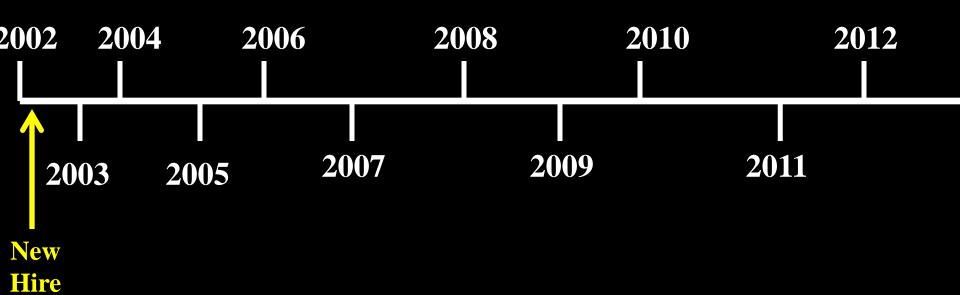
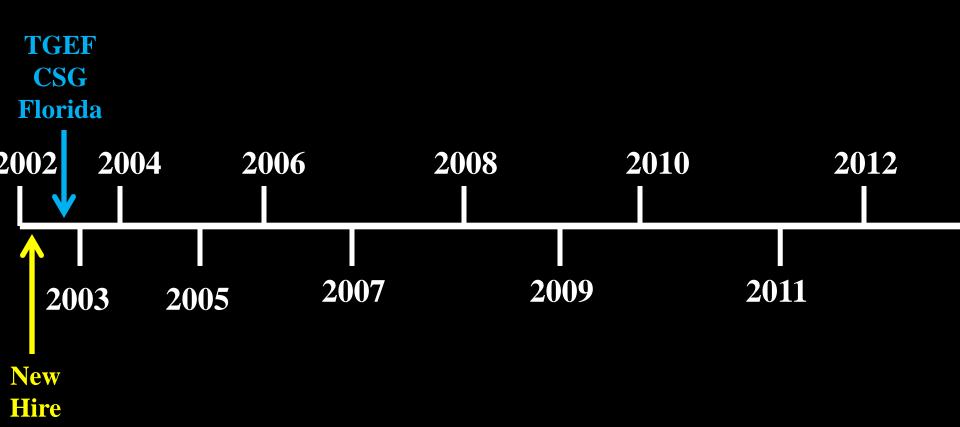
# Investigation of Crocodilian Innate Immunity:

# An EPSCoR-Supported International Research Program

Mark Merchant, Ph. D.
Professor of Biochemistry
McNeese State University
Lake Charles, LA







# Division of IUCN (International Union for Conservation of Nature)





**IUCN** 

Division of IUCN (International Union for **Conservation of Nature**)



Crocodile Specialist Group

Division of IUCN (International Union for Conservation of Nature)
One of 114 Species Survival Commissions



Responsible for setting international policies concerning trade in crocodile products

**Set Import/Export Limits** 

Determine the level of protection (stable, endangered, critically endangered, etc)





Division of IUCN (International Union for Conservation of Nature)
One of 114 Species Survival Commissions



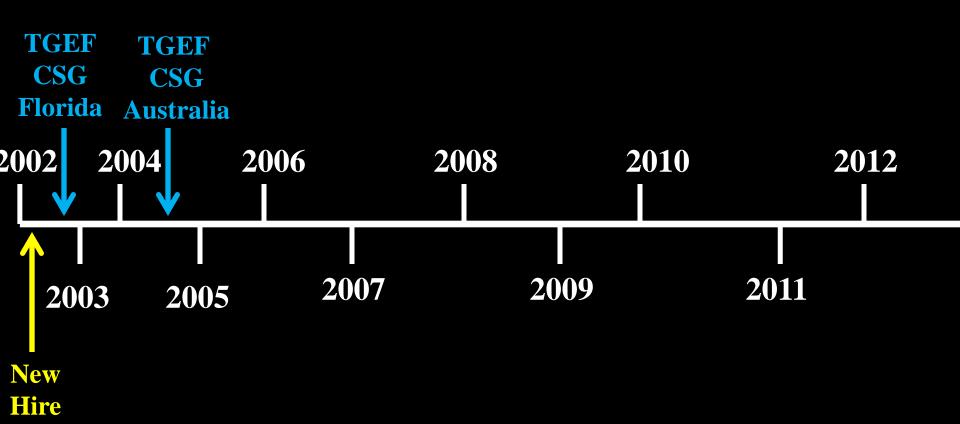
Responsible for setting international policies concerning trade in crocodile products

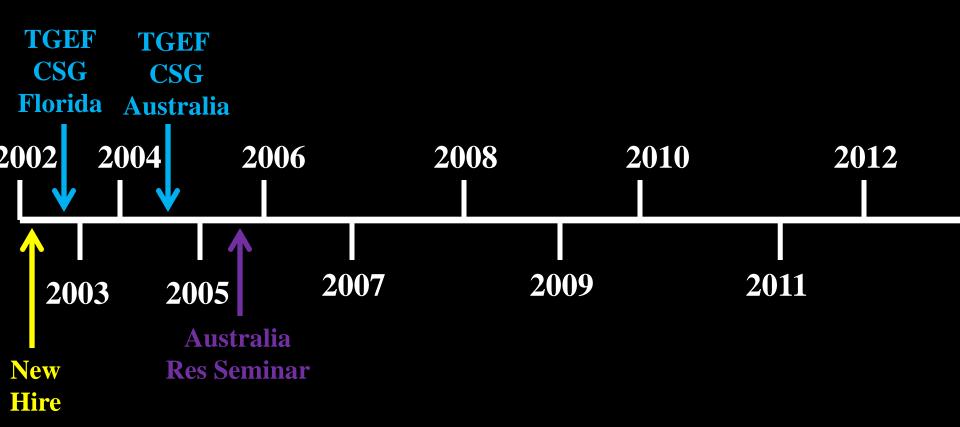
**Set Import/Export Limits** 





Meetings held every two years Must be nominated for membership

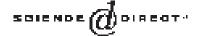








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Comparative Biochemistry and Physiology, Part A 143 (2006) 488-493

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### Characterization of serum complement activity of saltwater (Crocodylus porosus) and freshwater (Crocodylus johnstoni) crocodiles

Mark Merchant a,\*, Adam Britton b

Department of Chemistry, McNeese State University, Box 90455, Lake Charles, LA, 70609, USA Wildlife Management Incorporated, Darwin, Northern Territory, Australia

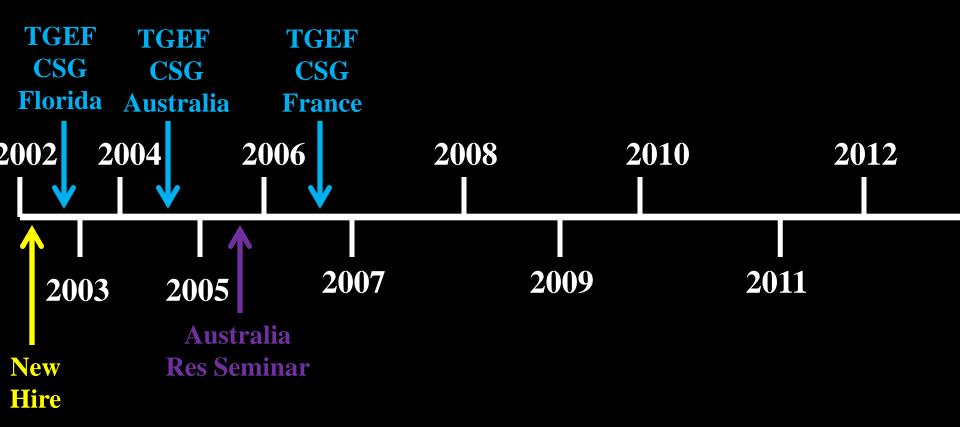
Received 12 October 2005; received in revised form 3 January 2006; accepted 8 January 2006 Available online 17 February 2006

#### Abstract

We employed a spectroscopic assay, based on the hemolysis of sheep red blood cells (SRBCs), to assess the innate immune function of saltwater and freshwater crocodiles in vitro. Incubation of serum from freshwater and saltwater crocodiles with SRBCs resulted in concentration-dependent increases in SRBC hemolysis. The hemolytic activity occurred rapidly, with detectable activity within 2 min and maximum activity at 20 min. These activities, in both crocodilian species, were heat sensitive, unaffected by 20 mM methylamine, and completely inhibited by low concentrations of EDTA, suggesting that the alternative serum complement cascade is responsible for the observed effects. The hemolytic activities of the sera were inhibited by other chelators of divalent metal ions, such as phosphate and citrate. The inhibition of SRBC hemolysis by EDTA could be completely restored by the addition of 10 mM Ca<sup>2+</sup> or Mg<sup>2+</sup>, but not Ba<sup>2+</sup>, Cu<sup>2+</sup> or Fe<sup>2+</sup>, indicating specificity for these metal ions. The serum complement activities of both crocodilians were temperature-dependent, with peak activities occurring at 25–30 °C and reduced activities below 25 °C and above 35 °C.

