* **did not** alter its behavior in response to water borne cues to *T. rutilus* predation risk (Kesavaraju and Juliano 2004); simultaneous experiments confirmed that *O. triseriatus* reduced movement, feeding, and time at the bottom (Kesavaraju and Juliano 2004). These changes are expected to reduce vulnerability to predation by *T. rutilus* (Juliano and Reminger 1992). Thus, *A. albopictus* should be more vulnerable to predation from *T. rutilus* when compared to *O. triseriatus*. (Griswold and Lounibos 2005) present data from the laboratory that also support this hypothesis.

ii) Species specificity of cues: We have shown that *O. triseriatus* reduces movement, feeding, and time at the bottom in water that has held crushed conspecifics, crushed cricket nymphs, or *T. rutilus* feeding on *A. albopictus,* indicating that the water-borne cues to which they respond are very general (Kesavaraju and Juliano 2004, Kesavaraju et al, Unpublished data). *Toxorhynchites rutilus* are voracious predators that feed on any moving organism, including their own kind (Steffan and Evenhuis 1981). For such a generalist predator, responses to nonspecific cues would

seem to be advantageous for a species like *O. triseriatus* because they will elicit appropriate responses even when *T. rutilus* are feeding on other species (even non-mosquito species).

Proposed research

The objective of this research is to determine the importance of behavioral responses to the threat of predation in this biological invasion. We will test the hypothesis that lack of appropriate behavioral responses in *A. albopictus* creates a trade-off of low vulnerability to predation vs. competitive ability for *A. albopictus* and *O. triseriatus*, and that this trade-off affects *A. albopictus* invasion success and prevents the displacement of *O. triseriatus* in habitats where *T. rutilus* are abundant. We have designed experiments and field surveys that will enable us to test whether predation plays a role in determining which species wins competitive interactions between *A. albopictus* and *O. triseriatus*, whether *O. triseriatus* and *A. albopictus* abundance in nature, whether there are inter-population differences in *A. albopictus* response to water-borne cues to *T. rutilus* predation risk, and finally, whether there is potential for evolution of antipredatory behavioral responses by *A. albopictus* if it is subjected to consistent selection by predation.

Methods for the proposed research

Field Survey: Do abundances of *A. albopictus* and *O. triseriatus* vary with the abundance of *T. rutilus*?

Aedes albopictus, O. triseriatus, and *T. rutilus* are container dwelling mosquitoes and are found most commonly in water-filled natural containers like tree holes, and in artificial containers like tires and cemetery vases in Florida (Lounibos 1983). Female *T. rutilus* are thought to prefer containers in shaded areas (e.g., tree holes) for oviposition. Multiple field surveys will be conducted in Florida at 4 tree hole sites (Myakka River State Park, Sarasota Co.; Sherwood Hammock, Ft. Pierce; Indrio Road, Ft. Pierce; and Highland Hammock State Park, Highland Co.), 4 cemetery vase sites (Oak Hill, near Bartow; Rose Hill, Tampa; Joshua Creek, near Arcadia; and Fort Myers City, Fort Myers), and 4 tire sites (auto salvage yards in Vero Beach, Apopka, Orlando, and Titusville) for the presence and absence of *T. rutilus*. These sites were chosen based on both published work (Lounibos et al. 2001; Juliano et al. 2004) and unpublished data on presence of *T. rutilus* (S.A. Juliano, *personal observation*). Containers from each of the sites will be sampled a minimum of 3 times between the months of July to December (the rainy season in south Florida, http://www.srh.noaa.gov/mlb/enso/ensoeducational4.htm)

Data Analysis: Mosquitoes found in the sampled containers will be identified and counted. The volume of water from each of the containers will also be recorded. We will analyze monthly data using Pearson correlation coefficient to test whether mean abundances (absolute and relative) of *A. albopictus* and *O. triseriatus* are negatively and positively correlated, respectively, with *T. rutilus* abundance.

Experiment 1: Does *T. rutilus* predation play a role in the outcome of competition between *A. albopictus* and *O. triseriatus*?

There will be 4 treatments that are combinations of two factors: *T. rutilus* (present, absent), and test water (Predation, Control). Each treatment will have 6 replicates and one

hundred fifty first instar larvae of both *A. albopictus* and *O. triseriatus* will be added to each replicate. Each replicate will be held in covered 400ml cups with 400ml of water.

