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# RECURSOS

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# RECURSOS NATURALES Y SOCIEDAD



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# Editorial

**E**n este nuevo número de Recursos Naturales y Sociedad se evidencia, ya, el inicio de la internacionalización de esta revista digital de divulgación científica.

Así, en el artículo escrito por el Doctor O'hara, y colaboradores, se presentan de manera muy didáctica los resultados de los monitoreos realizados en Alaska sobre grupos de animales, invertebrados y vertebrados, que fungen como centinelas de contaminación y de enfermedades infecciosas. Para ello, tal como se describe en esta contribución, se aplican diferentes aproximaciones metodológicas, que inclusive se comparan entre ellas: químicas, inmunológicas y moleculares, todo este aprendizaje está siendo aplicado ahora en Baja California Sur en colaboración con Investigadores del CIBNOR, y desde luego sus resultados serán de la más alta importancia para nuestra sociedad.

**I**n this new issue of Natural Resources and Society, the beginning of the internationalization of this digital journal for scientific dissemination is already evident.

Thus, the article written by Dr. O'hara and collaborators presented didactically the results of the monitoring carried out in Alaska on groups of animals, invertebrates and vertebrates, which serve as sentinels for contamination and infectious diseases. As described in this contribution, different methodological approaches were applied, and even compared among them: chemical, immunological and molecular. All this learning is now being applied in Baja California Sur in collaboration with CIBNOR researchers, and its results will be of the highest importance for our society.



Por su parte la Doctora Zenteno, y colaboradores, exponen las contribuciones de los ponentes de la primera Conferencia de una Salud en las Américas, iniciativa que conjuntó a personal clave del continente americano, con conocimientos y enfoques diversos, para identificar los temas prioritarios de salud que requieren atención. Como resultado de esta importante iniciativa, tal como lo exponen los autores de la misma, se exploraron las posibilidades de formar una Red de Una Salud en las Américas. Las resultantes de estas posibilidades se describen en el mismo.

En la tercera contribución el Doctor Herrera, y colaboradores, se describen los resultados generados por la Red de Monitoreo Ambiental de la Bahía de La Paz. Esta Red está compuesta por estaciones meteorológicas y sensores de la temperatura del mar ubicados en la zona costera y marina de esta Bahía. En esta contribución los autores enfatizan la importancia de estos datos, como la base para la toma de decisiones, fundamentadas, tanto en la prevención de riesgos, como en el aprovechamiento de energías renovables y para el Desarrollo Sustentable de esta región.

Finalmente, la licenciada Castro y el Doctor García reseñan del libro de Dean Haner "The God Gene". Libro en el cual se analizan las

On the following manuscript, Dr. Zenteno, and collaborators presented the contributions of the speakers of the first Conference of Health in the Americas, an initiative that brought key specialists of the American continent together, with knowledge and diverse approaches to identify priority health issues that require attention. As a result of this important initiative, as expressed by the authors, the possibilities of forming One Health Network in the Americas were explored. The results of these possibilities are described in this contribution.

In the third contribution, Dr. Herrera, and collaborators, described the results generated by the Environmental Monitoring Network of the Bay of La Paz. This Network is composed of meteorological stations and sea temperature sensors located in the coastal and marine areas of this Bay. In this contribution the authors emphasized the importance of these data as the basis for decision-making, for risk prevention, use of renewable energies and for the sustainable development of this region.

Finally, B.Sc. Castro and Dr. Garcia reviewed Dean Haner's book "The God Gene." Book in which the biological bases of spirituality, its potential evolutionary implications and

bases biológicas de la espiritualidad, de sus potenciales implicaciones evolutivas y de los aspectos resultantes en la naturaleza humana

Este quinto número de Recursos Naturales y Sociedad es entonces una invitación a reflexiones sumamente profundas, que los autores de estas cuatro contribuciones que lo integran, generosamente nos comparten

Muchas gracias a todas y todos ellos, así como a los miembros del Comité Editorial, del Cuerpo Editorial y a los Editores Asociados y Revisores Anónimos que hicieron posible la aparición de este quinto número.

the resulting aspects in human nature were analyzed.

This fifth number of Natural Resources and Society is an invitation to carry out very deep reflections, which the authors of these four contributions generously shared with us.

Our appreciation to all of them, as well as to the members of the Editorial Committee, the Editorial Board, the Associate Editors and the Anonymous Reviewers that made this fifth issue possible.

**Dr. Alfredo Ortega-Rubio**

Invierno/Winter, 2017



# One Health in the Americas Conference

Conferencia sobre Una Salud en Las Americas

## Abstract

The First One Health in the Americas Conference was held on November 15-16, 2016 in Todos Santos, Baja California Sur, Mexico, just after the first global One Health Day. The Conference was hosted by Colora-

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do State University's Todos Santos Center. The academic program of the Conference included lectures from nine invited speakers, one panel with seven experts, one facilitated discussion, and nine poster presentations. The Conference was attended by 40 invited participants from seven countries from throughout the Americas. The objectives of the Conference were to bring together key personnel from the Americas, with diverse backgrounds and focus, to identify priority topics for research towards One Health in the Americas, and to explore the creation of a One Health in the Americas Network. This manuscript summarizes the contributions from the

speakers at the Conference, as well as the outcomes of the panel and the facilitated discussion about the Network. Taken together, the activities at the Conference highlight shared challenges, interests, and opportunities for scientific projects, training and collaborations, as well as the need to increase community participation towards achieving the goals of One Health in the Americas.

**Keywords:** Americas, One Health, Transdisciplinary

## Resumen

La primera Conferencia sobre Una Salud en Las Américas se llevó a cabo del 15 al 16 de noviembre del 2016 en Todos Santos, Baja California Sur, México, justo después del primer día mundial de Una Salud. El centro educativo de la Universidad Estatal de Colorado en Todos Santos fue el anfitrión de esta Conferencia. El programa académico de la Conferencia incluyó ponencias de nueve invitados, un panel con siete expertos, una discusión facilitada, y nueve presentaciones en modalidad cartel. Cuarenta participantes de siete países de las Américas asistieron a la Conferencia. Los objetivos de la Conferencia fueron reunir a personal clave de las Américas, con conocimientos y enfoques diversos, para identificar los temas prioritarios sobre Una Salud en Las Américas, y explorar la creación de una Red de Una Salud en las Américas. Este manuscrito resume las contribuciones de los ponentes, así como los resultados del panel y la discusión facilitada. En conjunto, las actividades durante la Conferencia enfatizan retos, intereses y oportunidades para proyectos científicos, entrenamiento y colaboraciones compartidos, así como la necesidad de aumentar la participación de la comunidad para lograr las metas de Una Salud en Las Américas.

**Palabras clave:** Americas, Transdisciplinario, Una Salud

The concept of One Health is based on recognizing that human health is intrinsically related to the health of animals, plants and ecosystems (One Health Global Network; Centers for Disease Control and Prevention). The term "One Health" was adopted from the "One Health, One World™"

(Centers for Disease Control and Prevention) concept presented during the Wildlife Conservation Society's symposium "Building Interdisciplinary Bridges to Health in a 'Globalized World'" in 2004 (Wildlife Conservation Society), and which called for interdisciplinary, international approaches to disease prevention and health promotion.

The first "One Health in the Americas" Conference was held on November 15-16, 2016 in Todos Santos, Baja California Sur, Mexico, and was hosted by Colorado State University's Todos Santos Center (<http://todosantos.colostate.edu/>). The academic program of the Conference included lectures from nine invited speakers, one panel with seven experts, one facilitated discussion, and nine poster presentations. The Conference brought together 40 participants, from 17 institutions from seven countries throughout the Americas. The objectives of the Conference were to bring together key people from the Americas, with diverse backgrounds and focus, and to collectively identify regional needs, common goals and priority topics for One Health in the Americas.

Dr. John Spencer from the Department of Microbiology, Immunology & Pathology, College of Veterinary Medicine and Biomedical Sciences at Colorado State University, Fort Collins, CO, USA, along with his colleague Dr. Claudio G. Salgado from Laboratório de Dermato-Imunologia at Universidade Federal de Pará, Pará, Brazil, delivered the opening talk entitled "Evidence of zoonotic leprosy in Pará, Brazilian Amazon, and increased anti-PGL-1 titer in individuals who consume armadillos in their diet", thus inaugurating the first "One Health in the Americas" Conference.

Drs. Spencer and Salgado presented their long-term, on-going international collaboration studying leprosy in the Brazilian Amazon. Leprosy is a zoonotic disease in humans caused by *Mycobacterium leprae* transmitted from armadillos (*Dasypus novemcinctus*), the only other known natural reservoir of *M. leprae*. Drs. Spencer and Salgado reported a 63% prevalence of leprosy among the tested human population, and 58.3% infection prevalence among armadillos, in the Brazilian Amazon. Although they did not find a statistically significant difference in serum titers between human residents with hunting, manipulation, or moderate con-

sumption of armadillo meat and those with low contact with armadillos, they did find that individuals who consumed armadillos more than once per month had a statistically significantly higher titer than those who ate armadillos less frequently.

In addition, in the talk "Are we really eliminating leprosy or is it absence of diagnosis?", Dr. Salgado reported on finding leprosy among school children living in the Brazilian Amazon region of Pará State. Dr. Salgado highlighted insufficient diagnostic approaches, scarce official reports by the municipality, and not enough treatment applications as important barriers to children's health. Dr. Salgado suggested that disability rates and contact tracing capabilities are some of the most important indicators within the leprosy areas worldwide.

The series of talks for the first "One Health in the Americas Conference" were divided into three sessions, according to the program areas of One Health as co-designed by the One Health Institute of Colorado State University (<http://onehealth.colostate.edu>). These

programs are: Urbanization and Health, Environmental Change and Health, and Foodscapes and Health. In the Urbanization and Health Session, Dr. Katherine Mella from MIT Community Innovators Lab in Boston, Massachusetts, USA, delivered the talk “Wellness based development in the Bronx, New York”. Dr. Mella addressed the connections between poor health outcomes and the lack of opportunities for quality education, high-road jobs, and affordable housing. She mentioned that access to and provision of healthcare are not enough to narrow the gap on existing health inequities, and pointed out that the factors that most impact health outcomes are social, environmental, and economic (i.e., the ‘social determinants of health’).

Dr. Mella explained that MIT Community Innovators Lab and the Bronx Cooperative Development Initiative are working together towards building a sustainable, equitable, and democratic economy in the Bronx with the ultimate goals of improving health outcomes and providing the foundation for shared wealth, owners-

hip, and wellness

Next, Dr. Carlota Monroy from the School of Biology at Universidad de San Carlos in Guatemala City, Guatemala, spoke of “An ecosystem approach for the Prevention of Chagas Disease in Rural Guatemala”. Chagas disease is caused by a tropical parasite (*Trypanosoma cruzi*) that is transmitted by blood-sucking insects. Chagas disease is prevalent in various rural areas in the Americas. Dr. Monroy reported that because of the insects’ migration patterns, as well as the deterioration of the environment, conventional methods of vector control (insecticide-based) are ineffective, expensive, and/or require repeated applications. Working in rural Guatemala with local communities, Dr. Monroy’s transdisciplinary research and education team assessed and prioritized risk factors for the transmission of Chagas disease. Dr. Monroy reported that among the designed interventions that proved effective against vector infestation are house improvement (via the intentional use of local materials for building, cement flooring) and domestic animal management (chicken relocation into coops, vaccination); she stressed that community participation and education are key factors for the continued success of the project.

In her talk “The salmon people, a family of beavers, and a grey whale in False Creek: biophilic stories in urban regeneration”, Dr. Meg Holden from the Department of Urban Studies and Geography at Simon Fraser University in Vancouver, British Columbia, Canada, addressed the rise of ecologically friendly neighborhoods as a lifestyle choice for people in urban areas to promote healthier lives. Dr. Holden summarized case studies from Seattle, Vancouver, and Victoria, and emphasized the importance of construction policies and planning and building codes in the design and implementation of eco-urban sustainable developments.

**Session 2**, “Environmental Change and Health”, of the One Health in the Americas Conference was opened by Dr. Todd O’Hara from the Wildlife Toxicology Laboratory of the Department of Veterinary Medicine in the University of Alaska Fairbanks at Fairbanks, Alaska, USA. Dr. O’Hara spoke about “Rural Alaska monitoring for environmental agents of disease: linking local residents to disease specialists” (O’Hara et al., 2018). The





on-going Rural Alaska Monitoring Program utilizes invertebrate and vertebrate species as sentinels to monitor disease agents with active participation of the Alaska Native Tribal Health Consortium. Dr. O'Hara commented on a novel method implemented in Alaska to assess for antibody titers to *Toxoplasma gondii*, *Coxiella burnetii*, *Brucella* spp., and *Francisella tularensis*, as well as to monitor concentrations of heavy metals and contaminants in a range of wildlife species, vectors (e.g., mosquitoes) and humans. Results from this study are further detailed in the accompanying paper (O'Hara et al., 2018).

Dr. Hugo A. Ruiz-Piña from the Centro de Investigaciones Regionales of the Universidad Autónoma de Yucatán, Mérida, Yucatán, México, spoke of "Research experiences on wildlife hosts and transmission risk of zoonotic diseases in Yucatán, México". Dr. Ruiz-Piña reported on studies of toxoplasmosis in the Yucatán peninsula. Toxoplasmosis is a zoonotic parasitic disease with a worldwide distribution that is caused by the protozoan *Toxoplasma gondii*, which can infect birds and mammals, including humans. Dr. Ruiz-Piña reported that the opossum, *Didelphis virginiana*, can act as a source of infection to humans by consumption in tropical forest and rural areas.

In the final talk "Not such a bird-brained idea? Biodiversity, ecosystem integrity and One Health" of this session, Dr. Kathryn P. Huyvaert from the Department of Fish, Wildlife, and Conservation Biology at Colorado State University at Fort Collins, Colorado, USA, remarked that One Health is characterized by the interrelationships among people, places, and animals and that these interactions are often overlooked by metrics of health. Using a case study of a tropical seabird as a bioindicator of marine ecosystem health in the Americas, Dr. Huyvaert emphasized the value of ecological integrity, acknowledging that impacts of perturbations on one component of a system flow to the others. She concluded that acknowledging the interconnectedness of people, animals, and the environment is paramount for addressing the effects of environmental change on the health of humans, animals, plants, and the ecosystems we share.

**Session 3** of the One Health in the Americas Conference focused on

Foodscapes and Health (called Food Systems and Health at that time). Dr. Rafael Ortiz from the Environmental Defense Fund of Mexico, A.C. from La Paz, Baja California Sur, México, delivered the talk "Rights-based management and healthy fisheries- the case of Gulf Corvina". Meeting the global demand for nutrients is a challenge to food systems that is complicated by the degradation of the world's oceans. Using the case of gulf corvina (*Cynoscion othonotus*), Dr. Ortiz spoke of the benefits from scientific research-based catch limits and rights-based approaches to fisheries management, and recognizing the value of local cultures in improving fisheries performance. Other benefits of this participatory approach to the corvina fisheries include lower accident rate among fishermen and increased profits from the fisheries, which lead to better human health, lower corvina catch, and healthier fish stocks. Combined, these results suggest that rights-based approaches to fisheries management will eventually lead to healthier ecosystems.

In the final talk of the theme

sessions of the first One Health in the Americas Conference, Mr. Philip Sambol from Oasis Community Partners and Good Food Markets in Washington, D.C., USA, presented the case study of his own company. Good Food Markets is a mission-driven grocer that aims to provide fresh food to people living in communities that have very limited access to fresh produce (i.e., “food deserts”). Mr. Sambol spearheaded the launching of a stakeholder-based local food movement, Oasis Community Partners, a non-profit that provides education, data analysis, program development, and consultation within marginalized communities, such that assets are locally produced to match community needs. By designing and continuously updating food sourcing, funding, and pricing models, Good Food Markets and Oasis Community Partners allow fresh food to be available, affordable, and sustainable in a Washington, D.C. food desert. This model provides an option to decrease health disparities and to increase social coherence.