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Keywords: Crocodilian; Immunology; Innate immunity; Humoral immunity

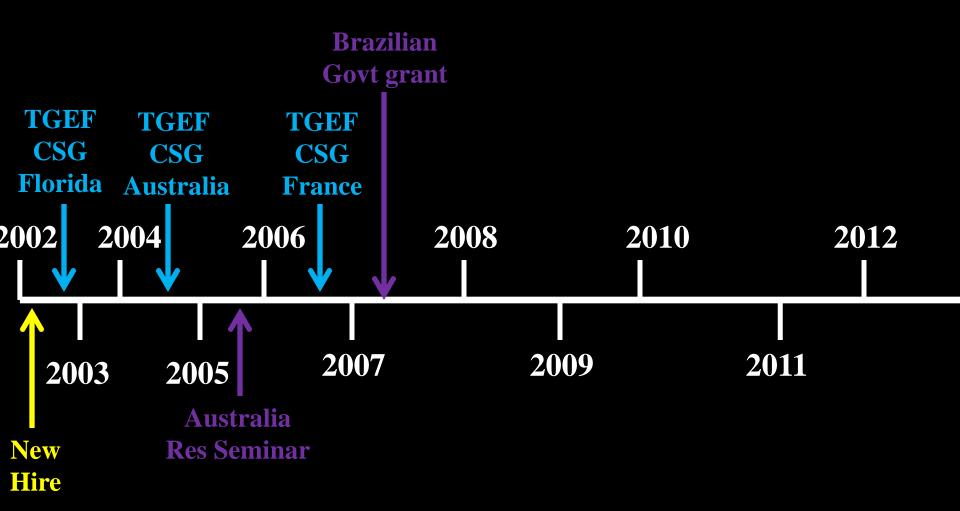






### **Fall of 2006**

Nominated and accepted into the CSG











#### Plasma Activity of the Broad-snouted Caiman (Caiman latirostris)

Pablo A. Siroski<sup>1,2,\*</sup>, Carlos I. Piña<sup>1,3</sup>, Alejandro Larriera<sup>1,2,4</sup>, Mark E. Merchant<sup>5</sup>, and Jose Di Conza<sup>6</sup>

(Accepted June 19, 2008)

Pablo A. Siroski, Carlos I. Piña, Alejandro Larriera, Mark E. Merchant, and Jose Di Conza (2009) Plasma activity of the Broad-snouted Calman (Calman latirostris). Zoological Studies 48(2): 238-242. Crocodillans exhibit well-defined social behaviors, which frequently result in serious wounds as a consequence of social disputes including the loss of entire limbs. Despite the severity of many wounds, there is typically little sign of infection. A common question is how these animals survive with serious wounds without showing obvious signs of lilness, particularly when living in environments containing potentially pathogenic microbes. In this study we determined in vitro plasma antibacterial activity of the Broad-snouted calman (Calman latirostris) against Escherichia coll and compared it to that in hen (Gallus gallus) and human plasma. Colony forming units were measured at different exposure times (0, 1, 3, and 6 h). The antibacterial activity of Broad-snouted calman plasma was consistently superior to those of human and hen plasma, and hen plasma had greater activity than human plasma except at 3 h of exposure. Only C. latirostris plasma completely inhibited E. coll proliferation at 6 h. http://zoolistud.sinica.edu.tw/Journals/48.2/238.pdf

Key words: Crocodylla, Immune system, Broad-snouted calman, Antibacterial activity, Calman latirostris.

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<sup>&</sup>lt;sup>1</sup>Proyecto Yacaré (Convenio Gobierno de Santa Fe/MUPCN), A. del Valle 8700, Santa Fe, 3000, Argentina

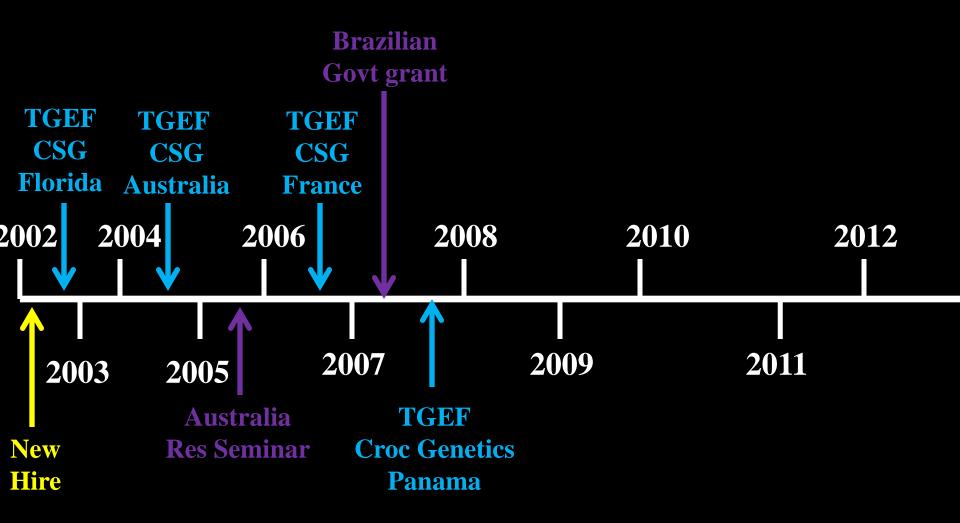
<sup>&</sup>lt;sup>3</sup>Secretaria de Estado de Medio Ambiente y Desarrollo Sustentable de la Provincia de Santa Fe, 3000, Argentina

<sup>&</sup>lt;sup>3</sup>CIC y TTP-CONICET, Proyecto Yacaré, Dr. Matterl y España, Diamante, Entre Rios, 3105, Argentina

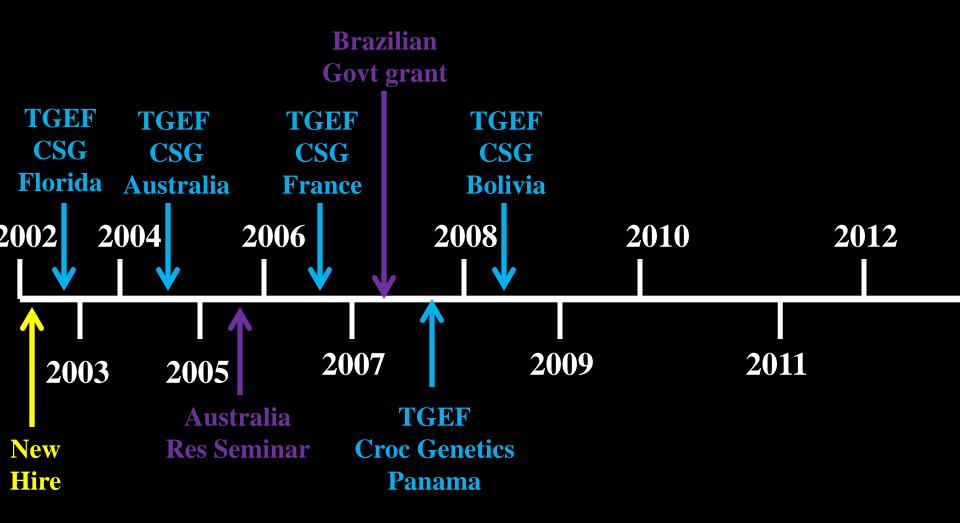
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Department of Chemistry, McNeese State University, Box 90455, Lake Charles, LA 70609, USA