Predation water will be prepared by holding a 3rd or 4th instar *T. rutilus* and 10 prey larvae for 4 d, with prey species alternating daily between *A. albopictus* and *O. triseriatus*. That is, if *A. albopictus* are added on the first day, all the survivors will be removed the next day and then 10 *O. triseriatus* larvae will be added. Control water will be prepared in the similar way, but without the predator. Before the experiment, prey larvae and the predator used for preparing the test water will be removed, so that test water contains only the water-borne cues (presumably chemical) to the actions of the previous occupants. *Ochlerotatus triseriatus* respond to such water-borne cues by reducing movement (Juliano and Gravel 2002), whereas *A. albopictus* do not (Kesavaraju and Juliano 2004, see above).

A constant amount of liver powder will be added to the cups as food for larvae. Both control and predation waters will be changed every 4 days. In those treatments that involve addition of predator (*T. rutilus*), the prey larvae will be given a head start of 5 days. *Toxorhynchites rutilus* are voracious predators and they will consume a minimum of 10 prey larvae per day, hence the initial addition of large numbers of prey, and a five-day head start for the prey larvae. Any dead *T. rutilus* will be replaced with an individual of the same instar. The experiment will run for 30 days with eclosed adults collected daily. Predictions for this experiment are summarized in Table 1. Predation water is expected to induce behavioral change in *O. triseriatus* but not in *A. albopictus*. With the predator absent, this advantage should be reversed due to superior competitive ability of *A. albopictus* and the costs of behavioral change (Table 1). Growth and development rates should always be greater for *A. albopictus*, but that difference should be accentuated by changes induced by predation water (Table 1). In control water, we expect a smaller survival advantage for *O. triseriatus* (Table 1).

Treatment	Behavior	Surivorship	Growth &
			Development
Predation water	tris: Reduced movement & foraging	tris >> albo	tris << albo
+ T. rutilus	<i>albo</i> : No change		
Predation water	tris: Reduced movement & foraging	tris < albo	tris << albo
- T. rutilus	<i>albo</i> : No change		
Control water	tris: No change	tris > albo	tris < albo
+ T. rutilus	<i>albo</i> : No change		
Control water	tris: No change,	tris < albo	tris < albo
- T. rutilus	albo: No change,		

Table 1. Predictions survivorship, growth, and development rates of *A. albopictus (albo)* and *O. triseriatus (tris)* for Experiment 1.

Data analysis: Upon eclosion adults will be identified and will be stored in the oven at 60°C. If the pupa dies without eclosing the sex and species will be determined via microscopic examination. Based on these data, development rate (time⁻¹), mass at pupation (a measure of growth), and survivorship to adulthood will be recorded for each species in each replicate. Effects of water treatment (Control water, predation water), predator (Present, absent) will be analyzed by MANOVA on development rate, survivorship, and mass at pupation for the two

prey species (*A. albopictus, O. triseriatus*), using sex as a block variable (I both species, males are smaller and develop more rapidly than females). Thus there will be 6 dependent variables and 3 response variables in the MANOVA. Standardized canonical coefficients will be used to determine the contribution of response variables (Scheiner 2001). A significant interaction effect on the MANOVA would indicate that predation risk plays a role in the outcome of competition.

Experiment 2: Are there inter-population differences in *A. albopictus* behavioral response to water-borne cues to *T. rutilus* predation risk?

As is true in other taxa (e.g., Relyea 2002c), both species are likely divided into separate populations with low levels of inter-population dispersal. *Toxorhynchites rutilus* abundance varies from abundant to absent in many of the sites in Florida (Lounibos et al. 2001). We need to know whether *A. albopictus* that invaded areas where *T. rutilus* are abundant have evolved behavioral or other responses to water-borne cues to risk of *T. rutilus* predation.

We will use *A. albopictus* larvae collected from the field sites we have sampled (see above), so that we will have estimates of the abundances of *T. rutilus* associated with each population. We will establish colonies from each one of the sites described in the previous experiment (Total=12 colonies; 4 tree hole, 4 Cemetery vase, and 4 tire sites). F_1 eggs collected from those colonies will be used for this experiment.

Behaviors of one-day-old 4th instar *A. albopictus* larvae from each of the sites will be digitally recorded while they are held in water treated in one of two ways. Control water will have held larval A. albopictus larvae alone, whereas Predation water will have held T. rutilus feeding on A. albopictus larvae. For the predation treatment, one T. rutilus 4th instar larva will be held for 5 days in 50 ml cups with 50 ml of water and 10 A. albopictus larvae. Larvae offered as prey for water preparation will be counted daily and any missing larvae will be replaced. For the control treatment, 10 A. albopictus larvae will be held without food. Any larvae that die will be replaced (Kesavaraju and Juliano 2004). Test A. albopictus from the different sites will be hatched and held individually in 18 ml vials with 10 ml of water, and will have no previous experience with T. rutilus or cues from this predator. These larvae will be fed with liver powder suspension (LPS) prepared by mixing 0.3 gm of Bovine Liver powder with one liter of water. This food suspension will be dispensed via pipetting from a beaker held on a stirring plate to ensure homogeneous delivery of food to larvae (Juliano and Gravel 2002). Once the larvae attain the 4th instar, they will be held individually in 50-ml cup with 30 ml of water with no food for 24 h in order to standardize hunger. Then the test larvae will be transferred to the treatment water (Control and Predation) and their behavior recorded for 30 minutes. The behavior of at least 15 larvae will be recorded for each treatment per site. In total there will be 30 replicates per site (Control ñ 15 replicates, Predation ñ 15 replicates) and overall there will be 360 replicates (30 per site times 12 sites).