The panel of the One Health in the Americas Conference included researchers, clinicians, program managers, and administrators from different institutions and countries. The international and transdisciplinary approach to discussion of the topics presented during the program sessions and the poster presentations provided a unique framework to the benefits of involving all sectors (i.e., academic, NGOs, government, community, private sector, funders), in promoting economically feasible, data-based programs that allow for actions, awareness, and surveillance of One Health issues in the Americas. The main concern identified during this panel was the need for workers from the different sectors to act in concert to maintain the health of humans, animals, plants and ecosystems. To achieve this common One Health goal, prevention programs need to be designed that recognize and respect the regional cultures, so that local communities are empowered to promote sustainable One Health actions in their homes, families, and communities.

The One Health in the Americas Conference ended with a facilitated discussion in which all participants joined in a conversation lead by Dr. Brian Dunbar, Executive Director of the Institute for the Built Environ-

ment, Colorado State University, and Dr. Aines Castro-Prieto, Colorado State University's Todos Santos Center. During the discussion, participants shared their concerns, perspectives and experiences in One Health, which were then used to identify and prioritize main needs and common goals relevant to One Health in the Americas. This facilitated discussion provides the foundations for the creation of a One Health in the Americas Network.

We feel that this first One Health in the Americas Conference succeeded in exploring critical issues of regional interest with global impacts. The main outcomes of this Conference were: (1) planting the seed for the creation of a One Health in the Americas Network that includes researchers, educators, stakeholders, funders, and decision-makers; (2) the reaffirmation that, in order to maintain and improve human health, actions to preserve the health of animals, plants, and ecosystems need to be considered and coherently implemented; and (3) the recognition that direct, engaged, and sustained participation of the



affected communities is imperative for the success of any action or activity that aims towards promotion of wellness and health of humans, animals, plants, and ecosystems.

### Acknowledgements

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# Developing a Sentinel-based Baja California Sur Rural Mexico Monitoring Program: Lessons learned from Alaska

Desarrollo de un programa de monitoreo basado  
en centinelas para el área rural de Baja California Sur:

**Lecciones aprendidas en Alaska**

## **Abstract:**

Alaska organizations [Alaska Native Tribal Health Consortium (ANTHC; main partner) and the University of Alaska Fairbanks (UAF)] participate in the Rural Alaska Monitoring Program

(RAMP). The RAMP represents a major One Health project in the Department of Veterinary Medicine at the UAF and many units within ANTHC. The ANTHC has received most of the funding for RAMP from the US Department of Interior and the US Environmental Protection Agency. We utilize invertebrate and vertebrate sentinels to monitor environmental agents of diseases. Mosquitoes and whole blood soaked filter

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papers (FP) from vertebrates are collected throughout Alaska, and elsewhere, by a network organized by our groups. Eluates (saline extracts) are prepared from dried FP collected by hunters and biologists for assessing infectious agent-specific antibody titers and other measures. We provide data on use for *Toxoplasma gondii* and *Brucella* spp. specific antibody titers via this standard technique and for a centrifugation method to concentrate eluate antibody for assay enhancement. Dried FP are also used for determining contaminants (mercury, polychlorinated biphenyls (PCBs)) and nutrients (selenium, Se) concentrations.

Mosquitoes sent to UAF were tested by polymerase chain reaction (PCR) for presence of DNA from *Francisella tularensis* using three different genes. This technique can be easily adapted to other microbial organisms of interest. We report on these efforts to further develop our collaboration with colleagues in Baja California Sur (BCS) to implement similar procedures in remote areas with respect to agricultural and wildlife monitoring and research considering common concerns related to environmental change. The subsistence foods similarities and needs for environmental monitoring of agents important to Public Health fit well within this One Health paradigm whether one is addressing the subarctic or subtropic; thus enabling technology transfer and standardization as an overall trans-Americas One Health effort. The technology utilized in RAMP was presented in November of 2016 at the One Health of the Americas conference held in Todos Santos, BCS, Mexico so that we can develop a similar R(A)MMP (where one M represents Mexico and the A Alaska for our proposed collaboration).

### Resumen:

Organizaciones de Alaska (Consortio de Salud Tribal de Nativos de Alaska (Alaska Native Tribal Health Consortium, ANTHC), socio principal, y la Universidad de Alaska Fairbanks (UAF)) participan en el Programa de Monitoreo en el área Rural de Alaska (RAMP, por sus siglas en inglés). El RAMP representa uno de los proyectos principales abordando el tema de Una Salud en el Departamento de Medicina Veterinaria UAF y varias unidades dentro del ANTHC. El ANTHC ha recibido la mayor parte de los fondos para el RAMP del Departamento del Interior y la Agencia de Protección Ambiental de Estados Unidos. Utilizamos centinelas vertebrados e invertebrados para monitorear agentes ambientales de enfermedades. A lo largo de Alaska y otras zonas, se colectan mosquitos y filtros de papel (FP) empapados con sangre de vertebrados con apoyo de una red organizada por nuestros grupos. Se prepara un eluato (extracto salino) a partir del FP seco colectado por cazadores y biólogos para evaluar títulos de anticuerpos contra

agentes infecciosos específicos y otras variables. Proveemos datos del uso para títulos de anticuerpos específicos contra *Toxoplasma gondii* y *Brucella* spp a través de esta técnica estandarizada y para un método de centrifugación que concentra el eluato para mejorar el análisis de anticuerpos. El FP seco también se usa para determinar concentraciones de contaminantes (mercurio, PCBs) y nutrientes (selenio). Los mosquitos enviados a UAF se analizan por PCR para la presencia de DNA de *Francisella tularensis* usando tres genes diferentes. Esta técnica puede adaptarse fácilmente a otros microorganismos de interés. Reportamos sobre estos esfuerzos para desarrollar nuestra colaboración con colegas en Baja California Sur (BCS) para implementar procedimientos similares en áreas remotas con respecto al monitoreo e investigación en agricultura y vida silvestre considerando preocupaciones comunes asociadas al cambio climático. Las similitudes en alimentos de subsistencia y las necesidades de monitoreo ambiental de agentes importantes para la Salud Pública son acordes

al paradigma de Una Salud (One Health) bien sea que nos enfoquemos en zonas subárticas o subtropicales; por lo tanto, permiten la transferencia y estandarización de la tecnología como un esfuerzo transversal en las Américas. La tecnología utilizada en RAMP se presentó en Noviembre de 2016 en el Congreso de Una Salud en América en Todos Santos, BCS, México para que podamos desarrollar un R(A)MMP (donde una M representa México y la A Alaska para nuestra propuesta de colaboración) similar.

## Introduction

### *One Health*

Most health practitioners are traditionally trained to work with a patient (the individual).

The individual is often the unit of concern and management. We do recognize there are those trained in family and population (herd) health that take a broader view and interventions. We are taking an even broader perspective when we approach health problems and solutions as “One Health” as practiced by the Rural Alas-

ka Monitoring Program (RAMP) and proposed for this Baja California Sur (BCS) based Rural (Alaska) Mexico Monitoring Program (R(A)MMP), using invertebrates (mosquitoes) and dried vertebrate blood on filter paper (FP)<sup>1</sup> for monitoring environmental agents of disease. This approach very much fits within the Public Health infrastructure of BCS as there are many overlaps in mission and desired outcomes (pers. comm. Dr. R. Gaxiola-Robles). For this report “One Health” is recognized as:

“health for people, non-human animals, and environment is deeply connected and inseparable.”

(<http://source.colostate.edu/one-health-institute-opens-doors-colorado-state-university/>)

Any person or group that is a steward of any of the components in the One Health paradigm is a potential “practitioner”. One of our mutual concerns and collaborative research efforts between Alaska and BCS is the presence of mercury (Hg) in the fish-based food web (Gaxiola-Robles et al., 2014; Bentzen et al., 2014). We have va-

<sup>1</sup>Nobuto; <http://www.advantecmfs.com/filtration/EnvironMonitor.shtml#nob>



validated the use of FP in the monitoring of Hg in blood of piscivores (Hansen et al., 2014) and look forward to using this technique in BCS. Alaskan and BCS colleagues would also exploit the collection of FP from BCS vertebrates for other agents of disease including some outlined in this paper.

Alaskan and BCS colleagues have been working together for many years on many One Health related research and monitoring efforts that include plants, invertebrates, and vertebrates; including human subjects. Many of these have focused on environmental contaminants and feeding ecology (Hernandez-Almaraz et al., 2016; Gaxiola-Robles et al., 2014; Bentzen et al., 2014; Barrera-García et al., 2013, 2012; Zenteno-Savin et al., 2013) but are easily amenable to monitoring other agents of disease, including infectious agents. We build on these efforts to propose a R(A)MMP for BCS by combining our experiences in Alaska with those in Mexico. We provide our method development and validation data here to kick start R(A)MMP; and propose foundation of

Rural (Alaska) Mexico Monitoring Program (RAMMP).

The BCS-based entities for which we propose to build this relationship include Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Hospital General de Zona No. 1, Instituto Mexicano del Seguro Social (IMSS), El Centro Interdisciplinario de Ciencias

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Marinas (CICIMAR), Universidad Autónoma de Baja California Sur (UABCS), and Colorado State University, Todos Santos Center (CSU TSC). The combination of these institutions and their associated expertise and resources makes R(A)MMP feasible. The likely inclusion of other groups would strengthen the proposed R(A)MMP. Those listed above are examples and we will encourage participation across many groups.

*Focus on zoonotic agents (vector and food) and chemical contaminants (food)*

Within the R(A)MMP, the emphasis for assessing FP (blood) will be food-based pathways (organisms) of environmental agents to people; thus monitoring of species and matrices that are consumed. This includes wild caught and farm raised animals. For most of the chemicals of concern, we can utilize experts in chemistry and biomedical sciences at CIBNOR for measuring certain elements as conducted in Hansen et al. (2014) for total mercury (THg). In addition, we also propose to develop measures of essential nutrients so we can track the nutritional status of monitored animals (e.g., copper, zinc). Commercially available serologic assays (detection of disease agent specific antibodies) can easily be established at CIBNOR, UABCS, or CSU TSC for the specific agents of interest. We provide validation of FP collection and laboratory processing for use in traditional (standard) diagnostic assays, including a centrifugation approach to enhance sensitivity.

The emphasis on mosquito monitoring will be based on determining the best capture and

sampling methods for the region and the proximity to human and animal areas. This requires proof of principle testing for our colleagues in BCS. Once successful captures can be assured we will monitor these invertebrates for infectious agents of concern based on the availability of PCR-based methods for such agents as zika, chikungunya, dengue and yellow fever. Compared to the serologic tests mentioned above, establishing PCR-based methods may be more demanding on the facility resources in BCS (La Paz). Thus, we need to explore further where this can best be established for monitoring hundreds of invertebrate samples in an efficient manner with attention to high quality assurance (sequencing and informatics). One could consider use of marine invertebrates (e.g., shellfish) as well (Quakenbush et al., 2016).

As an example of the potential community-based scope of agents to consider, Sampasa-Kanyinga et al. (2012) documented exposure to zoonotic agents (*Toxoplasma gondii*, *Toxocara canis*, *Echinococcus granulosus*, *Leptospira* spp., *Coxiella burnetii*, and *Francisella tularensis*) in sera from 267 residents of Cree communities of James Bay (Canada). High seroprevalence rates were documented for *Leptospira* spp. (23%), *Francisella tularensis* (18%), and *Toxoplasma gondii* (9%). Seroprevalence rates of less than 5% were observed for *Coxiella burnetii*, *Echinococcus granulosus*, and *Toxocara canis*. It should be emphasized this relates to exposure only, and no obvious adverse outcomes were associated with these findings. However, Public Health officials should recognize exposure potential and that continued hygiene and food safety awareness are critical to prevention of disease. This effort has enhanced value in the face of climate change and the enhanced movement of agents among the Americas (e.g., trade, biota, military operations, resource extraction industries, tourism).

#### *Shared pathogens of concern*

There are numerous agents and organisms of mutual concern for our subarctic and subtropical environments. For example, *Brucella* spp. is a zoonotic agent present in wildlife and agricultural species for which the

FP technique has been validated and used (Curry et al., 2011A, B) and is a pathogen of interest in BCS. This organism represents a significant wildlife and livestock agent of disease that can impact health and reproductive outcomes and the management of wildlife and agricultural resources between states and countries. We plan to develop this *Brucella* spp. antibody monitoring capability, including reconstituting the eluate to a protein concentration similar to plasma or serum, as an initial effort in BCS.

We present data on *Toxoplasma gondii* as well. As viruses are a global concern, the FP technique has been used to detect antibodies to agents including distemper and parvovirus (Kamps et al., 2015), West Nile Virus and five bovine viruses (Curry et al., 2014), and Avian Influenza Virus (Dusek et al., 2011) and thus is proven in this regard and can be applied to specific viral agents of concern.

We present basic methods, preliminary data, and some discussion of the approaches planned for establishing a disease agent monitoring program for BCS that

utilizes invertebrates and blood soaked Nobuto FP based on our success in our Alaska-based RAMP.

This approach will include a One Health perspective that must involve graduate and veterinary medical students from the “Americas” so we can advance our understanding of disease issues in a rapidly changing world (e.g. climate, technologically, culturally, politically, economically, etc.).

## Methods

### *Basic methods of capture and collection (vertebrates)*

Samples are easily collected once blood is recovered from the animal by simply using a syringe (apply blood to the Nobuto filter paper strip so as to absorb the estimated 100 µl). One can also soak the FP in the tube of blood. It is important to assure the blood is homogenous and that the FP is properly soaked and air dried. Some pinniped samples were collected for diagnostic purposes for animals admitted to The Marine Mammal Center (TMMC) for rehabilitation. Sample collection methods and handling are reviewed in Hansen et al. (2014) and Van Hooymissen et al. (2015).

Samples from wild pinnipeds brought to TMMC (Sausalito, California, USA) were sampled under authority of Marine Mammal Protection Act permit 932-1905/MA-009526. Harbor seals (*Phoca vitulina*) from Alaska were sampled in accordance with approval of Institutional Animal Care and Use Committees at the University of Alaska Fairbanks (protocol 07-37) and the State of Alaska Department of Fish and Game (Gail Blundell, protocol 07-16), as well as a permit from the National Oceanographic and Atmospheric Administration under the Marine Mammal Protection Act (Permit 358-1787-00 to Gail Blundell) as reported in Hueffer et al. (2011 and 2013). For Steller sea lions (*Eumetopias jubatus*) samples were collected in a manner similar to that reported in Peterson et al. (2016) as part of research efforts to assess range wide concentrations of mercury and selenium in sea lion pups permitted under MMPA permit 14326-02 issued by NOAA, and IACUC protocols at UAF (protocol 594759-2) and NOAA (protocol NW2103-2). Bottlenose dolphins (*Tursiops truncatus*)

were live-captured, sampled, and released after health assessments in Sarasota Bay, Florida during May and July 2012 by staff from the Chicago Zoological Society (National Marine Fisheries Service Scientific Research Permit 15543, Institutional Animal Care and Use Committee 11-09-RW1) under supervision of principal investigator Dr. Randall Wells.

For recently dead animals, collection of free flowing, or other forms of uncoagulated blood, is feasible. Simply soaking the FP should accomplish proper sampling if one avoids clotted blood or blood mixed with other fluids or sources of environmental contamination. The FP samples are then air dried and placed in paper envelopes.

