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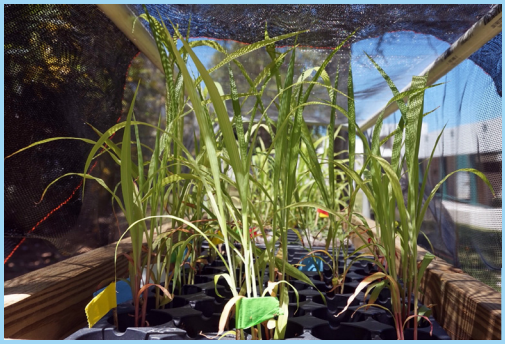
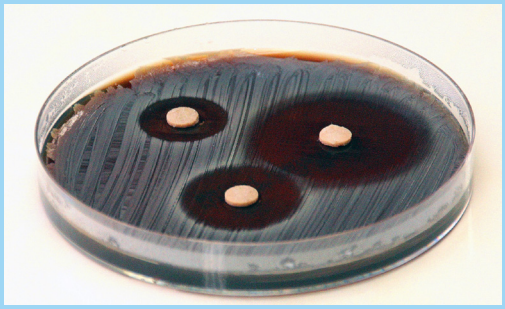


$\Delta H^{\circ}_{f,\,Et\text{-}R}$

UNIVERSIDAD INTERAMERICANA DE PUERTO RICO
RECINTO DE ARECIBO

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# Departamento de Ciencias y Tecnología

## Bachilleratos:

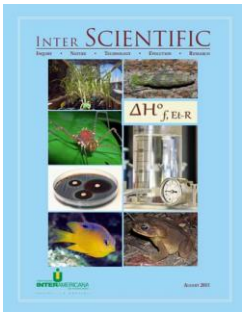
Biología · Biotecnología
Computadoras · Química

## Asociado:

Computadoras

**PORTADA/** COVER

Nuestra portada combina imágenes de los temas abordados en este ejemplar.

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Roberto Robles Miranda
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Dr. Alberto Puente-Rolón
Foto de sorgo: provista por la Prof. Arlyn Pérez
Foto del pez damisela: www.reefguide.org

Illustration of the diversity of topics covered in this issue.
**Design:**
Roberto Robles Miranda
**Photography:**
Rafael Colón González
Picture of the snail, harvestmen and frog: provided by Dr. Alberto Puente-Rolón
Picture of soghum: provided by Prof. Arlyn Pérez
Picture of the damselfish: www.reefguide.org

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## MENSAJE DEL RECTOR
## DR. RAFAEL RAMÍREZ RIVERA

Esta segunda publicación reafirma la investigación científica como el hilo conductor de la gestión académica universitaria. Este volumen presenta el conocimiento generado por estudiantes y sus mentores utilizando como herramienta estructural el método científico. Los diversos temas tratados son: bioquímica y biología molecular, genética, biotecnología de plantas, ecología, microbiología y química.

Definitivamente, la publicación de las investigaciones del claustro manifiesta el proceso inherente a las ciencias. Es por eso que este esfuerzo consciente de la academia ha permitido la amplitud y continuación de esta publicación científica.

## MESSAGE FROM THE CHANCELLOR
## DR. RAFAEL RAMÍREZ RIVERA

This second publication reaffirms the role of scientific research as the underlying common thread throughout the university's academic endeavor. This volume presents the knowledge generated by students under the guidance of their mentors through the use of the scientific method. The topics presented in this publication include biochemistry and molecular biology, genetics, plant biotechnology, ecology, microbiology and chemistry.

The publication of research conducted by our faculty definitely showcases the process inherent to science. It is through the conscious effort of the academia that the expansion and continuation of this scientific publication has been made possible.

## MENSAJE DE LA
## DECANA DE ASUNTOS ACADÉMICOS
## DRA. ANNETTE VEGA RODRÍGUEZ

Este segundo volumen de la revista Inter Scientific reafirma la necesidad de contar con un mecanismo para la divulgación del quehacer académico bajo la perspectiva de los proyectos de investigación realizados por estudiantes y facultad. Nos place presentarles en este volumen artículos científicos que pasaron por evaluación y arbitraje. Les exhortamos a compartir la publicación y la valiosa información que contiene.

## MESSAGE FROM THE DEAN OF ACADEMIC AFFAIRS
## DR. ANNETTE VEGA RODRÍGUEZ

This second volume of the journal Inter Scientific reaffirms the need for a means of disclosure of research within the category of academic research projects developed by students and faculty. We are pleased to present peer reviewed research articles in this volume, and we urge you to share this publication and the valuable information it contains.

## DESDE EL ESCRITORIO DE LA EDITORA
## DRA. LIZBETH ROMERO-PÉREZ

Con el fin de continuar la difusión de nuevos conocimientos producto de la investigación científica presentamos el segundo volumen de Inter Scientific.

En la sección de Artículos de Investigación, encontrará el trabajo del Dr. Vásquez titulado *Estimación teórica de las entalpías estándar de formación de 1,4 Benzodioxan-2-R* en el que se comparan datos de modelos establecidos con los resultados de la investigación. Estudiantes bajo la mentoría de la Profesora Pérez estudiaron diversas especies de sorgo y presentan sus resultados en el artículo, *Detección de polimorfismos en cultivos de sorgo utilizando microsatélites* Este volumen incluye una amplia gama de artículos de investigación en el campo de la Ecología generados por estudiantes de nuestro Recinto y de la Universidad de Massachussetts. Entre ellos se incluyen trabajos con el caracol *Nenia tridens*, los opiliones, el sapo de caña y la variedad del pez damisela en los arrecifes de coral de la isla de Vieques. El artículo *Evaluación de las propiedades antibacterianas del ajo (Allyum sativum)* presenta el efecto inhibidor observado contra bacterias patógenas. Como artículo de revisión se presenta *Pruebas de pureza requeridas para la aprobación final de un producto biológico: Producción de anticuerpos de ratón (mouse antibody production), producción de anticuerpos de hámster (hamster antibody production), transcriptasa inversa y HPLC,* desarrollado por estudiantes de Biotecnología. Esperamos con este segundo volumen continuar contribuyendo al campo de las Ciencias.

## FROM THE EDITOR'S DESK
## DR. LIZBETH ROMERO-PÉREZ

In order to continue the diffusion of knowledge produced through scientific research, we present the second volume of Inter Scientific.

In the Research Articles section you will find the work of Dr. Vásquez, *Theoretical estimation of the standard energies of formation of 1,4 Benzodioxan-2-R* in which data product of established models is compared to the results of the investigation. Under the direction of profesor Pérez, students study diverse species of sorghum and present their results in *Detection of polymorphism in sorghum cultivars applying* single sequence repeat markers. This volumen also includes several research articles on Ecology written by students from our campus and from the University of Massachussetts. Among them we find studies on the snail Nenia tridens, harvestmen, the cane toad and the variety of damselfish in the coral reef of Vieques island. *Evaluation of the antibacterial properties of garlic, (Allyum sativum)* presents the inhibitory effect observed against pathogenic bacteria. As a review article, we present *Purity tests required for the final approval of a biological product: mouse antibody production, hamster antibody production, reverse transcriptase, HPLC* written by biotechnology students. With this second volume we hope to continue contributing to the field of science.

# Estimación teórica de las entalpías estándar de formación de 1, 4 Benzodioxan-2-R

## Theoretical estimation of the standard enthalpies of formation of 1, 4 Benzodioxan-2-R

**Vásquez Moll, V.D.**

*Departamento de Ciencias y Tecnología, Universidad Interamericana de Puerto Rico, Recinto de Arecibo*

**ABSTRACT**

**The proposition of isodesmic reactions in the formation of 1,4 –benzodioxan- 2-R and the applications of semi-empirical methods such as PM3, MP2 and B3LYP allowed for the estimation of the standard enthalpies of formation of the reaction molecules at 298 K in their gas phase. In some cases, the calculated values showed a good agreement with the experimental data and revealed that the method that best reproduced the enthalpies of formation was the PM3. The general form for this type of isodesmic reaction is described by equation 1: 1,4-benzodioxan [g] + $CH_3CH_2R$ [g] → 1,4-benzodioxan-2-R [g] + $CH_3$ $CH_3$ [g], where R = {-COOH, -$CH_2OH$, -$CH_2COOH$, -OH, -$COCH_3$, -CHO, -$CH_3$, -CN, -$NO_2$}. After obtaining the standard enthalpies of formations, a multiple linear regression was carried out as a function of descriptors such as dipoles moment, molar mass, unsaturation index, molar refractivity and LUMO energy. Nine compounds resulting from the reaction described by equation 1 were included in the system under study.**

**RESUMEN**

La proposición de reacciones isodésmicas en la formación de 1,4 –benzodioxan- 2-R y la aplicación de métodos semi-empíricos como PM3, MP2 y B3LYP, permitieron estimar las entalpías estándar de formación de estas moléculas a 298 K en fase gaseosa. Los valores calculados presentan, en algunos casos, una buena concordancia con los datos experimentales encontrándose que el método que mejor reproduce las Entalpías de Formación de estos isómeros es el PM3. La forma general para este tipo de reacción isodésmica es descrita por la ecuación 1: 1,4-benzodioxan [g] + $CH_3CH_2R$ [g] → 1,4-benzodioxan-2-R [g] + $CH_3$ $CH_3$ [g], donde R = { -COOH, -$CH_2OH$, -$CH_2COOH$, -OH, -$COCH_3$, -CHO, -$CH_3$, -CN, -$NO_2$ }. Luego de obtener los valores de las entalpías de formación estándar se realizó una regresión lineal múltiple en función de descriptores tales como Momento dipolar, Masa molar, Índice de no saturación, Refractividad molar y Energía Lumo. Nueve compuestos resultantes de la reacción descrita por la ecuación 1 se incluyen en el sistema estudiado.

**KEYWORDS** isodesmic reaction, enthalpies of formation, bond dissociation energy, benzodioxan

PALABRAS CLAVE reacción isodésmica, entalpías de formación, energía de disociación de enlace, benzodioxan

## INTRODUCCIÓN

El 1,4 –benzodioxan ha sido ampliamente utilizado en el diseño de agentes terapéuticos como bloqueadores adrenergéticos (Fourneau, E. y Bovet, 1933; Rapela, C. E. y Green H. D, 1961; Giardina, D., Bertini, R., Brancia, E., Brasili, L., y Melchiorre, 1985), como también en la función hepática (Vogel, G. et al., 1975), ansiolíticos, (Mir, A. K., et al., 1988) espasmolíticos, (Ertan, R., Göker, H. y FABAD, 1987) etc.

La siguiente investigación presenta el cálculo teórico de propiedades termoquímicas como las entalpías estándar de formación y las energías de disociación de enlace en fase gaseosa de sustancias del tipo 1,4-benzodioxan-2-R, ya que muchos de estos compuestos no se pueden obtener con un 100 % de pureza.

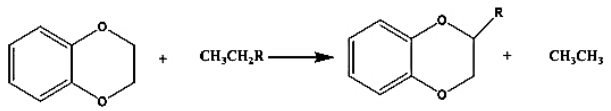



**Figura 1:** Esquema de reacción isodésmica para 1,4 –benzodioxan-2-R

También se evaluaron estas propiedades termoquímicas en función de ciertos descriptores como, el momento dipolar, la masa molar, el índice de no saturación, la refractividad molar y la energía Lumo, con el fin de obtener ecuaciones a través demétodos de regresión lineal múltiple. La figura 1 representa un esquema de reacción isodésmica para los distintos enlaces que forman un átomo de carbono con el radical R.

## MÉTODOS

### Determinación de cambios de entalpía estándar de formación en fase gaseosa.

Para determinar el calor de formación de los distintos isómeros de 1,4-benzodioxan-2-R se utilizó el siguiente esquema de reacción, basado en el conocido concepto de reacciones isodésmicas (Figura 1).

El cambio de Entalpía Estándar de Reacción para el esquema anterior se puede definir como:

$$\Delta H_r^0 = \Delta H_{f,Bz-R}^0 + \Delta H_{f,Et}^0 - [\Delta H_{f,Bz}^0 + \Delta H_{f,Et-R}^0] \qquad [1]$$

donde $\Delta H_r^0$ es el calor estándar de reacción, $\Delta H_{f,Bz-R}^0$ es el calor estándar de formación de 1,4 Benzodioxan-2-R, $\Delta H_{f,Et}^0$ es el calor estándar de formación de Etanol, $\Delta H_{f,Bz}^0$ es el calor estándar de formación de Benceno y $\Delta H_{f,Et-R}^0$ es el calor estándar de formación de Etanol-Radical.

Todas las moléculas involucradas en las distintas reacciones estudiadas fueron optimizadas con el propósito de alcanzar su geometría más estable.

Por otra parte, cálculos teóricos del cambio de la Entalpía Estándar de Reacción, se pueden obtener utilizando el software computacional Gaussian 03, siguiendo los procedimientos propuestos en los manuales de este software (J. B. Foresman and AE. Frish):

$$\Delta H_r^0 = H_{Bz-R} + H_{Et} - [H_{Bz} + H_{Et-R}] \qquad [2]$$

donde, $H_i = U_i^0 + f^{ctH}$ siendo $U^0$ la energía electrónica y $f^{ctH}$ el factor de corrección térmico en la entalpía.

Al igualar ecuaciones [1] y [2], se obtiene el calor de formación, en este caso, del 1,4-benzodioxan-2-R, considerando los calores de formación estándar experimentales presentados en Tabla 1 (Engel, T., and Reid, P., 2005).

### Cálculo de energía de disociación de enlace.

Las curvas de energía potencial en función de la longitud de enlace, en el caso de dos átomos vecinos, se puede seguir a través de la función potencial de Morse teniendo la siguiente forma (Vásquez Moll, V.D., 2014): $E_P = D_e[1.0 - e^{-\beta[L-L_e]}]^2$ [3]

donde $E_p$ es la energía potencial, $D_e$ es la energía de disociación de enlace, β es un parámetro, L es la longitud de enlace y $L_e$ es la longitud de enlace en el equilibrio o en la posición de mínima energía. Además, se usarón algunos descriptores, físicos y termodinámicos, para intentar relacionarlos con los resultados que se obtengan, tanto para la entalpía de formación como la energía de disociación de enlace.

**Tabla1.** Entalpías estándar de formación experimentales

| Molécula | $\Delta H_f^0[kJ/mol]$ |
|---|---|
| Etano | -84 |
| Ácido propanoico | -455.8 |
| Propanol | -256 |
| Ácido butanoico | -475.9 |
| Etanol | -234 |
| Butanona | -238.6 |
| Propanal | -188.7 |
| Propano | -104.7 |
| Cyanoetano | 51.5 |
| Nitroetano | -87.51 |
| Benzodioxan | -178.3 |

## RESULTADOS Y DISCUSIÓN

### Obtención de cambios de entalpía estándar de formación de 1,4-Benzodioxan-2-R

Utilizando el método semi-empírico PM3 de Gaussian y realizando el trabajo de Opt + Freq, se obtuvieron los siguientes resultados para $H_i$ expresada en unidades de energía en Hartree [Ha] y que se presentan en tabla 2.

Reemplazando los valores de tablas [1] y [2] en ecuaciones [1] y [2] se obtienen los cambios de entalpía estándar para las distintas reacciones isodésmicas que están relacionadas con la formaciónde Radicales 1,4-benzodioxan-2-R, que se presentan en la Tabla 3.

### Regresión lineal múltiple para $\Delta H_{f,BenzoR}$

Utilizando el software Workspace y ProyectLeader (Scigress Workspace. Scigress Explorer) se obtuvieron algunos descriptores (Tabla 4) que permitieron realizar tanto regresiones lineales como regresiones múltiples. Un ejemplo de regresión lineal está representado en la figura 2, que muestra una gran dispersión.

Es por ello que se propone a continuación una regresión lineal múltiple que da como resultado la ecuación [4] y la figura 3 para

el calor estándar de formación en función de varios descriptores. En la ecuación [4] p es el momento dipolar, M es el peso molecular, $U_i$ es el índice de no-saturación, $R_M$ es la refractividad molar, y $E_{Lumo}$ es la energía Lumo.

**Tabla 2.** Valores computacionales de la suma de la energía electrónica y térmica.

| Molécula | $H_i$ [Ha] |
|---|---|
| 1,4 –benzodioxan | 0.077248 |
| $CH_3CH_3$ | 0.049626 |
| $CH_3CH_2COOH$ | -0.069589 |
| $CH_3CH_2CH_2OH$ | 0.013896 |
| $CH_3CH_2CH_2COOH$ | -0.04825 |
| $CH_3CH_2OH$ | -0.009424 |
| $CH_3CH_2COCH_3$ | 0.048513 |
| $CH_3CH_2CHO$ | 0.011783 |
| $CH_3CH_2CH_3$ | 0.070091 |
| $CH_3CH_2CN$ | 0.108394 |
| $CH_3CH_2NO_2$ | 0.04974 |
| 1,4 –benzodioxan- 2-COOH | -0.036601 |
| 1,4 –benzodioxan- 2-$CH_2OH$ | 0.041681 |
| 1,4 –benzodioxan- 2-$CH_2COOH$ | -0.01733 |
| 1,4 –benzodioxan- 2-OH | 0.007598 |
| 1,4 –benzodioxan- 2-$COCH_3$ | 0.07596 |
| 1,4 –benzodioxan- 2-CHO | 0.045125 |
| 1,4 –benzodioxan- 2-$CH_3$ | 0.098041 |
| 1,4 –benzodioxan- 2-CN | 0.144392 |
| 1,4 –benzodioxan- 2-$NO_2$ | 0.083002 |

**Cálculo de energías de disociación de enlace**

Utilizando Método PM3 y haciendo un Scan para enlace C-N se obtienen resultados típicos dados por el software Gaussian 03[8] y que se muestran en figura 4.Utilizando el programa computacional GrapadPrism (Graph Pad Prism 4 for Windows 2003) se hicieron regresiones no lineales con el propósito de obtener la energía de disociación del enlace que une al 1,4 Benzodioxan con el Radical.

**Regresión lineal múltiple para la energía de disociación de enlace [ $D_e$]**

Utilizando el software Workspace y ProyectLeader se obtuvieron algunos descriptores que permitieron realizar regresiones lineales y múltiples, donde algunos ejemplos se muestran en figura 6.

Los gráficos 3 y 7 muestran regresiones lineales de la energía de disociación de enlace como función de la refractividad molar o del momento dipolar.