Observation Protocol: From the video recording, activity and position of the each larva will be recorded every minute for 30 minutes in instantaneous scan censuses (Juliano and Gravel 2002, Kesavaraju and Juliano 2004). Activities will be classified into four categories 1) Browsing ñ the larva moving along the surfaces, propelled by feeding movements of the mouth parts; 2) Resting ñ the larva completely still and not feeding; 3) Filtering ñ the larva drifting through the water column, propelled by feeding movements of mouth parts; and 4) Thrashing ñ the larva propelling itself through the water by vigorous lateral flexion of the body (Grill and Juliano 1996, Juliano and Reminger 1992, Juliano and Gravel 2002, Kesavaraju and Juliano 2004). Positions will be classified into four categories: 1) Surface ñ the larva's spiracular siphon

in contact with the surface; 2) Bottom ñ the larva within 1 mm of the bottom of the cup; 3) Wall ñ the larva within 1mm of the sides of the cup; and 4) Middle ñ the larva not in contact with the surface, and more than 1 mm from the cupis surfaces. For *T. rutilus* preying upon *O. triseriatus*, Juliano and Reminger (1992) showed that among positions, the surface is the least likely to lead to predation, the bottom is the most likely to lead to predation, and middle and wall are intermediate, and among activities, resting is the least likely to lead to predation, thrashing is the most likely to result in predation, and the two feeding behaviors are intermediate.

Data analysis: The activities and positions will be converted into proportions. There will be 4 activities and 4 positions making it a total of 8 variables. To reduce the number of variables and to obtain uncorrelated descriptors of behavior, a principal component analysis on activities and positions will be done (SAS Institute Inc. 1990, Juliano and Gravel 2002, Kesavaraju and Juliano 2004). Principal component scores will be analyzed using MANOVA with site type, population within type, and individual within population as effects. Standardized canonical coefficients will be used to determine the relative contribution of each of the principal components for producing the significance effect, and significant effects will be further analyzed by using multivariate contrasts (Scheiner 2001). We will also test for correlations and canonical correlations of behavioral variables with estimated *T. rutilus* abundances at the associated field sites. We predict differences among populations in behavior and behavioral response to waterborne predation cues, and that responsiveness will be positively associated with *T. rutilus* abundances.

Experiment 3: Will *A. albopictus* evolve behavioral responses to avoid predation if subjected to consistent selection by predation?

Controlled selection experiments (Conner 2003) are done by manipulating the environmental factor hypothesized to cause selection. The target organisms are allowed to reproduce in these manipulated environments for several generations. Samples of each generation are placed in the treatment environments (e.g., Predation) and the traits of interest (e.g., behavioral responses) are measured. These controlled experiments test both **whether** an organism can evolve in response to a specific agent of selection, and **how** they evolve in response to that agent (i.e., what trait or traits change, and in what directions; Conner 2003, Fry 2003). We have used a controlled selection experiment to investigate evolution of behavioral responses of *O. triseriatus* to water-borne cues to *T. rutilus* predation (Juliano and Gravel 2002) and we will use a similar experimental design to investigate potential for evolution of *A. albopictus* behavior and life history in response to predation by *T. rutilus*.

Based on the data from the field survey, *A. albopictus* will be collected from a site where the abundance of *T. rutilus* is lowest or where the behavior of *A. albopictus* changes least in response to *T. rutilus* predation (Experiment 2). Field collected *A. albopictus* will be reared in the laboratory and 2^{nd} generation offspring will be used to begin the experiment in order to reduce maternal effects. Sixteen experimental cohorts each containing 1000 larvae will be established from the 2^{nd} generation offspring. The larvae will be reared with liver powder in 1000 ml containers (26° C, 14:10 L: D). After 5 days 8 cohorts will be subjected to predation with a single *T. rutilus* until the numbers are reduced to approximately 50% of the original number. Because of stochastic mortality among larvae, there is likely to be variation in the final number surviving exposure to predation, but care will be taken to ensure that each cohort remains above 300 individuals. Every other day, the number of larvae surviving in each of the cohorts will be reduced at