### *Analytical Chemistry (Hg and Se; PCBs)*

The methods for measuring concentrations of total mercury ([THg]) and total selenium ([TSe]) are well described in other publications, thus for the sake of space we refer readers to those more detailed documents: Hansen et al. (2014), McGrew et al. (2015), Co-

rea et al. (2015), and Van Hoomissen et al. (2015). The blank FP samples (Nobuto strips, not blood soaked) were provided to the Applied Science, Engineering, and Technology (ASET) Laboratory at the University of Alaska for polychlorinated biphenyls (PCBs) analysis by gas chromatography (GC; Agilent 7890 GC) utilizing Electron Capture Detection (ECD). This method provides determination of 18 PCB congeners.

### *Mosquito Sampling*

Mosquitos were obtained using battery operated CO<sub>2</sub> generating traps (Mosquito Magnet), placed in proximity to freshwater bodies, within 0.25 km of rural Alaska Native villages on the Bering Sea coast, during the months of May-September. The device desiccates the trapped mosquitos. Samples were stored in Whirlpaks at ambient temperature until being shipped for PCR analysis.

### *Screening for Francisella DNA in mosquitoes*

We screened for Francisella in mosquitoes collected in rural western Alaska using Polymerase Chain Reaction (PCR) based methods. We established three real time quantitative PCR methods at UAF designed to detect DNA of three Francisella genes: lpnA2, fopA, and iQFt1. These three assays have average detection limits of 36, 825 and 2680 genome copies per reaction for lpnA2, iQFt1, and fopA, respectively. Based on these findings we pool 5 mosquitoes into one DNA extraction and screen the obtained DNA with the most sensitive assay (lpnA2) and confirm positive samples with the other assays. Based on results, mosquitoes remaining from the original sample can be screened and positive samples can be identified to species using DNA barcoding technique as described previously (Triebenbach et al., 2010).

### *Serology*

#### *Toxoplasma*

The Georgia Veterinary Diagnostic Laboratory tested serum using their standard latex agglutination test. This test detects IgG and IgM antibody

to *Toxoplasma gondii*. The positive threshold value used is a titer of 32 or greater. However, we do not attempt any diagnostic interpretation as we are simply using this test (measured titers) to determine the efficacy of our centrifugal eluate reconstitution technique that is described elsewhere.

#### *Brucella (Card and Plate tests)*

For the card test we used 3 categories of responders: 1) Strong Positive (++) - large, numerous clumps. Nearly identical to positive control, 2) Moderate Positive (+) - clumping, but not as numerous or as large as full positive results, and 3) Minor agglutination/Negative (-) - either no agglutination present or fine, slight agglutination present but not enough for a moderate positive designation.

This allowed for some assessment of "strength" of response but is not as discrete or useful as determination of titers as conducted in the plate test. For further details, see Hueffer et al. (2013). Positives on the plate test were determined by the presence of any agglutination at each titer level tested (25, 50, 100, 200, 400). For further details, see Hueffer et al. (2013).



### *Eluate and centrifugal reconstitution (protein concentrating)*

#### *Elution Methodology*

The serum or whole blood soaked FP were eluted similar to Hansen et al. (2014). Each FP was cut into five to seven pieces and placed in a 2ml cryogenic tube then 400µl of PBS with 1% penicillin–streptomycin was added. The tubes were then agitated using a vortex mixer to ensure that each FP segment was soaked in the solution then eluted overnight (~16 hours) at 4°C.

Each tube, containing one FP, yielded approximately 200 µl of eluate. According to the FP manufacturer's specifications elution of whole blood by this method has been estimated to be a 1:10 dilution from serum. When we used FP soaked with serum instead of whole blood, the dilution of our eluate was estimated to be about 1:5 from serum. Two FP eluates from each animal were combined, to be used in a non-concentrated form in the subsequent testing.

#### *Reconstitution Methodology (centrifugal)*

The goal is to concentrate the eluate to match original serum composition as closely as possible. We are mostly interested in reconstituting the activity (titer level) of antibody reactivity in commonly used immunoassays. For example, by concentrating about 1000µl of eluate (eluates of 5 FP strips combined) to about 250µl, we would aim to approximate the original serum. Prior to beginning the concentration protocol, 300µl of MES (2-(N-morpholino) ethanesulfonic acid) was added to each Amicon centrifugal filter (Amicon® Ultra Filter) and left to incubate overnight at 4°C. Further processing followed manufacturer instructions.

## **Results and Discussion**

### *Mosquito based monitoring*

Mosquitoes were tested for *Francisella tularensis* (Ft) for two reasons: First the bacterial pathogen, suspected to be present in rodent species which have extended their range north in Alaska, as the tree line has moved north, can be transmitted by mosquitoes; and secondly as

blood feeding arthropods mosquitoes contain host blood that may contain pathogens regardless of the competency as a vector for the pathogen in question. Human infection (tularemia, mild to life-threatening based on route of exposure and expression in host) with *Francisella tularensis* has been occasionally documented in the north; transmission by mosquitoes has never been investigated in North America but has been documented in Europe (Hansen et al., 2011). Especially with environmental change and expansion of beavers and other possible reservoirs of Ft, this pathogen is of special concern in the subarctic. However, our methods can be easily adapted to subtropical climates by adjusting the pathogen assayed for including zika, dengue, chikungunya, malaria, and many others that are DNA-based and found in the circulation.

### *Blood sampling benefit*

Assessment of whole blood in itself can provide a great deal of information about the health of an animal and when used collectively for a group (e.g., herd, flock, stock,

haul-out) can be used to assess population health. Whole blood can provide information outside of the traditional clinical uses. Here we consider whole blood as a matrix to assess “exposure”. We are referring to “exposure” to certain chemicals such as contaminants or essential nutrients.

In addition, we assess historic, and current, exposure to certain infectious agents of disease as well as specific antibodies generated to these agents. The detection of agent specific RNA and DNA indicates current presence whereas agent specific antibodies can relate to past exposure, possibly ongoing exposure as well.

Components of blood are often used to provide more specific information. In some cases, we only need the fluid compartments. Serum (clotting factors removed) and plasma (anticoagulant treated, clotting factors present) are common, but different representatives of the circulating fluid (acellular) fraction. The cellular fraction of blood can be quite useful as well and can be assessed as whole blood but in some circumstances the cells, including specific cell types, are removed for assessment. We do not go into detail here but want the reader to appreciate the complex nature of blood (e.g., various cell types and multiple fluid components). This is important when considering the use of whole blood soaked FP (cells present), or using serum soaked FP (cells removed), as presented in this report.

For whole blood soaked FP we can no longer separate the compartments identified above. Cells have lysed and their contents are now a part of any extraction from the dried FP; except for those bound to the paper. Thus, when we prepare the whole blood eluate in PBS we must consider it is liquid but does NOT represent plasma or serum. Thus, the utility of the eluate is not directly comparable to the commonly used fluid compartments of blood (serum, plasma) for many analytes and assays. Depending on the analyte or endpoint the use of the eluate or the blood soaked FP directly can be considered. For some measures, the presence of lysed cells contents is not conducive to this sampling technique whereas for others, such as antibodies, we can effectively use the eluate to measure antibody titers. Thus, some of our efforts have focused on

validation of these procedures for this complex eluate.

#### *Non-blood FP sampling benefit*

Above we describe the use of Nobuto FP for use with whole blood or serum. We will provide examples from our work in Alaska below. One of our key missions in BCS is to validate the use of this technology on non-blood matrices for use in agricultural, and possibly wildlife, scenarios. There are key diagnostic tests that use non-blood fluids such as the chemistry or microbiology of urine or milk.

We collected intact fluids and fluid soaked FP as matched samples from individual animals for parallel testing using the intact fluid as the gold standard to determine if the FP sample provides similar information. For milk, we can assess nutrients and contaminants using standard chemical tests, and for zoonotic agents of interest we can explore antibody based tests and direct measure of microorganism constituents (e.g., DNA or RNA). This is proposed as student-based research projects that would be based out of La Paz (BCS).

### *PCR-based methods for FP*

We used three different genes (*lfnA2*, *fopA*, and *iQFt1*) to determine the presence of *Francisella* DNA in these insect pools. In a survey of pooled mosquitoes from the Fairbanks area, 30% of the pools were PCR positive for Ft. For rural Alaska, 9 pools out of 56 total pools (5 mosquitoes/pool) consistently tested positive for *lfnA2* (best detection level at 36 genome copies/reaction of the three genes). We conducted validation studies and we are analyzing samples from field efforts as part of further establishing this monitoring effort. This technique is ready for transfer to BCS scenarios.

Even though it is not a part of RAMP, we point out that host DNA can be characterized from these samples as well. This might be important in some situations. However, using these specific FP types likely has limited use in gene expression studies as RNA is not easily stabilized and preserved as compared to DNA. With respect to future efforts and validation we are investigating the use of blood soaked materials for assessing genomic and transcriptomic assessments.

### *Serological-based methods for FP*

We are not compelled to do an expansive discussion on the serologic use of the FP as that is what they are designed for and many researchers have shown their value in this regard (Curry et al., 2011A, B, 2014; Kamps et al., 2015; Dusek et al., 2011). However, we do emphasize that we have introduced a technique that can reconstitute the eluate to approximate antibody titer levels of serum/plasma (Amicon® Ultra Filter).

Using assays of pathogen specific antibodies, we have shown increased titers in concentrated eluates that are close, or identical to, matched serum samples (the saline extracted eluate is dilute relative to blood, serum or plasma).

This post eluate processing allows for more relevant testing for assays that determine positivity at relatively low titers; such 1:2, 1:4, or 1:8, or where one wants to optimize detection. We use two agents of concern (*Toxoplasma gondii* and *Brucella* spp.) and standard diagnos-

tics tests (see below) to compare a gold standard (serum or plasma) to the eluate from the FP and eluate that is centrifugally treated to concentrate protein to approximately what would be expected in serum or plasma. We were graciously provided samples from populations known to be exposed to these microorganisms so that we could investigate this approach with a high expectation to find pathogen specific reactive antibody in the samples.

### **Toxoplasma**

Titers for antibodies to *Toxoplasma gondii* from bottlenose dolphins (*Tursiops truncatus*) from Sarasota, Florida (USA), a population known to be exposed to this organism, are listed in Table 1 for each individual dolphin tested (n=16 individuals, with duplicate plasma and WB FP eluates prepared for 9 of the 16; i.e., 25 total matched pairs of plasma and WB FP eluates). These are presented with respect to our gold standard matrix, the WB FP eluate, and the WB FP eluate post centrifugation (reconstituted). We highlight that 13 were negative as plasma sam-

ples (10 individuals with at least one negative sample) with 12 WB FP matched eluates as negative (9 individuals with at least one negative sample; one positive at 1:16, FP 057 and dolphin ID FB# 252). Within Table 1 we highlight as bold (FP0XX) animals that indicated reconstitution of the gold standard titer to within one dilution post centrifugation (e.g., successful reconstitution).

**Table 1.** Titers for antibodies to *Toxoplasma gondii* from bottlenose dolphins (*Tursiops truncatus*) for gold standard (Plasma Titer), the whole blood (WB) FP eluate (WB Eluate titer), and the WB FP eluate post centrifugation (Concentrated WB Eluate Titer). Highlighted as bold (FP0XX) animals that indicated reconstitution of the titer to within one dilution post centrifugation (e.g., successful reconstitution). Two values for a matrix indicate duplicate samples from the same animal.

| Sample                   | Dolphin ID FB# <sup>a</sup> | Plasma Titer | Concentrated WB Eluate Titer | WB Eluate titer |
|--------------------------|-----------------------------|--------------|------------------------------|-----------------|
| FP 054, 078 <sup>b</sup> | 142                         | Neg, Neg     | Neg, Neg                     | Neg, Neg        |
| FP 051                   | 164                         | Neg          | Neg                          | Neg             |
| FP 059                   | 173                         | Neg          | Neg                          | Neg             |
| FP 060                   | 232                         | Neg          | Neg                          | Neg             |
| FP 052, 064              | 233                         | Neg, Neg     | Neg, Neg                     | Neg, Neg        |
| FP 046, 070              | 274                         | Neg, Neg     | Neg, Neg                     | Neg, Neg        |
| FP 061                   | 278                         | Neg          | Neg                          | Neg             |
| <b>FP 049, 075</b>       | 20                          | Neg, 16      | Neg, 16                      | Neg, Neg        |
| <b>FP 057, 073</b>       | 252                         | Neg, 16      | 16, 16                       | Neg, Neg        |
| FP 050, 076              | 159                         | Neg, 64      | Neg, 16                      | Neg, Neg        |
| FP 047, 079              | 113                         | 16, 16       | Neg, Neg                     | Neg, Neg        |
| <b>FP 048, 074</b>       | 258                         | 16, 64       | 16, 32                       | Neg, Neg        |
| <b>FP 058</b>            | 7                           | 32           | 32                           | Neg             |
| <b>FP 056</b>            | 221                         | 64           | 64                           | Neg             |
| <b>FP 053</b>            | 242                         | 2048         | 4096                         | 512             |
| <b>FP 055, 077</b>       | 276                         | 4096, 8192   | 4096, 4096                   | 512, 512        |

<sup>a</sup> ID number assigned to each individual dolphin

<sup>b</sup> For some individuals (e.g. FB# 142) there were duplicate pairs of plasma and WB FPs prepared (e.g. FP 054, FP 078). Titers from matched plasma and FP eluates are listed in the order of the corresponding FP number for that individual.

This represents 9 samples out of the 12 total number of samples with a measured titer in plasma (7 out of 9 individuals), or 75% of samples were returned to a titer close to, or the same as, the gold standard. Dolphin FB# 159 (FP 076) did not regain the reactivity based on plasma results; plasma samples from dolphin FB# 113 (FP079 and FP047) were at the lowest recorded titer for plasma thus results are inconclusive for the

concentrated eluates as negatives. We conclude that the centrifugation technique enhances the use of blood soaked FP with respect to measuring *T. gondii* specific antibodies and that this would likely apply to other agent specific antibodies.

## Brucella

Prevalence of *Brucella* reactive antibody is approximately 13/80 (16.3%) and 14/79 (17.7%) for the card and plate test, respectively, for this group of harbor seals from Alaska (Table 2A). *Brucella* card and plate agglutination tests are not amenable to using WB FP eluate so these comparisons were conducted using eluates of serum soaked FPs. However, other assays can likely be used that do not rely on agglutination as a measure.

**Table 2A:** *Brucella* card and plate test results for harbor seal serum.

|       | Positive | Negative |
|-------|----------|----------|
| Card  | 13       | 80       |
| Plate | 14       | 79       |

For the card test (Table 2B), the 7 serum negative samples were also negative for the FP Eluate-concentrated and FP Eluate samples (control). For the 5 samples



classified as Serum ++ [serum samples showing strong reactivity]: 2 of 5 were reactive (1 at ++ and 1 at +) for FP Eluate and 4 of 5 were reactive (1 at ++ and 3 at +) FP Eluate-concentrated. Thus, reconstitution (centrifugation) resulted in 4 of the 5 being (remaining) positive. For the 8 weaker serum responders (+) only one sample remained reactive as FP Eluate whereas 5 samples remained + for FP Eluate-concentrated. This indicates concentrating these samples helps to retain antibody reactivity as compared to eluate alone for the card test. Although the FP method would still provide a lower prevalence estimate as compared to the serum samples, it would still show an exposure to a zoonotic agent is very likely for this group of harbor seals at the population level. In summary for the card test, serum showed 13 positives of which eluate only detected 3 of 13 while the centrifugally concentrated eluate detected 9 of 13 (7 negative controls remained so). Thus, we tripled the number of detectable samples with centrifugation and we conclude this technique enhances Brucella-specific antibody detection for the card test when using blood soaked or serum soaked FP.