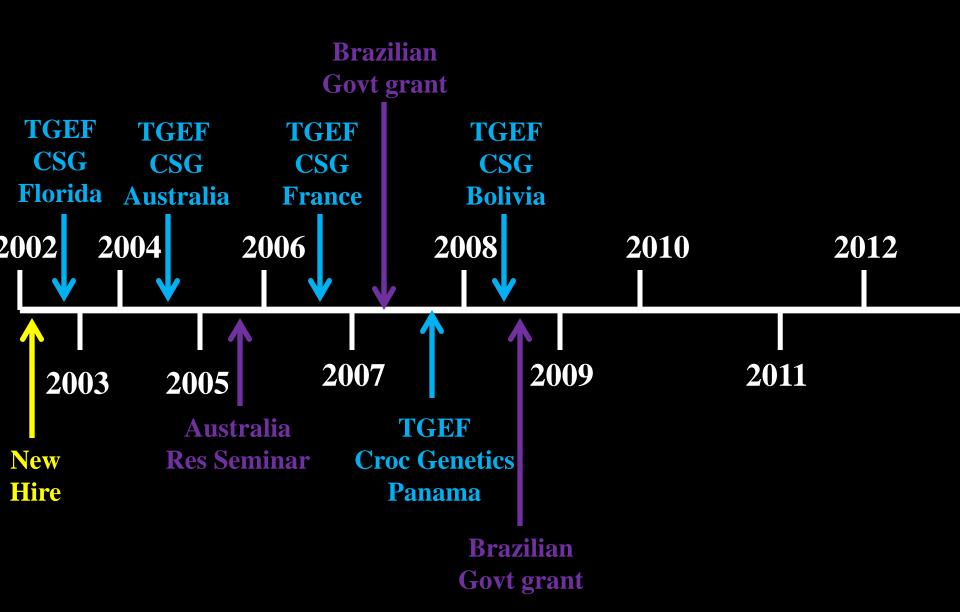
<sup>&</sup>lt;sup>4</sup>Microbiologia General, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, 3000, Argentina















#### Characterization of innate immune activity in Phrynops geoffroanus (Testudines: Chelidae)

Bruno O. Ferronato 1, 3; Mark E. Merchant 2; Thiago S. Marques 1 & Luciano M. Verdade 1

E-mail: mmerchant@mcneese.edu

ABSTRACT. The innate immune activity of the freshwater turtle Phrynops geoffroanus (Schweigger, 1812) was investigated, using a sheep-red-blood cell hemolysis assay. The time- and concentration-dependent hemolytic activity of the turtle plasma was low compared to that reported for other reptiles. However the plasma of P. geoffroanus exhibited higher activity at elevated temperatures, resulting in temperature-dependent hemolysis. The sensitivity of turtle plasma to temperature could be interpreted as a mechanism by which freshwater turtles use basking behavior to elevate body temperature, thus enhancing the innate immune response. However, we cannot discard the possibility that environmental contaminants could be affecting the turtle's immune response, since the animals in this investigation were captured in a polluted watercourse.

KEY WORDS. Chelonian; Immunology; plasma.

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<sup>\*</sup> Corresponding author.



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#### Microchemical Journal

journal homepage: www.elsevier.com/locate/microc



### Determination of heavy metals by inductively coupled plasma-optical emission spectrometry in fish from the Piracicaba River in Southern Brazil

Andrew Meche <sup>a</sup>, Manoela C. Martins <sup>b</sup>, Bruna E.S.N. Lofrano <sup>b</sup>, Carey J. Hardaway <sup>a</sup>, Mark Merchant <sup>a,\*</sup>, Luciano Verdade <sup>b</sup>

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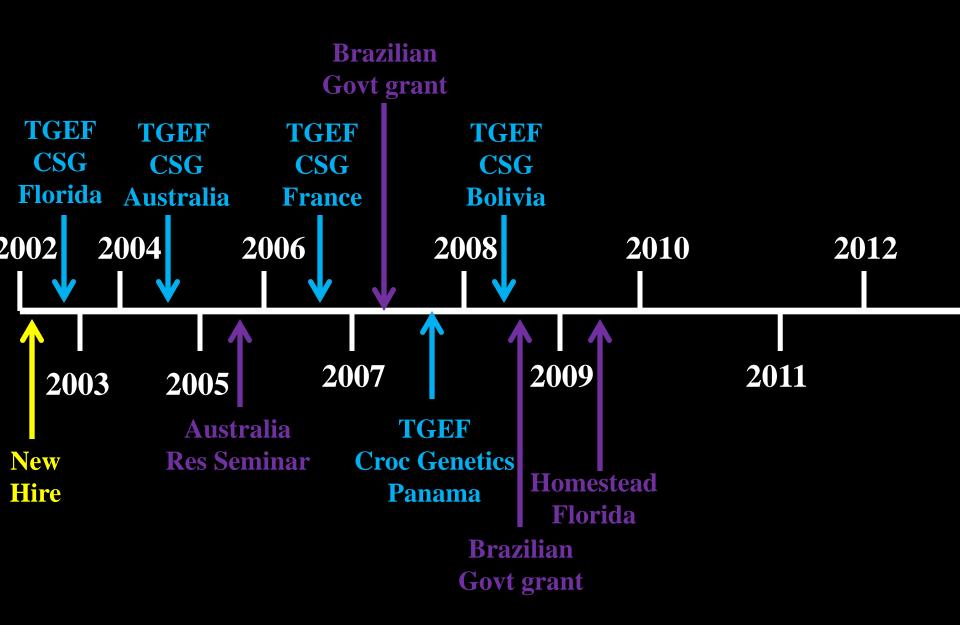
#### ABSTRACT

A total of 202 fish, representing 16 species, were collected during 2008 (March-October) in the Tanquan region of the Piracicaba River using nets. Flesh samples were collected and analyzed, using inductively coupled plasma-optical emission spectroscopy for Al, As, Cd, Co Cr, Cu, Mn, Mo, Ni, Pb, Se, Sn, Sr, and Zn. The results showed that the flesh of these fish all contained extremely high levels of Al and Sr, and moderately high levels of Cr, As, Zn, Ni, Mn and Pb. The metals were higher in these fish during rainy season, with fish collected during the months of March and October being the highest. In addition, the accumulation of metals was species-dependent. Cascudos (Hypostomus punctatus) and piranhas (Serrasalmus spilopleura) exhibited high levels of almost all of the metals, while curimbata (Prochilodus lineatus) had moderate levels. A few species, including pacu (Piaractus mesopotamicus) and dourado (Salminus maxillosus), had very low levels of most metals. The results show that the Piracicaba River Basin is widely contaminated with high levels of many toxic heavy metals, and that human consumption of some fish species is a human health concern.

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#### Research paper

### Identification and characterization of dipeptidyl peptidase IV enzyme activity in the American crocodile (Crocodylus acutus)

Mark Merchant a,\*, Stephanie Mead a, Charles McAdon a, Justin McFatter a, Joe Wasilewski b

#### ARTICLE INFO

#### Article history: Received 1 September 2009 Received in revised form 21 January 2010 Accepted 21 January 2010

Keywords: Crocodilian Dipeptidyl peptidase IV Innate immunity

#### ABSTRACT

Serum from the American crocodile was assayed for dipeptidyl peptidase IV (DPP4) activity. We measured the DPP4-mediated hydrolysis of Ala–Pro–ARC. The generation of AFC was dependent on the titer of serum, with significant DPP4 activity (0.20  $\pm$  0.03 nmol product formed) measured using only 2  $\mu$ L of crocodile serum, with maximum activity measured using 500  $\mu$ L of serum. The hydrolysis of substrate was inhibited in a concentration-dependent manner by diprotin A, a specific inhibitor of DPP4 activity, indicating that this activity was due to the presence of DPP4. The crocodile serum DPP4 exhibited classical Michaelis–Menten kinetics, with  $K_m$  and  $V_{max}$  extrapolated, by double-reciprocal plot, to be 14.7  $\pm$  1.3  $\mu$ M and 75.5  $\pm$  4.3 nmol/min, respectively. Crocodile DPP4 catalyzed the hydrolysis of Ala–Pro–ARC rapidly, with substantial activity measured within 5 min of the addition of substrate. After an initial rapid increase in activity, near maximal activity (7.43  $\pm$  0.24 nmol product formed) measured at 180 min. Crocodile serum DPP4 activity was temperature-dependent, with steadily increased activity from 5 to 40 °C.