**Tabla 3.** Entalpías estándar de formación de radicales 1,4-Benzodioxan-2-R a T = 298.15 K.

| Molécula | $\Delta H_r$ [Ha] | $\Delta H_r$ [kJ/mol] | $\Delta H^0_{f,BenzoR}$ [kJ/mol] |
|---|---|---|---|
| 1,4-Benzodioxan-2-COOH | 0.00537 | 14.088 | -536.01 |
| 1,4-Benzodioxan-2-$CH_2OH$ | 0.00016 | 0.428 | -349.87 |
| 1,4-Benzodioxan-2-$CH_2COOH$ | 0.00330 | 8.659 | -561.54 |
| 1,4-Benzodioxan-2-OH | -0.01060 | -27.83 | -356.13 |
| 1,4-Benzodioxan-2-$COCH_3$ | -0.00018 | -0.459 | -333.36 |
| 1,4-Benzodioxan-2-CHO | 0.00572 | 15.018 | -267.98 |
| 1,4-Benzodioxan-2-$CH_3$ | 0.00033 | 0.861 | -198.14 |
| 1,4-Benzodioxan-2-CN | 0.00838 | 21.991 | -20.81 |
| 1,4-Benzodioxan-2-$NO_2$ | 0.00564 | 14.808 | -167.00 |

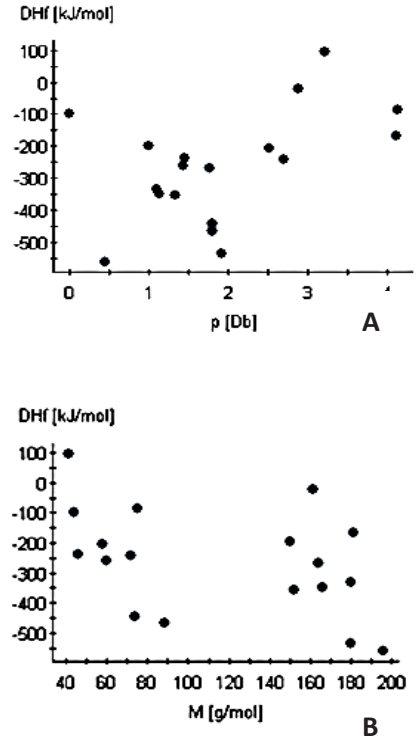
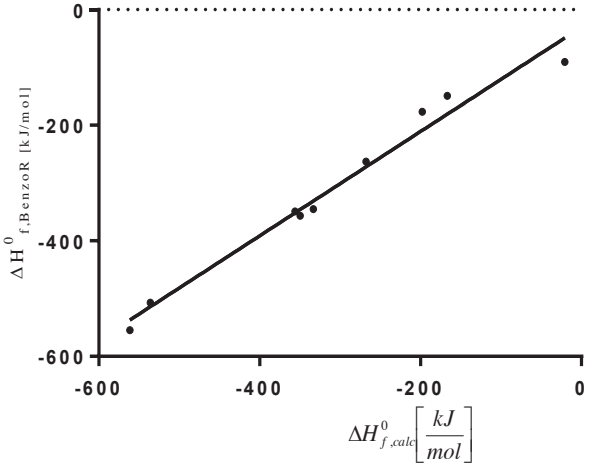



**Figura 2**: Variación del calor de formación con el momento dipolar y la masa molar [ Tabla 2].

$$\Delta H^0_{f,calc} = 102.4p - 17.3M + 181.7U_i + 54.8R_M + 63.4E_{Lumo} - 102.1pE_{Lumo} + 7.8p^2E_{Lumo} + 21.75p^2E^2_{Lumo} - 2.116pR_ME_{Lumo} - 424.4 \quad [4]$$




| $\Delta H^0_{f,calc}$ [kJ/mol] | $\Delta H^0_{f,BenzoR}$ [kJ/mol] |
|---|---|
| -554.398 | -561.540 |
| -356.433 | -349.870 |
| -176.534 | -198.140 |
| -262.734 | -267.990 |
| -90.259 | -20.810 |
| -345.087 | -333.360 |
| -507.179 | -536.010 |
| -149.208 | -167.010 |
| -349.092 | -356.130 |

$$\Delta H_{f,calc} = 102.4p - 17.3M + 181.7Ui + 54.8R_M + 63.4E_{Lumo} - 102.1pE_{Lumo} + 7.8p^2E_{Lumo} + 21.748p^2E^2_{Lumo} - 2.116pR_ME_{Lumo} - 424.4$$

**Figura 3.** Comparación entre el calor de formación de Benzodiozan-2-R presentado en la tabla [3] y el calor de formación calculado utilizando la ecuación [4].

**Tabla 4**: Descriptores para moléculas de 1,4-Benzodioxan-2-R.

Los datos computacionales anteriores fueron tratados mediante un método de ajuste de mínimos cuadrados cuyos resultados se presentan en figura 5 y tabla 5 (Graph Pad Prism 4 for Windows, 2003).

Los resultados muestran una gran dispersión. Es por ello que se propone a continuación una regresión lineal múltiple que da como resultado la ecuación [5] de la energía de disociación de enlace como función de varios descriptores. En esta ecuación p es el momento dipolar, Hy es el factor hidrofílico, MLOGP2 es el coheficiente de partición octanol-agua, M es la Masa Molar y Uy es el índice de no saturación.

Los resultados obtenidos para la energía de disociación de enlace

| Molécula | p [Db] | M [g/mol] | $U_i$ | $H_y$ | $R_M$ | TPSA | MLOGP2 | $E_{Lumo}$ [eV] |
|---|---|---|---|---|---|---|---|---|
| 1,4-Benzodioxan-2-COOH | 1.909 | 180.16 | 3 | -0.127 | 42.845 | 55.76 | 0.34 | 0.211 |
| 1,4-Benzodioxan-2-$CH_2OH$ | 1.127 | 166.18 | 2.807 | -0.164 | 42.978 | 38.69 | 0.448 | 0.279 |
| 1,4-Benzodioxan-2-$CH_2COOH$ | 0.448 | 196.20 | 3 | -0.166 | 48.907 | 55.76 | 0.786 | 0.27 |
| 1,4-Benzodioxan-2-OH | 1.329 | 152.15 | 2.807 | -0.119 | 38.045 | 38.69 | 0.575 | 0.149 |
| 1,4-Benzodioxan-2-$COCH_3$ | 1.097 | 180.20 | 2.807 | -0.748 | 47.729 | 27.69 | 0.946 | 0.291 |
| 1,4-Benzodioxan-2-CHO | 1.76 | 164.16 | 3 | -0.727 | 41.92 | 35.53 | 0.335 | 0.087 |
| 1,4-Benzodioxan-2-$CH_3$ | 1.003 | 150.177 | 2.807 | -0.79 | 41.434 | 18.46 | 2.265 | 0.337 |
| 1,4-Benzodioxan-2-CN | 2.872 | 161.16 | 3 | -0.727 | 41.825 | 42.25 | 0.355 | 0.022 |
| 1,4-Benzodioxan-2-$NO_2$ | 4.105 | 181.148 | 3.17 | -0.598 | 42.372 | 64.28 | 1.589 | -0.141 |

$$D_{e,calc} = 22.1p + 66.8H_y + 38MLOGP2 - 4145.2H_yMLOGP2 - 1897\,MLOGP2H^2_y + 354H_yMLOGP2^2 + 4.21MU_yH_yMLOGP2 - 0.38 \quad [5]$$

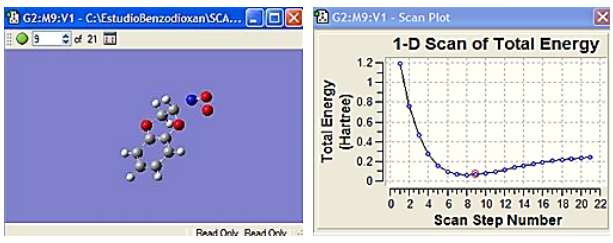



**Figura 4:** Scan de enlace C-N utilizando método PM3.

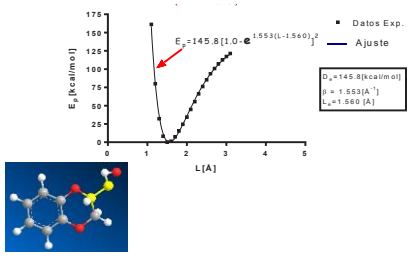



**Figura 5:** Scan de enlace C-C (amarillo) utilizando método PM3 $U_y$ es el índice de no saturación (HyperChem; Talete srl DRAGON Professional).

[$D_e$] desde ecuación [5] y ecuación [3] son comparados tanto en la tabla 6 como en la figura 7.

**Tabla 5**: Parámetros de Función Potencial de Morse.

| Molécula | $D_e$ [kcal/mol] | β [$Å^{-1}$ ] | $L_e$ [Å] |
|---|---|---|---|
| 1,4-Benzodioxan-2-COOH **[C-C]** | 109.1 | 1.436 | 2.296 |
| 1,4-Benzodioxan-2-$CH_2OH$ **[C-C]** | 156.4 | 1.512 | 1.58 |
| 1,4-Benzodioxan-2-$CH_2COOH$ **[C-C]** | 164.9 | 1.467 | 1.58 |
| 1,4-Benzodioxan-2-OH **[C-O]** | 179.4 | 1.713 | 1.409 |
| 1,4-Benzodioxan-2-$COCH_3$ **[C-C]** | 194.8 | 1.599 | 1.442 |
| 1,4-Benzodioxan-2-CHO **[C-C]** | 145.8 | 1.553 | 1.56 |
| ,4-Benzodioxan-2-$CH_3$ **[C-C]** | 179.7 | 1.482 | 1.546 |
| 1,4-Benzodioxan-2-CN **[C-C]** | 180.9 | 1.568 | 1.484 |
| 1,4-Benzodioxan-2-$NO_2$ **[C-N]*** | 140.3 | 1.743 | 1.492 |

*El paréntesis [C-N] indica el enlace formado por átomo de C de Benzodioxan y el átomo de N del radical R.

El cálculo de las entalpías estándar de formación mediante el método de reacciones isodésmicas y el proceso de cálculos teóricos a traves de PM3, permitieron encontrar resultados aceptables para las moléculas estudiadas, como se muestra en la figura 3.

**Tabla 6**: Comparaciónde las energías de disociación de enlace obtenida por regresión no lineal de ecuación [3] y la energía de disociación de enlace calculada por ecuación [5].

| Molécula | $D_{e,Morse}$ [kcal/mol] | $D_{e,calc}$ [kcal/mol] |
|---|---|---|
| 1,4-Benzodioxan-2-COOH | 109.1 | 111.4 |
| 1,4-Benzodioxan-2-$CH_2OH$ | 156.4 | 156.3 |
| 1,4-Benzodioxan-2-$CH_2COOH$ | 164.9 | 168.4 |
| 1,4-Benzodioxan-2-OH | 179.4 | 174.1 |
| 1,4-Benzodioxan-2-$COCH_3$ | 194.8 | 194.9 |
| 1,4-Benzodioxan-2-CHO | 145.8 | 142.5 |
| 1,4-Benzodioxan-2-$CH_3$ | 179.7 | 179.9 |
| 1,4-Benzodioxan-2-CN | 180.9 | 184.1 |
| 1,4-Benzodioxan-2-$NO_2$ | 140.3 | 139.7 |

La ecuación [3] de Potencial de Morse se ajusta a los datos computacionales obtenidos y permite obtener resultados aceptables para la energía de disociación de enlace, como se observa en la Tabla 6 y Figura 7.

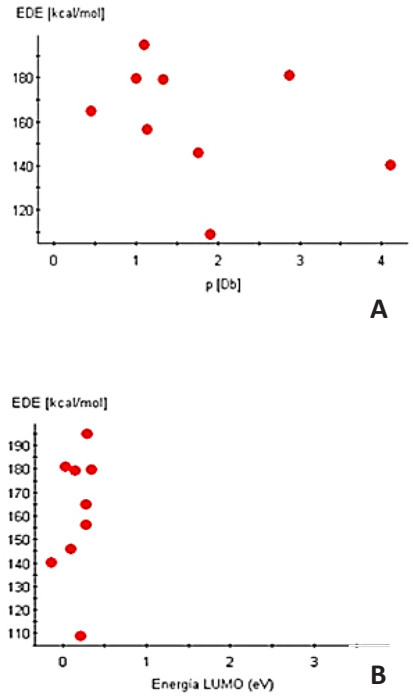



**Figura 6**: Variación del Calor de Formación con el momento dipolar (A) y la energía LUMO (B) [ Tablas 4 y 5].

**AGRADECIMIENTOS**

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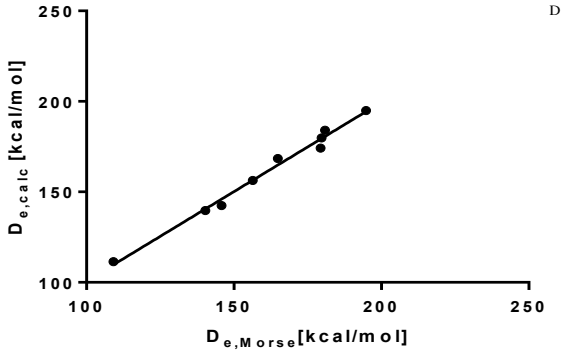


| $D_{e,Morse}$[kcal/mol] | $D_{e,calc}$[kcal/mol] |
|---|---|
| 164.9 | 168.395 |
| 156.4 | 156.344 |
| 179.7 | 179.852 |
| 145.8 | 142.465 |
| 180.9 | 184.104 |
| 194.8 | 194.939 |
| 109.1 | 111.38 |
| 140.3 | 139.696 |
| 179.4 | 174.125 |

$$D_{e,calc} = 22.1p + 66.8Hy + 38.0MLOGP2 - 4145.2HyMLOGP2 - 1896.98MLOPGP2\,Hy^2 + 354.1HyMLOGP2^2 + 4.21MUyHyMLOGP2 - 0.38$$

**Figura 7**: Comparación de las energías de disociación de enlace obtenida a través de la Función Potencial de Morse y la energía de disociación de enlace calculada por ecuación [5].

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# Detection of polymorphism in *sorghum* cultivars applying Single Sequence Repeat markers

## Detección de polimorfismos en cultivos de sorgo utilizando microsatélites

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**ABSTRACT**

**Sorghum collections of germplasm have increased throughout the years from different environments and origins. Simple Sequence Repeats (SSR) or microsatellites can identify genetic variation or polymorphisms between landraces and wild types of Sorghum. Polymerase Chain Reaction was used to determine the repetitive regions of SSRs in the plants genome. Sixteen SSR markers detected polymorphisms in cultivars of Sorghum bicolor with a total of 52 alleles. In the present study, a phylogenic tree was constructed using the frequency distance. Three main clusters were observed with each dividing in two sub-clusters. Based on the Polymorphic Information Content, we established a low genetic diversity among the seven cultivars studied.**

RESUMEN

Las colecciones de germoplasma de sorgo han aumentado a lo largo de los años a partir de diferentes ambientes y procedencias. Las secuencias de repeticion sencilla (SSR) o microsatélites pueden identificar variaciones genéticas o polimorfismos entre razas y tipos silvestres de sorgo. La Reacción en Cadena de la Polimerasa (PCR) se utilizó para determinar las regiones repetitivas de SSR en el genoma de las plantas. Dieciséis marcadores SSR detectaron polimorfismos en cultivos de *Sorghum bicolor* con un total de 52 alelos. Utilizando la distancia de frecuencia se construyó un árbol filogenético. Tres grupos principales se observaron y cada uno se dividió en dos sub-grupos. En base al contenido polimórfico se pudo determinar una baja diversidad genética entre los siete cultivos estudiados.

**KEYWORDS** SSR markers, sorghum, polymorphism, PCR

PALABRAS CLAVE marcadores SSR, sorgo, polimorfismo, PCR

## INTRODUCTION

Sorghum [(*Sorghum bicolor (L) Moench*)] is a drought-tolerant plant that originated on the African continent and since then has been exported throughout the world, to places like India, China, Pakistan, and North America (Kimber, C.T., Dahlberg, J.A., and Kresovich, S., 2013; Bekele, W.A., Wieckhorst, S., Friedt, W., and Snowdon, R.J., 2013). Today, it is the fifth most important cereal in the world (Casa et al., 2005; Billot et al., 2013) just below rice, wheat, maize, and potatoes in world consumption. It is consumed for fiber, energy and it does not contain gluten, and can also be used as animal feed, building materials and biofuel (Upadhyaya, H.D., Wang, Y., Sharma, S., Singh, S., and Gustafson, P., 2012).

There are five major cultivated races of *Sorghum bicolor* (bicolor, caudatum, durra, guinea, and kafir) which differ in panicle and grain shape (Casa et al., 2005; Kimber et al., 2013; Billot et al., 2013). There are also intermediate races that have been developed by hybridization or interbreeding of races. This genetic diversity of sorghum is preserve in different germplasm collections. The International Crops Research Institute of the Semi-Arid Tropics (ICRISAT) in India, the United States Department of Agriculture-Agriculture Research Station (USDA-ARS), and the National Center for Genetic Resources Preservation in the USA (Kimber et al., 2013; Billot et al., 2013) have the largest worldwide sorghum collection with more tham 40,000 accessions.

Since sorghum's domestication around 4,000 to 6,000 years ago many cultivar lineages have been developed (Billot et al., 2013; Evans et al., 2013). A method to distinguish the diversity between cultivars is the use of molecular markers like simple sequence repeats (SSR), single-nucleotide polymorphisms (SNP), restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), and randomly amplified polymorphic DNA (RAPD) (Wu, Y.Q., and Yinghua, H., 2007; Billot, et al., 2013). Targeting specific

regions is an effective strategy for differentiation and analysis of cultivars. Therefore, SSRs can be used to genotype accessions from the different composite germplasm collections.

Single sequence repeats, also known as microsatellites (Brown et al., 1996), are nuclear DNA regions that consist of repeating units of bases. They are widely distributed throughout the genome. The repeating units of bases can be mononucleotide, dinucleotide, trinucleotide, tetranucelotide or compound repeats. These regions can be amplified by polymerase chain reaction (PCR), and polymorphism detected in agarose gel electrophoresis. Briefly, the specific primers sets utilized during the amplification process attach themselves to either side of the repeat portions of the SSR, and depending on the size of the microsatellite repeat segments, different bands may be visible in the electrophoresis gel.

Recently, nine SSR markers were used to quantify the genetic diversity among *S. bicolor* genotype of different origin cultivated in Egypt (El-Awady, M., Youseff, S.S., Selim, E.E., and Ghonaim, M.M., 2008). The results indicated that 58% of the SSR markers were polymorphic. Like wise, Casa et al., (2005) used 98 SSR loci to compare 73 landraces (cultivated lines) and 31 wild sorghum. The study showed that cultivated lines retained 86% of the diversity observed in the wild sorghum. The landraces and wild lines were moderately differentiated and that there was little evidence of population differentiation among racial groups of cultivated sorghum.

Billot et al. (2013) studied a large collection of sorghum including 3300 accessions that were genotyped with 41 SSRs markers across all 10 chromosome pairs in the nuclear genome. The highest number of alleles (86.8%) was detected in the accessions of African origin. In addition, among the 783 alleles detected, 35% were observed only in cultivated accessions and 5% only in wild/weed accessions.

The objective of this study was to use 16 SSR markers to determine genetic diversity among seven cultivars of *Sorghum bicolor*.

## MATERIALS AND METHODS

### Plant Materials

Seeds from eight different cultivars: Keller, Acme Broom, QL3 India, SC 929, Dorado, BTx642, SC 66, and Rio, were subjected to the SSR analysis. The seeds were provided by Dr. Hugo E. Cuevas (USDA-ARS: Tropical Agriculture Research Stations, Mayaguez), planted by sets of three in rows of Jiffy-7 pellets, and grown in an exterior environment until the development of the third true leaf.