**Tables 2B and 2C:** Comparison of serum with serum soaked FP eluate and concentrated eluate for detection of Brucella reactive protein using Card Test (Serum 13++ or +, 7-) and Plate Test (Serum 11++ or +, 9-). Positives on the Card Test were determined by degree of agglutination, while positives on the Plate Test were determined by the presence of any agglutination at specific titer levels (25, 50, 100, 200, 400+).

| Table 2B Card Test    |                | # Occurrences                     |           |
|-----------------------|----------------|-----------------------------------|-----------|
| <sup>a</sup> Serum ++ | Serum          | FP Eluate <sub>concentrated</sub> | FP Eluate |
|                       | 5 ++           | 1 ++                              | 1 ++      |
|                       |                | 3 +                               | 1 +       |
|                       |                | 1 -                               | 3 -       |
|                       | Total Positive | 4/5                               | 2/5       |
| <sup>b</sup> Serum +  | 8 +            | 1 ++                              | 0 ++      |
|                       |                | 4 +                               | 1 +       |
|                       |                | 3 -                               | 7 -       |
|                       | Total Positive | 5/8                               | 1/8       |
| <sup>c</sup> Serum -  | 7 -            | 0 ++                              | 0 ++      |
|                       |                | 0 +                               | 0 +       |
|                       |                | 7 -                               | 7 -       |
|                       | Total Positive | 0/7                               | 0/7       |

For the Brucella plate test (Table 2C), the 9 negative samples used remained negative for both the FP Eluate-concentrated and FP Eluate. For the 5 ++ samples 5 of 5 and 2 of 5 samples remained positive for the FP Eluate-concentrated and FP Eluate, respectively. This indicates that reactivity was maintained post FP processing for both approaches. However, for the 6 serum + samples reactivity was lost in that only 1 and 0 retained reactivity for the FP Eluate-concentrated and FP Eluate, respectively. This indicates a post FP processing loss of reactors (Table 2C). In summary, serum showed 11 positives of which eluate only detected 2 of 11 while the concentrated eluate detected 6 of 11 (9 negative controls remained so) for the plate test. We conclude this concentration technique enhances Brucella-specific antibody detection for the plate test as we tripled the number of detectable samples with centrifugation while no decrease in specificity was detected as all negative samples remained negative after concentrating.

| Table 2C Plate Test             | # Occurrences  |                                   |           |
|---------------------------------|----------------|-----------------------------------|-----------|
| <sup>d</sup> Serum ++ (≥400)    | Serum          | FP Eluate <sub>concentrated</sub> | FP Eluate |
|                                 | 5 ++           | 2 ++                              | 1 ++      |
|                                 |                | 3 +                               | 1 +       |
|                                 |                | 0 -                               | 3 -       |
|                                 | Total Positive | 5/5                               | 2/5       |
| <sup>e</sup> Serum + (25≤x<400) | 6 +            | 0 ++                              | 0 ++      |
|                                 |                | 1 +                               | 0 +       |
|                                 |                | 5 -                               | 6 -       |
|                                 | Total Positive | 1/6                               | 0/6       |
| <sup>f</sup> Serum -            | 9 -            | 0 ++                              | 0 ++      |
|                                 |                | 0 +                               | 0 +       |
|                                 |                | 9 -                               | 9 -       |
|                                 | Total Positive | 0/9                               | 0/9       |

<sup>a</sup> Card test - Strong Positive (++) - large, numerous clumps. Nearly identical to control.

<sup>b</sup> Card test - Moderate Positive (+) - clumping, but not as numerous or as large as full positive.

<sup>c</sup> Card test - Minor agglutination/Negative (-) - Either no agglutination present or fine, slight agglutination present but not enough for a moderate positive designation (i.e. +).

<sup>d</sup> Plate test - Strong Positive (++) - agglutination at titers ≥ 400

<sup>e</sup> Plate test - Moderate Positive (+) - agglutination at titers between 25 and 400

<sup>f</sup> Plate test - Negative (-) - no agglutination present at titer = 25

### Chemical-based methods

Our group, and many others, have established the value of using blood soaked filter paper (Nobuto and other) for determining presence and concentrations of some chemicals of interest (Hansen et al., 2014; Jantos et al., 2011; Li et al., 2010; Alfazil and Anderson et al., 2008; Stanton et al., 1999; Spliethoff et al., 2012; Stove et al., 2008; Burse et al., 1997). In our laboratory at the UAF, we successfully showed that we could reliably measure total Hg concentrations ([THg]) in 3 FP products. Considering we were able to directly analyze the blood soaked FP (directly burn the FP and measure the Hg content with no processing), that matrix was deemed the most efficient and accurate measure that matched very well with corresponding whole blood samples (Hansen et al., 2014). We continue to validate this sampling technique for other chemicals of interest (selenium, stable isotopes of C and N) that we have not yet publi-

shed. We provide additional data here on continued work with THg, preliminary data on PCBs and total Se concentrations ([TSe]). For our R(A)MMP efforts we plan to include essential elements in the scheme so that we can also address nutritional adequacy or deficiency. Based on our success in measuring elements (e.g., Hg) on FP (Hansen et al., 2014) we anticipate success with other elements. Regardless, we will conduct the needed validation studies for this application in BCS, Mexico.

### Preliminary PCB findings FP alone

We hypothesized that Advantec Nobuto filter paper can also be used to quantify PCB concentrations in whole blood. We selected this as an easily measured organohalogen for this initial assessment. Our goal was to determine whether the FP had low enough blank background [PCB] for this use, indicating promise for this approach when soaked with blood and then extracted. If the strips provided inherently high background levels, then there is no reason to pursue this method further for the relatively low concentrations of PCBs

one could anticipate in blood. The majority of the PCB recovered from the blank strips (data not presented) detected only PCB 31 across the samples analyzed. In general, the higher the numbers of blank strips in the sample, the higher PCB 31 concentration detected. For example, the 20 strips sample recovered the highest amount of PCB31 levels out of all of the other samples. This general trend occurs with PCB 52 yield as well; whereas only 15 FP blanks and 20 FP blanks measured PCB 52 above the detection limit (using fewer strips were below detection but we can assume PCB52 is still present). Based on the results of these extractions, we can conclude that FPs do have a low enough background (exceptions PCB 31 and PCB 52) and might recover detectable concentrations of PCBs. However, we caution that a single FP holds approximately 100µl of whole blood and this low volume is likely inadequate for reliable concentration measurements that are derived from whole blood. Thus, we are presented with a dilemma of using multiple strips to achieve a reasonable volume (e.g., 200 to 500 µl) to detect what is in whole blood while simultaneously increasing the background levels by using more strips as shown for PCBs 31 and 52 (data not presented). We continue to explore how we can determine organohalogen contaminants in whole blood soaked FP without introducing a high background level or requiring an unreasonable number of FPs for each analysis.

#### *Total selenium concentrations ([TSe]) for FP and Whole Blood*

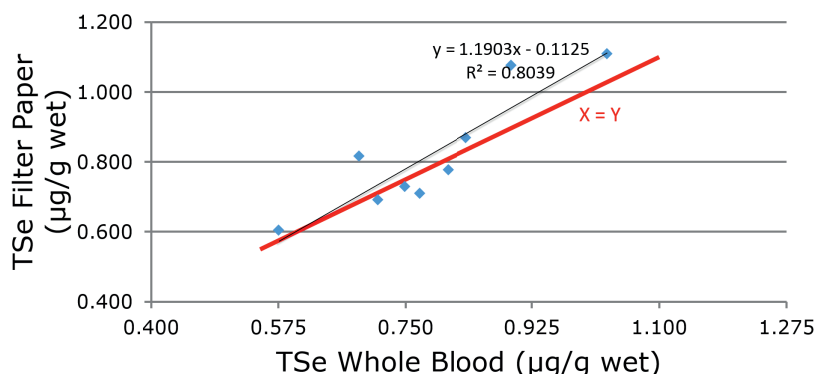
Preliminary data using matched whole blood and WB soaked FP from Steller sea lions (SSL) suggest that WB FP may be useful for assessing whole blood Se status ([TSe], Figure 1. The linear relationship is strong ( $r^2$  of 0.8039) and very much parallels unity (slope of 1.1903). As expected, there is more innate variability in the analysis of [TSe] than [THg], with recoveries from certified reference material typically from 85-115% (compared to 95-105% for [THg]). This analytical variability will result in more variability when comparing [TSe] in samples from the same individual as compared to [THg]. Nevertheless, these preliminary data show a strong relationship between [TSe] in whole blood and WB FP. We will

follow up using whole blood and WB FP samples from harbor seals, northern fur seals (*Callorhinus ursinus*) and other pinnipeds to determine if this strong relationship persists. We plan to directly apply this method to measure of [TSe] in blood of animals sampled in BCS as well as attempt to measure [TSe] in other matrices.

#### *Comparison of whole blood, blood soaked filter paper (FP), and hair [THg]*

We have validated use of FP based assessments of [THg] by comparing it to matched whole blood samples (Hansen et al., 2014) so one can estimate circulating levels of Hg. In our next phase of validation, we are assessing Se (results presented above) as it is well known to be associated with [THg] and is a key antioxidant. Also, we are determining how whole blood and FP-based [THg] compares to hair [THg] as both matrices are important for assessing Hg exposure but integrate Hg in different ways that affect interpretation. We are promoting the sampling of full length hair and FP as a part of RAMP and R(A)MMP. Some of our

previous work related to hair and blood [THg] comparisons are presented in Peterson et al. (2016). Thus, combining these matrices will allow for a more complete temporal assessment where hair integrates Hg over time (about 1cm of growth every month for humans) and whole blood Hg assessments represent current status and likely recent dietary exposure.



**Figure 1:** Comparison of total selenium (TSe) concentration between whole blood (TSe Whole Blood (µg/g wet)) and blood soaked filter papers (TSe Filter Paper (µg/g wet)) in Steller sea lions.

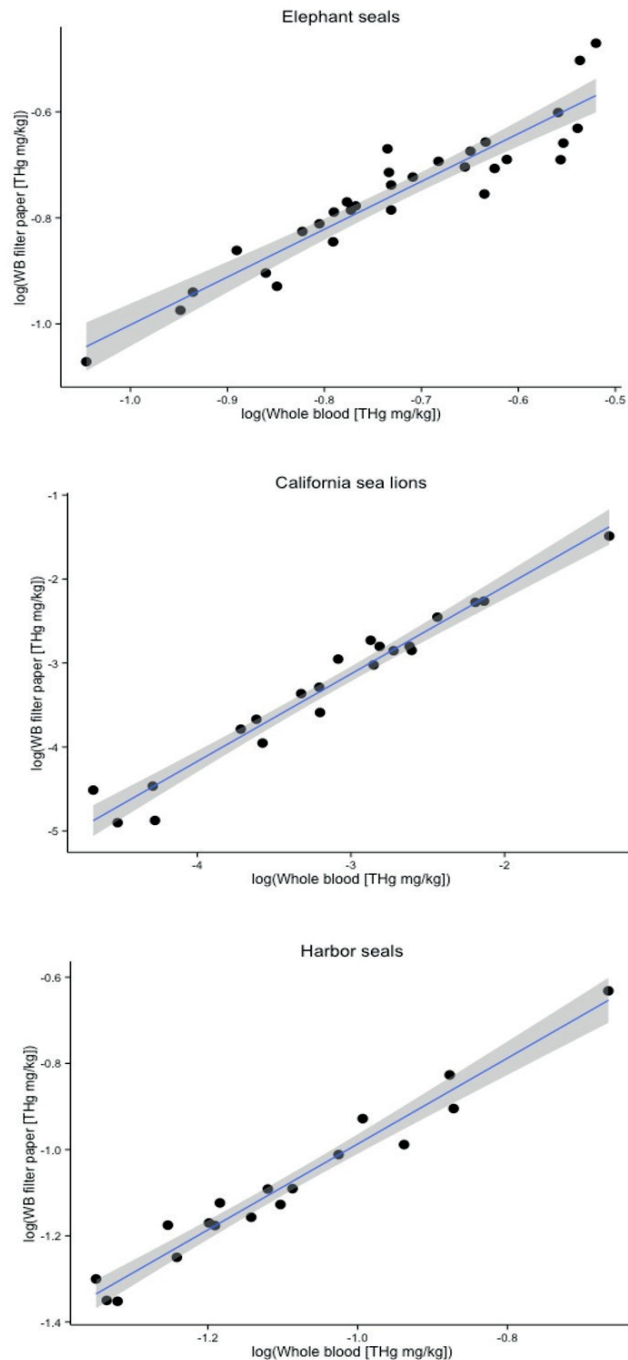
Each has value and when combined gives a much more complete picture of the Hg exposure status. Collection of full length hair and blood soaked FP is likely to be an important One Health monitoring approach that is inexpensive and logistically feasible under most field conditions.

By validating THg and TSe measures in blood soaked FP we can compare the determination of the TSe:THg molar ratio in blood (not hair) that we often use to relate Se status to Hg concentrations for assessing potential “imbalances” for these interacting elements (Correa et al., 2015; Quakenbush et al., 2016). These approaches will be implemented in BCS (Mexico) and have particular value to fish eating organisms including humans.

We have extended validation studies of [THg] in blood soaked filter papers in additional pinniped species using matched blood soaked FP and whole blood from California sea lions (*Zalophus californianus*), elephant seals (*Mirounga angustirostris*) and additional harbor seal samples from The Marine Mammal Center (TMMC) in Sausalito, CA (Figure 2).

TMMC colleagues have strict clinical protocols for sampling that

allow us to do high quality diagnostics and validation assessments that we can then combine with more remote sampling by biologists and hunters in Alaska (e.g. data presented above for *Brucella* serology). These data confirm the conclusions of Hansen et al. (2014) and validate the use of blood soaked filter papers for [THg] assessment across a range of species that we plan to integrate with use of hair samples (e.g., short and long term assessments of Hg exposure) in a future publication. The [THg] linear relationships (whole blood compared to FP) were quite impressive for harbor seals and California sea lions ( $r^2 = 0.9655$  and  $r^2 = 0.9826$ ; respectively) while elephant seals show a promising relationship based on  $r^2$  ( $r^2 = 0.8038$ ,  $p < 0.05$ ) that may require more investigation with all 3 species showing significant linear relationships ( $p < 0.05$ ).



**Figure 2:** Linear regression comparing total mercury concentrations ([THg]), converted to wet weight between whole blood and blood soaked filter papers (FP) from three pinniped species.

## Conclusion

The PCR-based methods have shown utility in our vector sampling scheme to detect bacteria that we will expand in scope in Alaska and ini-

tiate in BCS. We have validated the use of these blood soaked strips to detect agent specific antibodies that was enhanced with a centrifugation technique to reconstitute the samples back to concentrations found in the gold standard matrix (serum).

This indicates we can achieve a reasonable level of sensitivity to determine the presence of exposure to certain agents of disease. We will implement this in BCS in the very near future. Chemical analyses of blood soaked FP for elements in blood shows great promise if we use Hg and Se as our prototypical elements. We will explore use in other fluids such as milk and urine in BCS for some of the measures validated with use of blood and serum. The Alaska experience (RAMP) should allow for establishment of R(A)MMP in BCS based on common interests in addressing climate driven changes in the environment in a One Health context.