random to the mean number of survivors from the predation group. To remove larvae at random from control cohorts, container contents will be poured through a screen that will retain larvae. The screen will be marked with a numbered grid, and a random number table will be used to determine the grid squares from which larvae are removed. After sufficient numbers are culled, remaining larvae will be reared to adulthood and propagated in 20L cages in environmental chambers. The procedure will be continued for 4 generations, which was sufficient for O. triseriatus to show divergence of behavioral responses in a similar experiment (Juliano and Gravel 2002). In each generation a sample of eggs will be taken from each cohort, hatched, and resulting larvae will be tested for their behavioral responses to water-borne cues to predation by analyzing their behavior in control and predation water treatments (prepared as described in previous experiments) using principal component analysis and MANOVA as described in previous experiments. We predict that the predation cohorts will show significant reduction in activity levels, particularly feeding, in predation water when compared with control cohorts. Further we predict that we will observe a trend across the generations for the predation cohorts to show progressively greater reductions in activity levels, and that such a trend will be absent in the control cohorts. The potential for constitutive responses (i.e., stable differences between behavior of predation and control lines regardless of test water) will also be tested in these analyses. Evolution of such a constitutive response was observed in O. triseriatus (Juliano & Gravel 2002).

Feasibility

Our lab has been maintaining colonies of mosquitoes for well over a decade. We have developed techniques and have all necessary equipment (e.g., environmental chambers, digital video equipment). Both the PI and doctoral student have experience in experiments involving mosquito behavior and have published multiple papers on both mosquito behavior and predator-prey interactions (Grill and Juliano 1996, Juliano and Reminger 1992, Juliano 1996, Nannini and Juliano 1998, Juliano and Gravel 2002, Kesavaraju and Juliano 2004, Yee et al. 2004). Our lab has been collaborating with Dr. Phil Lounibos, Florida Medical Entomology Laboratory (FMEL), Vero Beach, FL for over a decade. We have permission to stay in the FMEL bunk house (accommodation for visiting researchers) and to use laboratory space in Florida during the field survey. All the field sites mentioned are close to FMEL, and we have secured permission from the site owners/manager.

Significance

The roles of competition and predation in communities are widely studied, and the tradeoff between competitive ability and vulnerability to predation is believed to be wide-spread and theoretically important for community organization (Grill and Juliano 1996, Sih et al. 1985, Werner and Anholt 1996, Werner 1991, Wellborn 2002). Only in a relatively few cases, however, has the mechanism producing the trade-off, and its costs and benefits, been determined. Our work is novel as we test rigorously the role of one mechanism ñ flexible predator avoidance behavior -- in determining the trade-off, and because our work addresses the role of this tradeoff in a recent biological invasion. We hope to be able to contribute to our general knowledge of the biological bases of invasive capabilities of animals. Investigations regarding native predatoris ability to prevent invasion of exotic species are not common and the information will be a valuable addition to our knowledge of conservation.

Timetable

We did preliminary site identification and field sampling of *A. albopictus* in autumn 2004. The field survey will resume in July 2005 (next rainy season). We will also begin behavioral comparisons of *A. albopictus* from different locations in August 2005. We will start the competition experiment in February 2006. We will start the selection experiment during Autumn 2005. The behavior videos will be viewed and scored beginning in December 2005.

Broader Impacts

This study will form the Ph.D. dissertation of **Banugopan Kesavaraju**, co-PI, and its completion is important in developing his career in mosquito ecology. The grant, if funded, will make a substantial contribution to the training of an ecologist from a developing Asian country (India) working on an important group of organisms. Invasive mosquitoes (*A. albopictus*, *Ochlerotatus japonicus*) of USA have been introduced from Asia (Hawley et al. 1987, Peyton et al. 1999). The ecology of these mosquitoes in their native habitats may play an important role in understanding their invasion potential and vector capabilities, but has been poorly studied. The main reason for this lack of investigation of ecology of Asian mosquitoes has been lack of trained mosquito ecologists, language barriers, and hesitation on the part of the developing Asian countries to give permits to western universities (Gillis 2004). Banugopan Kesavarajuís career goals include such ecological research in Asia with collaboration from western universities which will both benefit the developing countries in Asia and provide enhanced understanding of invasion biology of these species.

Undergraduate workers assisting in this project will gain valuable research experience. The PI has had considerable success as a mentor of undergraduate research students, and many of these students have been authors on publications (see **Biographical Sketch**).