### Genomic DNA Extraction

Plant tissue was weighted to obtain 0.08 to 0.1g and grinded. Genomic DNA (gDNA) extraction was done using: cell lysis buffer (100mM Tris HCl pH 8.0, 50mM EDTA pH .0, 500mM NaCl, 2% SDS, and 1% polyvinylpyrrolidone-360 (PVP), RNAse (10mg/mL) and 5M KAc pH 6.5 and temperature of 65°C. Proteins were removed using chloroform: isoamil 24:1. DNA precipitation was obtained using isopropanol, -20ºC incubation period and 96% ethanol. gDNA was dried in a chemical fume hood (Captair, Erlab) and stored at -20 ºC. Concentration and purity (ratio 260/280) of gDNA were determined by spectrophotometric analysis (NanoDrop Lite, Thermo Scientific). To confirm the presence of gDNA a 1% agarose gel electrophoresis was run.

### SSR Primers

Sixteen SSR primers distributed across the sorghum genome, and previously used to characterize sorghum germplasm (Billot et al., 2013) were provided by Dr. Hugo E. Cuevas from USDA-ARS: Tropical Agriculture Research Station (TARS) in Mayagüez, Puerto Rico. Forward and reverse primers sequence and repeats can be seen in Table 1.

### DNA Amplification

The PCR reaction was performed using gDNA with a concentration of 25ng/µL and 2X Go Taq Green Master Mix (1.5U Taq polymerase, 3mM MgCl2, 400µM dNTPs, 2X Buffer pH 8.5) (Promega). Forward and Reverse primers had a final concentration of 0.45µM. Each reaction was carried out in a final volume of 8µL. PCR reactions were placed in the thermal cycler (GeneAmp PCR System 2700, Applied Biosystems).

**Figure 1.** *Sorghum bicolor* cultivars and third true leaf.

Cycling initiated with denaturation for 5 minutes at 95ºC. It was followed by, 39 cycles of denaturation for 1 minute at 95ºC, annealing at 60 ºC for 1 minute and extension at 72 ºC for 30 seconds. The final extension was carried out at 72ºC for 5 minutes. A negative control was used to determine DNA contamination. Analysis of the PCR products was realized by a 4% agarose gel electrophoresis. A 100 bp marker was used to

estimate the size of the PCR products.

**Data Analysis**

Genetic diversity (allele number, gene diversity, and polymorphic information content) of the sorghum cultivars was achieved by the use of Power Marker v.3.25 software. Phylogenic tree data was produced based on the Neighbor Joining Method and visualized by the web tool Interactive Tree of Life (iTOL).

**RESULTS**

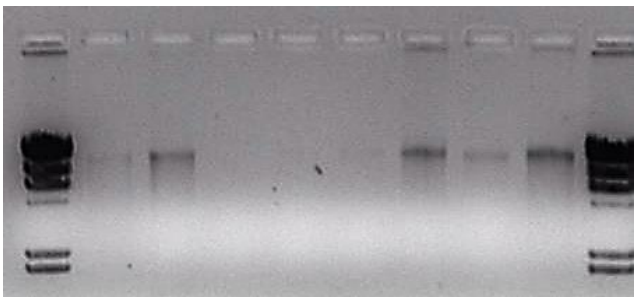

**Figure 2.** Electrophoresis 1% agarose gel of gDNA extraction of *Sorghum bicolor* cultivars. Lamba/HindIII marker (lane M), Keller (lane 1), Acme Broom (lane 2), QL3 India (lane 3), SC 929 (lane 4), Dorado (lane 5), BTX 642 (lane 6), SC 66 (lane 7), and Rio (lane 8).

**Table 1.** gDNA extraction concentration and 260/280 ratio.

| Sorghum | Weight (g) | Initial concentration (ng/µL) | 260/280 Ratio |
|---|---|---|---|
| Keller | 0.1066 | 24.5 | 1.80 |
| Acme Broom | 0.1251 | 47.0 | 1.74 |
| QL3 India | 0.0541 | 13.3 | 1.88 |
| SC 66 | 0.1130 | 54.1 | 1.86 |
| SC 929 | 0.1046 | 57.4 | 1.83 |
| SC 317 | 0.0834 | 40.4 | 1.76 |
| BTX-642 | 0.0836 | 531.4 | 1.90 |
| Dorado | 0.1025 | 12.7 | 1.70 |
| Rio | 0.0811 | 184.8 | 1.80 |

**DISCUSSION**

Genomic DNA was obtained from all cultivars. BTX 642 cultivar had the highest concentration (531.4ng/µL) and purity (1.90). The second highest concentration (184.8ng/µL) with a 260/280 ratio of 1.80 was Rio. Dorado (12.7ng/µL) and India (13.3ng/µL) were the cultivars with lowest concentration. India was the cultivar with the slowest growth in Jiffy 7.

All sixteen SSR markers detected polymorphism in individuals as seen in previous studies of sorghum (Casa et al., 2005; El-Awady et al., 2008,; Billot et al., 2013). By quantifying the number of resulting alleles from the polymorphic data a statistical matrix was produced. A total of 52 alleles were detected with an average of 3.25 allele per locus. The number of alleles per marker ranged from 2 (Xcup 62) to 4 (mSbCIR 248, mSbCIR 276, gpsb123, Xcup53, and Xcup63). The PIC value depends on the number of alleles of the marker and its relative frequency. PIC values ranged from 0.32 in Xcup 62 marker to 0.64 in mSbCIR 248, gpsb123 and Xcup63 markers with an average across all markers of 0.53.

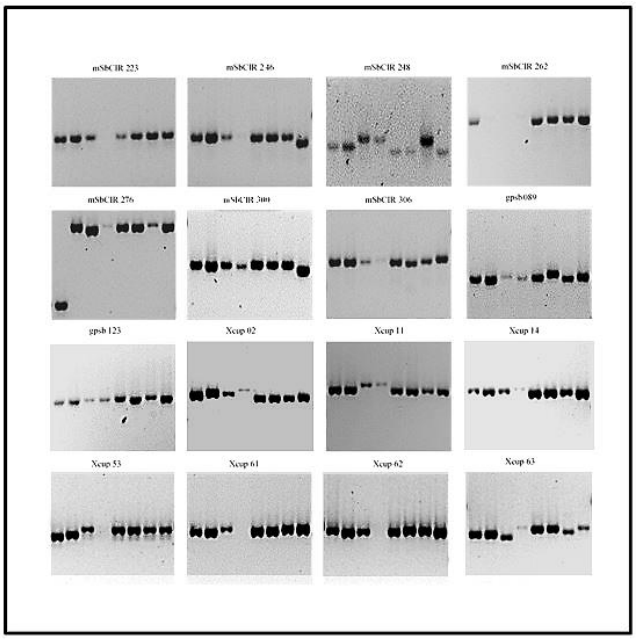


**Figure 3.** Electrophoresis 4% agarose gel of SSR markers products in *Sorghum bicolor* cultivars. Keller (lane 1), Acme Broom (lane 2), QL3 India (lane 3), SC 929 (lane 4), Dorado (lane 5), BTx 642 (lane 6), SC 66 (lane 7), and Rio (lane 8). Polymorphism is detected among the eight cultivars using sixteen SSR primers.

Phylogenic tree data was produced based on the Neighbor Joining Method and using the frequency distance matrix. In the present study, three main clusters were clearly observed. The first cluster has two sub-clusters which contain the Keller and Acme Broom cultivars. The second main cluster also has two subclusters divided into SC 66 and QL3 India. The third main cluster contains two sub-clusters with the first one being of BTx 642 and the second sub-cluster has two branches of Rio and Dorado.

We confirmed gDNA extraction from the different cultivars using agarose gel electrophoresis and spectrophotometric analysis. Polymorphism in *S. bicolor* cultivars was detected using all 16 SSR markers. Phylogenetic relationships were established between cultivars.

**Table 2.** SSR markers characteristics and statistical analysis.

| Marker | Sequence | Repeat | Allele No. | Gene Diversity | PIC |
|---|---|---|---|---|---|
| mSbCIR223_F | CGT TCC AAT GAC TTT TCT TC | (AC)6 | 3 | 0.6531 | 0.5798 |
| mSbCIR223_R | GCC AAT GTG GTG TGA TAA AT | | | | |
| mSbCIR246_F | TTT TGT TGC ACT TTT GAG C | (CA)7.5 | 3 | 0.5714 | 0.5015 |
| mSbCIR246_R | GAT GAT AGC GAC CAC AAA TC | | | | |
| mSbCIR248_F | GTT GGT CAG TGG TGG ATA AA | (GT)7.5 | 4 | 0.6939 | 0.6414 |
| mSbCIR248_R | ACT CCC ATG TGC TGA ATC T | | | | |
| mSbCIR262_F | GCA CCA AAA TCA GCG TCT | (CATG)3.25 | 3 | 0.6531 | 0.5798 |
| mSbCIR262_R | CCA TTT ACC CGT GGA TTA GT | | | | |
| mSbCIR276_F | CCC CAA TCT AAC TAT TTG GT | (AC)9 | 4 | 0.6122 | 0.5698 |
| mSbCIR276_R | GAG GCT GAG ATG CTC TGT | | | | |
| mSbCIR300_F | TTG AGA GCG GCG AGG TAA | (GT)9 | 3 | 0.449 | 0.4065 |
| mSbCIR300_R | AAA AGC CCA AGT CTC AGT GCT A | | | | |
| mSbCIR306_F | ATA CTC TCG TAC TCG GCT CA | (GT)7 | 3 | 0.5714 | 0.5015 |
| mSbCIR306_R | GCC ACT CTT TAC TTT TCT TCT G | | | | |
| gpsb089_F | ATC AGG TAC AGC AGG TAG G | (TG)9 | 3 | 0.5714 | 0.5015 |
| gpsb089_R | ATG CAT CAT GGC TGG T | | | | |
| gpsb123_F | ATA GAT GTT GAC GAA GCA | (CA)7 + (GA)5 | 4 | 0.6939 | 0.6414 |
| gpsb123_R | GTG GTA TGG GAC TGG A | | | | |
| Xcup02_F | GAC GCA GCT TTG CTC CTA TC | (GCA)6 | 3 | 0.449 | 0.4065 |
| Xcup02_R | GTC CAA CCA ACC CAC GTA TC | | | | |
| Xcup11_F | TAC CGC CAT GTC ATC ATC AG | (GCTA)4 | 3 | 0.5714 | 0.5015 |
| Xcup11_R | CGT ATC GCA AGC TGT GTT TG | | | | |
| Xcup14_F | TAC ATC ACA GCA GGG ACA GG | (AG)10 | 3 | 0.6531 | 0.5798 |
| Xcup14_R | CTG GAA AGC CGA GCA GTA TG | | | | |
| Xcup53_F | GCA GGA GTA TAG GCA GAG GC | (TTTA)5 | 4 | 0.6122 | 0.5698 |
| Xcup53_R | CGA CAT GAC AAG CTC AAA CG | | | | |
| Xcup61_F | TTA GCA TGT CCA CCA CAA CC | (CAG)7 | 3 | 0.5714 | 0.5015 |
| Xcup61_R | AAA GCA ACT CGT CTG ATC CC | | | | |
| Xcup62_F | CGA GAA GAT CGA GAG AAC CC | (GAA)6 | 2 | 0.4082 | 0.3249 |
| Xcup62_R | TGA AGA CGA CGA CGA CAG AC | | | | |
| Xcup63_F | GTA AAG GGC AAG GCA ACA AG | (GGATGC)4 | 4 | 0.6939 | 0.6414 |
| Xcup63_R | GCC CTA CAA AAT CTG CAA GC | | | | |
| **Total** | | | 52 | - | - |
| **Mean** | | | 3.25 | 0.5893 | 0.5280 |
| **Min** | | | 2 | 0.4082 | 0.3249 |
| **Max** | | | 4 | 0.6939 | 0.6414 |

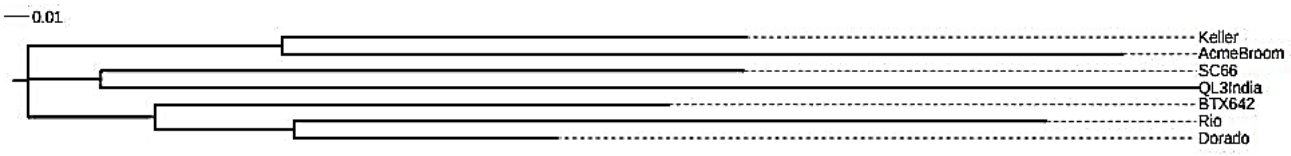


**Figure 4.** Dendrogram of seven *Sorghum bicolor* cultivars constructed from 16 SSRs markers using Power Marker and interactive Tree of Life (iTOL).

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# Habitat use of harvestmen (*Neocynortoides sp.)* at Mata de Plátano Reserve in Arecibo, Puerto Rico

## Uso del Hábitat por parte de los opiliones *(Neocynortoides sp.)* en la Reserva de Mata de Plátano en Arecibo, Puerto Rico

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**ABSTRACT**

***Neocynortoides sp.* is a poorly studied species found in Puerto Rico that belongs to class Arachnid, order Opiliones. Among many aspects of the ecology and natural history of this organism, little prior research has been conducted on *Neocynortoides sp.* habitat use. In our project, we studied the substrate use of this harvestman species in different areas of Mata Plátano Natural Reserve in Arecibo, Puerto Rico. We chose to contrast substrate use in three different areas: a haystack hill, a reforested area, and an open area. The study was conducted during January 12 and 13, 2015 approximately around 8:00-11:00 pm. Five transects of 20 m² were made in each area. We searched by hand the litter, bush, buttress roots, rock, and trees. We observed an average of 10.2 *Neocynortoides sp.* in the haystack hill, and their primary use was the substrate trees. In the reforested area we observed an average of 1.25 *Neocynortoides sp.* using the litter. In the open area we detected an average of 2.4 harvestmen that used bushes as a primary shelter. Consequently, we concluded that *Neocynortoides sp.* are more abundant in the haystack hill since that area has higher humidity percentage and has a higher canopy cover, which harvestmen tend to use for shelter.**

RESUMEN

*Neocynortoides sp*. pertenece a la clase de los Arácnidos, orden Opiliones. Es una especie poco estudiada que se encuentran en Puerto Rico. Entre los muchos aspectos de la ecología y de la historia natural de este organismo, poca investigación previa se ha realizado sobre *Neocynortoides sp*. relacionado al uso del hábitat. En nuestro proyecto, hemos estudiado el uso de sustratos de esta especie de Opilión en diferentes áreas de la Reserva Natural Mata de Plátano en Arecibo, Puerto Rico. Elegimos para contrastar el uso de sustrato tres áreas diferentes: un mogote, un área reforestada, y un área abierta. El estudio se realizó el 12 y 13 de enero de 2015 entre las 8: 00-11:00pm. En cada área se realizaron cinco transectos de 20 m². La búsqueda se llevó a cabo a mano en la hojarasca, arbustos, raíces contrafuerte, rocas y tronco de árboles. Se observó un promedio de 10.2 *Neocynortoides sp*. en el mogote y su substrato principal fueron los árboles. En el área reforestada se observó un promedio de 1.25 *Neocynortoides sp*. y fueron observados mayormente sobre la hojarasca. En la zona abierta se detectó un promedio de 2.4 organismos y el substrato más utilizado fueron los arbustos. En conclusión, *Neocynortoides sp*. fue más abundantes en los mogotes que posee un porcentaje de humedad mayor y tiene una cubierta de dosel superior, en la que los opiliones pueden utilizar como refugio.

**KEYWORDS** *Neocynortoides sp*, canopy cover, substrate, Opilionids

PALABRAS CLAVE *Neocynortoides sp*, cobertura de dosel, substrato, Opiliones

**INTRODUCTION**

Karst is a limestone region that covers a 58% of Puerto Rico (Ewel and Whitmore, 1973). The northern karst region is covered by sub-tropical moist forest life zone. This region harbors rich flora and fauna, with many endangered and migratory species, freshwater aquifer, and caves. The harvestmen (Arachnida: Opiliones) have been richly studied in other regions, but relatively little research on this order has been conducted in Puerto Rico. Sometimes known as 'daddy long legs,' harvestmen is a species rich group consisting of four suborder, 45 families, approximately 1,500 genera, and between

six and ten thousand species, depending on the account (Pinto-da-Rocha, Machado, and Giribet, 2007).

In general, harvestmen are known to forage nocturnally. They are very selective in their habitat use and seek shelter in moist areas. For this reason, by most accounts during the day, they take refuge in moist areas (Curtis and Machado, 2007). The most common areas to find the harvestmen are litter, shrubs and, trees. These areas provide protection from winds, sun exposure and, rainfall (Burns, Hunter, and Townsend, 2007). Adults are usually found on trunks, buttress roots, and branches and spend from weeks to months in these areas finding food and mates for reproduction. Harvestmen mostly feed on insects, worms, and plant material, such as fruits and flowers.

One of harvestmen species found in Puerto Rico and relatively common in our research area is the species *Neocrynortoides sp*. (Figure 1). The purpose of this study is to compare substrate use of *Neocrynortoides sp*.in different habitat types within the northern mesic karst region of Puerto Rico. We conducted all our investigation at the Mata de Plátano Reserve in Arecibo, Puerto Rico.

## MATERIALS AND METHODS

### Study site

Mata de Plátano Natural Reserve is a private nature reserve managed by the Inter American University of Puerto Rico, Bayamón Campus and located in the north-central karst in Arecibo, Puerto Rico. The reserve has a total annual rainfall of approximately 1400mm (Ewel and Whitmore, 1973). The reserve is composed of 130 acres and has areas with multiple successional stages of moist forest, including mature forest, young forest, and open areas.

### Data collection

We collected the data for this study on January 12 and 13, 2015, between the hours of 8:00-11:00 pm at Mata de Plátano Natural Reserve in Arecibo. We conducted five transects of 20 $m^2$ (10 m in length, and 2 m wide) at each sample site. Our sampling areas were as follows (and in order of decreasing forest maturity): haystack hill (mogote), reforested area, and open area. We surveyed each transect by intensely searching the litter, buttresses, rock, and trees. Any trees present were examined throughout all three sites up to a height of 2 m. For each individual *Neocynortoides sp*. that was observed on a tree, we measured the diameter at breast height (DBH) of the tree. We also measured canopy cover three times for each transect (at 0 m, 5 m, and 10 m) with a spherical densitometer. We also recorded the temperature and relative humidity once at each sample site.

### Data analysis

We used the R software to conduct all of our statistical analyses. We used analysis of variance (ANOVA) to test the hypothesis that harvestman abundance differed between different habitat types. We used analysis of covariance (ANCOVA) to determine if there is was any relation between harvestman abundance and canopy cover, controlling for habitat type.

**Figure 1**. Harvestmen (*Neocrynetoides sp*.) on a fern.

## RESULTS

We encountered a total of 77 *Neocynortoides sp*. in all our sampling transects. In general, we found that harvestmen were more abundant in the haystack hill site than in our other habitat types (Figure 2). Summarizing all transects in each habitat type, we found 43 *Neocynortoides sp*. in the haystack hill, 12 in the open area, and 19 in the reforested area. The temperature for the haystack hill was 22.9°C and a relative humidity of 96%, the reforested area a temperature of 23.9°C and a relative humidity of 88.30%, and open area a temperature of 23.9°C and relative humidity of 82.70%. The average number of *Neocynortoides sp*. per 20$m^2$ transects was approximately 7 at the haystack hill, 1.25 at the reforested area, and 2.4 at the open area. We observed harvestmen using different substrates at each study site.