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b Natural Selections of South Florida Inc., Princeton, FL, USA



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Comparative Biochemistry and Physiology, Part B 143 (2006) 133 - 137

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# Comparisons of innate immune activity of all known living crocodylian species

Mark E. Merchant a,\*, Kaili Mills a, Noelle Leger a, Erin Jerkins a, Kent A. Vliet b, Nola McDaniel c

Department of Chemistry, McNeese State University, Box 90455, Lake Charles, LA, 70609, USA
 Department of Zoology, University of Florida, Gainesville, FL, USA
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Received 11 June 2005; received in revised form 12 October 2005; accepted 13 October 2005 Available online 20 December 2005

#### Abstract

Serum samples from all twenty-three known living members of the Crocodylia were tested for antibacterial activity against eight bacterial species. These data were used to generate an immune profile for each crocodylian species. Statistical analyses revealed that the three living lineages of crocodylians, Alligatoroidea, Crocodyloidea, and Gavialoidea, were distinguishable by their immunological activities. For instance, species within the Alligatoroidea and Crocodyloidea exhibited remarkable immune activity similarities to others in their own lineages. Comparisons of the members of the different lineages, however, revealed substantial differences in immune profiles. Furthermore, species that are in the same genus were shown to exhibit more immune similarities to each other than to members of other genera within the same family. Finally, our immunological analyses reveal that *Tomistoma schlegelii* aligns more closely with the Gavialoidea than the Crocodyloidea.

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#### Veterinary Immunology and Immunopathology





Research paper

#### Identification and characterization of serum complement activity in the American crocodile (Crocodylus acutus)

Mark Merchant a,\*, Justin McFatter a, Stephanie Mead a, Charles McAdon a, Joe Wasilewski b

#### ARTICLE INFO

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Keywords: Crocodylus acutus Innate immunity SRBC hemolysis

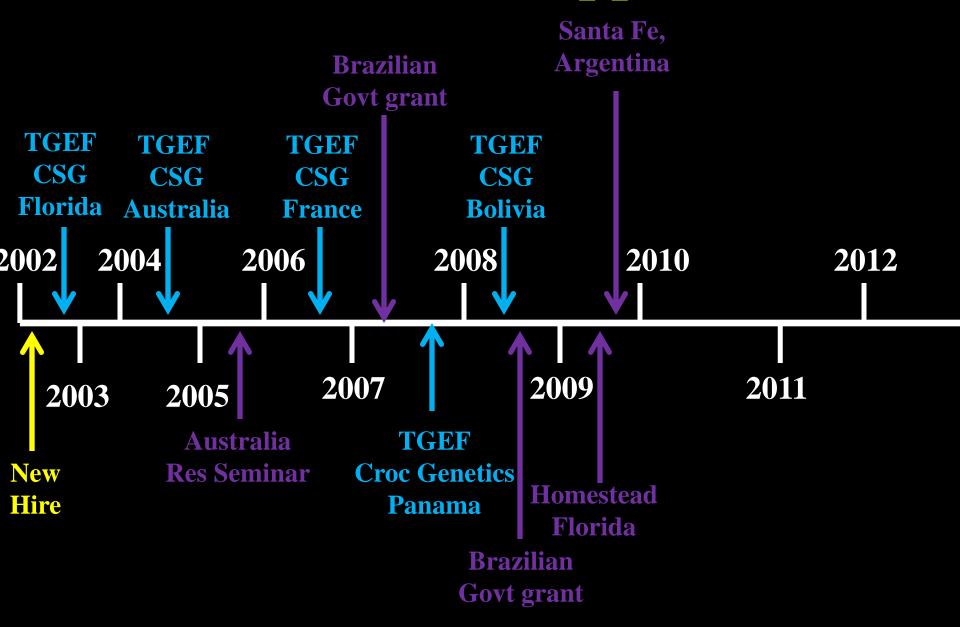
#### ABSTRACT

Incubation of unsensitized sheep red blood cells with serum from the American crocodile (Crocodylus acutus) resulted in a concentration-dependent hemolysis. The hemolytic activity was heat-sensitive, and inhibited by EDTA in a concentration-dependent manner. The EDTA-inhibited SRBC hemolysis could be restored by the addition of excess Ca<sup>2+</sup> or Mg<sup>2+</sup>, but not Ba<sup>2+</sup> or Cu<sup>2+</sup>, revealing the specificity of this activity for these two divalent cations. The hemolytic activity of crocodile serum was titer-dependent, with 329 µL producing 50% of maximal SRBC hemolysis. The complement activity was also temperature-dependent, with decreased activity at lower temperatures (5–15 °C) and maximal activity occurred at 30–40 °C. The hemolysis occurred relatively slowly, with near zero activity after 10 min, 40% of activity observed within 15 min of exposure to SRBCs, and maximal activity at 30 min.

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#### Characterization of the Serum Complement Activity of the Broad-Snouted Caiman Caiman latirostris (Crocodilia: Alligatoridae)

Pablo A. Siroski<sup>1,2,4</sup>, Mark Merchant<sup>3</sup>, María Virginia Parachú Marcó<sup>1,4</sup>, Carlos I. Piña<sup>1,4</sup>, and Hugo H. Ortega<sup>2</sup>

(Accepted April 28, 2009)

Pablo A. Siroski, Mark Merchant, María Virginia Parachú Marcó, Carlos I, Piña, and Hugo H, Ortega (2010)

Characterization of the serum complement activity of the broad-shouted calman Calman latinostris (Crocodilia: Alligatoridae). Zoological Studies 49(1): 84-70. The sheep red blood cell (SRBC) hemolysis assay was used to detect and characterize complement-system activity of broad-shouted calman (Calman latinostris) serum. The hemolytic activity of calman serum was inhibited by 2 classic inhibitors (EDTA and heat) suggesting the existence of complement-system activity. In addition, we found that the capacity of C. latinostris serum to disrupt SRBCs was concentration, temperature, and kinetics dependent. Hemolytic activity was detected from a very low concentration (< 10%) of calman serum and increased until 100%. Temperature influenced the activity of the serum by disrupting SRBC membranes. The serum showed a peak of hemolysis between 30 and 40°C, within which lies the optimal temperature calmans prefer during thermoregulation for normal physiological processes. Hemolytic activity rapidly occurred at 2 min, and maximum activity was detected at 60 min. These observations reflect previously reported findings in other crocodilian species (Alligator mississippiensis, Crocodylus johnstoni, and Cro. porosus), thus adding to the knowledge of the role of the complement system in immunological activities of crocodilians. http://zoolstud.sinica.edu.tw/Journals/49.1/84.pdf

Key words: Crocodillans, Ectothermics, Hemolytic assay, Immunology, Innate immunity.

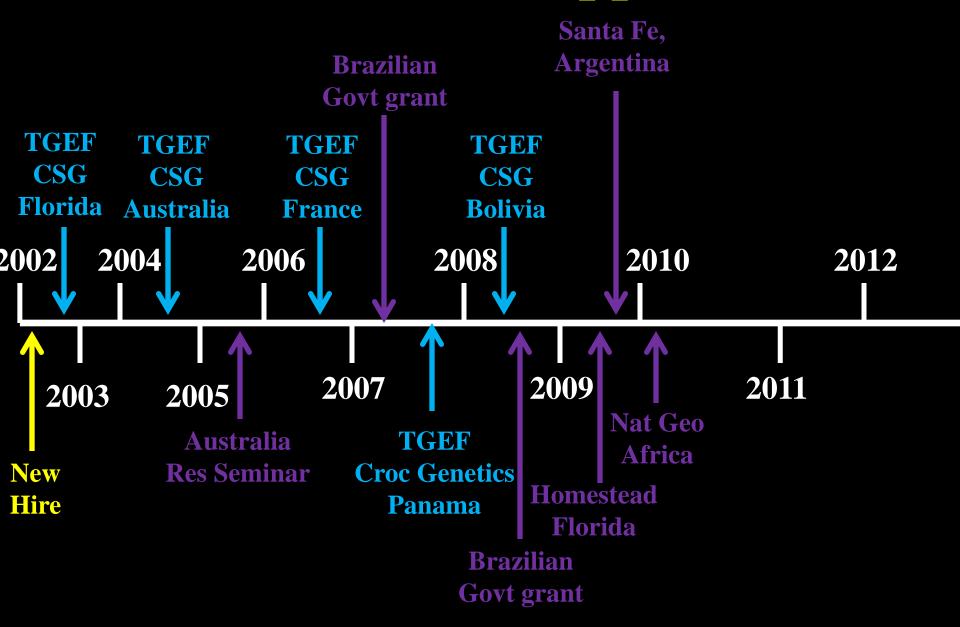
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<sup>&</sup>lt;sup>2</sup>Secretaria de Medio Ambiente de la Provincia de Sante Fe. CP 3000. Sante Fe. Arcentina.