Both *A. albopictus* and *O. triseriatus* are vectors of arboviruses. *Aedes albopictus* is a known vector of Dengue and West Nile viruses (Yuill 1986, Sardelis et al. 2002). *Ochlerotatus triseriatus* is a known vector of La Crosse encephalitis virus (Eldridge et al. 2000). Understanding interactions of *A. albopictus* with predators should aid in understanding what will ultimately limit the range of *A. albopictus* in North America. Investigations of the ecology of the larval stages of these species will contribute to our basic knowledge of the processes determining distribution and abundance of these medically important species.

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Yuill, T. M. 1986. The ecology of tropical arthrond-borne viruses. Annual Review of Ecology and Systematics 17:189-219.

BIOGRAPHICAL SKETCH:

BORN: 30 August 1955

Department of Biological Sciences Illinois State University, Normal, IL 61790-4120 Phone: (309) 438-2642 sajulia@mail.bio.ilstu.edu

a. Professional Preparation

B.A. Biology, Kalamazoo College, Kalamazoo, MI. 1977
M.S. Entomology, Cornell University, Ithaca, NY. 1979
Ph.D. Zoology (Minor Statistics), Pennsylvania State University, State College, PA. 1985
NATO Postdoctoral Fellow, University of York, York, U.K. 1987-88

Steven A. Juliano

b. Appointments

Faculty Positions

Illinois State University, Department of Biological Sciences: Assistant Professor 1986-92, Associate Professor 1992-96, Professor 1996-present

Pennsylvania State University, Department of Statistics: Instructor 1985-86

Concurrent Positions

University of Florida, FMEL: Courtesy Assoc. Professor 1993-94; Courtesy Professor 2000-01

c. Publications (graduate student coauthors <u>double underscored</u>; undergraduate coauthors <u>underscored</u>)

Most relevant

- Juliano, SA, LP Lounibos, & GF O'Meara. 2004. A field test for competitive effects of Aedes albopictus on Aedes aegypti in South Florida: Differences between sites of coexistence and exclusion? Oecologia 139:583-593.
- Juliano, SA, GF O'Meara, <u>JR Morrill</u>, & MM Cutwa 2002. Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitoes. *Oecologia* 130:458-469
- Juliano, SA & <u>ME Gravel</u>. 2002. Predation and the evolution of prey behavior: An experiment with tree hole mosquitoes *Behavioral Ecology* 13:301-311
- <u>Kesavaraju, B &</u> Juliano, SA 2004. Differential behavioral responses to water-borne cues to predation in two container-dwelling mosquitoes. *Annals of the Entomological Society of America* 97:194-201
- Juliano, SA 1998. Species introduction and replacement among mosquitoes: Interspecific resource competition or apparent competition? *Ecology* 79:255-268.

Other

- Juliano, SA, JR Olson, EG Murrell, & JD Hatle 2004. Plasticity and canalization of insect reproduction: testing alternative models of life history transitions. In press -- *Ecology*.
- <u>Daugherty, MP</u> & SA Juliano. 2002. Testing for context-dependence in a processing chain interaction among detritus-feeding aquatic insects. *Ecological Entomology* 27:541-553.
- <u>Aliabadi, BK</u> & **SA Juliano.** 2002. Escape from Gregarine Parasites Affects the Competitive Impact of an Invasive Mosquito. *Biological Invasions* 4:283-297.
- <u>Frankino, WA</u> & SA Juliano. 1999. Costs of reproduction and geographic variation in the reproductive tactics of the mosquito *Aedes triseriatus*. *Oecologia* 120:59-68

<u>Hechtel, LJ</u> & **SA Juliano** 1997. Effects of a predator on prey size at and time to metamorphosis: plastic response by prey or selective mortality? *Ecology* 78:838-851.

d. Synergistic activities

Mentor: Research mentor for 5 Elementary & Jr. High Teachers 1995-97, ISU.
 Research mentor for 1 Pre-service High School teacher 2003, Fermi Lab.
 Academic mentor, Louis Stokes Alliance for Minority Participation, Illinois, for 3 students 2002-04.

Research mentor for over >50 undergraduate research students; 17 of these students have been authors on 16 peer reviewed publications; 7 have been first authors.

Subject Editor: Ecological Society of America (Ecology, Ecological Monographs) 1994-97.

Grant Review Panel Member: NSF/NIH panel ñ Ecology of infectious disease, 2000, 2002 NSF panel ñ Population Ecology, 1993

Symposium co-organizer: Using ecological theory to understand container mosquito communities Entomological Soc. America, Nov. 2004 (with graduate student Don Yee).