At the haystack hill the species used trees, rocks, leaf litter, and bushes. In the reforested area they used leaf litter and trees, and in the open area they were found on bushes and trees (Figure 3). The canopy cover in the three areas did not have any significant effect on the number of harvestmen sampled ($F= 3.85$, $p= 0.08$).

Based on a one way-analysis of variance (ANOVA) with all the data, we found no significant differences in an abundance between habitat types (ANOVA, $F=2.095$, $p=0.1658$). However, after identifying one outlier in harvestman abundance in the open area transects (Figure 4) and removing that datum, we were able to see a strong effect of habitat type on harvestman abundance (ANOVA, $F=11.087$, $p=0.00290$). Canopy cover has no

influence in the numbers of harvestmen observed (ANCOVA, F= 3.85, p= 0.08).

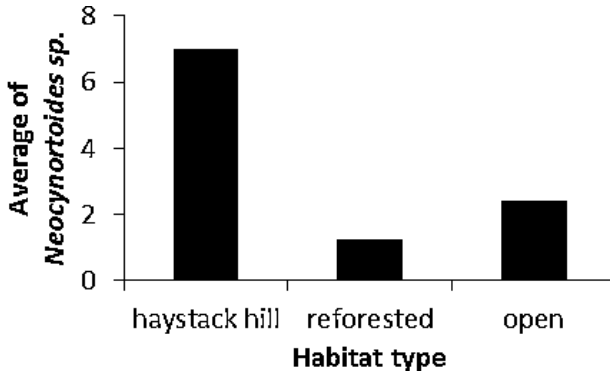


**Figure 2.** Average Number of *Neocynortoides sp*. in haystack area, reforested area, and open area**.**

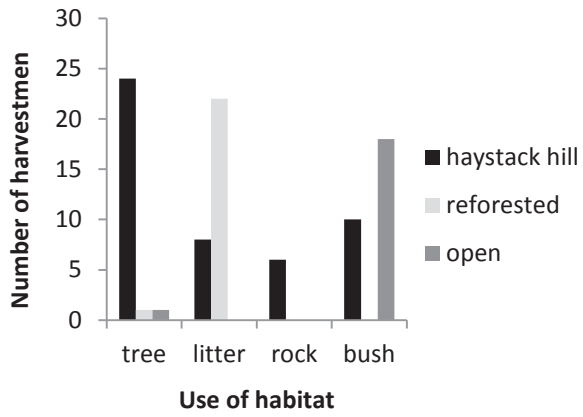


**Figure 3.** Numbers of *Neocynortoides sp*. observed in substrates at different habitats.

## DISCUSSION

Harvestmen, although better studied in other regions, have been subject to relatively little research on the island of Puerto Rico. In this project, we studied substrate use and abundance of the harvestman *Neocynortoides sp*. in different types of habitat at the Mata de Plátano Reserve in northwestern Puerto Rico. We found that abundance differed significantly between habitat types. In particular, we found that harvestmen were more abundant in the more mature forests of the haystack hills than in open areas or in younger secondary forest (Burns et al., 2007).

We found that in open areas the harvestmen tended to use bushes as substrates. This is a behavior not found in the other habitat types; however, we hypothesize that it is due to differential habitat availability (trees are absent from open areas entirely) rather than active habitat selection. Another unique aspect of open areas in our study was that it was the only site in which species other than our focal taxon were encountered. This should be investigated in future research, as it is possible that these species may compete with *Neocynortoides sp*. In reforested areas, we observed harvestmen using litter. It's possible that this behavior, which we did not see in our other habitat types, may have been an idiosyncratic effect of the weather conditions of the sampling day.

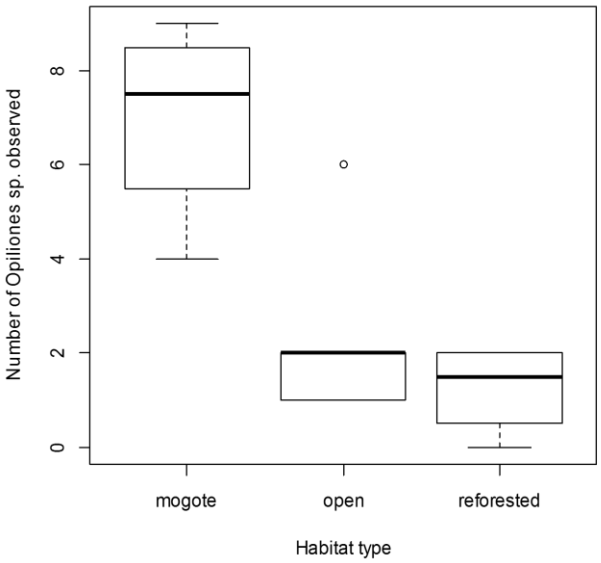


**Figure 4.** Boxplot showing the number of *Neocynortoides sp*. in different habitat type.

In conclusion, we found high abundance of *Neocynortoides sp*. in the haystack hill habitat, which may due to the high humidity of this area creating a favorable microclimate for the harvestmen. By contrast, there was no significant difference in abundance found between open and reforested areas. Future research should focus on other factors which might affect abundance and distribution of *Neocynortoides sp*., including the effects of interspecific competition with other harvestmen.

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Son estudiantes de la Universidad Interamericana de Puerto Rico, Recinto de Arecibo y de la Universidad de Massachuseetts en Boston, respectivamente. Participaron del curso de Ecología Tropical Caribeña en Puerto Rico durante el invierno de 2015. Pueden ser contactados a través de sus mentores el Dr. Liam Revell o el Dr. Alberto Puente-Rolón al: apuente@ arecibo.inter.edu

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# Influence of canopy cover and leaf litter depth on the tree snail, *Nenia tridens*, in El Yunque National Forest, Puerto Rico

## Influencia del dosel y de la densidad de hojarasca en el caracol terrestre, *Nenia tridens*, en el Bosque Nacional El Yunque, Puerto Rico

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**ABSTRACT**

**Although some prior studies have investigated the influence of disturbance and leaf litter density on the abundance of the terrestrial snail, *Nenia tridens*, much is still to be learned about what factors influence the relative abundance of this species across the landscape. In this study, we investigated the abundance of *N. tridens* in different sites consisting of mature and young forest. We also measured canopy cover and leaf litter depth, to attempt to determine if these factors might influence the relative abundance of this species. We found no evidence of influence of forest maturity, leaf litter depth, or canopy cover on *Nenia tridens* in our survey.**

RESUMEN

Aunque estudios previos han investigado la influencia de la perturbación y la densidad de hojarasca en la abundancia del caracol terrestre, *Nenia tridens*, todavía queda mucho por aprender acerca de los factores que influyen en la abundancia relativa de esta especie a través del paisaje. En este estudio, se determinó la abundancia de *N. tridens* en bosque maduro y joven. También se midió la cobertura del dosel y la profundidad de la hojarasca, para tratar de determinar si estos factores pueden influir en la abundancia relativa de esta especie. No se encontró relación entre la madurez del bosque, la profundidad de la hojarasca, o cubierta del dosel con la densidad de *Nenia tridens*.

**KEYWORDS** land snails, canopy cover influence, density, leaf litter depth effect

PALABRAS CLAVE caracoles terrestres, influencia del dosel, densidad, efecto de la profundidad de hojarasca

## INTRODUCTION

Puerto Rico is a tropical island in the Greater Antilles harboring a range of habitats of a variety of types. The wettest terrestrial ecosystem found on the island is tropical rainforest, found in the El Yunque National Forest in the Luquillo mountain range of northeastern Puerto Rico. El Yunque is characterized by very high rainfall of up to 5,000 mm per year, typical for tropical rain forests, and shares many other attributes in common with its continental counterparts. El Yunque, however, though rich in animal life, does not support the same vertebrate animal abundance as would a rainforest in Costa Rica or South America. El Yunque is rich in invertebrates and other species, such as 34 species of terrestrial snail species, many of which are endemic to the island or the region (Miller and Lugo, 2009). Snails are a vital part of this tropical ecosystem. They are largely detrivores, recycling the dead matter of plants. One species, *Nenia tridens* (Figure 1), is particularly abundant in the El Yunque forest. This is an arboreal terrestrial snail, which is active at night as is typical of most other species found in this area (Bloch and Willig, 2009). Since *N. tridens* is a detritivore that feeds on decomposing plant material, we hypothesized that factors which might influence plant species composition or productivity would also likely influence the health and success of this species.

Disturbances in this region are varied in nature. For instance, the forest is subject to continued irregular disturbance by major hurricanes. In addition, human disturbance to the island of Puerto Rico is widespread and intense. Our study area, however, has long been protected as part of the Luquillo Experimental Forest, limiting anthropogenic disturbance to the area by removing the effect of human land use. Prior study of this species showed that hurricanes, as one would expect given their capacity to influence the vegetative attributes of an area, can also influence the distribution and abundance of plant detritivores, such as *Nenia tridens* (Alvarez and Willig, 1993). For instance, prior research

following Hurricane Hugo, which caused intense defoliation throughout the El Yunque Forest, found that *Nenia tridens* was significantly more abundant in canopy gaps then under a closed canopy (Bloch, 2012; Willig et al., 1998). Two possible explanations for this observation come immediately to mind. One possibility is that *N. tridens* prefer an open canopy, but a second is that this finding is merely a correlative effect of canopy gap areas harboring higher leaf litter abundance. The objective of this study, therefore, is to try and disentangle the effects of leaf litter depth, canopy cover, and forest maturity on the abundance of this common terrestrial rainforest snail, *Nenia tridens*.

**Figure 1**. *Nenia tridens*.

## MATERIALS AND METHODS

We conducted the data collection for this study from El Verde Field Station in the Luquillo Experimental Forest (El Yunque National Forest) in northeast Puerto Rico. All data collection was conducted between the 6$^{th}$ and the 9$^{th}$ of January, 2015. Average temperature during the data collection period of our study was around 19 $^{O}$C, and the humidity was approximately 78 %. The elevation of El Verde Station is approximately 500 m and the forest type of the area is characterized as Tabonuco (*Dacryodes excels*) forest, although *D. excels* does not dominate the tree species composition of the area. We collected data on snail abundance in two distinct sites: mature forest containing mostly older trees; and a younger secondary forest.

Our data collection design consisted of 20 transects of dimension 10 m by 2 m (20 m$^2$). We performed 13 of these transects through mature forest and the remainder (seven) in young forest. We measured each transect with a metric transect tape and then marked the beginning and the end of it with colored tape. This way we could easily walk the transect to search all trees for *Nenia tridens* individuals.

As we encountered trees with *N. tridens* during our transect survey, we proceeded to count all the *N. tridens* encountered. We also measured the canopy cover and leaf litter depth. To measure canopy cover, we used a spherical densitometer, taking four measurements facing in four different, orthogonal directions of the tree. We measured the leaf litter once by each tree with a standard plastic ruler. In addition, we used a basic temperature and hydrometer to measure the temperature and humidity once during each transect. For those transects in which we did not find any *N. tridens,* we measured the temperature, humidity, canopy cover, and leaf litter once for the transect. For each transect, we averaged the canopy cover and leaf litter measures, and computed a cumulative total of *N. tridens* abundance for the transect.

We conducted all data analysis using the R statistical software. We conducted one-way ANOVA, linear regression, and ANCOVA to test for the effects of forest type, leaf-litter, canopy cover, and all three factors combined on *N. tridens* abundance on our transects.

## RESULTS

We encountered a total of 69 *Nenia tridens* over all of our 20 transects of this study. We found no evidence that forest type influenced *N. tridens* abundance [mean (mature) = 3.69, mean (young)= 2.85, F= 0.23, P= 0.64; Figure 2]. Furthermore, neither did we find a significant effect of either canopy cover [β= -0.03, F= 0.17, P= 0.68; Figure 3], or leaf litter depth [β= -0.12, F= 0.54, P= 0.47; Figure 4] on *N. tridens* relative abundance across transects of this study. In addition, a linear (ANCOVA) model with all three factors included failed to find any significant explanatory power for *N. tridens* abundance (F= 0.39, P= 0.76).

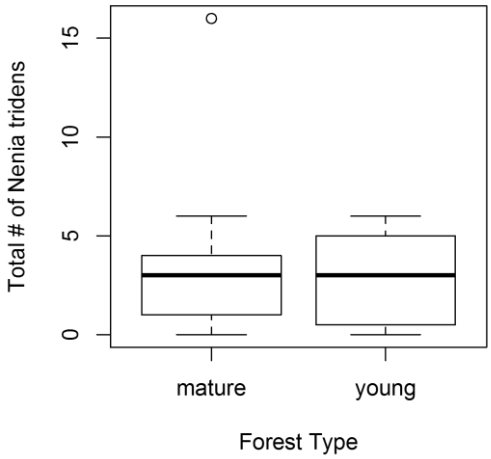


**Figure 2.** *Nenia tridens* abundance by forest type.

## DISCUSSION

In the present study, we wanted to understand what environmental and ecological factors might influence variability in *Nenia tridens* abundance across sites. Specifically, we wanted

to determine if canopy cover, leaf-litter abundance, and/or forest maturity might influence *N. tridens* density. Our impetus for collecting data on these variables was the fact that a prior study had been published showing that *N. tridens* abundance was increased in defoliated areas after Hurricane Hugo.

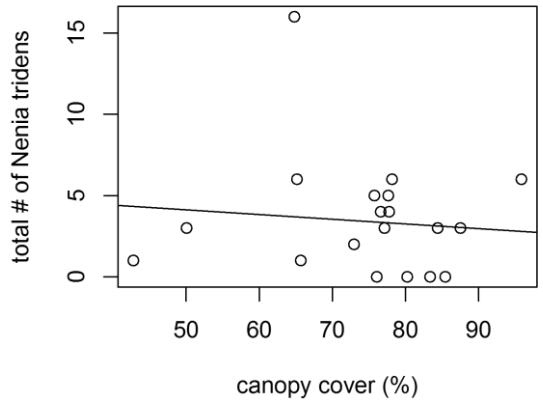


**Figure 3.** *Nenia tridens* abundance is weakly negative, but non-significantly, related to canopy cover density.

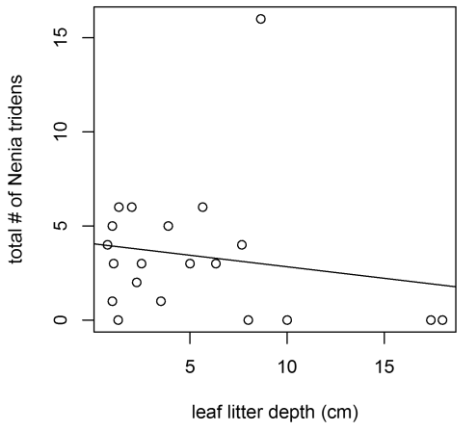


**Figure 4.** *Nenia tridens* abundance is weakly negatively, but once-again non-significantly, related to leaf litter depth.

We hypothesized that this change in abundance might be due to increased leaf litter abundance in defoliated areas or due to the canopy opening itself, and our study was designed specifically to differentiate these alternatives.

In our study we were neither able to affirm or refute prior research. Specifically, we did not find evidence for an influence of forest maturity, canopy cover, or leaf litter depth on local *N. tridens* abundance in our transects. Although we were somewhat surprised by this result it is possible that it shows that forest maturity differences between two areas we chose for this study are not of great significance to this species. This finding might change if we were to compare forests of more dramatically different maturity. We also failed to find an effect of canopy cover. This might be due to measuring canopy cover on a narrower range than full defoliation to 100% canopy cover, as in the Hurricane Hugo case. Finally, we did not find any effect of leaf litter depth. Once again, this may be because leaf litter depth is inconsequential to this species, or simply because our transects did not vary sufficiently in leaf litter abundance to influence *N. tridens* success.

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# Effect of location and an abundant food resource on body condition of the cane toad (*Rhinella marina)* in Puerto Rico

## Efecto de la localización y el recurso abundante de alimentos en la condición corporal del sapo de la caña (*Rhinella marina*) de Puerto Rico

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**ABSTRACT**

**Body condition is an easily obtained measurement which can be computed from an animal's mass and length and used as a proxy measure of health and vigor. It is a convenient tool to use when conducting field work because data on body condition are inexpensive and relatively simple to gather, and in most cases, particularly for reptiles and amphibians, the data collection technique poses little to no risk to animal well-being. In this study, we compared body condition between cane toads, *Rhinella marina*, found in two different areas of the Karst region of Puerto Rico. We collected a sample of 83 toads from in and around a cave harboring a large population of bats of multiple species and from a second sample site consisting of a large open area surrounding a small pond. We found that, controlling for other confounding factors such as sex, toads collected in or near the cave had significantly higher body condition than those collected from the non-cave site. An untested but sensible explanation for this pattern may be that the extremely high abundance of cockroaches found in the cave and nearby may be supplying a highly accessible, nutrient rich food source to the toads using this habitat. Understanding the behavior of *R. marina* is important to help control the exploding populations of this exotic species on the island of Puerto Rico and to help protect the endemic and native species of the island which are being threatened by the presence of the cane toad.**

**RESUMEN**

La condición corporal es una medida fácil de obtener y que se puede calcular a partir de la masa de un animal y su longitud. Esta medida se utiliza como una medida aproximada de la salud y el vigor. Es una herramienta fácil de usar al llevar a cabo el trabajo de campo porque los datos sobre la condición corporal son de bajo costo y relativamente fáciles de recoger. En la mayoría de los casos, en particular para los reptiles y los anfibios, la técnica de recolección de datos plantea poco o ningún riesgo para el bienestar animal. En este estudio se comparó la condición corporal entre individuos de sapos de caña, *Rhinella marina*, en dos áreas diferentes de la región caliza de Puerto Rico. Se tomó una muestra de 83 sapos dentro y alrededor de una cueva que alberga una gran población de múltiples especies de murciélagos y un segundo lugar muestreado consistió de un área abierta cubierta de pastos y con un pequeño estanque. Se encontró que los sapos recogidos en o cerca de la cueva tenían una condición corporal significativamente mayor que los recogidos en el área abierta. Una posible explicación para el patrón observado, puede ser presencia de una extremadamente alta abundancia de cucarachas en el interior de la cueva y sus cercanías. Esto representa una rica fuente de nutrientes muy accesible para los sapos que utilizan este hábitat. Entender el comportamiento de *R. marina* es importante para poder desarrollar estrategias de manejo y control de esta especie exótica en la isla de Puerto Rico y para contribuir a proteger las especies endémicas y nativas de la isla que se encuentran amenazadas por el sapo de Caña.

**KEYWORDS** exotic species, cane toad, body condition index, invasive species

PALABRAS CLAVE especies exóticas, sapo de la caña, índice de condición corporal, especie invasivas

**INTRODUCTION**

Many different parameters can be used to summarize a wild animal's health and physical condition. Some of the techniques available to determine an animal's physical health can be expensive and time consuming, which may make them impractical in a field setting. While something like a blood sample could more accurately determine specifics about the condition of an animal, this requires an invasive procedure and could be detrimental to the health of the animal or stressful (Wilemsen and Hailey, 2002). One way to help solve this problem is by using a measurement of body condition.