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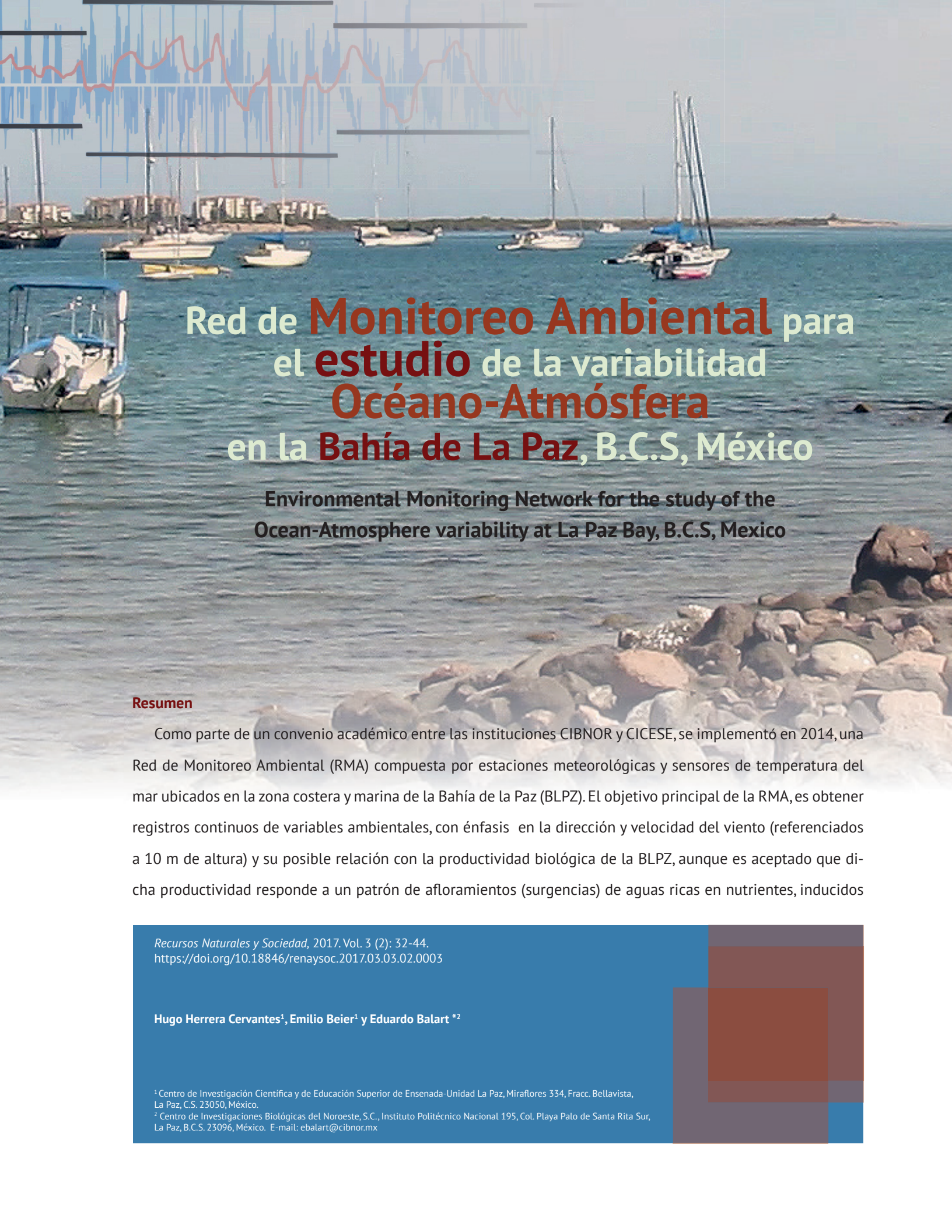
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# Red de **Monitoreo Ambiental** para el **estudio** de la variabilidad **Océano-Atmósfera** en la **Bahía de La Paz**, B.C.S, México

**Environmental Monitoring Network for the study of the  
Ocean-Atmosphere variability at La Paz Bay, B.C.S, Mexico**

## **Resumen**

Como parte de un convenio académico entre las instituciones CIBNOR y CICESE, se implementó en 2014, una Red de Monitoreo Ambiental (RMA) compuesta por estaciones meteorológicas y sensores de temperatura del mar ubicados en la zona costera y marina de la Bahía de La Paz (BLPZ). El objetivo principal de la RMA, es obtener registros continuos de variables ambientales, con énfasis en la dirección y velocidad del viento (referenciados a 10 m de altura) y su posible relación con la productividad biológica de la BLPZ, aunque es aceptado que dicha productividad responde a un patrón de afloramientos (surgencias) de aguas ricas en nutrientes, inducidos

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por el forzamiento del viento y la presencia de estructuras de mesoescala (giros, filamentos, meandros), durante invierno y verano. Mediciones del viento superficial, basadas en imágenes del satélite Sea Wind-QuickScat, muestran para el suroeste del Golfo de California un patrón de vientos del noroeste durante el invierno, que cambia su dirección durante el verano del sur y sureste (comportamiento monzónico de verano). Los registros del viento en San Evaristo, ubicado en el norte de la BLPZ,

mostraron el efecto orográfico que induce la presencia de la sierra de El Mechudo. Durante el invierno el viento predominante fue del noroeste, con velocidades de 5-10 m/s, y durante el verano, el viento fue del sureste con velocidades de 4-6 m/s. El Islote Ballena, ubicado en el archipiélago Espíritu Santo, mostró durante el invierno vientos del norte con velocidades de 8-12 m/s, cambiando durante el verano su dirección a la del sur-suroeste con velocidades de 6-12 m/s. En ambos puntos se contó con observaciones del paso del huracán Odile (2014), donde se registraron rachas de viento de ~70 m/s. Las observaciones ambientales registradas por la RMA en la BLPZ y mostradas en este trabajo, aportan información valiosa para la toma de decisiones gubernamentales, para la prevención de riesgos, la sustentabilidad y en el aprovechamiento de energías renovables.

**Palabras clave:** Variables meteorológicas, hidrografía, Golfo de California

## Abstract

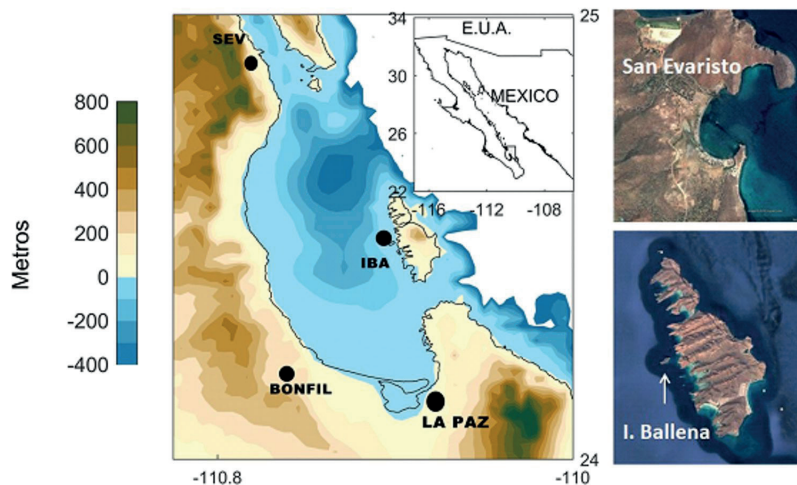
An Environmental Monitoring Network (RMA) composed of meteorological stations and sea temperature sensors located in the coastal and marine area of La Paz Bay (BLPZ) was implemented as part of the academic agreement between CIBNOR and CICESE in 2014. The main objective of the RMA is to obtain continuous measurements of environmental variables with emphasis on wind speed and direction (referenced to 10-m height) and its relationship with hydrographic conditions and high biological productivity of the bay; this productivity responds to an upwelling pattern of nutrient-rich waters induced by wind stress and the presence of mesoscale structures (gyres, filaments, meanders) during winter and summer though. Based on SeaWind-QuikSCAT satellite images for the southwestern part of the Gulf of California, wind measurements have shown a pattern of strong northwest winds during the winter that change its direction from south to southeast and intensity from moderate to weak during the

summer (summer monsoon behaviors). The wind records in San Evaristo, located in the northern portion of the BLPZ, have shown the orographic effect induced by the presence of the Sierra El Mechudo. During the winter, the predominant wind was northwest with maximum speeds of 5-10 m/s while the wind was southeast with speeds of 4-6 m/s during the summer. The Islote Ballena station, located in the Archipiélago Espíritu Santo, showed north wind with speeds of 8-12 m/s during the winter while wind direction was south-southwest with speeds of 6-12 m/s during the summer. In 2014, the passage of Hurricane Odile was recorded at both points with wind gusts of ~70 m/s. The environmental observations registered by the RMA in the BLPZ and reported in this work provide valuable information for government decision-making, risk prevention, sustainability and the use of renewable energy.

**Key Word:** Weather variables, hydrography, Gulf of California

## Introducción

La Bahía de La Paz (BLPZ) se ubica en la margen oriental de la península de Baja California, a 180 km de la boca del Golfo de California (Figura 1), delimitada al norte por las Islas San José, al sur por la Ensenada y Ciudad de La Paz, al este por el archipiélago Espíritu Santo y al oeste por la árida costa sur-oriental de la península de Baja California donde se ubica la Sierra El Mechudo, región montañosa de difícil acceso con un pronunciado gradiente altitudinal (~ 1000 m de altitud) y varios pasos de montaña donde los vientos del noroeste que cruzan la península durante el invierno, modifican sus características antes de interactuar con la BLPZ. El área de estudio presenta una diversidad de actividades, turismo ecológico, un atractivo potencial pesquero de especies demersales y pelágicos de importancia comercial (Arreguín-Sánchez *et al.*, 2004; Vázquez-Hurtado *et al.*, 2010), industria minera, granjas acuícolas y pequeños campos pesqueros.



**Figura 1.** Bahía de La Paz mostrando la ubicación de San Evaristo (SEV) e Islote Ballena (IBA). Las imágenes de la derecha muestran una ampliación de los sitios de observación ambiental utilizando la aplicación Google Earth (<http://earth.google.com/>), con resolución espacial de 15 m derivado de la Misión SRTM, de la NASA, utilizando mallas geográficas con 90 metros de resolución.

Las características hidrográficas de la BLPZ, son fuertemente influenciadas por el patrón estacional del viento: vientos del noroeste durante el invierno que cambian de dirección durante el verano (del sur-suroeste). Este cambio en la dirección del viento (con carácter monzónico) in-

fluye en la circulación superficial típica de la bahía asociada a la formación de fenómenos de mesoescala como giros, meandros y filamentos (Coria-Monter *et al.*, 2015), mientras que la causa principal de la variación diurna del viento cercano a la superficie es el sistema de brisas tierra-mar, presentando una alta correlación con el comportamiento de las corrientes observadas lo largo de las costas de la BLPZ (Turrent y Zaitsev, 2014). Observadas a lo largo y el inicio del verano, la bahía presenta una estratificación importante en la columna de agua, causada principalmente por tres factores: la intensa radiación solar recibida durante esta época del año, el arribo de agua del Golfo de California y el afloramiento de agua fría y densa asociada a la presencia de estructuras de mesoescala que enriquecen la zona eufótica (Coria-Monter *et al.*, 2015), mientras que durante el invierno se presentan surgencias y una significativa homogenización y enfriamiento de la columna de agua asociada al esfuerzo que ejerce el viento en la superficie y a la disminución de la radiación solar (Obeso-Nieblas *et al.*, 2008).

La riqueza biológica que presenta la BLPZ durante un ciclo anual, constituida por altos índices de biomasa fitoplanctónica (Obeso-Nieblas *et al.*, 2008; Sánchez-Velasco *et al.*, 2006; Kahru *et al.*, 2004), se mantiene gracias al aislamiento de la bahía, protegida de forma natural por grandes islas, islotes y cadenas montañosas cercanas a la costa, esto último hace que la bahía sostenga una diversa mega fauna, incluyendo al menos 16 especies de cetáceos con afinidades subtropicales y templadas (Urban *et al.*, 1997; Salvadeo *et al.*, 2009; Pardo *et al.*, 2013) y una producción pesquera basada exclusivamente en la pesca artesanal (Vázquez-Hurtado *et al.*, 2010). Los registros del viento mostrados en este trabajo corresponden a los observados en dos puntos de la RMA: San Evaristo (SEV), ubicado al norte de la BLPZ y el Islote Ballena (IBA), ubicado en el archipiélago Espíritu Santo.

### Antecedentes

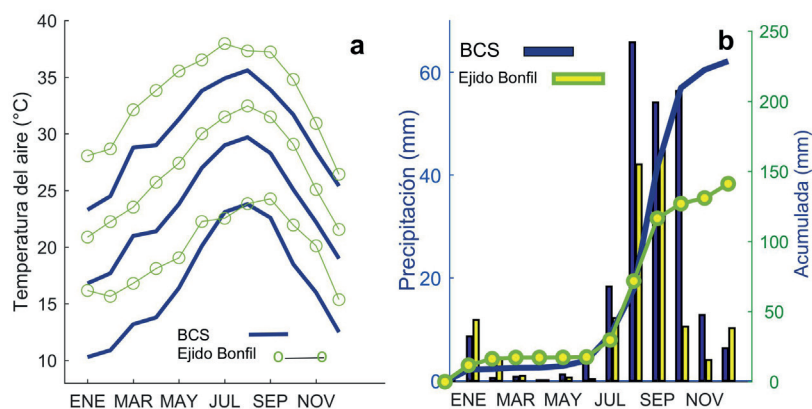
El clima en el Estado de Baja California Sur, corresponde a la clasificación climática tipo BW<sub>hs</sub>(x) de Köppen modificada por García (1964),

correspondiente a climas muy secos y semi-cálidos. La Figura 2 muestra el comparativo del ciclo anual (valores máximos, promedio y mínimos) de (a) la temperatura del aire y (b) la precipitación para el estado de Baja California Sur y para una estación cercana a la BLPZ (Estación V. Bonfil; Figura 1). Los datos corresponden a los registros de la Comisión Nacional del Agua (CNA) para el año 2013. Las temperaturas de invierno cercanas a la BLPZ varían de 15°-28° C, mientras que para verano varían de 24°-37.0°C (Figura 2a, curvas punteadas). La precipitación registró valores menores a 36 mm durante el invierno y de 130-150 mm durante el verano (Figura 2b, barras y curva punteada en amarillo), estas últimas asociadas al paso de tormentas y ciclones tropicales. En relación al viento, el sistema de alta presión del Pacífico Norte y la baja presión sobre el desierto de Sonora, producen un extenso período de vientos energéticos del noroeste, asociados con el paso de frentes fríos que ingresan al Golfo de California y provocan un descenso de la temperatura y algunas lluvias invernales. El clima árido

y caliente de la región, genera un incremento en la evaporación y el inicio del monzón durante el verano, generando vientos predominantes del sur y suroeste (de  $\sim 3$  a  $6 \text{ m s}^{-1}$ ) moduladas por la actividad sinóptica de sistemas ciclónicos (huracanes y tormentas tropicales con rachas  $> 30 \text{ m/s}$ ) que se presentan durante el verano.

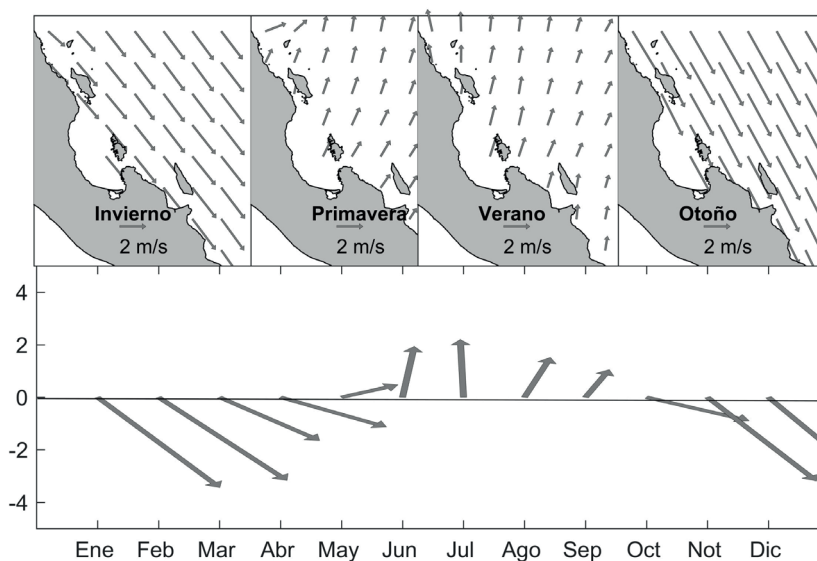
La Figura 3 presenta el patrón estacional del viento en el suroeste del Golfo de California generado a partir imágenes mensuales obtenidas por el satélite QuikSCAT, level 3, JPL PO.DAAC (<https://winds.jpl.nasa.gov/missions/quikscat/>), donde se observa la variabilidad estacional del viento y una baja cobertura espacial para la BLPZ.

