Building capacity and determining disease threats to endemic Galapagos fauna

Darwin project: 162-12-017

Annual report 2004/2005

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Darwin Initiative for the Survival of Species
Annual Report

1. Darwin Project Information

Project Ref. Number	162-12-017
Project Title	*Building capacity and determining disease threats to endemic Galapagos fauna*
Country(ies)	*United Kingdom, Ecuador*
UK Contractor	*Institute of Zoology, Zoological Society of London, and School of Biology, University of Leeds*
Partner Organisation(s)	*Galapagos National Park; Program of Biotechnology, University of Guayaquil, Ecuador*
Darwin Grant Value	*£195,381 (£47,097 for 2004/5)*
Start/End dates	*Start 1st October 2004, End 30th September 2006*
Reporting period (1 Apr 2004 to 31 Mar 2005) and report number (1,2,3..)	*Report to 31st March 2005* *Annual Report Number 2*
Project website	*http://www.biology.leeds.ac.uk/ggepl/*
Author(s), date	*Dr. Simon Goodman, Dr. Andrew Cunningham and Dr. Virna Cedeño, 30th April 2004*

2. Project Background

This project is based in the Galapagos archipelago, Ecuador and is a partnership between the Institute of Zoology (Zoological Society of London), School of Biology - University of Leeds, The Galapagos National Park Service and the Programme of Biotechnology, University of Guayaquil, Guayaquil, Ecuador. The project was established in response to the urgent need for an assessment of disease threats to the Galapagos fauna and for capacity within the Galapagos to determine and address these threats to the archipelago's unique biodiversity.

3. Project Purpose and Outputs

Purpose

To establish the ability of researchers and managers in the Galapagos National Park to determine the nature and prevalence of disease threats to endemic fauna stemming from the introduction of novel pathogens and vectors, and to build a capacity for the continued monitoring of introduced diseases in these populations.

Outputs

- Identity and prevalence of key pathogens and vectors that threaten endemic species determined.
- A management plan for endemic species in relation to disease threats.
- Establishment of a wildlife disease laboratory and a continuing monitoring programme with trained personnel.
- Educational events and materials (locals & tourists).
- Media representation

Progress and Achievements against logical framework – see Annex 1.

Neither the outputs nor the operational plan have been modified over the last year. However, the project leader, Dr. Simon Goodman moved to the School of Biology, University of Leeds in November 2004 to take up a position as lecturer. Dr. Goodman continues to manage the project and no major alterations in light of this move are anticipated.

4. Progress

The project started on 1st October 2003 following a deferral of 6 months at the request of the Darwin Initiative. In the first year (October 2003 to September 2004) the project activities focused on establishing the laboratory, identifying project staff members, devising training programs, purchasing and shipping equipment, and making links with stakeholders for wildlife disease issues in Galapagos. The project had the opportunity to greatly increase its capacity thanks to a much bigger than anticipated investment by the University of Guayaquil (>$110,000) that permitted complete refurbishment of a major building in Galapagos for the laboratory, and allowed equipping of the laboratory to a higher standard than originally planned. Additional financial support was also obtained from the Galapagos Conservation Trust and the British Embassy in Quito. Work in the second year of the project (October 2004 to present) has focused on implementing the management role of the laboratory for the Galapagos National Park. This has involved staff training, development of the molecular and pathology assay techniques for pathogen diagnosis and surveillance, and by conducting risk analyses to assess the likelihood of introduction and impacts of new diseases to Galapagos such as West Nile Virus (WNV). The project has potentially discovered novel pathogens in giant tortoise and sea lions which are currently being characterised to confirm initial results. Following recommendations by a project workshop on WNV introduction, lobbying by stakeholders has resulted in changes to Ecuadorian law being made such that fumigation/disinsection is now required for all transport to Galapagos to minimise the risk of transport of infectious arthropod vectors (e.g. mosquitoes) to the archipelago.