Measuring body condition is an inexpensive and less invasive way to estimate the relative health of the animal. To measure body condition, the mass of an animal is measured and then this quantity is regressed on overall body size. The (residual) deviation of mass regressed on size, with both measured normally on a logarithmic scale, gives us a measure of the physical robustness of any individual compared to the population average.

Although this measurement only gives a snapshot of the animal's overall health, it can be a great indicator of past and future food foraging success and the animal's ability to deal with environmental pressures (Jakob, Marshall, and Uetz, 1996). An animal with better overall body condition might correlate well with total reproductive rate, particularly if reproduction is energetically costly (Jakob et al., 1996). For example, in *Rhinella marina*, commonly known as the cane toad, larger body size in males may help the males to outcompete other males during the competition for a female, and may also help the male keep the female in place during amplexus (Bowcock, Brown, and Shine, 2013). Higher body condition for females may mean that she has more energy to dedicate towards the production of eggs.

*Rhinella marina* was introduced to the island of Puerto Rico between the years of 1920 and 1926 as a biological control agent. The intended purpose of releasing this exotic species to the island was to attempt to reduce the number of white grubs present on the island, which were responsible for the destruction of sugar cane plants (Rivero, 1998). Although they did help decrease the number of white grubs, as is the case in many intentional introductions, there were unforeseen consequences (Rivero, 1998). Since their initial introduction, the cane toads have become an extremely invasive species and are out-competing many endemic and native species of Puerto Rico, such as the highly endangered Puerto Rican crested toad, *Peltophryne lemur*. Due to the toxic paratoid glands on the dorsal surface of its back, *R. marina* has very few natural predators and has been able to invade many different habitats, from highly disturbed to relatively pristine, throughout the island.

In the present study, we will compare the body condition of cane toads found at two different sites in the northern mesic karst region of northwestern Puerto Rico in the municipality of Arecibo. We will measure body condition from individuals found in and around a cave, Cueva de los Culebrones, well known to harbor a large population of some 300,000 or more bats belonging to multiple species native to the island. Bats produce abundant guano where they roost, and abundant guano can support very large populations or macro-invertebrates, such as insects and in particular cockroaches (order Blattodea) that constitute the majority of the diet of cane toads. We also measured body condition from individuals found in and around a relatively nearby open area containing a small pond in the El Tallonal forest reserve. We hypothesized that, controlling for other confounding factors such as sex, individuals collected in or around the cave would have higher body condition on average than individuals encountered in other areas.

Learning about which environments allow the cane toad to flourish is an important area of study to help understand their biology and use this information to control the population and work to save the endemic species of Puerto Rico presently threatened by the highly invasive, *Rhinella marina*.

**Figure 1**. Adult Cane Toad (*Rhinella marina*). (Wimborne, T)

## MATERIALS AND METHODS

We collected a random sampling of 83 *Rhinella marina* from the two different locations in the mesic northern karst region of Puerto Rico. This region is characterized by large areas of soluble limestone which has been dissolved away by water over time, creating a unique landscape called a Karst Zone or 'haystack hill' landscape consisting of dramatic hills and sinkholes (Miller and Lugo, 2009). The dissolution of calcium carbonate has also created a system of many caves and underground streams with their own unique characteristics and microclimates (Miller and Lugo, 2009).

The two sites used to collect cane toads were chosen purposefully and each had its own unique characteristics. The first site chosen was the area in and around a cave called *Cueva de los Culebrones* (cave of the snakes). This particular cave was chosen due to the abundance of cane toads found in this area and the marked and quite dramatic abundance of cockroaches found both inside and around the mouth of the cave. We hypothesized a priori that this high cockroach abundance might represent an unusually concentrated and nutrient-rich food source for the toads. Inside the cave it is extremely hot and humid and there are a few species living in the cave including cockroaches, the Puerto Rican boa, bats and the cane toad. The estimated abundance of bats for this cave has been reported to be around 300,000 individuals of several species. This number of bats produces a huge amount of guano which is a food source for

cockroaches. At this site, we collected a total of 41 toads from both inside the cave itself, as well as from the trail and area surrounding the cave entrance. The area around the cave is comprised of a secondary forest.

The second site chosen was a private natural reserve named *El Tallonal*. Here, we collected toads from an open area around a small pond. This pond is used actively by *Rhinella marina* for reproduction. *El Tallonal* reserve is comprised of a more mature, though still secondary forest. We collected a total of 42 toads from this site. At both *Cueva de los Culebrones* and *El Tallonal* we collected all samples in the few hours after sunset, as this is the time period during which the cane toads are most active, and each toad was marked with a number in white out before being released to ensure that the same animal was not sampled multiple times.

After each toad was captured we first determined the sex based on the appearance and texture of the dorsal surface (which is quite distinctly different between males and females of this species), the presence of nuptial pads on the forefeet, and whether or not a release call was produced. Next, we weighed the toad to the nearest gram using a 1 kilogram Pesola spring scale. After weighing each individual, we measured the snout to vent length (SVL) to the nearest 0.1 centimeter using a clear plastic ruler pressed against the ventral side of the toad and measuring from the tip of the snout to the cloacal opening. After the measurements were made the toads were marked and then released back to the location from which they were caught.

We entered all data using Microsoft Excel, and then conducted analyses using the R statistical software. We built two separate linear models to analyze our data. In model 1 we asked how body mass varied as a function of sex, snout vent length (SVL), and site (cave or non-cave). In the second model, we first conducted a linear regression of body mass on SVL and computed the residuals to estimate body condition, and then we used these derived body condition values to fit a model in which body condition varied as a function of sex and site.

**RESULTS**

At the first site, *Cueva de los Culebrones*, we caught a total of 41 cane toads, with 34 females and 7 males. For both sexes, the average mass of all toads caught at the cave was 353.5g and the average SVL was 13.9 cm. At *El Tallonal*, we sampled a similar number of individuals, 42 toads in total. Of these, 17 were females and the remaining 25 were males. For all the toads of both sexes caught at *El Tallonal* the average mass was 195.4g and the average SVL was 11.8cm.

In model 1, we tested if body mass varied among the individuals as a function of SVL, sex, and collection site. Both the mass and SVL were log transformed. We found that site type [t=-3.139, p=0.00239], sex [t=2.348, p=0.02410] and SVL [t=14.974, p<2e-16], all had significant effects on mass. In particular, controlling for site and sex, we found that males had a mass that was 9.7% larger than a females. This result is somewhat surprising because females are often noticeably larger than males (Figures 2, 3); however, our results show that they are not heavier, when size is taken into account. We also found that, when controlling for sex and SVL, toads found outside of the cave had a 17.4% smaller mass than a toad found near or in the cave.

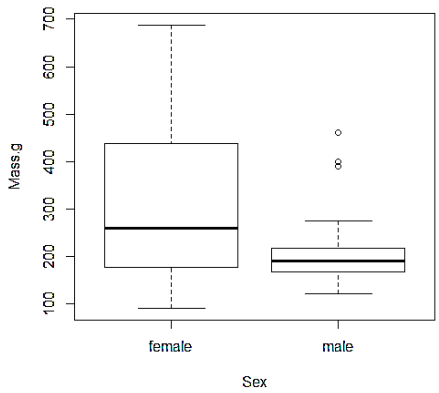


**Figure 2.** The differences in mass values between the sexes of toads, while controlling for SVL and location.

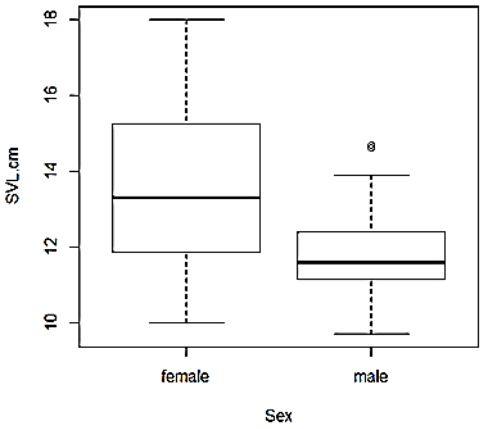


**Figure 3.** This figure shows the difference in SVL between the sexes of toads, while controlling for mass and location where the toad was found, both *El Tallonal* and *Cueva de los Culebrones*.

For the second model, we first computed a measure of body condition for each of the toads. To do this we regressed body mass on SVL and computed the residuals. Figure 4 shows the relationship (on a linear scale – we conducted the regression on a log scale) of SVL and body mass. With the residuals in hand, we next fit a model in which body condition varied as a function of location and sex (SVL and mass were already accounted for in the calculation of body condition). We found that both sex

[t=3.073, p=0.00290] and location [t=-3.054, p=0.00306] had a significant effect on body condition of the toads. Specifically, males had 11.9% better body condition than females, controlling for site (Figure 5). We also found that animals found far from the cave had 12.4% worse body condition on average (Figure 6).

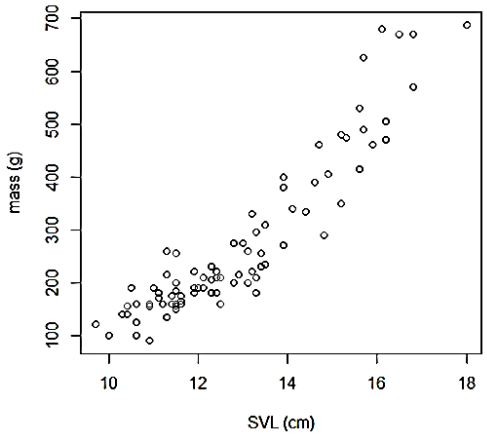


**Figure 4.** SVL as a function of mass. Body condition is computed from the residuals of log-log regression of mass on SVL. For a given SVL measurement, a higher mass value indicates a higher body condition.

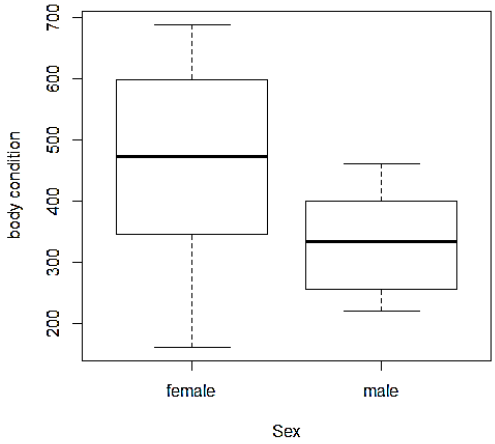


**Figure 5.** The differences in body condition between the sexes of toads found inside the cave only, while controlling for SVL.

## DISCUSSION

In this study, we examined the effects of multiple factors on body condition and overall body mass in the cane toad, *Rhinella marina*. First, we explored the effect of sex on body mass and condition. Though females are often larger than males, we found that (controlling for the effect of overall size and for differences in body condition between sites), males actually had higher body condition and higher mass controlling for size than did females.

In addition, we were interested in testing the hypothesis that overall body condition might differ between localities – due primarily to differences in the availability of food between sites. Specifically, we predicted that toads found in and around a highly active bat cave would have higher body condition on average than individuals found far from this cave. Overwhelmingly, our data supported this prediction. We hypothesize that the markedly greater abundance of food in the form of Blattodea (cockroaches) around the cave is likely responsible for this difference in body condition between site (although we note that we have not provided direct evidence of this assertion).

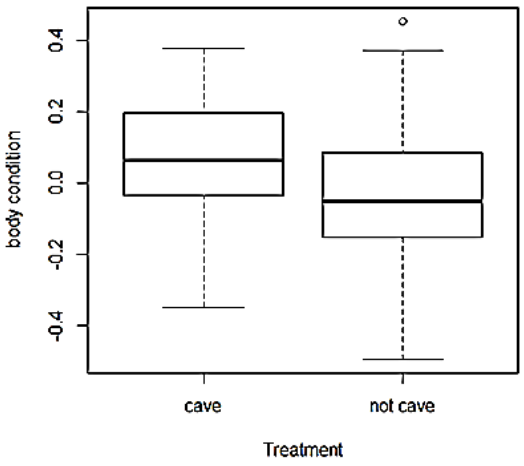


**Figure 6.** The differences in measurements of body condition between the two sites where the toads were found, while controlling for sex.

An interesting finding of this study was that while females had larger mass on average than males, males in fact had better average body condition than females. Although many of the females, both inside and outside the cave had higher masses than the males found in these same areas, the females had lower measurements of SVL than would be expected for their body mass. This means that although many of the females weighed more than the males, the males had a larger SVL given their mass, and thus a higher body condition. This result was found to be significant in both the cave population and non-cave population of toads. One possible reason for this could have to do with the differing energy demands of males and females. Females could have higher energy requirements than males due to reproductive needs and thus have to go out and forage for food more often which could decrease their body condition. This could be another interesting area of study to help understand the cane toad's behavior.

Another interesting point brought up by this study is the matter of proximity to water for reproduction. Cane toads have a tadpole stage of development and a body of water is necessary

for this phase of life. The toads found outside the cave were much closer to a body of water, while the toads found in the cave had no obvious body of water nearby. This could be another possible area of study to try and determine where these cave cane toads are reproducing and to see if this has any effect on body condition. Perhaps, for instance, toads found near the cave are effectively non-reproductive in that season, leading to energy conservation and elevated body condition.

In conclusion, this study did in fact support our initial hypothesis that body condition would be higher in or near an active bat cave, *Cueva de los Culebrones*, than at other sites. There are a number of different factors that could be responsible for this effect and we are not able to definitively disentangle these possibilities presently; however, we propose that the very high abundance of Blattodea near and in the cave may at least be partially responsible.

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# Coral reef biodiversity of Pomacentridae (damselfish) on Vieques Island

## Biodiversidad de Pomacentridae (pez damisela) en el arrecife de coral de la isla de Vieques

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**ABSTRACT**

**Biodiversity is important for ecosystem stability and overall function. Coral reefs are a highly diverse tropical ecosystem, and a prominent family of fish is Pomacentridae, with several different species represented in a typical reef. We conducted a series of visual encounter surveys along multiple transects to measure the biodiversity of pomacentrids within coral reef systems. We chose three different study sites on Vieques Island: one man-made site called Mosquito Pier, and two natural sites called Pata Prieta Beach and La Chiva Beach. We found that two species, the dusky damselfish (*Stegastes fuscus*) and the beaugregory (*Stegastes leucostictus*), were the most abundant overall across all sites. We estimated within-site (alpha) and between-site (beta) biodiversity using the Simpson index and Whittaker's formula, respectively. We found that both alpha and beta diversity was nearly equal for all three sites.**

RESUMEN

La biodiversidad es importante para la estabilidad y función del ecosistema global. Los arrecifes de coral son ecosistemas con una gran diversidad, y prominente familia de peces presentes en este ecosistema es la Pomacentridae conocidos como damiselas. En un arrecife típico se pueden observar varias especies de esta familia. Se realizó una serie de conteos visuales a lo largo de varios transectos para medir la biodiversidad de pomacentridos dentro de los sistemas de arrecifes de coral. Se eligieron tres sitios en la isla de Vieques. Uno de los lugares representa un hábitat hecho por el hombre llamado muelle Mosquito, y dos sitios naturales conocidos como llamados Pata Playa Prieta y Playa La Chiva. Se encontraron dos especies, la damisela oscuro (*Stegastes fuscus*) y la beaugregory (*Stegastes leucostictus*), que fueron las más abundantes en todos los sitios. Se estimó la biodiversidad por lugar y entre lugares utilizando el índice de Simpson y la formula de Whittaker. Se encontró que tanto la diversidad alfa y beta fueron similares para los sitios.

**KEYWORDS** Coral reefs, biodiversity index, damselfish, *Stegastes*

PALABRAS CLAVES Arrecifes, índice de biodiversidad, damiselas, *Stegastes*

## INTRODUCTION

Biodiversity refers to the genetic, species, and ecological variation of life, often within a given area (Miller, and Lugo, 2009). The tropics, the band centered on the equator and delimited by the Tropic of Cancer in the northern hemisphere and the Tropic of Capricorn in the southern hemisphere, is renowned for the relatively high species diversity of many of its ecosystems, such as rainforests and coral reefs. Many prior studies have shown the importance of biodiversity in ecosystem health and function. For instance, high biodiversity may allow for better tolerance of environmental disturbances. Additionally, ecosystem properties, processes, and goods derived from ecosystems are all functions that are affected by biodiversity, and can be negatively impacted by the lack of diversity. Recent studies have found that multifunctional ecosystems require greater species diversity in order to maintain its multifunctionality because different species occupy different niches and can perform different ecosystem roles. In many cases, mixtures of species are necessary to maximize ecosystem responses and can buffer ecosystems against perturbation. In a diverse ecosystem, multiple species can have the capacity to perform the same ecological function. So if one species declines and can no longer perform its role in the ecosystem, another species may take the place, and the ecosystem can persist (Gamfeldt, Hillebrand, and Jonsson, 2008).

Coral reefs are ecosystems that are well known to be extremely biodiverse. They constitute among the oldest stable marine systems on the planet, with contemporary reefs as much as 5,000 – 10,000 years old, suggesting a high level of ecosystem stability for many centuries. These ecosystems are one of the most diverse in animal species and among the most structurally complex in terms of habitat. Different types of coral reef fauna

include swimming fish, substrate fish, pelagic invertebrates, burrowing invertebrates, benthic invertebrates, and sessile invertebrates. Because of this high biodiversity, 50% of countries with coral reefs have put in place conservation efforts to protect these systems, and many coral reef systems would face extinction without protection (Miller, and Lugo, 2009). Additionally, coral reefs also protect other important marine ecosystems that serve as nursery grounds for many species, such as mangrove swamps and seagrass beds (Miller, and Lugo, 2009).

It has been shown in previous studies that coral reef fish species abundance, diversity, richness, distribution and biomass can differ between different types of reef environments (Roberts, 1987). Our study focuses on the fish species diversity of various coral reef habitats, both man-made and natural. Specifically, we focused on the diversity of species from the family Pomacentridae, commonly called damselfish, in different coral reef habitats on the Island of Vieques, Puerto Rico. Specifically, we measured diversity of damselfish in both man-made habitat (specifically, a rocky pier) compared to natural coral reef. We predicted *a priori* that we would find greater diversity in natural vs. man-made habitats, due to the high structural complexity of natural coral reef systems. We also computed beta diversity between sites, which is a measure of species similarity (or, conversely, turnover) between sites. We hypothesized that beta diversity would be higher between man-made and natural reef sites, than between our two natural coral reef habitats.

Pomacentrids (damselfish) are common, substrate dwelling tropical reef fish that occupy space in and around rock crevices (Robertson, Hoffman, and Sheldon, 1981). Damselfish are territorial, and engage in interspecific and intraspecific competition to defend their space around the substrate. Damselfish are typically benthic feeders, feeding on the substrate in which they have established their territory. It has been shown in prior studies that different damselfish species can occupy the same reef zone, or different reef zones. Many damselfish exhibit habitat partitioning, which is what allows them to coexist in the same ecosystem. This partitioning could be due to differing ecological requirements, habitat preferences, or competitive abilities. Coral reef ecosystems are structurally variable and complex and can contain many ways for species to intermingle and coexist (Waldner, and Robertson, 1980).