Research tools: Developed statistical methods for fitting and comparing nonlinear regressions. Regularly serve as a consultant to researchers who need to apply these techniques. Methods summarized in a chapter in Scheiner & Gurevitch (eds.) 2001 (see references cited)

e. Collaborators

i. Collaborators (Past 4 yr):

Illinois State Univ.: VA Borowicz, DW Borst, DW Whitman, CF Thompson, SK Sakaluk, WL Perry, RM Bowden, K Lind, C Horvath Univ. of Florida: LP Lounibos, GF OíMeara, MAH Braks, MM Cutwa, N Nishimura, RL Escher, Univ. of N. Florida: JD Hatle, Univ. of Illinois: U Kitron, M Lancaster, Inst. Osw. Cruz, Brasil: RL Oliviera, N Honorio. Indiana Univ.: M Tseng. Univ. of North Carolina: WA Frankino. Yale Univ.: LE Munstermann. Southern Illinois Univ.: MR Eskew. Michigan State Univ.: M Kaufman.

ii. Graduate and Post-graduate Advisors

M.S. Advisor:	CO Berg (deceased)
Ph.D. Advisor:	DL Pearson (Arizona State University)
Post Doctoral Advisor:	JH Lawton (Imperial College, U.K.)

iii. Student and Post-doctoral Advisees (Past 5 yr)

Current Graduate Students: B Kesavaraju, DA Yee,

Recent Students (Affiliation): KS Costanzo (Univ. Buffalo), MH Lee (Univ. Illinois) S Hegrenes (Carthage College), BW Aliabadi (San Jacinto Comm. Coll.), BW Alto (Univ. Florida), MP Daugherty (Univ. California, Berkeley), SL Van Rhein (State of Missouri), MA Nannini (EPA), AS Aspbury (Univ. Texas), ME Gravel (Memorial Univ., Canada), E Gunnawardene (Purdue), VL Flanagin (Max Plaanck Inst., Munich) J Olson (Ohio State Univ), E Murrell (Covance Labs), CA Jones (SUNY Albany) L Luker (Illinois College of Optometry) **Recent Post Doc (Affiliation):** JD Hatle (Univ. of North Florida)

TOTAL ADVISEES: Graduate students - 18; Post Doctoral - 2

BIOGRAPHICAL SKETCH: Banugopan Kesavaraju

BORN: May 21, 1975

Department of Biological Sciences Illinois State University, Normal, IL-61790-4120 Phone: 309 438 5278 Email: <u>bkesava@ilstu.edu</u>

a) Professional preparation

B.sc. Zoology, University of Madras, India M.S. Ecology, Pondicherry University, India

b) Appointments

Research Assistant	-	Illinois State University, Fall 2004 ñ Present
Teaching Assistant	-	Illinois State University, Fall 2003 ñ Spring 2004
Research Assistant	-	Illinois State University, Fall 2001 ñ Summer 2003
Junior Research Fellow	-	Salim Ali Centre for Ornithology and Natural
		History, India, June 1999 ñ July 2000
Research Assistant	-	Wildlife Institute of India

c) Publications

i) Most Relevant

- Kesavaraju, B., and S. A. Juliano. 2004. Differential behavioral responses to water-borne cues to predation in two container dwelling mosquitoes. *Annals of the Entomological Society of America* 97(1):194-201.
- Yee, D. A., B. Kesavaraju, and S. A. Juliano. 2004. Interspecific differences in feeding behavior and survival under food-limited conditions for larval *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *Annals of the Entomological Society of America* 97: 720-728.

Yee, D. A., **B. Kesavaraju**, and S. A. Juliano. 2004. Larval feeding behavior of three cooccurring species of container mosquitoes. *Journal of Vector Ecology* (In Press).

ii) Other

- Costanzo, K.S., **B. Kesavaraju**, and S. A. Juliano. *In review*. Condition-specific competition in container mosquitoes: the role of non-competing life-history stages. Submitted to *Ecology*.
- Bivash Pandav, **K. Banugopan**, D. Sutaria & B.C. Choudhry 2000. Fidelity of male Olive Ridley sea turtles to a breeding ground. *Marine turtle Newsletter* 87:9-10.
- Banugopan K., and Priya Davidar 1999. Status of sea turtles along the Pondicherry coast, India. *Hamadryad* 24 (1) 43.

d) Synergistic activities

Co-developed a laboratory exercise on predator-prey behavioral interactions for the basic undergraduate biology course (Biology 101) in Fall 2003 at Illinois State University, Department of Biology.

Supervised an undergraduate on a research project (Director: S. A. Juliano, Illinois State University) on differential mortality and survival of *A. albopictus* and *O. triseriatus* from predation by *Toxorhynchites rutilus* in the Spring of 2003. The undergraduate received 1 research credit for the project.