Progress and achievements against milestones for 2004-2005

Institutional Capacity Building and Training

The milestones for institutional capacity building and training set for this year have all been met. The project moved in to the new laboratory in late June 2004 with the bulk of the equipment arriving from the USA in August 2004. The last minor pieces of work (some upgrades of the electrical system and installation of new wireless internet connection) were completed in January/February 2005. These final pieces of infrastructure work were delayed by administrative disruption at the Park, but did not significantly impact the working capacity of the lab. The project staff have been employed full time since May 2004 and have participated in on the job, workshop and taught training programmes (**see Annex 3**). Workshop components include a risk analysis assessment for the introduction of West Nile Virus (April 2004), participation in assessment of feasibility of using vaccines to control Canine Distemper Virus (CDV) in the domestic dog population (August 2004), training in marine mammal (sea lion/fur seal) pathology, handling and sampling (December 2004), and a workshop on wildlife haematology (February/March 2005). In addition the project staff Marilyn Cruz (veterinarian) and Leandro Patiño continued to participate in and teach on the Masters programme in Molecular Biology that was established in Galapagos last year by the University of Guayaquil. The staff are on target to be able to work autonomously by the end of the project.

Research and disease monitoring programme

The research and monitoring programme is a multiyear activity and so most of the main outputs from this part of the project are expected to come in the final year. However, significant progress has been made in different components and milestones met. Two manuscripts have already been submitted to scientific journals for publication. The focal studies this year were:

Base line surveys of pathogen prevalence and distribution: This part of the project has a huge scope and so is being conducted in collaboration with a large number of other researchers working in Galapagos in order to extend the species, spatial and temporal scope of sampling. The aim is to collect basic information about pathogen prevalence and distribution for as many species as possible, in order to have a bench mark against which future change can be assessed, and to identify any current potential sources of concern. We are adopting two strategies, analysis of material from opportunistic necropsies, and directed sampling of live animals. Material for opportunistic necropsies is collected when project staff are in the field, or with material collected by Park rangers, tourist guides, international research teams working on other projects or members of the public. Project staff, rangers and guides visit key areas of the archipelago on a regular basis (often weekly) and so can provide good coverage for mortality at the sites visited. The aim of the necropsies is to describe causes of death where possible (which may or may not be disease related), plus any additional pathologies and pathogen burden. Results are archived in database and will be used for spatial statistical analyses (e.g. Geobugs) at the end of the project to assess distribution patterns and identify disease risk factors.

The current directed studies on pinnipeds, tortoise, and birds are described below.

a) *Pathogen assay development*: PCR (polymerase chain reaction) assays have been established for approximately 25 avian, mammalian, reptile and chelonian pathogens at the laboratory. These include tests for Canine Distemper Virus, leptospira, salmonella, herpes virus and mycoplasma among others (**see Annex 4**). We are now screening clinical and pathological material for some of these assays and have obtained positive results in several cases. However more investigation over the next year required before the results can be put into a larger epidemiological context.

b) *Avian sampling and testing programme*: This component of the work is being conducted in collaboration with partners from the Dept. of Biology, University of St. Louis-Missouri and St. Louis Zoo, USA. The work comprises regular surveillance of chickens on farms on the inhabited islands, and sampling of a range of sea and terrestrial bird species on other islands in the archipelago 3 to 4 times per year. Samples collected so far include material from Galapagos petrel, waved albatross, Galapagos hawk, Galapagos mocking birds and finches. The samples are being subjected to serological (e.g. ELISA) and PCR tests for a range of pathogens (**see Annex 4**). As before more work is required to put the results into a larger epidemiological context.

c) *Assessment of disease transfer from dogs to sea lions*: We have continued to collect blood samples from domestic dogs across the archipelago (we now have material from more than 200 individuals) in collaboration with the NGO WildAid which is running a sterilisation campaign. In addition we have also taken part in 2 research cruises around the archipelago (December 2004 & March 2005) in collaboration with other researchers (Fritz Trillmich, University of Bielefeld, Germany & David Aurioles, Autonomous National University Of Mexico) in order to collect blood and clinical samples from sea lions and fur seals. On each cruise up to 11 haul out sites on 9 islands were visited, and a minimum of 10 individuals sampled at each site. This material is currently being tested for a variety of pathogens, including CDV, by PCR and serological (ELISA) techniques to assess spatial and temporal patterns of disease transfer from domestic dogs in to the pinniped population. Initial PCR results for dogs indicate active CDV infections on