## MATERIALS AND METHODS

### Study sites

Since the primary goal of this study was to assess the species diversity of coral reef damselfish, we conducted an inventory of the species that occurred in habitats at specific sites. The study sites for this investigation included one man-made reef, Mosquito Pier, and two natural coral reefs, Pata Prieta beach and La Chiva beach (Figure 1). Mosquito pier is a mile long manmade structure that was constructed by the United States Navy in the 1940's, and was intended to serve as a breakwater. Pata Prieta beach is a natural rocky coral reef habitat along the southern central coast of Vieques. La Chiva beach is also a natural rocky coral reef habitat along the southern coast of Vieques, slightly east of Pata Prieta, and it has more structural complexity than Pata Prieta.

### Sampling

We established two 10 m long and 2 m wide transects at each site, and conducted a visual encounter survey along each transect. Transect tape was used to measure transect at 1-1.5 m depth. Two of the co-authors remained stationary in the water during each survey to hold the transect line in place. The transect locations that we chose had sufficient rocky substrate to ensure that we were sampling in damselfish habitat. We used the visual encounter survey technique which assumes that every individual of each species has equal chance of being observed, and that there is no bias due to coloration, size, behavior, weather, predators or competitors. This method also assumes that the recorder can keep track of all movement in order to avoid multiple records of the same individual (Heyer, Donnelly, McDiarmid, Hayek, and Foster, 1994).

The visual encounter method of surveying fish biodiversity is useful because it is non-destructive to the reef, and causes minimal disturbance. However, one disadvantage of this method is that it depends on the ability of the recorder to correctly identify the observed species (Sutherland, 2006). In order to compensate for this disadvantage, we used the website www.reefguide.org, as well as additional material, to study damselfish species identification prior to conducting the survey.

Using a snorkel, mask and fins, the principal author swam slowly and steadily along the transect line and identified and recorded, on an underwater writing slate, every individual of each species encountered (both juvenile and adult) (Figure 9). It was often necessary to stop and float over a section of the transect to wait for fish to emerge from their territories. Each site was surveyed one time, each on different days, with two transects at each study site. Mosquito Pier was surveyed on 1/18/2015 at approximately 3:30PM; Pata Prieta beach was surveyed on 1/19/2015 at approximately 3:30PM; and La Chiva beach was surveyed on 1/20/2015 at approximately 10:45AM. All days were clear with bright sun, which are optimal conditions for observation.

### Data analysis

Species diversity refers to the number of different species and their relative abundance in a given area (Miller, and Lugo, 2009). Measures of alpha (α) and beta (β) diversity were used to determine the overall diversity of each study site. Alpha diversity

represents the diversity within a community, and beta diversity represents the diversity between communities (i.e., species turnover; Sepkoski, 1988). Once data collection was complete, we analyzed the species counts and calculated the Simpson index to determine alpha diversity, and used Whittaker's formula to calculate beta diversity (Koleff, Gaston, and, Lennon 2003). The Simpson Index is computed as $\lambda = \sum_{i=1}^{R} p_i^R$, in which $p_i$ is the proportion of individuals of a given species (Heip, Herman, and Soetaert, 1998). The Simpson Index estimates the probability that two items taken at random will be the same type – in this case, the same species. This measure thus varies between 0 and 1, with 0 being the highest biodiversity, and 1 the lowest (complete dominance of one species).


**Figure 1:** Survey site locations on Vieques Island. Map courtesy of Google Maps.

The formula used to calculate beta is $\beta = \frac{a+b+c}{(2a+b+c)/2}$, where $a$ is the total number of species common to both communities, $b$ is the total number of species in community 1, and $c$ is the total number of species in community 2 (Koleff, et al., 2003).

**RESULTS**

We made some preliminary observations and learned the predominant damselfish species found in shallow fringing reef zones which were *Stegastes variabilis* (cocoa), *Stegastes leucostictus* (beaugregory), *Stegastes fuscus* (dusky), *Stegastes planifrons* (threespot), *Abudefduf saxatilis* (sergeant major), *Microspathodon chrysurus* (yellowtail), and *Stegastes partitus* (bicolor).

The cocoa damselfish juvenile is yellow ventrally and blue dorsally with vibrant blue stripes on the head with a dorsal and tail spot. The adult cocoa can be varied in color, but typically yellow and blue, with blue predominant, and the tail spot is retained (Figure 2). The juvenile beaugregory is almost identical to the cocoa juvenile, except it does not have the tail spot. The adult beaugregory is also very similar to the adult cocoa, but it has slightly more yellow, and no tail spot (Figure 3). The dusky damselfish juvenile is very distinct, having a blue body with a splash of orange on the head and a dorsal fin spot. The dusky adult is light to dark brown, with a dark edge along the anal fin, and sometimes the adults retain the dorsal spot (Figure 4). The juvenile threespot damselfish is bright yellow with a dorsal spot and tail spot. The adult threespot is brown-yellow, and retains the tail spot (Figure 5).

**Figure 2:** Cocoa juvenile (top) and adult (bottom). Pictures from www.reefguide.org).

**Figure 3:** Beaugregory juvenile (top) and adult (bottom) (Pictures: juvenile taken at Mosquito Pier, adult from www.reefguide.org)

The juvenile and adult sergeant major is white with black vertical stripes, and a yellow on the dorsal side (Figure 6). The

juvenile yellowtail damselfish is blue with light blue spots and as yellow tail. The adult yellowtail is brown or dark blue with a yellow tail, and it retains some light blue spots on the head (Figure 7). Lastly, the bicolor damselfish juvenile and adult both have a black front half and white back half, and there can be yellow beneath the black (Figure 8).

**Figure 4: Dusky juvenile (top) and adult (bottom).** Pictures from www.reefguide.org)

Overall, we found that the three most abundant damselfish species were the dusky damselfish, the beaugregory, and the sergeant major. The most abundant species at Mosquito Pier was dusky at 48%, and second most abundant was beaugregory at 27%. The least abundant damselfish species at Mosquito Pier were the yellowtail and bicolor damselfishes, as we found no individuals of either species in our survey. At Pata Prieta the most abundant damselfish species were the beaugregory and the dusky damselfish, which had highly similar abundances at 37% and 35%, respectively, in our surveys. The least abundant species at Pata Prieta were once again the yellowtail and bicolor damselfishes, of which we observed only 1 and 0 individuals, respectively, but in addition we also observed no threespot damselfish in our surveys of this site. At La Chiva the most abundant species were the dusky damselfish at 46% and the beaugregory at 34%. The lowest abundance at La Chiva were the yellowtail and bicolor damselfishes, with 0 and 1 individuals, respectively, and again the threespot damselfish with 0 individuals in our surveys.

The Simpson index measures were 0.328, 0.296, and 0.342 for Mosquito Pier, Pata Prieta, and La Chiva respectively (Table 1). Since the Simpson Index is a probability, its range is 0 to 1, with lower values indicating higher diversity. Consequently, all three sites had very similar, and fairly high biodiversity. The beta diversity values were 1.556, 1.579, and 1.524 for Mosquito Pier, Pata Prieta, and La Chiva respectively (Table 1).

**Figure 5.** Threespot juvenile (top) and adult (bottom) (Pictures: juvenile taken at Mosquito Pier, adult from www.reefguide.org)

**Figure 6:** Sergeant Major adult/juvenile. (Picture taken at Mosquito Pier).

**DISCUSSION**

In this short study we compared the within-site (alpha) diversity between two different types of habitat on the Island of Vieques. Specifically, we used multiple transects to estimate the diversity of damselfish species in man-made habitat (a former Navy pier and breakwater) and natural coral reef. We hypothesized that due to the high structural complexity of coral reefs, the alpha diversity might be higher in coral reefs than at the man-made pier. We also computed beta diversity between pairs of sites. We hypothesized that species composition and relative abundance might be more similar between different coral reefs than between

coral reef and pier. Our results revealed instead that species diversity of damselfish was highly similar across sites and that there was no notable or consistent difference in beta diversity among pairs of sites.

**Figure 7:** Yellowtail juvenile (top) and adult (bottom). (Pictures from www.reefguide.org)

Our sampling technique, visual survey, required a number of assumptions, such as equal probability of seeing different species in the environment. Although we cannot definitively rule out the possibility of bias, we have no reason to suspect that visual survey assumptions have affected our results. Additionally, since damselfish are territorial and remain close to their territory, we found that it was relatively simple to visually track the individuals in order to avoid double counting.

**Figure 8:** Bicolor adult/juvenile. (Pictures from www.reefguide.org)

Though we were somewhat surprised to discover that alpha diversity was comparable between natural and artificial reef habitat, this is an encouraging sign that, at least for damselfish, man-made substrates may provide suitable habitat to sustain some level of biodiversity. Future research on relative abundance, individual health, and the diversity and abundance of other reef using species should be conducted to further explore our relatively narrow result presented here

**Table 1.** Calculated diversity within each habitat (alpha diversity, Simpson Index); and diversity between each pair of sites (beta, Whittaker Index).

| **ALPHA DIVERSITY** | **Simpson** |
|---|---|
| Mosquito Pier | 0.328 |
| Pata Prieta | 0.296 |
| La Chiva | 0.342 |
| **BETA DIVERSITY** | **Whittaker** |
| Mosquito Pier vs. Pata Prieta | 1.556 |
| Mosquito Pier vs. La Chiva | 1.579 |
| Pata Prieta vs. La Chiva | 1.524 |

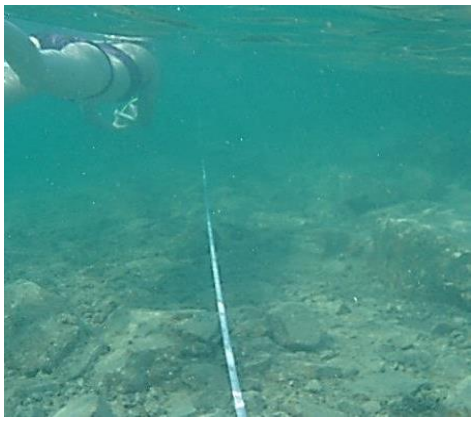

**Figure 9:** Photo of transect at Mosquito Pier.

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# Evaluación de las propiedades antibacterianas del ajo (*Allyum sativum)*

## Evaluation of the antibacterial properties of garlic (*Allyum sativum)*

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**ABSTRACT**

**Antibacterial, antifungal and antihypertensive properties have been attributed to garlic. Previous studies have shown the efficacy of garlic against malaria, amoebiasis, and even that it can inhibit proliferation of cancerous cells. These properties are attributted to the organosulfur allicin. Due to the increase in bacteria resistant to antibiotics, it is necessary to explore other alternatives. The present study evaluated garlic extract as an antibacterial agent against *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Alcaligenes faecalis and Staphylococcus epidermidis,* using the disk diffussion susceptibility test. Also the effect was compared to commercially available antibiotics erythromycin, doxycycline and bacitracin. Our results show that garlic was effective inhibiting *S. epidermidis, S. aureus* and *A. faecalis.* Also, garlic extract was able to inhibit, similarly or more than doxycycline, bacitracin and erythromycin, *A. faecalis, E. coli and S. aureus.* Future studies are required to study the effect of purified allicin and its molecular mechanism of action.**

RESUMEN

Al ajo (*Allyum sativum*) se le atribuyen propiedades como agente antibacterial, antifungal y antihipertensivo. Estudios previos han mostrado su efectividad en combatir la malaria, amebiasis e inclusive inhibiendo la proliferación de células cancerosas. Estas propiedades se le atribuyen a la presencia del organosulfuro alicina. Debido al aumento de bacterias resistentes a antibióticos, es necesario explorar otras alternativas. En el presente estudio se evaluó la efectividad del ajo como agente antibacterial en contra de *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Alcaligenes faecalis y Staphylococcus epidermidis*. También se comparó su efectividad con los antibióticos comerciales eritromicina, doxiciclina y bacitracina. Los resultados mostraron que el extracto de ajo fue efectivo inhibiendo a *S. epidermidis, S. aureus* y *A. faecalis*. De igual manera el extracto de ajo logró inhibir de manera similar o mayor que bacitracina, doxiciclina y eritromicina a *A. faecalis, E. coli y S. aureus*. Estudios futuros son necesarios para estudiar el efecto alicina purificada y su mecanismo de acción molecular.

**KEYWORDS** garlic, antimicrobial, allicin, bacteria

PALABRAS CLAVE ajo, antimicrobiano, alicina, bacterias

**INTRODUCCIÓN**

Muchos organismos como las plantas y los hongos han desarrollado mecanismos evolutivos que les permiten combatir agentes patógenos, y que a su vez se han convertido en una fuente de productos farmacológicos. Por ejemplo, la penicilina que se obtiene de *Penicillium chrysogenum*, la cefalosporina de *Cephalosporium gramineum* y la alicina que se obtiene del ajo. Durante siglos se le han conferido propiedades curativas al ajo y aunque esas propiedades han sido explotadas, no fue hasta 1944 que se investigó por primera vez su capacidad antimicrobiana con rigor científico (Caballito y Bailay, 1944).

La actividad antimicrobiana del ajo se le atribuye a un oganosulfuro llamado alicina (S-Alil-2-propentiosulfinato). Feldberg y sus colegas (Feldberg et al., 1988) demostraron el efecto bacteriostático de la alicina en *Salmonella typhimurium*. En dicho caso se observó inhibición lenta y parcial de la síntesis de ADN y proteínas, pero inhibición casi inmediata y total de la síntesis de ARN, lo que sugiere que este último es probablemente su mecanismo de acción (Feldberg et al., 1988). Por su parte Bakri y Douglas (2014) demostraron la efectividad del extracto de ajo en la flora bucal, con un efecto inhibitorio y bactericida tanto en organismos Gram-positivos, como Gram-negativos, especialmente *Streptococcus mutans* y *Porphyromonas gingivalis* (Bakri y Douglas, 2014).

En otro estudio se mostró la eficacia del aceite de ajo, polvo de ajo y extracto del ajo para tratar infecciones crónicas de *Helicobacter pilori* en el estómago (O'gara, Hill y Maslin,

2000). En 2013 se evaluó el efecto del ajo mezclado con miel de tanza (producida por la abeja *Apis mellipodae*). Se observó una zona de inhibición de 30-35 mm para *Salmonella, Staphylococcus aureus, Listeria monocytogenes* y *Streptococcus pneumoniae*, lo cual fue considerado inhibitorio y efectivo contra estos patógenos (Andualem, 2013).

Se ha observado que la propiedad antimicrobiana del ajo puede potenciarse cuando se mezcla con especias o con antibióticos. Su Cepas de *E.coli* resistentes a antibióticos mostraron un aumento en su susceptibilidad cuando fueron expuestas a una mezcla de extracto de limón, ajo y jengibre en una proporción 1:1:1 (Rahman, Parvez, Islam y Khan, 2011). Un efecto similar se observó para especies de *S. aureus* resistentes a ampicilina. Cuando la bacteria fue expuesta a una combinación de ampicilina y ajo, se observó la generación de una zona de inhibición amplia, dependiente de la concentración de ajo (Pillai, Trivedi y Bhatt, 2013). Experimentos realizados con enterococos resistentes a vancomicina, mostraron que soluciones de vancomycina y ajo aumentaron el efecto bacteriostático de vancomicina. (Jonkers, Sluimer y Stobberingh, 1999).

El efecto antimicrobiano del ajo se ha observado también para protozoarios patógenos como *Entamoeba histolytica* causante de la amebiasis. Se cree que la alicina inhibe las proteasas de cisteína y evitando así la destrucción de los riñones de hámsters (Ankri, Miron, Rabinkov, Wilcheck y Mirelman, 1997). Ratones infectados con malaria fueron tratados con alicina y se observó una disminución considerable en la infección. También se trató directamente a *Plasmodium* (organismo que causa malaria) con alicina y luego se inyectó a ratones. En esos casos la infección fue inhibida completamente (Coppi, Cabinian, Mirelman y Sinnis, 2006).

En un estudio sobre cáncer de seno realizado en el 2013, se demostró que las células cancerosas detenían su crecimiento luego de ser tratadas con extracto de ajo. (Modem, DiCarlo y Reddy, 2012). Mikaili y colegas, realizaron una extensa búsqueda de literatura relacionada con el efecto del ajo en diversas condiciones clínicas y concluyeron que el ajo exhibe efectos beneficiosos a la salud incluyendo efecto antibacterial, antifungal, antiparasítico, antiviral, reduce hipertensión, colesterol, inflamación, exhibe propiedades anti-cáncer, y puede actuar como modulador inmunológico (Mikaili, Maadirad, Molouddizargari, Aghajanshakeri y Sarahroodi, 2013).

En esta investigación evaluamos la efectividad del extracto de ajo contra distintos microorganismos patógenos y comparamos su efectividad con respecto a otros antibióticos comerciales utilizando el método de susceptibilidad (Kirby-bauer).

## MATERIALES Y MÉTODOS

### Preparación del extracto de ajo

Se utilizaron dos cabezas de ajo. Se removió la cáscara y se trituraron con un mortero de cerámica; se utilizó la parte líquida. Se prepararon soluciones al 100%, 50% y 25% utilizando buffer de fosfato (PBS).

### Microorganismos

Se utilizó *E. coli* (ATCC 25292) *K. pneumoniae* (ATCC 13883), *S. aureus* (ATCC 25923), *A. faecalis* (ATCC 35655) y *S. epidermidis* (ATCC 12228). Se prepararon diluciones de cada cultivo en PBS utilizando el estándar McFarland 0.5 y se esparcieron en platos de TSA (Triptic Soy Agar).

### Prueba de susceptibilidad

Se utilizó la técnica de Kirby-bauer: método de susceptibilidad por difusión (Capuccino, y Sherman, 2005). Para *S. epidermidis* se utilizaron discos de papel de filtro los cuales fueron sumergidos en las tres soluciones de ajo y se compararon con el antibiótico eritromicina. Para las otras bacterias se utilizó únicamente la solución de ajo 100% y se comparó con los antibióticos doxiciclina, bacitracina y eritromicina. Los platos fueron incubados a 37 ºC por 24 o 48 horas. Cada prueba se realizó en triplicados.

## RESULTADOS

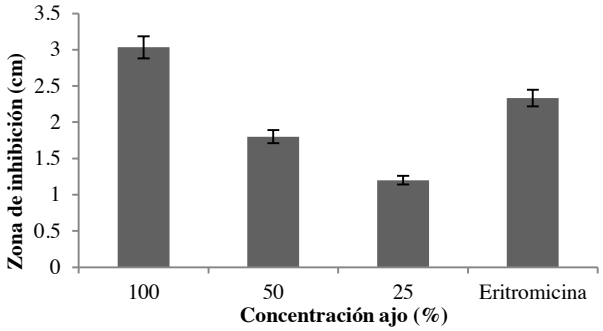


**Figura 1.** Zona de inhibición generada por las soluciones de ajo al 100, 50 y 25 % comparadas con el antibiótico eritromicina para *S. epidermidis* a las 24 horas de exposición.

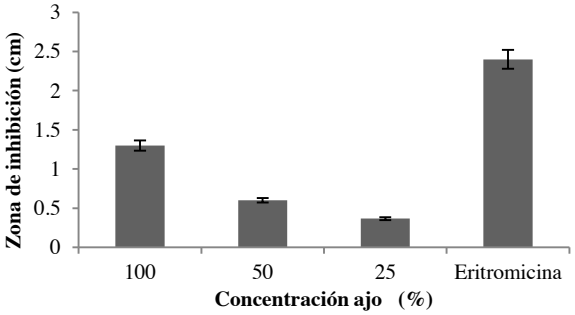


**Figura 2.** Zona de inhibición generada por las soluciones de ajo al 100, 50 y 25 % comparadas con el antibiótico eritromicina para *S. epidermidis* a las 48 horas de exposición.