I am currently involved in a research project with B. W. Alto (University of Florida) and L. P. Lounibos (University of Florida) at the Florida Medical Entomology Laboratory, Vero Beach, FL on behavior of *Corethrella appendiculata* and anti-predatory behavioral responses of *Aedes albopictus* and *Ochlerotatus triseriatus* in response to its predation.

e) Collaborators and affiliations

i) Collaborators

University of Florida
University of Florida
SUNY Buffalo
Illinois State University
Illinois State University

ii) Graduate and post graduate advisors

M.S. Advisor: P. Davidar, Pondicherry University, India Ph.D. Advisor : S.A. Juliano, Illinois State University

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2. Banugopan Kesavaraju - none	0.00	0.00	0.00		0	
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C *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

Budget Justification

Materials and supplies: We record behavior in MPEG2 format on computers. The digital clips average in size of about 1 GB per clip. We burn these clips on to DVDís which can be viewed later. We have computers and software that can record these digital files and burn them on to DVDís. But we need about 100 DVDís (\$100) to record all our proposed behavioral experiments. These digital files are of a higher resolution, less expensive and have a longer storage life than conventional S-VHS analog recording systems. Cages for holding adult mosquitoes will be constructed using wood and Plexiglas with nylon screening. We will use guinea pigs and mice to blood feed the mosquitoes (IACUC protocol **01-2004**, approved through January 2005, with renewal for two more years expected). For the controlled selection experiment alone we will need 16 cages (approximately \$800) and enough guinea pigs to blood feed the mosquito lines (\$200). Experiments will be conducted in plastic beakers and larvae to be used in behavioral trials will be held individually in 10 ml glass vials. An adjustable digital pipettor will be used to dispense liver powder suspension. These miscellaneous supplies are estimated to cost \$650. Field sampling supplies (containers, flagging, pumps, turkey basters) are expected to cost \$250.

Materials & Supplies = \$2000

Travel: Airfare to Melbourne, FL and the travel from there to Vero Beach, FL costs approximately \$300 (No direct flights to Vero Beach, FL). We will be going to Florida twice during the period of the project. Rental cars to go to field sites from Vero Beach cost about \$35 per day, with gasoline costs of about \$30 each time we rent a car. We will be renting the cars for about 10 days in a month for 4 months in the first year and 3 months in the second year. Renting cars on a daily basis, as needed, and flying to Florida is less expensive than renting cars on a monthly basis from the university and driving from Illinois (estimated costs \$1200 per month). Accommodation at the bunk house at FMEL, Vero Beach, FL will cost \$300 per month for seven months (First year = 4 months and second year = 3 months), and is thus less expensive than motel or apartment accommodation.

We plan to present the research related to this proposal at 2005 ecological society of America meeting, Montreal, Canada. We request funds for airfare (approximately \$400) and accommodation (approximately \$700) to attend the meeting. We have presented in the previous meetings and are confident that our paper will get accepted.

Air tickets, car rental, & accommodation for 2 years = \$8350

Personnel: The controlled selection experiment is very labor intensive, and extra personnel will be vital to its success. We will have 16 cohorts of 1000 larvae in each generation of this experiment and we will have to count each one of those cohorts every other day of the experiment. Also each one of those cohorts has to be propagated as adults in colonies for four generations. Undergraduate help especially during the period of this experiment will be crucial. So we request funds to have an undergraduate help for 10 weeks.

Hourly wages: \$8/hr, 10 hrs/week for 10 weeks, \$800/year for 2 years = \$1600

Current and Pending Support (See GPG Section II.C.2.h 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.
Other agencies (including NSF) to which this proposal has been/will be submitted. Investigator: Steven Juliano
Support: □Current ⊠Pending □Submission Planned in Near Future □*Transfer of Support Project/Proposal Title: CRUI: Physiology and fitness consequences of different life history phenotypes(Co-PI)
Source of Support: NSF CRUI Total Award Amount: \$ 912,719 Total Award Period Covered: 09/01/04 - 08/31/09 Location of Project: Normal IL Person-Months Per Year Committed to the Project. Cal:0.00 Acad: 2.25 Sumr: 2.00
Support: Current Pending Submission Planned in Near Future *Transfer of Support Project/Proposal Title: Container-dwelling mosquitoes and microbial productivity (PI)
Source of Support: NIAID AREA Program Total Award Amount: \$ 140,000 Total Award Period Covered: 06/01/03 - 05/31/06 Location of Project: Normal IL Person-Months Per Year Committed to the Project. Cal:0.00 Acad: 2.25 Sumr: 0.75
Support: □Current ⊠Pending □Submission Planned in Near Future □*Transfer of Support Project/Proposal Title: Invasion Biology of Aedes albopictus
Source of Support: NIAID subcontract from Univ. of Florida Total Award Amount: \$ 790,993 Total Award Period Covered: 02/01/05 - 01/31/10 Location of Project: Normal IL and Vero Beach FL Person-Months Per Year Committed to the Project. Cal:0.00 Acad: 3.00 Sumr: 1.50
Support: □Current □Pending □Submission Planned in Near Future □*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: □Current □Pending □Submission Planned in Near Future □*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: Summ:
*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.