En 2013, las instituciones CIBNOR y CICESE firmaron un convenio académico para implementar una Red de Monitoreo Ambiental (RMA) en la BLPZ, como parte de las actividades del Programa de Ecología Pesquera y del Observatorio Marino de Mares y Costas de CIBNOR. La RMA, está compuesta por estaciones Meteorológicas Autónomas Davis Vantage Pro2 en su versión inalámbrica que inclu-



**Figura 2.** (a) Ciclo anual (valores máximos, promedio y mínimos) de temperatura del aire para el estado de Baja California Sur (líneas gruesas en azul) comparados con los valores promedio de la Estación V. Bonfil (líneas con círculos). (b) precipitación (promedio y acumulada) para el estado de Baja California Sur (barras y línea gruesa en azul), comparados con los valores promedio de la Estación V. Bonfil (Barras y línea en amarillo y verde). Los datos se obtuvieron de los registros de la Comisión Nacional del Agua (CONAGUA).

ye sensores de temperatura, humedad relativa, colector de lluvia y un anemómetro además de sensores de radiación solar y radiación UV. La Temperatura del Mar se registró a partir de sensores de temperatura (HOBOS Water Quality Data Loggers). El objetivo de la RMA, es monitorear de forma continua variables ambientales de la atmósfera y el océano, haciendo énfasis en las mediciones del patrón de vientos y su relación con las condiciones hidrográficas, con la riqueza biológica, la di-



**Figura 3.** Ciclo anual de la velocidad y dirección del viento, calculados a partir de imágenes mensuales del viento generadas por el satélite SeaWind - QuickScat durante el período 1999-2009 para la región suroeste del Golfo de California. La Bahía de La Paz muestra una baja cobertura debido al tamaño de celda del sensor ( $0.25^\circ \times 0.25^\circ$ ;  $\sim 27 \text{ km}$ ). La punta de la cabeza de la flecha indica la dirección hacia donde se dirige el viento.

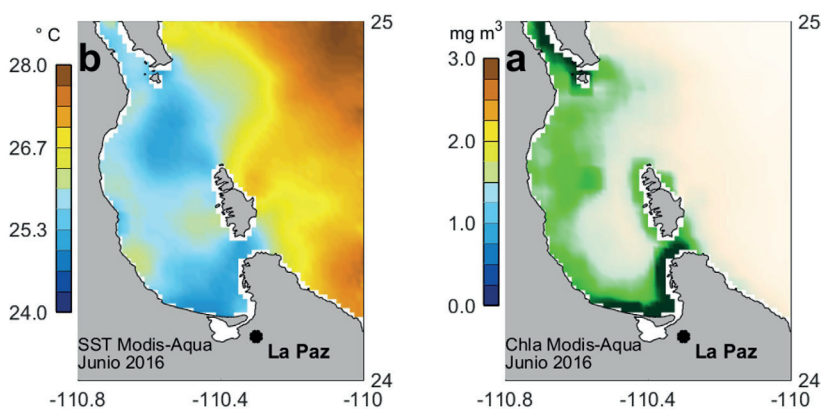


námica costera (erosión de playas) y la presencia de eventos algales nocivos (mareas rojas) en la Bahía de La Paz. El monitoreo continuo del viento en la BLPZ, servirá para generar bases de datos ambientales que sean útiles en la toma de decisiones gubernamentales, para la comunidad científica y a la sociedad en general.

### Área de estudio

La BLPZ se ubica entre las coordenadas 24.1°-25.0° de latitud norte y de 110.1°-111.0° de longitud oeste, está catalogada como la más grande bahía de la costa este de la Península de Baja California, presenta una dinámica importante intercambiando agua con el Golfo de California (Monreal-Gómez *et al.*, 2001) principalmente a través de la boca grande ubicada al norte de la Bahía (Obeso-Nieblas *et al.*, 2008). Las islas San José al norte, Espíritu Santo al este y Punta Coyote al sur, delimitan la bahía del resto del Golfo de California, dándole el carácter de bahía semi-cerrada con profundidades mayores a 400 m (Cuenca Alfonso). Debido a la baja incidencia de nubes en la atmósfera durante gran parte del año, el área

es ideal para el estudio de los procesos de interacción océano-atmósfera utilizando imágenes de satélite (Martínez-Flores *et al.*, 2006). La Figura 4, muestra un ejemplo de imágenes de satélite de promedio mensual en la Bahía de La Paz en junio del 2016: (a) Temperatura Superficial del Mar (°C) y (b) Clorofila superficial ( $\text{mg m}^{-3}$ ), donde se observa un patrón de enfriamiento superficial y una alta productividad biológica (asociada con el afloramiento de aguas profundas ricas en nutrientes), características diferentes comparadas con la zona profunda del Golfo de California.



**Figura 4.** Imágenes de promedio mensual del satélite MODIS-Aqua, (a) temperatura superficial del mar (°C) y (b) Clorofila a ( $\text{mg m}^{-3}$ ), para el mes de junio del 2016, (<http://coastwatch.pfeg.noaa.gov/erddap/griddap/erdMWSstdmday.html>) procesadas para el área de la Bahía de La Paz. Tamaño de celda de 4x4 km.

### Ubicación de las estaciones meteorológicas

El poblado de San Evaristo (SEV), se ubicada a ~170 km al norte de la ciudad de La Paz, comunicado vía terrestre por un difícil camino de terracería el cual sufre constantes deslaves principalmente en el ascenso y descenso de la sierra del Mechudo, sus habitantes se dedican principalmente a la pesca ribereña y la bahía sirve de refugio a la navegación. La estación meteorológica se ubicó a 100 metros de la costa y a ~10 metros sobre el nivel del mar (Figura 5, panel superior). El islote Balleina (IBA), ubicado en el extremo más al oeste del archipiélago Espíritu Santo, declarado por la UNESCO en 2005 como patrimonio natural de la humanidad, se presenta como un punto ideal para la observación, tanto ambiental como de las poblaciones de aves marinas que lo utilizan como zona de anidación,. En este islote se ubica el único faro que sirvió de guía



a la navegación durante muchas décadas (actualmente sin operar). La estación meteorológica (Figura 5, panel inferior), se ubica a ~10 metros sobre el nivel del mar y a 30 metros del borde sur del islote.



**Figura 5.** Vista panorámica de la ubicación de las estaciones meteorológicas en San Evaristo (panel superior) e Islote Ballena (panel inferior).

## Resultados

### Estación San Evaristo

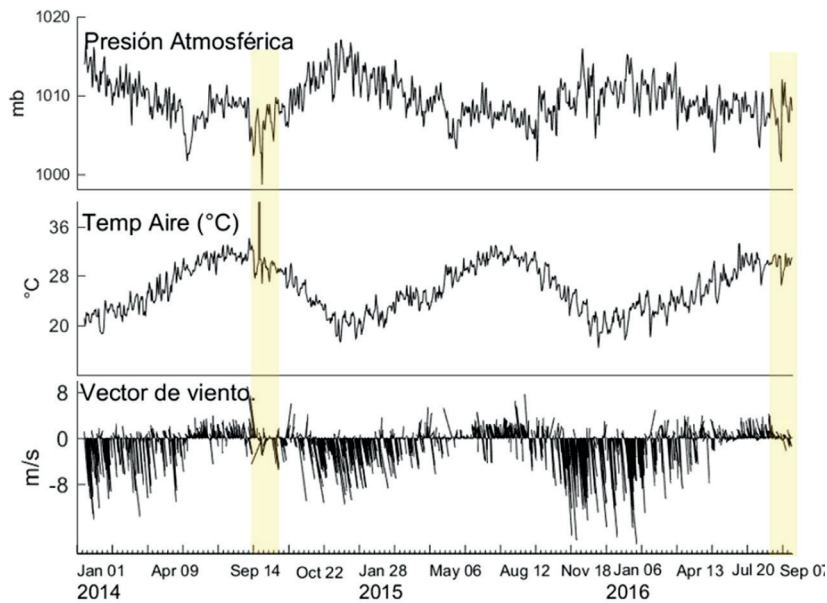
La Figura 6, muestra el promedio diario acumulado de algunas variables (temperatura del aire, presión atmosférica y vectores de viento) registradas en la Estación San Evaristo durante el período enero del 2014 a septiembre 2016.

El promedio diario sirvió para remover las fluctuaciones de menor duración a un día ya que el forzamiento atmosférico a escalas de tiempo superior a un día es más efectivo para forzar la circulación en zonas

semi-cerradas como la BLPZ. En la mayoría de las variables asociadas al viento, se observa el ciclo anual, la magnitud y dirección del viento, muestran los máximos valores (~10 m/s) durante el período otoño-invierno, mientras que durante la primavera-verano (monzón Norteamericano), se observa el cambio tanto en la magnitud (2-5 m/s) como en la dirección del viento (Sur-Suroeste). Durante el invierno, la temperatura del aire presenta bajos valores, y alta presión atmosférica, mientras que durante el verano las altas temperaturas se acompañan con el arribo de tormentas tropicales.

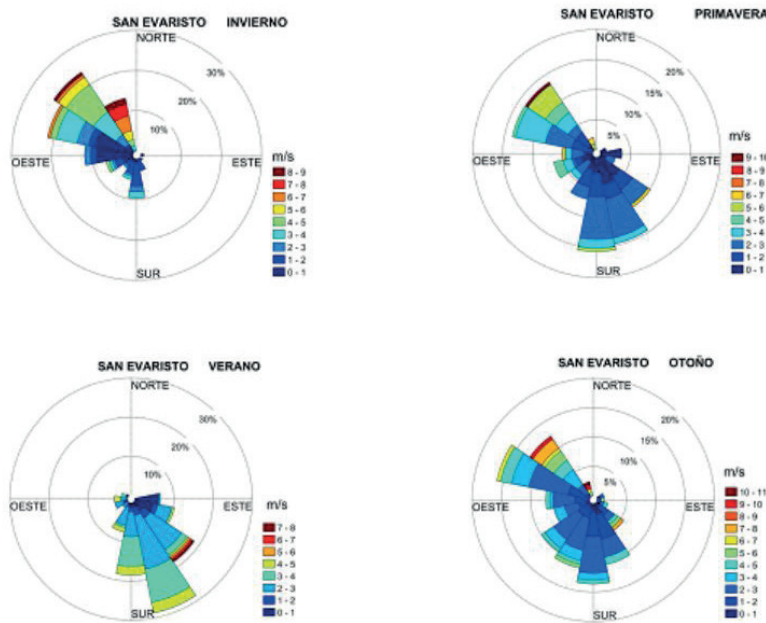
Los huracanes Odile (2014) y Newton (2016) mostraron durante el mes de septiembre (barras en amarillo), cambios pronunciados en la mayoría de las variables analizadas.

La Figura 7 muestra la distribución estadística de la magnitud y dirección de datos de promedio diario del viento (magnitud y dirección) para los períodos de invierno, primavera, verano y otoño (representación gráfica de la rosa de los vientos) en la Estación San Evaristo, la cual indica el porcentaje de tiempo en el que el viento sopla de diferentes direcciones.



**Figura 6.** Promedio diario de variables ambientales medidas en la Estación San Evaristo durante el período de enero-2014 a septiembre-2016: variables: presión atmosférica, temperatura del aire, y vectores del viento. Las barras en amarillo indican el periodo donde los huracanes Odile en 2014 y Newton en 2016 se acercaron a la BLPZ.

La gráfica consiste en utilizar barras o extensiones que van desde el centro de un círculo hacia un punto determinado que ilustra la dirección del viento; la longitud de cada extensión indicará el porcentaje de tiempo en el que el viento se dirigió hacia esa dirección (Ahrens, 1998). Se puede observar que la dirección y el porcentaje de mayor ocurrencia durante



**Figura 7.** Esquema de rosa del viento para la Estación San Evaristo para invierno, primavera, verano y otoño. Cálculos a partir de datos de promedio diario.

invierno es del noroeste con velocidades predominantes del orden de ~10 m/s, mientras que para la primavera el viento empieza a disminuir en intensidad (5-7 m/s) y a rolar hacia el sur. Durante el verano el viento se presenta franco del sureste con velocidades de 8 m/s. Para el período de otoño el viento empieza a rolar nuevamente hacia el noroeste con velocidades entre 10-11 m/s.

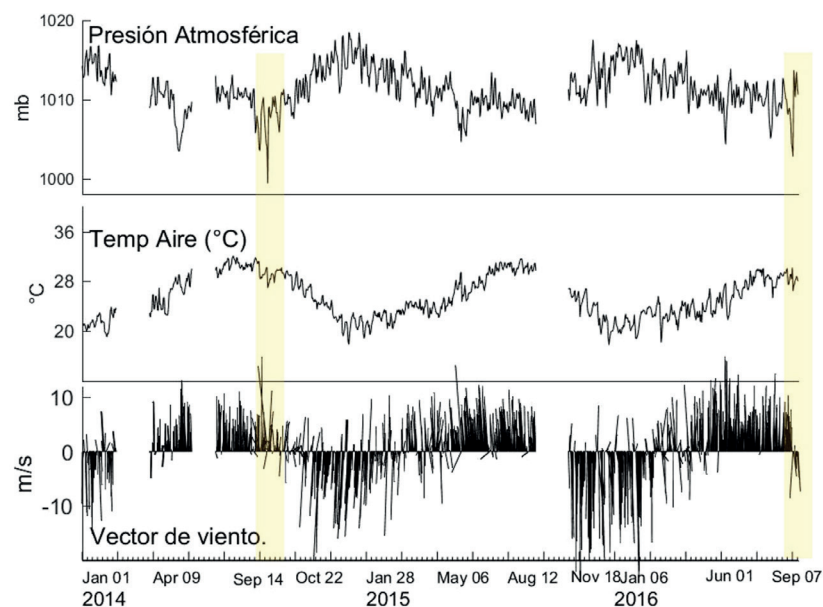
### Estación Islote Ballena

La Figura 8 muestra las variables ambientales registradas en Islote Ballena durante el período 01 de enero del 2014 a al 07 de septiembre 2016, las mismas variables analizadas para la estación San Evaristo. Al inicio de la series se observan períodos sin datos debido a errores y fallas en el equipo. Las variables asociadas al viento, muestran de forma clara el ciclo anual, la magnitud y dirección del vector viento muestra los máximos valores (>11 m/s) principalmente durante el período otoño-invierno, mientras que durante la primavera-verano (monzón Norteamericano) se observa el cambio en la magnitud (4-8

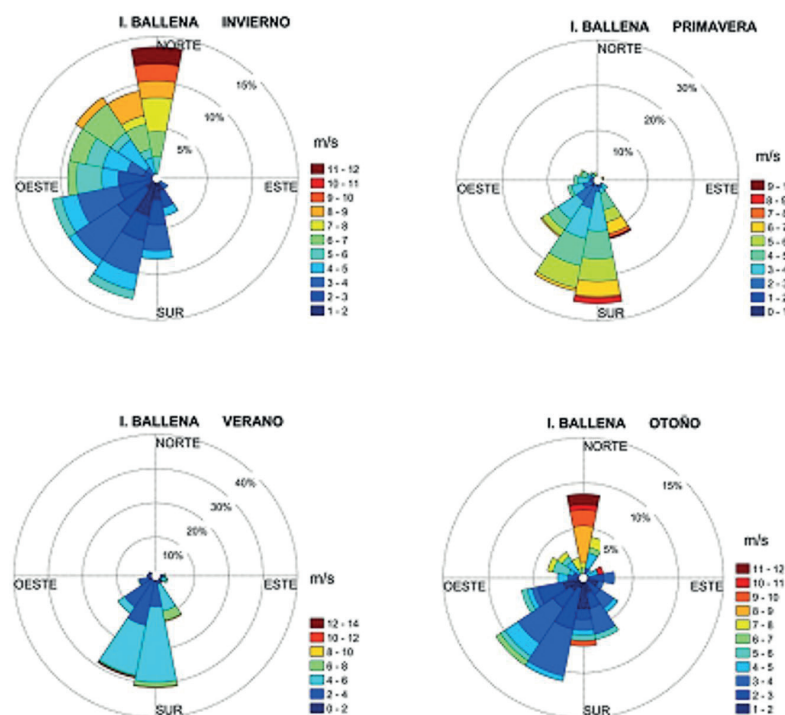
m/s) y dirección del viento (del sur-suroeste). La temperatura del aire presentó valores mínimos durante invierno ( $18.2^{\circ}\text{C}$ ) y máximos en el verano (de  $33.4^{\circ}\text{C}$ ). Durante el paso de los huracanes Odile y Newton (septiembre 2014, 2016: barras en amarillo) la presión atmosférica presentó sus valores más bajos y las rachas de viento sus valores más altos.