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<sup>\*</sup>Centro de Investigaciones Científicas y Transferencia de Tecnología a la Producción (CICyTTP-CONICET), CP 3105, Diamente, Entre Ríos, Arcentina

<sup>\*</sup>Departemento de Ciencias Morfológicas, Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, CP 3000, Sente Fe, Arcentina – CONICET







# Characterization of serum dipeptidyl peptidase IV activity in three diverse species of West African crocodilians

Mark Merchant<sup>1</sup>, Arian Royer<sup>1</sup>, Quintin Broussard<sup>1</sup>, Sarah Gilbert<sup>1</sup>, Rodolfo Falconi<sup>1</sup> & Matthew H. Shirley<sup>2</sup>

\*WoNesse State University, Department of Chemistry, Lake Charles, Louisiana, USA. 
\*University of Florida, Department of WildMe Ecology and Conservation, Gainesville, Florida, USA.

Serum dipeptidyl peptidase IV (DPPIV) activity was characterized in three divergent and sympatric species of West African crocodiles. The serum of the Nile crocodile (Crocodylus miloticus) exhibited higher DPPIV activity than that of the African dwarf crocodile (Osteolasmus tetraspis) and the slender-snouted crocodile (Mecistops cotaphractus). Kinetic analyses showed that the rate of product formation was higher in serum of C. miloticus with respect to time, and it was confirmed by double reciprocal plot analysis that the V<sub>max</sub> for serum of C. miloticus was higher than the other two species. However, the Michaelis constants were very similar for all three species, indicating that the C. miloticus DPPIV enzyme may be a more efficient catalyst. Thermal activity profiles demonstrated that the serum DPPIV activities of all three species increased substantially with temperature. Although activity of C. miloticus was higher than that of O. tetraspis and M. cataphractus at all temperatures investigated, linear increases of activity with temperature were noted for all three species. The results from this study show that three diverse species of West African crocodiliums express soluble serum DPPIV.

Key words: Crocodylus niloticus, DPPIV, innate immunity, Mecistops cataphractus, Osteolaemus tetraspis, reptilian, T-cell activation Hindawi Publishing Corporation Biochemistry Research International Volume 2011, Article ID 925012, 7 pages doi:10.1155/2011/925012

#### Research Article

#### Characterization of Serum Phospholipase A<sub>2</sub> Activity in Three Diverse Species of West African Crocodiles

#### Mark Merchant, 1 Kate Juneau, 1 Jared Gemillion, 1 Rodolfo Falconi, 1 Aaron Doucet, 1 and Matthew H. Shirley 2

Correspondence should be addressed to Mark Merchant, mmerchant@mcneese.edu

Received 27 March 2011; Revised 21 July 2011; Accepted 25 July 2011

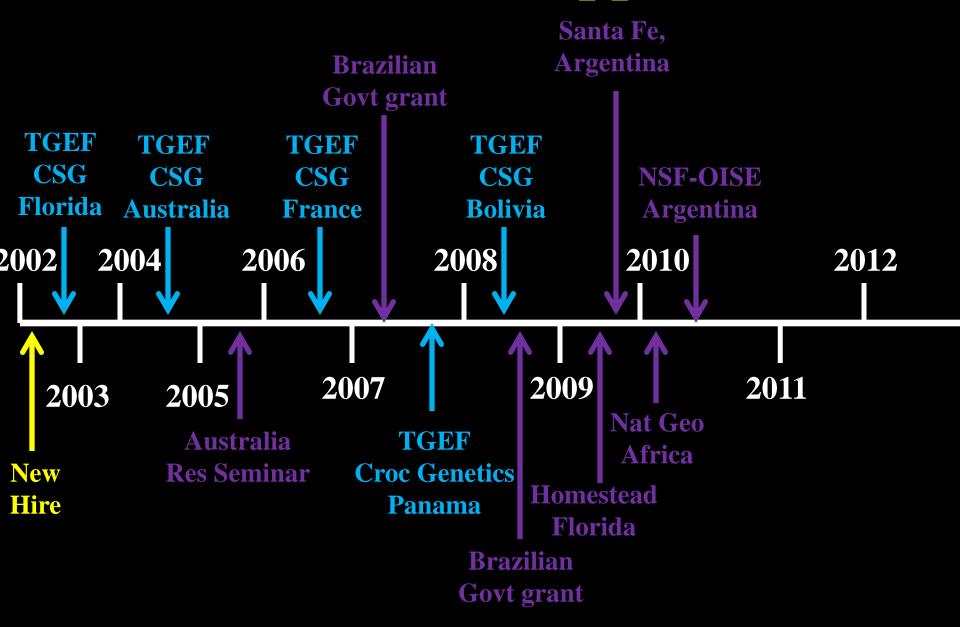
Academic Editor: Sanford I. Bernstein

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Secretory phospholipase A<sub>2</sub>, an enzyme that exhibits substantial immunological activity, was measured in the serum of three species of diverse West African crocodiles. Incubation of different volumes of crocodile serum with bacteria labeled with a fluorescent fatty acid in the sn-2 position of membrane lipids resulted in a volume-dependent liberation of fluorescent probe. Serum from the Nile crocodile (Crocodylus miloticus) exhibited slightly higher activity than that of the slender-snouted crocodile (Mecistops camphracius) and the African dwarf crocodile (Osteolaemus tetraspis). Product formation was inhibited by BPB, a specific PLA<sub>2</sub> inhibitor, confirming that the activity was a direct result of the presence of serum PLA<sub>2</sub>. Kinetic analysis showed that C. niloticus serum produced product more rapidly than M. cataphracius or O. tetraspis. Serum from all three species exhibited temperature-dependent PLA<sub>2</sub> activities but with slightly different thermal profiles. All three crocodilian species showed high levels of activity against eight different species of bacteria.

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#### Characterization of the Serum Complement Activity of the Broad-Snouted Caiman Caiman latirostris (Crocodilia: Alligatoridae)

Pablo A. Siroski<sup>1,2,\*</sup>, Mark Merchant<sup>3</sup>, María Virginia Parachú Marcó<sup>1,4</sup>, Carlos I. Piña<sup>1,4</sup>, and Hugo H. Ortega<sup>5</sup>

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(Accepted April 25, 2009)

Pablo A. Strocki, Mark Merchant, Maria Virginia Parachù Marcó, Carlos I. Piña, and Hugo H. Ortega (2010) Characterization of the serum complement activity of the broad-snouted calman Calman latirostris (Croccodila: Alligatoridae). Zoological Studies 48(1): 64-70. The sheep red blood cell (SRBC) hemolysis assay was used to detect and characterize complement-system activity of broad-snouted calman (Calman latirostris) serum. The hemolytic activity of calman serum was inhibited by 2 classic inhibitors (EDTA and heat) suggesting the existence of complement-system activity. In addition, we found that the capacity of C. latirostris serum to disrupt SRBCs was concentration, temperature, and kinetics dependent. Hemolytic activity was detected from a very low concentration (< 10%) of calman serum and increased until 100%. Temperature influenced the activity of the serum by disrupting SRBC membranes. The serum showed a peak of hemolysis between 30 and 40°C, within which lies the optimal temperature calmans prefer during thermoregulation for normal physiological processes. Hemolytic activity rapidly occurred at 2 min, and maximum activity was detected at 60 min. These observations reflect previously reported findings in other croccodillan species (Alligator mississippiensis, Croccodylus johnston), and Cro. porosus), thus adding to the knowledge of the role of the complement system in immunological activities of croccodillans. http://zoolstud.sinica.edu.tw/Journals/49.1/64.pdf

Key words: Crocodillans, Ectothermics, Hemolytic assay, Immunology, Innate Immunity,



Research Article

Ultraviolet radiation on innate immunity and growth of broad-snouted caiman (Caiman latirostris): implications for facilities design

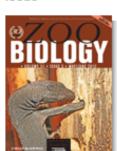
Issue

Pablo Ariel Siroski 1,\*, Gisela Laura Poletta<sup>1,2</sup>, Lucia Fernandez,<sup>1</sup>, Hugo Héctor Ortega,3, Mark Edwin Merchant4