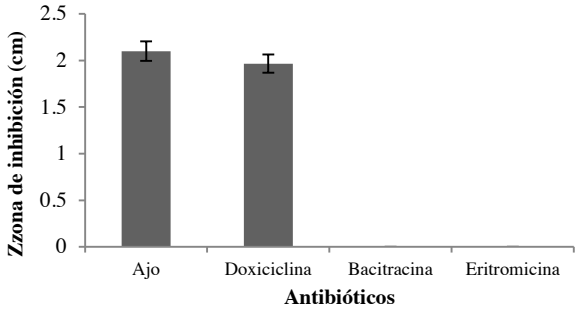


**Figura 3.** Zona de inhibición generada por el extracto de ajo y varios antibióticos comerciales en *E.coli*.

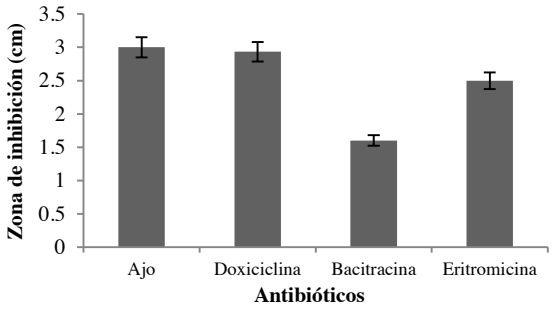


**Figura 4**. Zona de inhibición generada por el extracto de ajo y varios antibióticos comerciales en *S. aureus*.

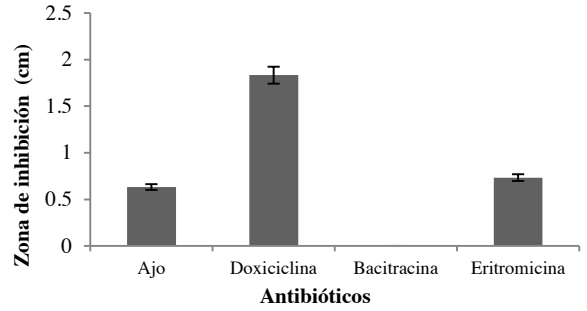


**Figura 5.** Zona de inhibición generada por el extracto de ajo y varios antibióticos comerciales en *K. pneumoniae*.

## DISCUSIÓN

Al cabo de 24 y 48 horas se observó el efecto del extracto de ajo en *S. epidermidis* y se midió su área de inhibición como se muestra en las figuras 1 y 2. A las 48 hrs se notó que el efecto inhibitorio del ajo en las tres concentraciones utilizadas para *S.epidermidis* se redujo.

En el caso de *E. coli* el extracto de ajo creó una zona de inhibición de 2.2 cm que fue similar a la generada por doxiciclina (figura 3), que es un antibiótico que se utiliza en contra de bacterias Gram positivas y Gram negativas. Un efecto similar se observó para *S. aureus*. El extracto de ajo inhibió creando una zona de 3 cm que fue similar a la generada por doxiciclina pero mayor a la generada por bacitracina y eritromicina (figura 4).

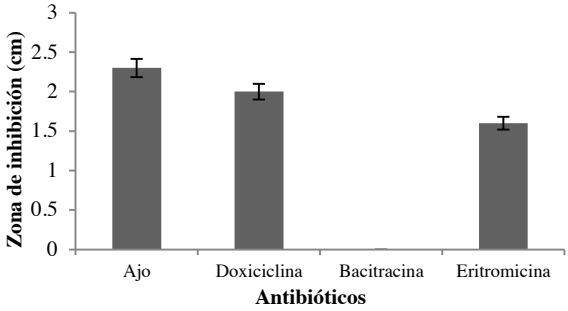


**Figura 6.** Zona de inhibición generada por el extracto de ajo y varios antibióticos comerciales en *A. faecalis*.

*K. pneumoniae* es un bacilo Gram negativo implicado en infecciones nosocomiales. En extracto de ajo no mostró un fecto inhibitorio marcado; doxiciclina fue el agente más efectivo logrando generar una zona de inhibición de 1.9 cm (figura 5). El efecto que se observó para *A. faecalis* fue similar al observado para *S. aureus* (figura 6). El extracto de ajo resultó ser el agente de mayor inhibición cuando se comparó con los tres antibióticos. Estos resultados demuestran que el extracto de ajo tiene una acción antibiótica en contra de bacterias de importancia médica como *E. coli, S. aureus y A. faecalis y S. epidermidis*. En el caso de *S. aureus y E.coli* los resultados son cónsonos con estudios previos (Andualem, 2013; Pillai et al., 2013; Rahman et al., 2011).

El descubrimiento de los antibióticos en 1928 por Alexander Fleming, marcó una nueva era en la medicina, pero su uso indiscriminado ha dado a lugar a la resistencia que desarrollan muchos microrganismos. *S. epidermidis* aunque pertenece a la flora normal de la piel, si logra penetrar las barreras físicas del cuerpo, puede causar infecciones en heridas, cistitis, septicemia y endocarditis entre otros (Prescott, Harley y Klein, 1999) y *E. coli,* causante de infecciones del tracto urinario, sistema digestivo, neumonía y peritonitis. *S. aureus* causa infecciones cutáneas, foliculitis, conjuntivitis, osteomielitis, meningitis, neumonía, entre muchas otras (Prescott et al., 1999), *K. pneumoniae* que afecta el tracto urinario, causa neumonías y sepsis y *A. faecalis* que afecta el tracto urinario. De todas estas bacterias se han registrado cepas resistentes a antibióticos. Por ello, se necesario explorar alternativas para poder contrarrestar infecciones bacterianas.

Nuestros resultados al igual que estudios previos muestran la eficacia del ajo para inhibir bacterias y lo presentan como una potencial y accesible alternativa natural antimicrobiana. Como

plan futuro sería recomendable estudiar las propiedades químicas de alicina purificada y evaluar su efecto directo en bacterias y otros microorganismos, al igual que poder establecer su mecanismo molecular de acción.

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# Pruebas de pureza requeridas para la aprobación final de un producto biológico: producción de anticuerpos de ratón (mouse antibody production), producción de anticuerpos de hámster (hamster antibody production), transcriptasa inversa y HPLC

## Purity tests required for the final approval of a biological product: mouse antibody production, hamster antibody production, reverse transcriptase, HPLC

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**ABSTRACT**

**The objective of this article is to discuss four different tests used to determine the purity and to achieve the approval of a final biological product. The Mouse Antibody Production test (MAP) is used to demonstrate the stability of cell lines, tissue samples or other materials of mouse origin, and that they are free of adventitious viral particles. Hamster Antibody Production (HAP) is another test based on the same concept as MAP for detection and identification of viral contaminants. Both tests use natural hosts (hamster, mouse) and specific detection methods like ELISA. When a virus, specifically retrovirus, infects a cell, the enzyme reverse transcriptase catalyzes the synthesis of DNA from a viral RNA template. A test based on Reverse Transcriptase can also be used to detect viral contamination in a biological product, particularly on cultures for monoclonal antibody production. Finally, High-Performance Liquid Chromatography (HPLC) allows the separation of components in a mixture based on different chemical interactions between the analyzed substance and chromatographic column.**

RESUMEN

Este artículo explora diversas pruebas de pureza que son requeridas para la aprobación final de un producto biológico. El ensayo de producción de anticuerpos de ratón, MAP (mouse antibody production), es utilizado para demostrar la estabilidad de las líneas celulares, muestras de tejidos u otros materiales de origen murino y que los mismos se encuentren libres de partículas virales. La prueba de producción de anticuerpos de hamster, HAP (hámster antibody production), se basa en el mismo concepto que MAP para la detección e identificación de contaminantes virales en muestras biológicas. Ambas pruebas se realizan utilizando huéspedes naturales altamente susceptibles (hámster, mouse) y métodos de detección como ELISA. Cuando un retrovirus infecta una célula la enzima transcriptasa inversa cataliza la síntesis de AND a partir de ARN. El método de transcriptasa inversa, también se puede utilizar para detectar contaminación viral en una muestra biológica, particularmente en cultivos para la producción de anticuerpos monoclonales. Por ultimo, la cromatografía líquida de alta eficacia, HPLC (High-performance Liquid Chromatography), permite separar componentes de una mezcla basándose en diferentes interacciones químicas entre la sustancia analizada y la columna cromatográfica.

**KEYWORDS** mouse antibody production, hamster antibody production, reverse transcriptase, HPLC

PALABRAS CLAVE producción de anticuerpos de ratón, producción de anticuerpos de hámster, transcriptasa reversa, HPLC

**INTRODUCCIÓN**

Al momento de lanzar un nuevo producto al mercado, el mismo debe ser previamente analizado mediante pruebas de pureza para su aprobación. Esto es necesario para asegurar que el product es seguro, efectivo y libre de cualquier patógeno, virus, pirógenos o alguna otra sustancia que pueda ser perjudicial a la salud del consumidor. La esterilidad se define como la ausencia de microorganismos vivos (Manual de OIE, 2008). Solamente manteniendo un control adecuado de los productos primarios utilizados y de los procesos que se siguen, así como del almacenamiento, se puede asegurar la esterilidad y la ausencia de contaminación (Manual de OIE, 2008).

Según el manual de la OIE (World Organization for Animal Health) se deben seguir unos procedimientos generales para

evitar contaminación en el producto. Los materiales originales deben obtenerse a partir de fuentes que estén libres de contaminación y que se manejen de tal modo que se reduzca la contaminación y la posibilidad de que se multiplique. Los materiales que puedan esterilizarse sin que sus propiedades biológicas resulten excesivamente afectadas deben esterilizarse. El método debe reducir el nivel de la contaminación hasta que esta sea indetectable. Si se utiliza un procedimiento de esterilización, este deberá validarse para demostrar su conveniencia y funcionamiento. De igual manera los lugares donde se realizan las manipulaciones asépticas deben mantenerse limpios, protegidos de fuentes externas de contaminación y controlados para evitar la contaminación interna.

Este artículo tiene como objetivo discutir la aplicación de algunas de las diferentes pruebas de purificación comúnmente utilizadas para la aprobación de diferentes productos. Dentro de estas pruebas se encuentra la de producción de anticuerpos de artón (MAP), la cual es utilizada para demostrar estabilidad de líneas celulares, muestras de tejido u otros materiales de origen murino (O'Garra, A., Stapleton, G., y Dhar, V., 1990). Producción de anticuerpos de hamster (HAP) es otra prueba de pureza basada en el mismo concepto de MAP. La prueba de transcriptasa inversa que es una enzima la cual puede catalizar la síntesis del ADN a partir del molde del RNA viral (Corona, A., Tramontano, E., y Esposito, F., 2012). Y por último, la cromatografía líquida de alta eficacia, HPLC (High-performance Liquid Chromatography), que se basa en la separación de componentes de una mezcla basándose en diferentes interacciones químicas entre las sustancias analizadas y la columna cromatográfica (Swartz, M., 2010)

**Producción de anticuerpos de ratón (MAP)**

Las pruebas de producción de anticuerpos de ratón (MAP por sus siglas en inglés) se han convertido en el estándar de ensayo para la detección de contaminación vírica murina en los materiales biológicos, tales como líneas celulares y tumores trasplantables. MAP es un método establecido para demostrar la estabilidad de las líneas celulares, muestras de tejidos u otros materiales de origen de ratón y que las mismas se encuentren libres de partículas virales adventicias. Para esta prueba, el material sospechoso se inyecta dos veces en ratones ingenuos, serológicamente negativos. Luego, se le permite a los ratones tiempo de seroconversión. Tres semanas después de la exposición inicial, se toman muestras de suero y son analizadas por el método indirecto de ELISA. Las muestras positivas son examinadas nuevamente para confirmar el resultado. Para analizar líneas celulares establecidas, dos alícuotas de células de 1 ml (5 x $10^5$ células / ml) deberán presentarse al laboratorio en un estado congelado o refrigerado, en su medio de cultivo típico pero sin ningún crioprotector. Adicional, 1.0 ml de medio de cultivo estéril también puede ser presentado para actuar como control.

Las pruebas MAP requieren ratones inmunocompetentes libres de anticuerpos contra los patógenos virales. Se deben tomar precauciones adicionales, tales como el uso de unidades de micro aislamiento y la realización de todo tipo de manipulación de los animales en una cabina de bioseguridad para evitar la infección por virus y para prevenir la infección de otros animales en las instalaciones (Blank, W.A., Henderson, K.S., y White, L.A., 2004). Los protocolos de pruebas MAP requieren un mínimo de 28 días después de la inoculación para que los animales puedan expresar una respuesta inmune detectable; la preparación de muestras y las pruebas serológicas contribuirán tiempo adicional a el total de tiempo requerido para este método (Blank et al., 2004). Algunos de los virus que se pueden detectar a través de esta prueba son: cytomegalovirus de ratón (MCMV), adenovirus de ratón (MAV) y virus de hepatitis de ratón (MHV). Materiales biológicos de rata o hámster también se pueden analizar de una manera similar, mediante la detección de la seroconversión en sus especies de origen.

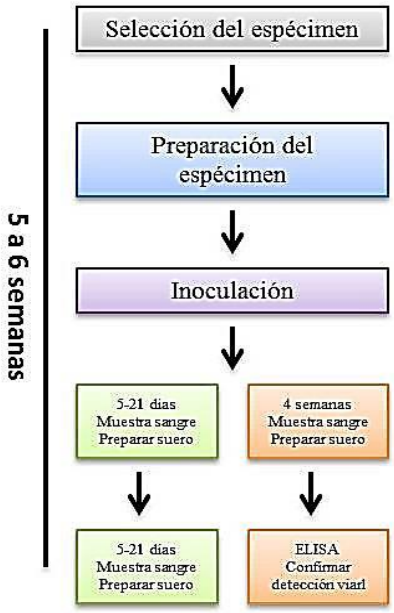


**Figura 1.** Pasos para la prueba de MAP.

**Producción de anticuerpos de hámster (HAP)**

La prueba de producción de anticuerpos de hámster (HAP por sus siglas en inglés) está basada en el mismo concepto que la prueba de MAP. Es un método indirecto para detectar virus contaminantes mediante prueba de inoculación en hámster (River, C., 2014). El suero de estos animales es posteriormente probado para la presencia de anticuerpos reactivos con un panel de virus específicos para el sistema del animal (River, C., 2014). Técnicas de inmunofluorescencia y ELISA son empleadas para evaluar los resultados de HAP (River, C., 2014). A diferencia de MAP, el ensayo de HAP es capaz de detectar otros virus como:

Virus de la neumonía de ratones (PVM), Reovirus tipo 3 (Reo3) y Virus Sendai. Los pasos de la prueba de HAP son los mismos que para MAP (figura 1).

**Transcriptasa Inversa**

La transcriptasa inversa es una enzima que puede sintetizar ADN utilizando como plantilla o molde ARN. La misma se utiliza para procesos de purificación, tecnología de ADN recombinante entre otros. La familia de retrovirus como el virus de inmnodeficiencia humana (VIH) tiene su genoma de RNA. Una vez el retrovirus infecta a una célula, la transcriptasa inversa cataliza la síntesis de ADN de doble hebra a partir del RNA viral (Morier D., 2014). Ese ADN de doble hebra se puede integrar al genoma de la célula huésped (infectada).

En cultivos de hibridomas murinos para la producción de anticuerpos monoclonales, se puede utilizar la transcriptasa inversa como método para detectar la presencia de retrovirus. En un estudio realizado por Brorson y colegas (Brorson, K., Swann, P.G, Lizzio, E., Maudru, T., y Stein, K.E., 2001) utilizaron una transcriptase reversa optimizada y fusionada a una sonda taq man para detector los niveles de actividad de la transcriptasa inversa y para demostrar la eficiencia con la que se puede eliminar de la muestra. Los resultados del estudio sugieren que la transcriptase optimizada es ideal para monitorear los cultivos de hibridoma y eliminar la presencia de retrovirus.

**HPLC (High-performance Liquid Chromatography)**

La cromatografía líquida de alta eficacia, mejor conocida como HPLC (por sus siglas en inglés) es una técnica utilizada para separar componentes de una mezcla basándose en diferentes interacciones químicas entre las sustancias analizadas y la columna cromatográfica. Este tipo de cromatografía está compuesta por dos fases: la fase estacionaria y la fase móvil. La fase estacionaria se basa en un cilindro o columna con pequeñas partículas redondeadas conocidas como esferas que tienen ciertas características químicas en su superficie. La fase móvil por otra parte, es un líquido que fluye a través de la columna que contiene a la fase estacionaria. Es junto a la fase móvil que la sustancia experimental es analizada. En general, se deben tener en consideración algunos parámetros al momento de realizar HPLC, estos parámetros incluyen: la naturaleza de la fase estacionaria, el tamaño de la partícula o partículas analizadas, el eluyente (composición y flujo), el pH, la dimensión de la columna y el detector (en el equipo) (Clark, J., 2007).

En la industria biotecnológica la técnica de HPLC tiene importantes aplicaciones para la separación y purificación de metabolitos, purificación y separación de enantiómeros, purificación de compuestos naturales y purificación y caracterización de enzimas y proteínas. Para la purificación de una muestra se debe tener en consideración que la migración a través de la columna, de la sustancia experimental y de los contaminantes, sea lo suficientemente distinta para que dicho compuesto pueda ser separado. Posteriormente, se puede identificar y cuantificar la sustancia (Ozores, M.I., y Carrera, N.).

Existen variantes de HPLC: de fase nomal y de fase inversa. En el HPLC de fase normal la columna se llena con partículas de sílica pequeñas, y el disolvente es, por ejemplo, no polar - hexano. Una columna típica tiene un diámetro interno de 4.6 mm (puede ser menor), y una longitud de 150 a 250 mm. Los compuestos polares en la mezcla que se pasa a través de la columna se pegaran más a la sílica polar a diferencia de los compuestos no polares. Por lo tanto, los no polares pasarán más rápidamente a través de la columna (Meyer, V.R., 1998).

Para análisis por fase inversa, el tamaño de la columna es el mismo que en fase normal, pero la sílica se modifica para que sea no polar. A dicha sílica se le adhieren cadenas largas de hidrocarburos de entre 8 a 18 átomos de carbono. Se utiliza un disolvente polar, por ejemplo, una mezcla de agua y alcohol como metanol. En ese caso, habrá una fuerte atracción entre el disolvente polar y las moléculas polares en la mezcla que se pasa a través de la columna. No habrá atracción entre las cadenas de hidrocarburo unidos a la sílica (la fase estacionaria) y las moléculas polares en la solución. Las moléculas polares en la mezcla pasarán la mayor parte de su tiempo en movimiento junto con el disolvente (Clark, J., 2007).

HPLC se puede acoplar a detectores como ultravioleta (UV), fluorescencia, espectrometría de masa, detección del índice de refracción (IR), entre otros (Swartz, M., 2010).