La Figura 9 muestra la representación gráfica de los vientos (rosa de los vientos) en la Estación Islote Ballena utilizando los datos de promedio diario de las series de tiempo del viento para los períodos de invierno, primavera, verano y otoño. El Islote Ballena mostró que la dirección y velocidad de mayor ocurrencia del viento durante el período invierno fue del tercer cuadrante (norte-noroeste) con velocidades predominantes de  $\sim 11\text{--}12\text{ m/s}$ , mientras que para el periodo primavera-verano, la dirección del viento fue del segundo y tercer cuadrante (sur-suroeste) con magnitudes de  $9\text{ a }14\text{ m/s}$ . El islote ballena presenta con mayor claridad el cambio del viento asociado a la presencia del monzón Norteamericano, a diferencia de lo

observado en San Evaristo donde el efecto orográfico es importante. La influencia de los huracanes *Odile* y *Newton* durante septiembre del 2014 y 2016, fueron suavizados por el promedio diario.



**Figura 8.** Promedio diario de variables ambientales medidas en la Estación Islote Ballena durante el período de muestreo de enero-2014 a septiembre-2016: variables: presión atmosférica, temperatura del aire, y vectores del viento. Las barras en amarillo indican el periodo donde se presentaron los huracanes Odile en 2014 y Newton.



**Figura 9.** Esquema de rosa del viento para la Estación Islote Ballena para invierno, primavera, verano y otoño. Cálculos a partir de datos de promedio diario.

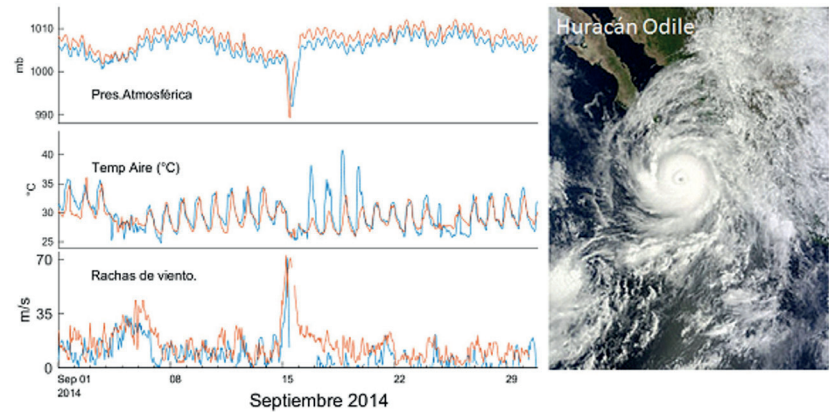


En otoño, el viento vuelve a soplar del noroeste con velocidades ~11 m/s.

### Influencia del Huracán Odile

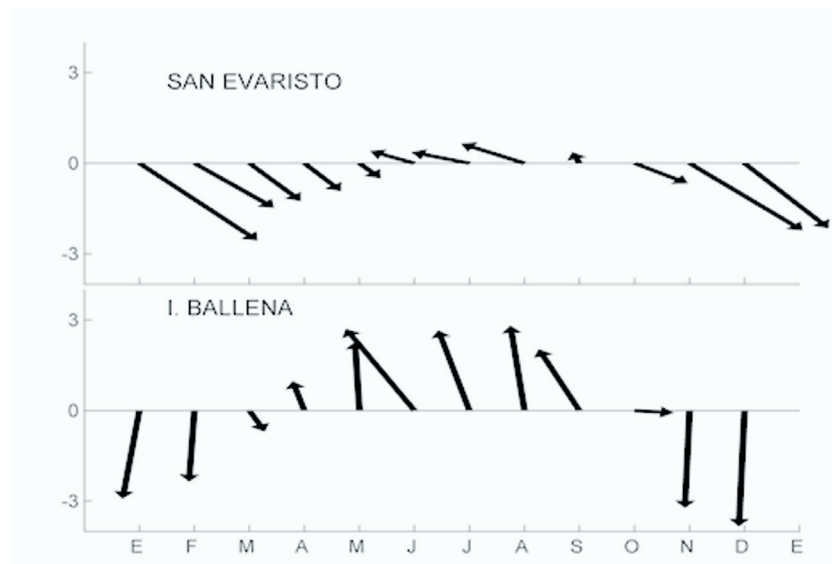
La Figura 10, muestra un comparativo entre series de tiempo de datos horarios de algunas variables observadas en San Evaristo (azul) e Islote Ballena (marrón) durante el mes de septiembre del 2014, cuando el huracán Odile tocó tierra en el sur de la Península de Baja California (14 de septiembre) para posteriormente, en la madrugada del 15 de septiembre, impactar directamente sobre la Bahía de La Paz. En la gráfica se observa en detalle el ciclo diario de la presión atmosférica y la temperatura del aire junto con el sistema de brisas tierra-mar en el viento durante septiembre del 2014 en la BLPZ (Turrent y Zaitsev, 2014). La temperatura del aire presentó valores de ~32° C previos al evento, cayendo hasta ~25° C el 15 de septiembre. La presión atmosférica mostro valores horarios por debajo de 990 milibares durante el periodo de mayor cercanía de Odile a los puntos de observación. El 15 de septiembre, las

rachas máximas de viento incrementaron de 10 m/s hasta valores máximos de ~70 m/s. Las estaciones ubicadas en San Evaristo e Islote Ballena se mantuvieron en pie durante los fuertes vientos de Odile.



**Figura 10.** Comparativo de series de tiempo de datos horarios de variables ambientales (presión atmosférica, temperatura del aire y rachas de viento) registradas durante el mes de septiembre del 2014, cuando el huracán Odile se desplazó por la Bahía de La Paz (14-15 de septiembre). San Evaristo e Islote Ballena (curvas en marrón) La imagen de satélite de la derecha fue obtenida del sitio <http://rapidfire.sci.gsfc.nasa.gov/cgi-bin/imagerly>.

La Figura 11 muestra el ciclo anual promedio de la dirección y velocidad del viento registrado en San Evaristo e Islote Ballena calculado para el periodo 2014-2016; San Evaristo muestra el efecto orográfico de la Sierra de El Mechudo, sobre el viento predominante del Noroeste desde



**Figura 11.** Vectores promedio del ciclo anual del viento en San Evaristo (panel superior) e Islote Ballena (panel inferior), obtenido a partir de las series de tiempo de datos horarios observados durante el periodo 2014-2016. La punta de la flecha del vector indica la dirección hacia donde sopla el viento.

el mes de octubre hasta mayo (otoño-invierno-primavera), mientras que durante el verano el viento muestra el comportamiento monzónico con los vientos del sureste. En el Islote Ballena, desde noviembre y hasta marzo (otoño-invierno), la dirección del viento se presenta casi franco del norte, mientras que a partir de abril (dos meses antes que San Evaristo) y hasta septiembre (primavera-verano), se observa el cambio de dirección del viento con carácter monzónico. Los patrones climáticos del viento obtenidos a partir de observaciones coinciden con los obtenidos en base a imágenes del satélite QuikSCAT (Figura 3). Las observaciones del viento a diferentes escalas de tiempo (de horas a meses), pueden aportar información valiosa sobre su relación con la hidrografía y el enriquecimiento biológico superficial reportado por otros autores en la BLPZ.

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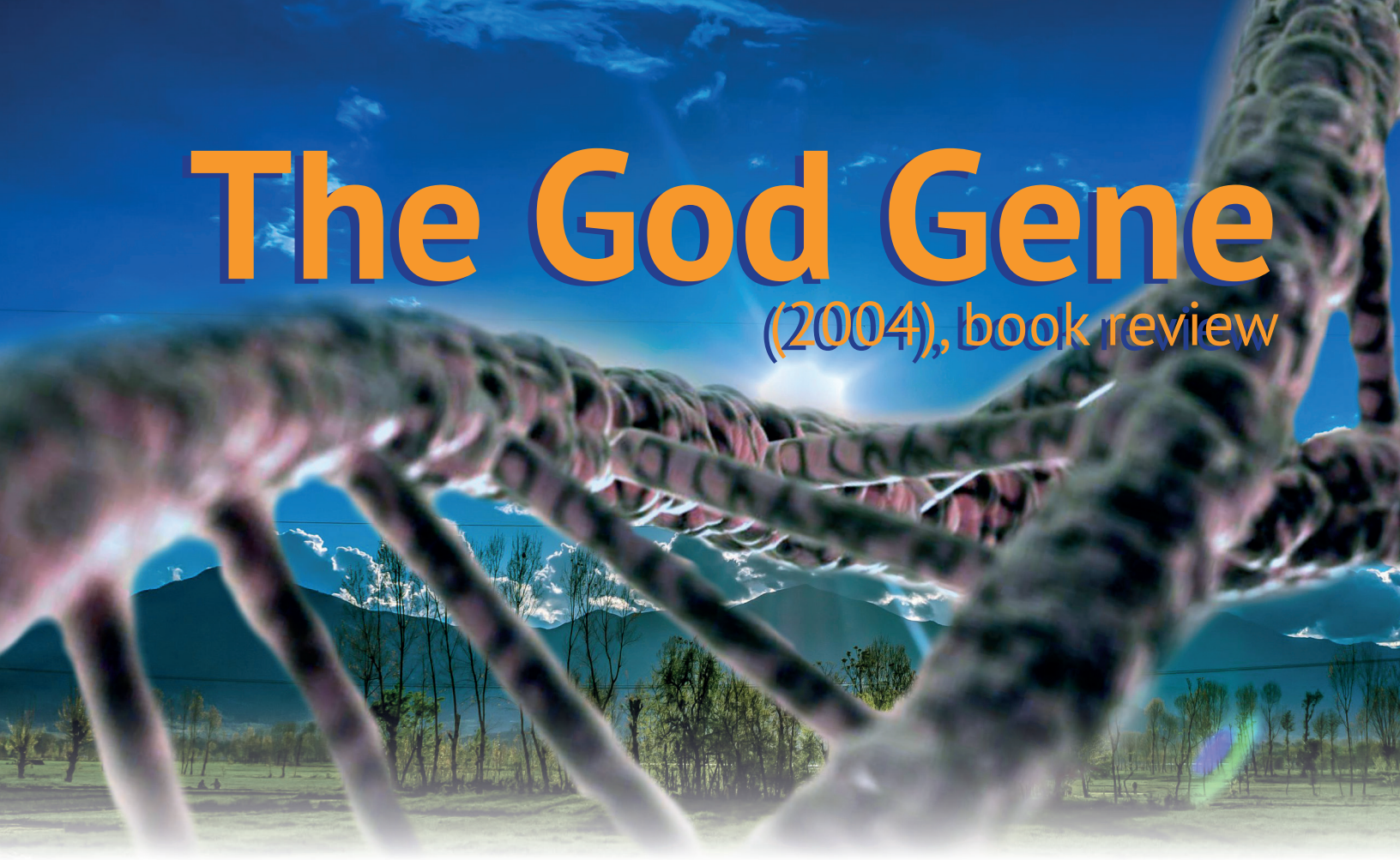
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# The God Gene

(2004), book review

**E**n el libro de Dean Hamer, genetista que colaboró hasta su retiro en el *National Institute of Health* de EE.UU., se analiza la base biológica de la espiritualidad, que de existir, debe ofrecer una ventaja evolutiva. El libro es ciencia popular descrita para que el lector entienda cómo se hace una investigación científica. Es un mini curso sobre ciencia y muestra la forma en que la ciencia, para serlo, debe cuantificar alguna característica o propiedad del sujeto de estudio, en este caso la espiritualidad. Busca entender la relación causa efecto entre principios biológicos y espiritualidad. El libro contribuye a reflexionar sobre la naturaleza humana analizando el instinto espiritual.

Tiene entre sus virtudes el poner sobre la mesa la posibilidad de diser-

**T**he *God Gene: How Faith Is Hardwired into Our Genes* is a nonfiction work authored by Dean Hamer, a molecular geneticist who collaborated at the National Institutes of Health until he retired. In *The God Gene*, Hamer analyzes the biological basis of spirituality and argues that if such a trait exists, it must offer an evolutionary advantage.

The book falls into the popular science category because it is written to help the reader understand how scientific research is performed. In that sense, it is a short science course that demon-

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strates how science, to be true science, must quantify a phenomenon, in this case spirituality. The author seeks to understand the cause-effect relationship between biological principles and spirituality. By analyzing the spiritual instinct, this book contributes to the study of human nature.

One of the virtues of Dr. Hamer's work is offering the opportunity to discourse about the eternal dialectic between science and religion. Secondly, his work adds insight to the description of the scientific method and thought.

The author introduces the reader to Tenkai, the adopted name of a German man educated

tar sobre la eterna dialéctica entre ciencia y religión, por un lado, y por otro, la plausible aportación que conlleva la descripción del método y el pensamiento científico.

El autor presenta a Tenkai, que es el nombre adquirido por un alemán que se educa en la tradición religiosa y el sistema de meditación Zen Budismo en Japón. A quien se unió en su búsqueda para investigar si hay una base biológica para la espiritualidad. El autor explica que el Zen es una religión única porque no se basa en teología, escrituras o rituales; no hay dioses o diablos; ni cielo ni infierno. Muy importante, no hay sacerdotes que reclamen divinidad. El Zen no es sobre saber, es sobre cómo percibir la naturaleza tal como es, es sentirse integrado al universo, vivir una realidad enfocada, espiritual, la que no depende de religión. Tenkai se encuentra en el extremo superior de una escala de espiritualidad; un continuo que abarca a todos los humanos, desde unos pocos con alto grado de espiritualidad, unos muchos con mediano grado y otros pocos con muy poca espiritualidad.

*El gen de Dios* propone brindar "autoría" a la genética para marcar la tendencia o la proclividad espiritual, que no religiosa, en algunos individuos. En resumen, abre una discusión científica que fundamenta y explica el origen de la conducta espiritual del hombre, mediante la exposición de argumentos profundos, que van de la revisión minuciosa y tipificación de variables en secuencias de ADN, hasta la compilación y confrontación de varios estudios sociológicos y (conductuales) que permiten afianzar una clara separación entre lo que entraña la religión, de lo que es dado a la naturaleza biológica en el ser humano: su capacidad instintiva.

Es una valiosísima aportación científica, ya sea para explicar su origen o para sentir el bienestar, o apreciar la dificultad para construir ese universo diferenciado entre seres genéticamente predispuestos, de los que solo aprenden a través de la religión. La espiritualidad es un instinto parcialmente heredable que se refuerza con las experiencias del individuo.