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Zoo Biology

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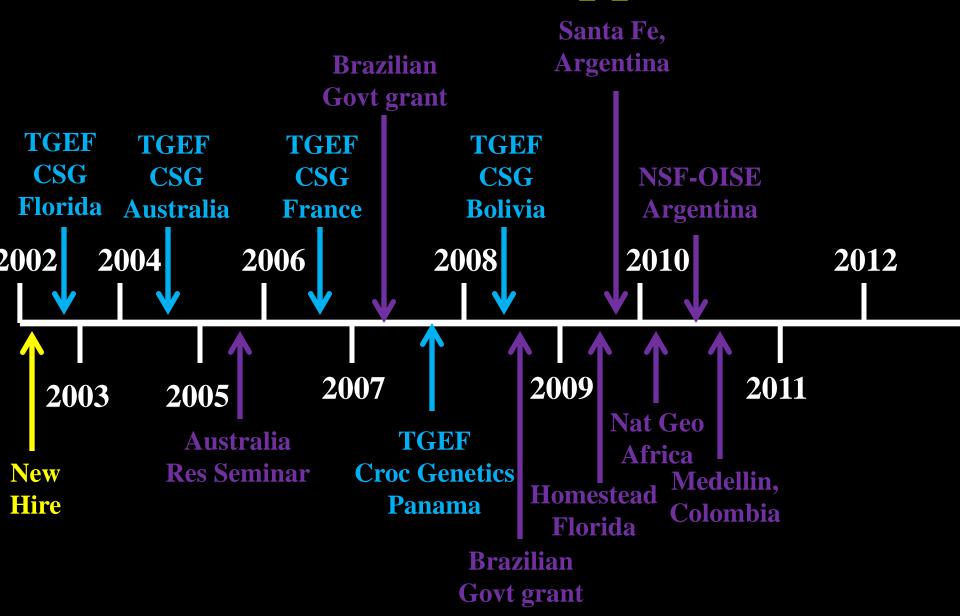
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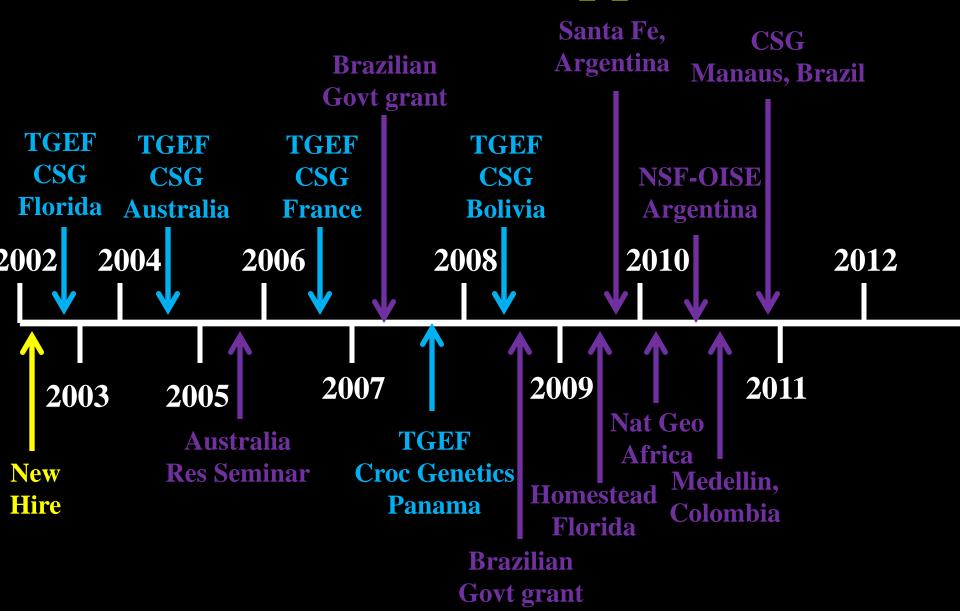
immunity; complement system; crocodilian husbandry; raising; UV radiations

#### Abstract

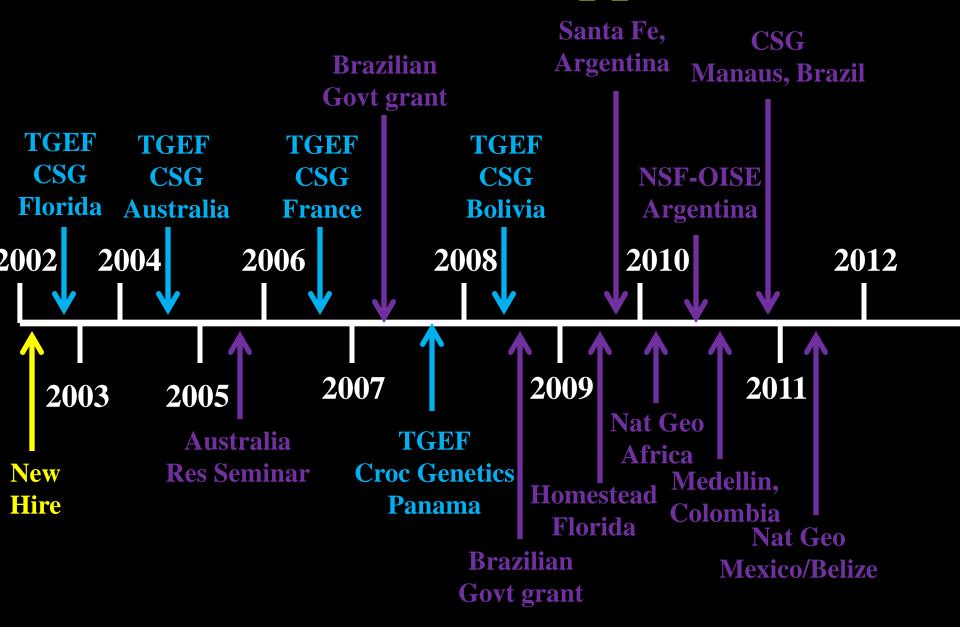
Sunlight is a key environmental factor in almost all ecosystems, and it is necessary for many physiological functions. Many vertebrates require ultraviolet (UV) radiation to perform different physiological processes. Artificial light is used to supplement UV in captive animals, through appropriate photoperiods and UV wavelengths. Previous studies reported that repeated exposure to artificial UV radiation may cause damage to the immune system. Taking into account the importance of UV effects and the serum complement system, the relationship between them was investigated. The study lasted 90 days and was carried out in plastic chambers. Ninety six broad-snouted caiman (C. latirostris) were assigned to four treatment groups with two replicates each; total darkness (TD), 8 hr per day (8 hr) and 16 hr per day (16 hr) of artificial UV/visible light exposure, and normal photoperiod of natural light (NP). Snout-vent length was measured to determine animal growth. Hemolytic assays were performed to evaluate the effects of artificial UV/visible light, TD, and NP on the serum





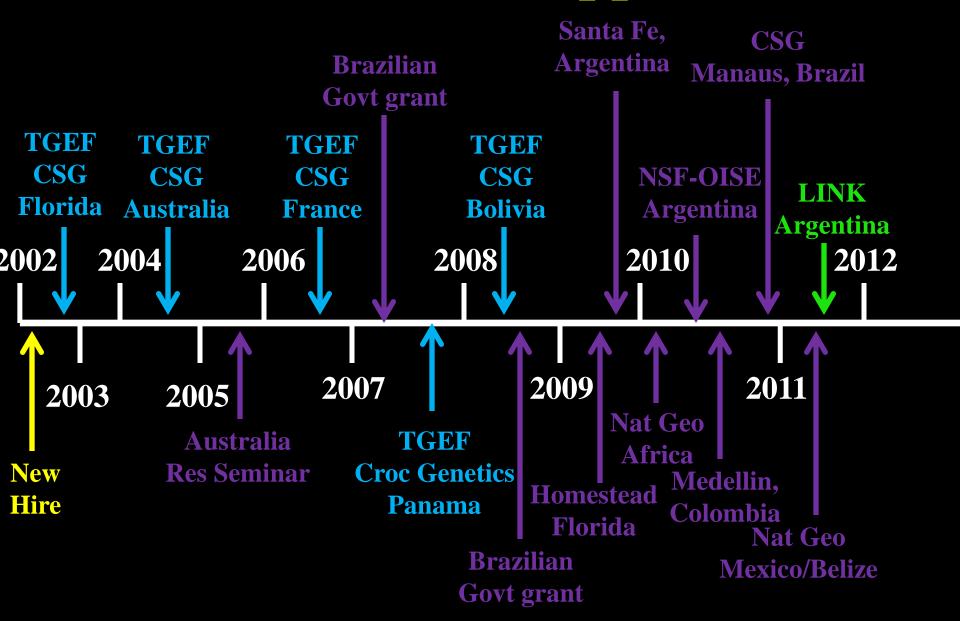


















## Comparison of plasma dipeptidyl peptidase IV activity in two caiman species: Caiman latirostris and Caiman yacare (Crocodylia, Alligatoridae)

Authors: Siroski, Pablo A.<sup>1</sup>; Merchant, Mark E.<sup>2</sup>; Marcó, María Parachú V.<sup>3</sup>; Poletta, Gisela L.<sup>4</sup>; Ortega, Hugo H.<sup>5</sup>