**DISCUSIÓN**

El presente artículo tiene como objetivo principal discutir algunas de las diferentes pruebas de purificación comúnmente utilizadas para la aprobación final de diferentes productos biotecnológicos. Las pruebas de purificación que se presentan en este artículo son: producción de anticuerpos en ratón, producción de anticuerpos en hámster, transcriptasa inversa y HPLC.

Para garantizar la calidad de los productos biotecnológicos es necesaria la implementación de regulaciones. Estas regulaciones sirven de control y tienen como propósito asegurar que se aprueben productos aptos para el consumo. En las industrias biotecnológicas las agencias reguladoras son las encargadas de establecer parámetros con el fin de obtener la integridad del producto final. Según Quintana y Apezteguí (2010) algunos parámetros establecidos para las buenas prácticas en la producción de productos biotecnológicos son: la organización y el personal, las instalaciones y el equipamiento, los insumos y su control, la producción y el control de proceso, el aseguramiento de la calidad en la producción y en los laboratorios analíticos, la

contratación de servicios productivos y de ensayo, así como cumplir con la calificación y validación en equipos, procesos y sistemas. Todos estos factores afectan la calidad del producto final.

Luego de haber presentado cada una de las pruebas previamente descritas se puede tener una idea concreta de algunos de los controles necesarios para la producción de medicamentos biotecnológicos. Se debe tener en consideración que cada prueba de pureza a pesar de ser diferente tiene un mismo fin, garantizar la calidad y eficacia del producto final. Una vez el producto sea aprobado estará listo para ser lanzado al mercado

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**Melissa Cabrera Santiago, Erika Lugo López y Keishla Méndez Guzmán.** Son estudiantes de la Universidad Interamericana de Puerto Rico, Recinto de Arecibo. Estudian Biotecnología y escribieron este artículo de revisión como parte del curso de Biotecnología Operacional II. Pueden ser contactadas a través de su mentora la Dra. Lizbeth Romero-Pérez al: lromero@arecibo.inter.edu

Students major in Biotechnology at the Inter American University of Puerto Rico, Arecibo Campus. They wrote this review paper as part of the Operational Biotechnology II course. They can be contacted through their mentor Dr. Lizbeth Romero-Pérez at: lromero@arecibo.inter.edu.

## LA INVESTIGACIÓN EN EL CAMPUS/ RESEARCH ON CAMPUS

**Resumen de las investigaciones realizadas por nuestros estudiantes en el Recinto durante el año académico 2014-2015 en los campos de bioquímica y biología molecular, genética, biotecnología de plantas, ecología, microbiología y química.**

Summary of research conducted on campus by our students during the academic year 2014-2015 in the fields of biochemistry and cell biology, genetics, plant biotechnology, ecology, microbiology and chemistry.

### BIOQUÍMICA Y BIOLOGÍA MOLECULAR/ BIOCHEMISTRY AND MOLECULAR BIOLOGY

**1-A Evaluation of proteasome activity in multiple myeloma and osteosarcoma cancer cells treated with resveratrol**

Roxanna Alicea, Lizmarie Cruz and Lizbeth Romero-Pérez

Studies propose that drinking a glass of wine helps cancer patients. In many cancer types, including multiple myeloma and osteosarcoma, proteasome activity is high. Proteasome is a multiprotein complex that degrades proteins in a highly regulated and specific way. Resveratrol, a compound extracted from grapes, is proposed to have anti cancer activity, and one of the mechanisms suggested for that action is by reducing proteasome activity. High proteasome activity is linked to the degradation of tumor suppressors and uncontrolled proliferation of cells, eventually generating tumors. In our study RMPI-8226 cells of multiple myeloma and U2-OS osteosarcoma cells were exposed to resveratrol for 24 hours at different concentrations. Concentrations of 5, 10, 25, 50, and 100µm of resveratrol were tested on multiple myeloma cells, and 25 uM for osteosarcoma cells. DMSO was used as a control. Extraction was performed to obtain cellular proteins including the proteasome. Proteins were quantified by Lowry. Proteasome activity was tested using modified casein as a substrate and TNBSA. Resveratrol was effective reducing proteasome activity at concentrations of 25 and 50µm. More studies are necessary to determine the best concentration of resveratrol. This is important because low or high doses of resveratrol can exhibit an inverse effect.

### GENÉTICA/ GENETICS

**2-A Extraction and analysis of DNA from harvestmen (Opiliones) found in Puerto Rico**

Jonathan Ayala, José García and Lizbeth Romero-Pérez

Harvestmen are known to be humidity indicators. They belong to the order of Opiliones and can be found in rocks and caves. In Puerto Rico, only 10 species have been identified. With the advancement of molecular techniques, phylogenetic relationships among them can be easily established. The objective of our study is to identify a method to isolate gDNA from harvestmen. Four specimens were collected at El Yunque Rainforest or El Karso in Arecibo. One leg, two legs or ¼ of the body were used. The extraction was carried out using the DNEasy blood and tissue kit (QIAGEN). Concentration and purity of the DNA was determined by spectrophotometry. PCR was performed using primers for 28S rDNA gene on a reaction of 35 cycles. Our results show that gDNA obtained from one leg is enough and has good quality to allow gene amplification. Further studies are required to amplify other genes in order to identify unknown species of harvestmen found in Puerto Rico.

**2-B Expression of miRNA 21 in multiple myeloma cells NCI H929 and RPMI 8226 exposed to berberine and resveratrol**
Emmanuel Arias, Nicole Figueroa, Saúl López, Rafael Olavarría, Jessica Santiago and Arlyn Pérez Samot

Multiple Myeloma is a malignancy characterized by the proliferation of plasma cells in the bone marrow. MicroRNAs are small and non-coding molecules that are responsible for the regulatory process of RNA molecules. MicroRNAs such as miR-21 can act as oncogenes and are over expressed in different types of cancer cells. Berberine is an alkaloid that suppresses the growth of multiple myeloma cancer cells by down regulating miR-21. Resveratrol is an antioxidant that induces apoptosis and inhibits cell proliferation of cancer cells. In this study it was determined by statistical analysis, that there was no change in the expression of miR-21 in multiple myeloma NCI-H929 cells after exposure to berberine. In a preliminary study, a down regulation of the expression of miR-21 was observed in multiple myeloma cells RPMI 8226 after exposure to resveratrol and berberine.

## BIOTECNOLOGÍA DE PLANTAS/ PLANT BIOTECHNOLOGY

**3-A Detection of *MT2a* and *MT2b* genes and the expression of *MT2a* after exposure to copper sulfate in *Arabidopsis thaliana***
Erika Lugo, Jesus Ramos, Adoniel Rivera, George Santiago and Arlyn Pérez Samot

The metallothioneins (MTs) are low molecular weight proteins that bind to heavy metals such as copper, zinc, and cadmium. A*rabidopsis* has four MT types: MT1, MT2, MT3 and MT4. They are involved in metal homeostasis, tolerance and distribution in the plant. High copper concentrations are toxic to the plant and low concentrations are used as cofactor for growth and development. MT2a and MT2b genes were detected successfully using cDNA as template by traditional PCR technique. In this preliminary study, an over expression of MT2a was detected after exposure to 30µM and 70 µM of copper sulfate by qT-Real Time PCR.

## ECOLOGÍA/ PLANT BIOTECHNOLOGY

**4-A Analysis of the heavy metal content on fish collected at Lago Dos Bocas and Río Tanamá**
Miguel Tapia and Lizbeth Romero-Pérez

Heavy metals are toxic metals whose density is five times the density of water. Regulatory agencies establish limits for the presence of these metals for human consumption. The objective of our study is to determine the level of Cu, Cd and Ni on fish collected at Lago Dos Bocas and Río Tanamá. Four species of fish were analyzed. Tissue used was muscle and liver and was processed by wet digestion. Heavy metals were detected by atomic absorption spectrophotometry. Nickel was not detected in any of the samples. Cadmium levels for fish Amphilophus labiatus were higher that the maximum permissible levels established by the Environmental protection Agency. Copper levels for all samples were below the maximum permissible. No difference was observed between muscle and liver. Our results suggest that consuming fish from these areas does not present a threat to human health. More studies are needed to investigate other species in the area and more heavy metals including mercury and lead.

**4-B Diet of the invasive *Boa constrictor* in Puerto Rico**
Jessica Maisonet and Alberto R. Puente-Rolón

Invasive species can impact negatively by causing ecological disturbances extirpating native species from their habitat, and competing with other native species. The *Boa constrictor* is an example of an invasive species well established, caused mainly by the illegal traffic of exotic animals in Florida, Aruba, Cozumel and recently in Puerto Rico. The diet of these species in its natural distribution (Central and South America), consist mostly of mammals (bats, rats, porcupines, mongoose, rabbits, deer), reptiles (iguanas) and birds. The objective of our study was to determine the diet of the invasive *B. constrictor*. A total of 90 individuals (44 females and 42 males) were captured by personnel of the Department of Natural Resources and Environment. Each individual was dissected to evaluate the stomach and gut content. Stables isotopes were also used to compare the muscle signatures of *B. constrictor* with the muscle signal of the Puerto Rican Boa (*Epicrates inornatus*). Prey items documented include rats, mice, chickens, iguanas, mongoose and cats among others. Rat and mice (90%) were the prey items most commonly consumed by the *B. constrictor.* No difference in isotopic signal was detected when we compare muscle samples of *B. constrictor* and *E. inornatus.* Previous research conducted in Guam, Aruba, Cozumel and Florida shows that invasive snakes are responsible of population declines of mammals, birds and reptiles. Therefore, long term monitoring of the diet of this new invasive species is important to identify potential threats to our biodiversity.

**4-C Body size and reproduction of the invasive *Boa constrictor* in Puerto Rico**
Jessica Maisonet, Félix Rivera-Marengo, and Alberto Puente Rolón

*Boa constrictor* is a species native to Central and South America, but has recently been reported as invasive in different countries, including Puerto Rico. The snakes achieve their sexual maturity when they reach 1.5-2 meters in length (~2-4 years). Each female has the capability of producing 20-60 hatchlings, thus generating vast populations wherever they proliferate, especially in the absence of predators. The objective of the study was to determine the size, distribution, and reproductive status of wild caught B. constrictor population established in Puerto Rico. Approximately 160 snakes were captured by personnel of the Department of Natural Resources and Environment. Snout vent length, tail length and body mass were documented, and each snake was dissected to determine its reproductive status. Average snout vent length for males was 132.6±43.5cm and 148.5±39cm for females. Males showed longer tails (19.1±6.8 cm) than females (17.6 ±4.3cm). Average mass for males was 2411 ±1911g and 3480±2754g for females. The smallest mature male (maturity determined by presence of thickened and convoluted sperm ducts) was 106.5 cm SVL, a typical size at maturity for male B. constrictor. Gravid females have an average of 21 eggs. Our data on size suggest that B. constrictors in Puerto Rico are not as strongly food-limited as some other populations of species that have been reported on the island. Continuous population monitoring of this invasive species is necessary in order to foresee possible impacts on our native biodiversity.

**4-D Screening of oxidoreductase activity in fungal strains isolated from Puerto Rico for biotechnological applications**
Juan G. Abreu Ramos, Luis J. Barrios Babilonia, David Gonzalez Vargas, Eliseo A. Lebron Burgos, Arnadldo Roman Acevedo, Omar M. Zayas Cruz and Richard L. Giles

Novel wild isolated fungal strains with aromatic depolymerization activity have the potential to significantly improve current bioremediation and biofuel production technologies. In this study, 10 fungal strains of varied taxonomic groups were isolated from Puerto Rico and screened for extracellular oxidoreductase activity. Macroscopic and microscopic characteristics were used to identify the collected specimens to

morphological species using standard taxonomic monographs and treatments. Solid medium containing the chromogen 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was used to screen fungi for the ability to degrade phenolic compounds. Isolates of *Trametes pubescens* and *Cladosporium* sp. exhibited both rapid growth and ABTS oxidizing activity suggesting oxidoreductase activity across varied taxonomic groups. This study is the first to report oxidoreductase activity of fungi isolated from Puerto Rico for biotechnological applications.

## MICROBIOLOGÍA/ MICROBIOLOGY

### 5-A Bactericidal activity of copper against nosocomial pathogens

Kathie Sánchez, Julishka Sotero, Jonathan Vélez and Yolanda Jové

It has been estimated that between 15% and 30% of nosocomial infections could be prevented by compliance with infection control practices and appropriate hygiene measures. Environmental contaminants are responsible for nosocomial infections in health care facilities. Metallic copper (Cu) surfaces have excellent antimicrobial properties against a variety of different microorganisms. Rapid killing of copper surface exposed bacteria has been documented. Copper causes extensive membrane and envelope damage to bacteria. In this study, we exposed *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* to a copper coupon and a stainless steel coupon for different exposure times. The number of colony forming units was determined and results showed a decrease in viable cell numbers in those exposed to copper. We concluded that copper surfaces can reduce and eliminate bacteria in health care facilities.

### 5-B Antimicrobial activity of copper on water borne bacterial pathogens

Melissa Cabrera, Sigfredo Rivera, Karianis Torres and Yolanda Jové

Water is vital for all living beings on this planet. Water contaminated with bacterial pathogens can lead to a variety of diseases in humans. Using copper as an antimicrobial agent on water has been demonstrated in several studies. The objective of this study was to determine the effect of copper on water samples contaminated with bacterial pathogens. The bacteria used in this study were *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*. To investigate the effect of copper, water samples with and without a copper piece were inoculated with a cell suspension of the above mentioned bacterial pathogens. Pour plates on PCA were prepared from the water samples at different time intervals for 3 hours. The results revealed that *E. coli* and *S. typhimurium* are more susceptible to the antimicrobial effect of copper than *S. aureus.* The findings prove the antimicrobial properties of copper in bacteria contaminated water.

### 5-C Antimicrobial properties of copper sulfate, copper nitrate and cupric chloride

Celeny Ríos and Yolanda Jové

Copper is a reddish-brown nonferrous mineral, which has been used for thousands of years by many cultures. Archaeological evidence suggests that copper is among the earliest metals used by humans. In the modern healthcare setting one of the most widespread and successful applications of copper is its antimicrobial potential, specifically on the control of nosocomial pathogens in hospitals. Our objective is to evaluate the antimicrobial properties of copper sulfate, copper nitrate and cupric chloride on bacteria of medical importance including *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus, Enterococcus faecalis* and *Salmonella typhimurium*. Each compound was tested at 5

mg/ml, 10 mg/ml and 15 mg/ml. Our results show that *P. aeruginosa*, *E. aerogenes*, *S. typhimurium* and *E. faecalis* were more susceptible to cupric (II) chloride. Instead, *B. cereus* was more susceptible to copper sulfate and *E. coli* to copper (II) nitrate**.** Copper chloride showed the highest degree of inhibition. Our findings were consistent to previous studies, but the amount of research using copper in solutions is limited. Further studies are needed to test other concentrations of copper and more species of bacteria.

**5-D Study of the antibacterial properties of copper**
Celeny Ríos and Lizbeth Romero-Pérez

Copper is an important metal that is distributed throughout nature and whose discovery dates from around 5000 BC. Pure copper is soft and malleable; a freshly exposed surface has a reddish-orange color. It has been used in a variety of applications ranging from construction of household utensils, clothing accessories and even as an electric conductor. History has shown a health related use for copper in ancient civilizations. Copper is a powerful antimicrobial with proven rapid, board spectrum efficacy against pathogens threatening public health and wire community. In this study, we want to evaluate and compare, at two different times, the effect of solid copper on *Staphylococcus aureus* and *Enterobacter aerogenes.* The antibacterial effect of copper will be compared to plastic and stainless steel. Our results show that copper inhibits the three tested bacteria at 1 and 3 hrs of exposure. Copper surfaces show complete inhibition of growth in contrast with the effect observed in plastic and stainless steel. We also tested, using *S. aureus* and *Pseudomonas aeruginosa*, the antibacterial effect of copper in solution. Inhibition of copper was observed on *S. aureus* at a concentration of 15 ug/ml. Our results demonstrate the antibacterial properties of copper on bacteria of medical importance. Further studies are required to test more concentrations of copper in solution and other strains of the tested bacteria.

## CHEMISTRY/ CHEMISTRY

**6-A Determinación simultánea por HPLC de cafeína, teobromina y teofilina en los alimentos, bebidas, productos a base de hierbas y plantas medicinales**
Félix Negrón y Arnaldo Bravo

La cafeína (CF) 1,3,7-trimetilxantina, teobromina (TB), 3,7-dimetilxantina, teofilina (TF), y 1,3 - dimetilxantina son tres xantinas obtenidas de plantas de distribución. Estas poseen propiedades farmacológicas similares y se encuentran en el cacao, chocolate y otras plantas. La cafeína es un estimulante del sistema nervioso, el exceso de consumo puede causar nerviosismo, angustia, insomnio y temblores. La teobromina que también se encuentra en el cacao estimula el sistema nervioso de una manera leve y es sensible para algunos animales y es la responsable de provocar una pequeña adicción al chocolate. Por otro lado, el exceso de teobromina afecta al embarazo y otras funciones del cuerpo humano. El método desarrollado usando cromatografía líquida de alta resolución (HPLC) tiene como objetivo principal separar, detectar y cuantificar la composición de una mezcla de cafeína, teobromina y teofilina analizando los cromatogramas obtenidos y generando una curva de calibración con mezclas de composición conocida de cada reactivo . La cromatografía es realizada en una columna Zorbax Eclipse XDB-C18 (4.6 x 150 mm id, 5-micras de tamaño de partícula) a 25 °C, con una fase móvil de agua-THF (0.1% de THF en agua, pH 8) acetonitrilo (90:10, v/v). La velocidad de flujo es de 0.25 mL/min, y la detección es por UV a 273 nm. El desarrollo y aplicación del método nos permite la determinación simultánea de cafeína, teobromina, y teofilina en 17 muestras que incluyen alimentos, bebidas, productos a base de hierbas y plantas medicinales. Las curvas de calibración obtenidas dan aproximadamente una correlación de 0.99 para cada xantina analizada.

**6-B Determinación de la composición de mezclas de polímeros usando el método del estándar interno por espectroscopía FTIR-ATR**
Edibal Guilloty y Arnaldo Bravo

El método del estándar interno puede utilizarse para determinar la composición de una mezcla de polimeros (etileno-co-acetato de vinilo) cuando ésta es analizada por espectrometría del infrarrojo utilizando transformada de Fourier (FTIR). Utilizando estándares de poli (etileno-co-acetato de vinilo) a diferentes concentraciones se determinaron los espectros en la región del infrarrojo y se elaboró una técnica para la preparación de las muestras y obtención de los espectros. Con estos datos se estableció una correlación de 0.997 entre la razón de la absorbancia de los picos de acetato de polivinilo (PVA) a 1020 $cm^{-1}$ y polietileno (PE) a 720 $cm^{-1}$ y la razón de la composición de ambos polímeros, comparable a resultados publicados. En adición, se pudo demostrar que esta correlación también se presenta con los picos de PVA a 1737, 1370 y 1238 $cm^{-1}$ aspecto no publicado en referencias relacionadas a este tema. Esta metodología y correlaciónes obtenidas permiten determinar el contenido de uno de estos polímeros en materiales comerciales si se le añade el otro polímero como estándar interno. Cuatro materiales comerciales se analizaron obteniendo resultados con errores típicos menores al 1%.

## POLÍTICAS EDITORIALES/EDITORIAL POLICIES

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