Current and Pending Support

(See GPG Section II.C.2.h 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. Other agencies (including NSF) to which this proposal has been/will be submitted.
Investigator: Banugopan Kesavaraju
Support: ⊠ Current □ Pending □ Submission Planned in Near Future □ *Transfer of Support Project/Proposal Title: Evolution of antipredatory behaviors in Aedes albopictus: response to Toxorhynchites predation
Source of Support:Phi Sigma Honor Society, Illinois State UniversityTotal Award Amount:\$ 600 Total Award Period Covered:08/01/04 - 07/31/05Location of Project:Illinois & FloridaPerson-Months Per Year Committed to the Project.Cal:0.00Acad: 4.50Sumr: 3.00
Support: □Current ⊠Pending □Submission Planned in Near Future □*Transfer of Support Project/Proposal Title: Natural abundances of Aedes albopictus and Ochlerotatus triseriatus in relation to Toxorhynchites abundances
Source of Support: Sigma Xi Total Award Amount: \$ 950 Total Award Period Covered: 05/01/05 - 04/30/06 Location of Project: Florida Person-Months Per Year Committed to the Project. Cal:0.00 Acad: 2.00 Sumr: 3.00
Support: □Current □Pending □Submission Planned in Near Future □*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: □Current □Pending □Submission Planned in Near Future □*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: □Current □Pending □Submission Planned in Near Future □*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: Summ: *If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.

FACILITIES

Laboratory

Steven Juliano occupies an ecology laboratory with a 25'X32' work area and a 25'X12' office area in Felmley Science Annex (FSA). Three laboratory benches, 2 sinks, refrigerator/freezer, drying oven, and a hood are available in the laboratory. A 2^{nd} adjacent room (25'X13') houses equipment, primarily 7 environmental chambers and a drying oven. A windowless basement room (25'X30') with photoperiod control is used to maintain insects and, because it is isolated and has limited access, for video recording of behavior trials. Steven Juliano also has access to a walk-in environmental room (10'X7') with photoperiod and temperature control.

Laboratory equipment available for experiments includes seven temperature and photoperiod controlled environmental chambers, one with humidity control, an sVHS video recording system (video camera, macro-zoom lens, digital recording software, sVHS recorder, monitor), an image analysis system including a 3-chip digital video camera and *Scion Image 4.0.2* software (ideal for measurement and counting), two Wild M3 dissecting microscopes with camera attachment, one Nikon compound microscope with camera attachment, a Cahn C21 Ultra-microbalance, an analytical balance, three top loading balances (one large range), Hach DR/800 spectrophotometer, and two drying ovens. All of this equipment is located in Steven Juliano's laboratory and is dedicated to his research program.

Clinical (not applicable)

Animal

Animal facilities for maintaining mice and guinea pigs are available in FSA. A full time animal keeper is employed. The facilities conform to regulations for housing mice and guinea pigs.

Computer

Seven IBM compatible pentium computers (2 laptops, 1 office, 4 lab), with printers, CD/RW burners, scanner, WWW access. Statistical software (*PC/SAS 8.2, RT*), Image analysis (*Scion Image*), modeling Software (*Stella Research 5.1.1, PC/SAS 8.2*), and graphics software (*Sigmaplot, Excel, DeltaGraph*) are adequate for all data analyses.

Office

PI Steven Juliano has adequate office space (12'X18') in FSA. CoPI Banugopan Kesavaraju has office space in the laboratory in FSA (see above).

Other

We have secured owner permission to conduct experiments and sampling at our field sites in Florida. We are collaborators with investigators at Florida Medical Entomology Laboratory, Vero Beach, FL, and have access to lab space at the lab during periods of field work in Florida.

University/Departmental facilities

A machine shop for insect cage construction is located in an adjacent building. Departmental staff includes 3 full time secretaries and an administrative aide, a full-time ordering specialist, and a departmental equipment manager. The department has two vans for field research. The Department provides phone, e-mail, regular mail, FAX, and office supplies. Milner Library provides on-line abstract searches, on-line access to many journals in ecology and entomology, and is linked to other university libraries in Illinois. Milner receives 356 journals; interlibrary loan within Illinois makes rapid acquisition of publications feasible.