Source: Animal Biology, Volume 61, Number 2, 2011, pp. 199-210(12)

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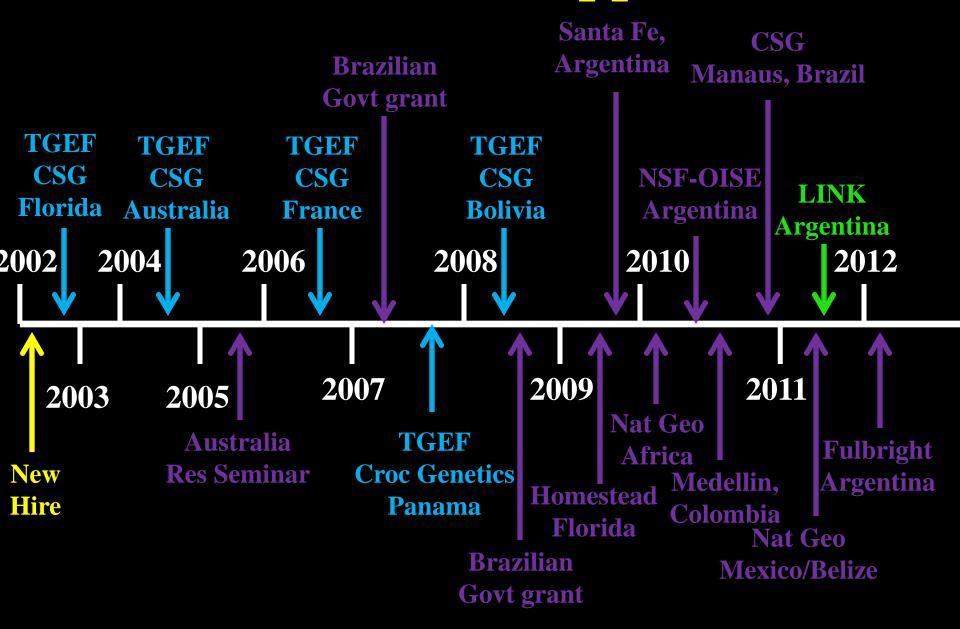
Biology

Mark item

#### Abstract:

Dipeptidyl peptidase IV (DPPIV) is a well-characterized protease with broad substrate specificity, functionally-related to the activity of many bioactive peptides. It plays an important role as physiological regulator of a number of peptides that serve as biochemical messengers within the immune system. Plasma DPPIV activity was characterized with respect to temperature, kinetics and concentration dependence in two species of caiman, the broad-snouted caiman (*Caiman latirostris*) and the black yacare (*Caiman yacare*). DPPIV activity showed a significant positive correlation from titrations carried out in the presence of different plasma concentrations. DPPIV activity was lower in *C. yacare* than in *C. latirostris* at all temperatures tested. *C. yacare* DPPIV activity showed a significant increase only at higher temperatures whilst *C. latirostris* plasma demonstrated a strong positive correlation starting at the lowest temperature, probably due to an adaptation for the tolerance of lower temperatures. Exposure of *C. latirostris* and *C. yacare* plasma at different time points showed that plasma DPPIV activities were time-dependent, and that the titer-dependent curves were different for the two species. These results revealed that plasma DPPIV activities were different between these two crocodilian species, which could contribute to the differences in susceptibility to infection between them.

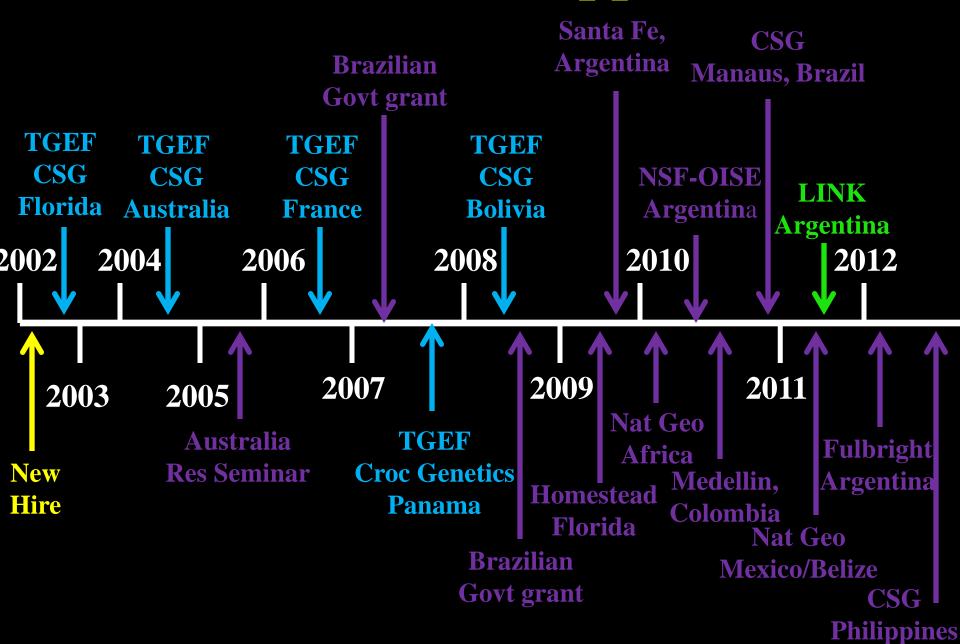
Keywords: DPPIV; PEPTIDASES; BROAD-SNOUTED CAIMAN; YACARE CAIMAN; CROCODILIAN IMMUNE SYSTEM









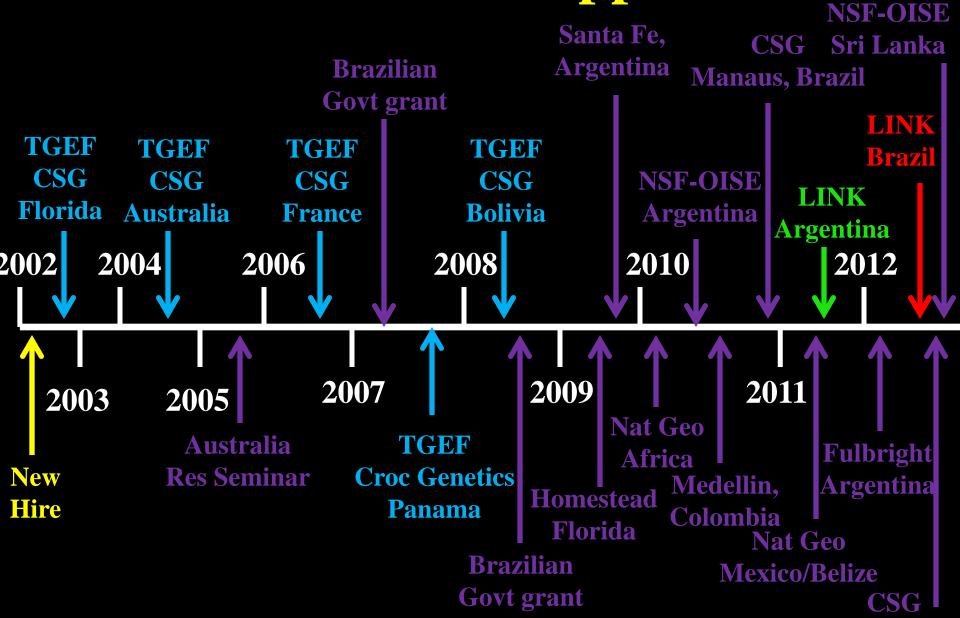


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- 2004 Darwin, Australia
- 2006 Montelimar, France
- 2008 Santa Cruz, Bolivia
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**Philippines** 

## Press

National Geographic "Ultimate Crocodile" 2008



National Geographic "Ultimate Crocodile"
National Geographic "Zombie Alligators"
National Geographic "Jurassic CSI: T-Rex Trauma"
National Geographic – not yet titled

Discovery Channel "Project X" CBC "???"
TNN Canada "Eds up"

**Good Morning America Fox and Friends in the Morning** 

Who wants to be a Millionaire?