En la hipótesis de *El gen de Dios* se propone que la espiritu-



in the Zen Buddhism religious tradition and meditation system in Japan. Hamer joined Tenkai to investigate if there is a biological basis for spirituality. The author explains that Zen is a unique religion because it is not based on theology, scriptures or rituals. In Zen, there are no gods or devils; neither heaven nor hell. Furthermore and of greater relevance, there are no priests who claim divinity. The Zen practice is not about knowing, it is about perceiving nature just as it is. The goal is to feel integrated with the universe and to live a focused, spiritual reality, independent of religion. Tenkai has reached the highest point of a spirituality scale; a continuum that embraces all humans. In this continuum, some individuals possess a high degree of spirituality while others rate moderately or low.

*The God Gene* points out that genes influence the capability of the brain, which becomes the basis for spiritual (not religious) experiences. In summary, the book opens a scientific discussion around the source of our spiritual behavior. It presents deep arguments, ranging from a meticulous review and classification of the variables found in DNA sequences to the compilation and comparison of several sociological and behavioral studies. These arguments strengthen the notion of a clear separation between the elements of religion and what is intrinsic to the biological nature of human beings: their instinctive ability.

The findings are an invaluable scientific contribution to the understanding of the origin of spiritual behavior and its associated feeling of well-being. In addition, *The God Gene* has helped to appreciate the difficulty in building a differentiated universe between genetically predisposed individuals and those who only learn through religion. Hamer states that spirituality is a partially hereditary instinct that is reinforced by the experiences of the individual.

The hypothesis of *The God Gene* postulates that spirituality depends on a biological, neuronal, cerebral mechanism, similar to that of bird-song, which is a genetic predisposition. Thus, religion expresses itself in response to personal and cultural experiences. One of the chapters that helps understand the author's hypothesis is the one about *self-transcendence*.

alidad depende de un mecanismo biológico, neuronal, cerebral, similar al del canto de las aves, el cual es una predisposición genética. Así, la religión se expresa en respuesta a experiencias personales y culturales.

Uno de los capítulos para lograr la comprensión de lo expuesto, es el relativo a la *autotranscendencia*, como eje de medición del grado de espiritualidad en el individuo. Una forma cuantitativa de aproximación.

Con una serie de ejercicios que involucraron incluso lazos consanguíneos y relaciones como las establecidas en gemelos idénticos, Nicholas Martin y Lindon Eaves, logran ubicar ese *rasgo* a través del registro de un importante número de pares de gemelos, a quienes se les indagó, entre otras cuestiones, acerca de vivir la inspiración de la trascendencia o la iluminación (para el caso de los orientales). El resultado para los factores ambientales (incluyendo o considerando la crianza misma del individuo) fue del 52%, mientras que para la genética misma un 48%.

El gen influye en el cerebro

para la capacidad de grados de conciencia que es la base de la espiritualidad. El autor acepta que el título *El Gen de Dios* es una sobre simplificación; varios genes participan a la par que los factores ambientales. Entonces, la espiritualidad es más que aceptar la existencia de dios o dioses. Quienes tienen grados altos de espiritualidad no basan su vida en una deidad. El título del libro es solo una abreviación, una simplificación con fines de mercadotecnia.



Siendo el autor un científico, propone explorar diferentes líneas de razonamiento para mostrar lo instintivo, biológico, de la espiritualidad. Como en toda investigación científica, se debe definir y cuantificar la característica a indagar. Para demostrar la relación causa efecto de un fenómeno, biología-espiritualidad, se debe generar una hipótesis de trabajo, la cual permite definir qué variables se investigarán experimentalmente para demostrar si la hipótesis explica el fenómeno con base en los paradigmas de la ciencia. Para que sea hipótesis, la espiritualidad debe ser cuantificada, medible. Es aquí en donde reside la premisa de la investigación científica y del libro.

Se presentan al lector algunas evidencias que justamente derivan en lo que el autor ha denominado: el instinto espiritual, dejando para otro momento una explicación contundente sobre si el mismo gen, que compartiendo múltiples funciones como la producción de adrenalina, tiene que ver con algo tan lejano como el refugio que ofrece la religión para el 41 por ciento y hasta 52 por ciento de individuos analizados.

*Self-transcendence* is the axis measurement of an individual's degree of spirituality; a quantitative approximation.

Hamer references the studies on twins performed by two scientists who based their research on genetic behaviors as key variables. Nicholas Martin and Lindon Eaves conducted studies involving subjects with consanguineous ties, specifically identical twins. The studies allowed Martin and Eaves to locate *the self-transcendence trait*, by recording the answers given by a considerable number of twins. The twins were asked, among other questions, if they had experienced transcendence or enlightenment (as known by Asians). The data demonstrated that environmental factors (including the individual's upbringing) account for 52% while genetics accounts for 48%. The gene influences the consciousness degree of the brain, which is the basis of spirituality.

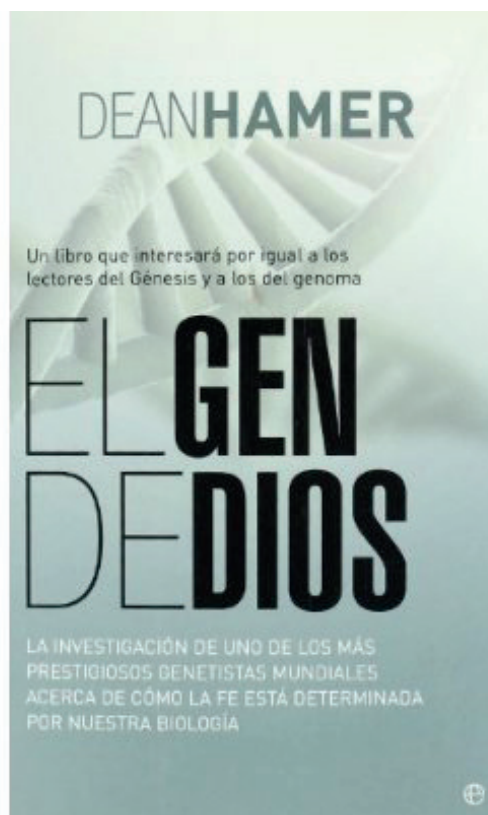
Hamer acknowledges that the title of his book, *The God Gene* is an oversimplification, given that in reality, several genes participate alongside environmental factors to determine spirituality. Hamer

also states that spirituality goes beyond accepting the existence of god or gods; those with high degrees of spirituality do not base their lives on a deity. The title of the book is just an abbreviation, a simplification for marketing purposes.

As a scientist, the author explores different lines of reasoning to show the instinctive, biological side of spirituality. As in all scientific research, the variable to be analyzed must be clearly defined and quantified. To demonstrate the cause-effect relationship of biology-spirituality, a working hypothesis was required. It allows the researcher to determine the variables to be studied experimentally to prove if the hypothesis explains the phenomenon based on scientific paradigms. To become a hypothesis, spirituality must be quantifiable. The entire scientific premise of the research and the book lies on this fact.

Hamer presents evidence of what he coined *The Spiritual Instinct*. However, he does not go into a forceful explanation about whether this gene, which among other functions is also involved in

El autor expone cómo logra encontrar relación -a partir de la asociación del gen VMAT2- de conductas como la autotranscendencia. Y afirmar sobre atributos y conductas religiosas no heredadas a través de su ADN, pero sí *impulsados* por el gen de la espiritualidad.



La hipótesis se comprueba y valida: existe relación entre la biología, la genética de los individuos y la espiritualidad. Esto gracias a poder cuantificarla. Hay un dicho que se atribuye a Galileo Galilei que dice: “en ciencia cuantifica lo cuantificable y haz cuantificable lo que no lo es”. Por mucho tiempo se aceptó que el dominio de la ciencia y las religiones no coincidían. Ahora la ciencia estudia a las religiones desde varias disciplinas, sociología, antropología y recientemente de la psicología evolutiva. Esta última ha permitido cuantificar la autotranscendencia y con ello a la espiritualidad

El autor usa la escala numérica de la *autotranscendencia* de otro investigador, el psiquiatra y genetista, Robert Cloninger. La autotranscendencia es la capacidad de sentirse unido, unificado, en armonía con el todo, la

the production of adrenaline, has something to do with other phenomena. For example, why does 41% of the general population find solace in religion just as 52% of the individuals analyzed in the study also do?

Furthermore, the author explains how he discovered behaviors such as self-transcendence through the VMAT2 gene. Hamer states that some religious attributes and behaviors are not associated with our DNA but are driven by the spirituality gene. His hypothesis is verified and validated: there is a relationship between biology, the genetics and spirituality of individuals and it is quantifiable.

It is said that Galileo Galilei stated: "in science, quantify what is quantifiable and make quantifiable what is not." For a long time, it was accepted that religion and science could not coincide. Now, science studies religion from various disciplines: sociology, anthropology and recently evolutionary psychology. The latter has made it possible to quantify self-transcendence and thus spirituality.

The author also employs a self-transcendence numerical scale devised by another researcher, Robert Cloninger, a psychiatrist and geneticist. Self-transcendence is the ability to feel united, in harmony with the whole, nature and the universe. It measures what in western cultures we call faith. Eastern cultures describe it as a state of mind characterized by the absence of desire or suffering.

Hamer's research is successful at separating spirituality from religion, not an easy task. Religion is based on spiritual beliefs and spiritual beliefs are usually expressed using the language and rituals of religion. The separation is achieved by studying those individuals who, oblivious to any form of organized religion, have a high degree of self-transcendence.

The research strengthens its findings by analyzing if spirituality is acquired from our parents, in other words, if it is inherited. In addition, it studies the effect of the environment on the individual's level of spirituality. It is now known that there is a genetic predisposition to spirituality. The next step belongs to molecular genetics, which found that individuals rating high on the scale of self-transcendence have an associated gene. The gene contains the code for a monoamine transporter protein, a brain neurotransmitter.

naturaleza, el universo. Mide lo que en las culturas occidentales llaman fe, y las culturas orientales llaman estado de la mente caracterizada por la ausencia de deseo o de sufrimiento.

El logro de esta investigación es poder separar espiritualidad de religión, lo que no es fácil, ya que las religiones están basadas en creencias espirituales y las creencias espirituales generalmente se expresan usando lenguaje y rituales de las religiones. La separación se logra al estudiar a aquellos individuos que, ajenos a cualquier forma de religión organizada, tienen un alto grado en la escala de autotranscendencia.

La investigación se complementa y auto afirma estudiando si la característica de espiritualidad se adquiere de los padres, se hereda, así como cuál sería el efecto del medio ambiente. Ahora se sabe que hay una predisposición genética a la espiritualidad. Y estando involucrada la genética, el siguiente paso en la investigación es tarea de la genética molecular; la que encontró que individuos con alto grado en la escala de autotranscendencia poseen un

gen asociado. El gen codifica para una proteína transportadora de monoaminas, neurotransmisor cerebral.

Característico de una investigación científica bien llevada es confirmar, por varias vías, el fenómeno. Por lo que el autor estudió el mecanismo cerebral de la espiritualidad. Las monoaminas influyen en la espiritualidad al alterar la conciencia, que en ciencia se define como la capacidad de sentir la realidad, cómo nos vemos a nosotros y al universo por medio de pensamientos, memorias y percepciones.

La religión, aparece claramente delimitada como algo aprendido, algo que surge del constructo heredado culturalmente, algo transferido en el *ambiente social* del individuo, incluidos los momentos de su crianza. Es justamente en este episodio del texto cuando el lector podría inferir que la multiplicidad de factores que inciden en la *tendencia* hacia los aspectos de carácter espiritual, tendrían una forma gradual de aparición en ciertos individuos y si esa *graduación* es posible determinarla, mediando entre lo aprendido y lo heredado. Se posibilita de igual manera una reflexión más detenida sobre si ese gen es motor o no para el diseño de ese constructo cultural llamado religión, o si ello implica solamente la conciencia de ser parte de un todo (cualquiera) como lo afirma el autor, sin que prive el dogma como intermediación.

Un genetista ruso trabajando en EE.UU. sobre la síntesis evolutiva moderna afirma: “Nada tiene sentido en biología si no es a la luz de la evolución”. La teoría de Darwin sobre evolución y ventaja competitiva se aplica a cualquier característica, incluyendo espiritualidad, o a la forma de los picos en las aves, o la habilidad para cazar del león. Por lo que el autor se pregunta, ¿qué ventaja evolutiva tiene poseer este gen?, ¿es un efecto colateral de la evolución?, ¿ofrece una ventaja evolutiva directa? como cohesión social, soporte en dificultades, reducción de estrés, prevención de enfermedades, mantener el ánimo y extensión de la vida, ¿es entonces un comportamiento que evolucionó?

El que la espiritualidad, la base de las religiones, haya sido favorecida por selección natural, solo prueba que hay una ventaja en ello, y ni confirma ni desaprueba la existencia de dios(es).

La espiritualidad depende de un mecanismo biológico similar al canto de las aves, aunque mucho más complejo y matizado. Predispone a los humanos a “creer” aunque no a qué creer, de ahí la variedad de religiones. Como toda característica de los humanos, la espiritualidad ocurre en alto grado en unos pocos individuos, algo en la mayoría y muy poco en pocos individuos. Independientemente de qué religión profesen, si es que profesan alguna.

Hay que leer el libro para construir una opinión propia basada en argumentos científicos.

**Diseño gráfico editorial: Lic. Gerardo Hernández.**

Hay una traducción al español. El gen de Dios: la investigación de unos de los más prestigiosos genetistas mundiales acerca de cómo la fe está determinada por nuestra biología (En papel). Dean Hamer, 2006. 304 págs. Tapa blanda. Ed. La Esfera de los libros. ISBN: 9788497345552



A well-conducted scientific research confirms a phenomenon in several ways. In this case, the author studied the cerebral mechanism of spirituality. Monoamines influence spirituality by altering consciousness. For science, consciousness is defined as the ability to feel reality, to see ourselves and the universe through thoughts, memories and perceptions. On the other hand, religion is clearly defined as something learned, something that arises from our inherited culture, characteristics transferred to the individual by his environment, including his upbringing.

At this juncture, the reader could infer that the many factors that influence our tendency towards a spiritual nature would surface gradually in certain individuals. If that is true, we could mediate between what is learned and what is inherited. The book also invites the reader to reflect on whether that gene is behind the design of religion, or if the gene only aids in the awareness that we are part of a whole (any), as the author claims, free of intermediary dogmas.

A Russian geneticist working in the U.S.A. on modern evolutionary synthesis said: "Nothing in biology makes sense except in the light of evolution". Darwin's theory of evolution and competitive advantage applies to spirituality as much as it applies to the shape of a bird's beak, or the lion's hunting abilities. With that in mind, Hamer asks, what the evolutionary advantage of this gene is? Is it a collateral effect of evolution? Does it offer a direct evolutionary advantage?

Spirituality often translates into social cohesion and offers support in difficult times. Spirituality has also been associated with stress reduction, disease prevention, improved mood and longer life spans; is it then a behavior that evolved? The fact that spirituality, the basis of religion, has been favored by natural selection, only proves that it offers an advantage. However, it does not prove or disprove the existence of god(s).

Spirituality depends on a biological mechanism similar to a birds 'song but at a much more complex level. Spirituality predisposes humans to believe, but not to believe in something in particular, which explains the wide variety of religions. Like all human traits, a few individuals possess a high degree of spirituality while the majority possesses a moderate level of spirituality and a few individuals have a low degree of this trait. The level of spirituality is independent of the religion they profess, if any.

Read the book for yourself and build your own opinion based on the scientific arguments Dean Hamer offers us.

**Editorial Design by Lic. Gerardo Hernández**

**English Edition by D. Dorantes**

The book is available in Spanish: *El Gen de Dios: la investigación de unos de los más prestigiosos genetistas mundiales acerca de cómo la fe está determinada por nuestra biología* (En papel). Dean Hamer, 2006. 304 págs. Tapa blanda. Ed. La Esfera de los libros. ISBN: 9788497345552.