Yahoo Front Page New York Times, London Times, Houston Chronicle, Chicago Tribune, etc Rush Limbaugh

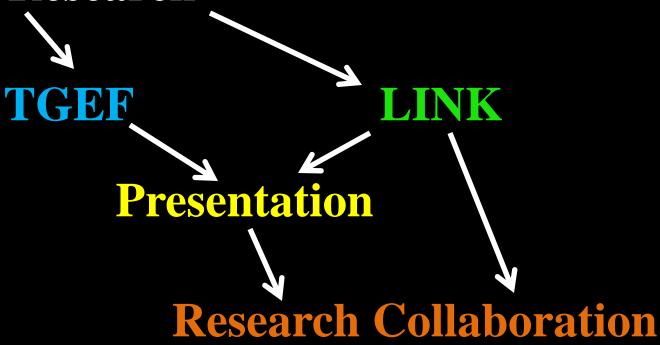
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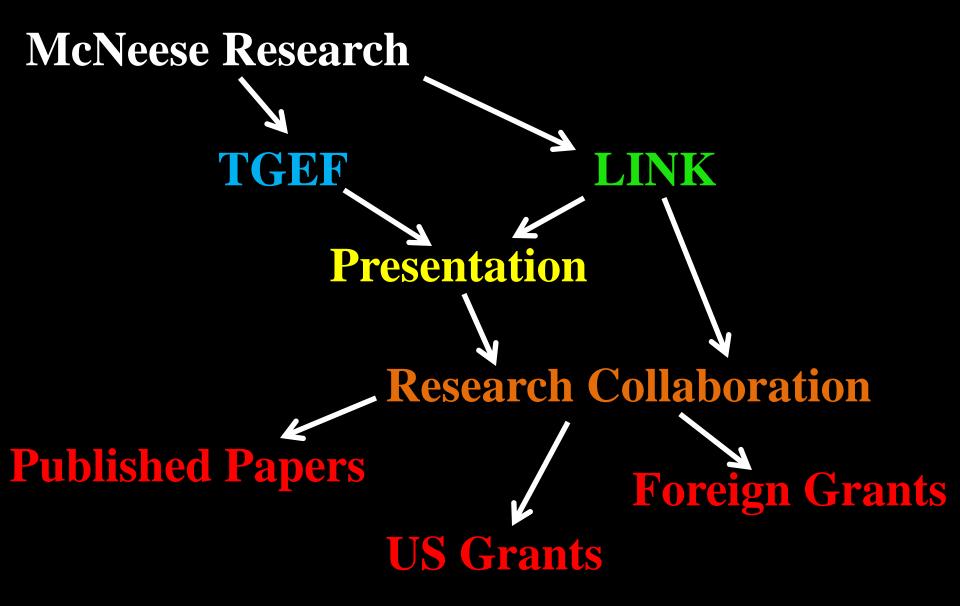
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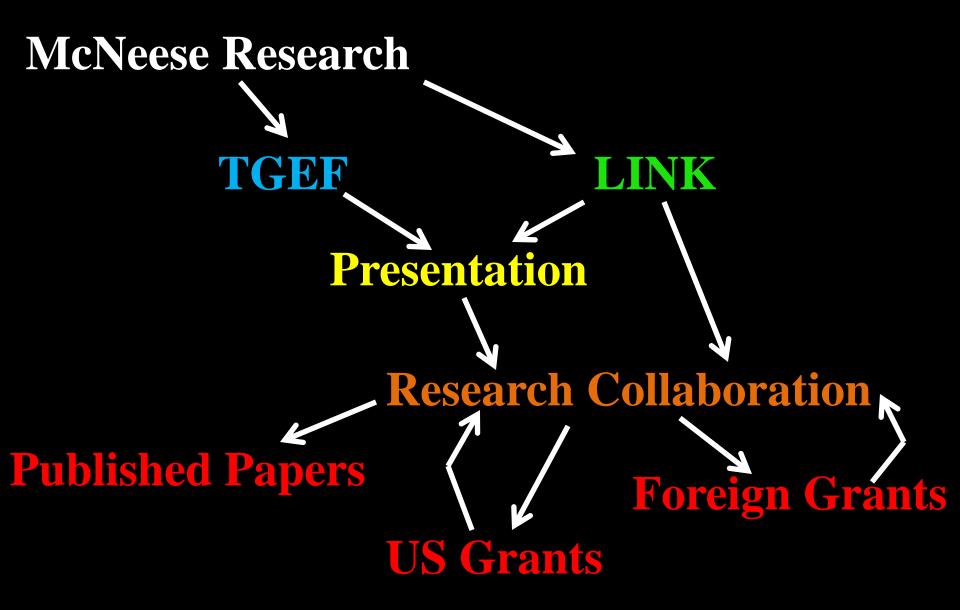
McNeese Research

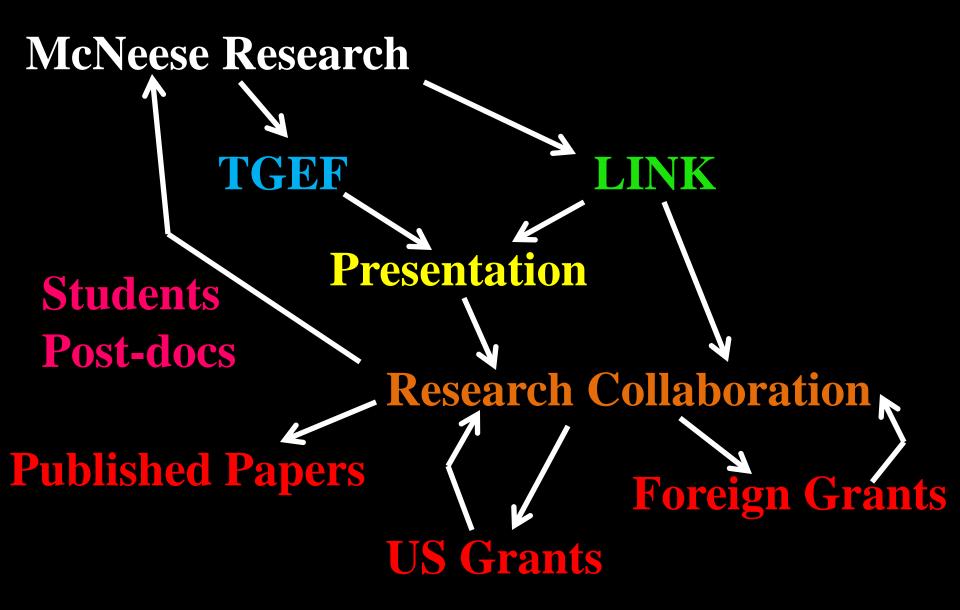


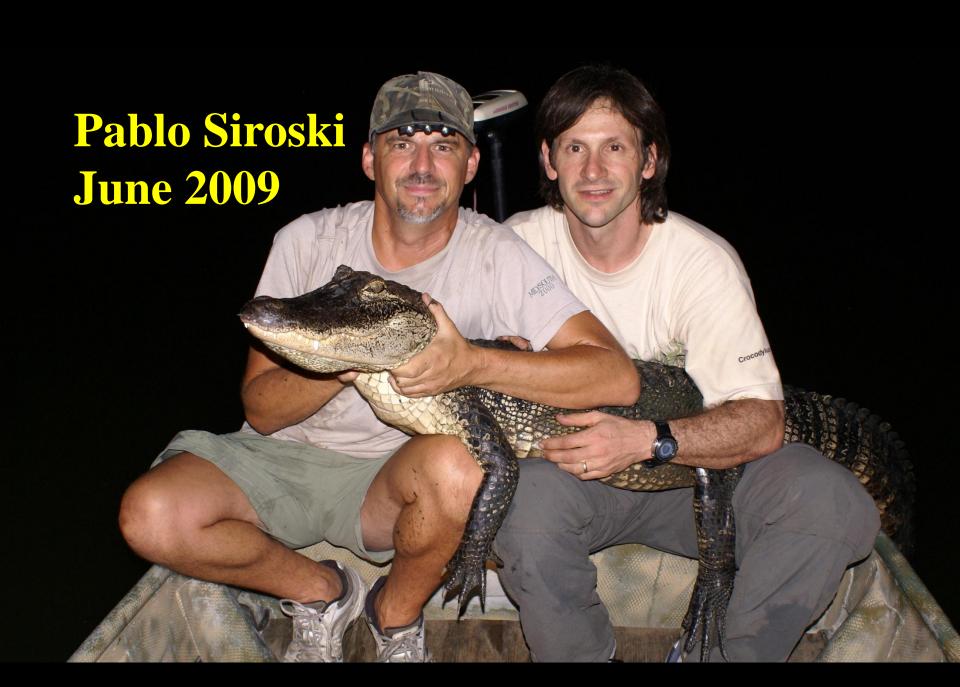
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Virginia Parachu Sept-Dec, 2011

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