Santa Cruz island. On the December 2004 research cruise we observed what appeared to be widespread "sealpox" infections in several colonies. We are currently processing material to confirm the identity and origin of the causative agent by PCR and DNA sequencing.

d) *Risk assessment for the introduction of West Nile Virus to the archipelago*: We conducted a pre-emptive risk assessment to evaluate the likelihood of introduction of West Nile Virus to Galapagos by different routes. We concluded that transport of infectious mosquitoes on aircraft posed the highest risk. Our conclusions were taken up by local stakeholders and lobbying of the national government has been successful in bringing into legislation the mandatory fumigation or disinsection of all transport to Galapagos to prevent transport of live insects. A scientific paper has been submitted to the journal "Conservation Biology" detailing this work. Full details of the methods and workshop report are given in **Annexes 5 and 6**).

e) *Giant tortoise health investigations*: We have begun a programme for the regular monitoring of giant tortoise health for captive and wild populations. This is in response to deaths among wild tortoises due to an as yet unknown pathogen over the last 10 years. The monitoring includes regular sampling of populations ranging from weekly to 6 monthly intervals depending on accessibility on 4 islands (Santa Cruz, Isabella, Santiago, and San Christobal). Blood, oral, ocular, nasal, anal and cloacal swabs, pathological material from lessions and faeces are collected for PCR screening (**see Annex 4**), plus serology, haematology, histological and parasitological investigations. We have built up a data base of lymphocyte counts from more than 150 tortoises. These investigations will establish base line values for basic health parameters and help with pathological investigations. Positive results have been obtained for sick and healthy tortoises for a 2 PCR based pathogen assays (herpes and mycoplasma), but further investigation is need to confirm these results (sequencing of PCR products). However, if confirmed these agents may be the source of past tortoise mortalities, and we will be able to suggest appropriate mitigation and treatment measures for future outbreaks.

f) *Arthropod disease vector distribution and abundance*: Mosquitoes are a key vector for transmitting a range of important wildlife diseases. Therefore we have begun a programme of systematic monitoring using oviposition and CDC light traps to assess the abundance, distribution and seasonal variation for the 3 three mosquito species present in Galapagos. Regular surveys are conducted on the four inhabited islands, trapping 4 four nights per week in one low land and one highland site, with a repeat 2 weeks later to account for the lunar cycle. The numbers of each species are recorded and specimens are kept for PCR analysis of pathogen load and identification of hosts from blood meals. In our first survey we made the first ever record of breeding *Culex quinquefasciatus* in Galapagos and confirmed it's continuing presence after 16 years of no reported sightings. A scientific paper has been submitted to the journal IBIS detailing some of this work (**see Annex 7**). We have also produced a guide to the identification of mosquitoes and relate insects on the islands (**see Annex 9**).

g) *Investigation of mass mortality of marine iguanas on San Christobal*: necropsies by project veterinarian Marilyn Cruz showed that deaths of large numbers of marine iguanas on San Christobal in February/March 2005 were due to attacks by feral dogs.

Education and conservation awareness

Information leaflets are now available from the project website (http://www.biology.leeds.ac.uk/ggepl/), and we have begun a programme of school visits to the laboratory for local children, and visits to local schools by project staff to explain important issues for Galapagos conservation and the role of the laboratory.

The main aim of this is to demonstrate that these are activities primarily being carried out by Ecuadorians, and therefore is something that is accessible to them. In addition we are working with local teachers to develop science and conservation courses for high school level children. However implementing this in the curriculum has proved more difficult than anticipated as initial education of the teachers is also required. We are investigating the possibility of taking on a full time education officer for the project.

Project staff participated in a local community workshop with local stakeholders in June 2004 to discuss the conservation implications of vaccination strategies for domestic animals in Galapagos. At present all domestic animal vaccination is band, but controlled vaccination for diseases such as CDV may prevent major wildlife impacts. A discussion document was prepared for local government consideration.

Dr. Cedeno has made a number of local presentations to other organisations in the islands such as the CDRS and municipal government explaining the role of the lab and further developing support and collaborations.

Five undergraduate and masters students are undertaking thesis research projects in the laboratory on disease and endangered plant propagation (**see annex 8)**.

The implementation of presentations to tourists has proved very difficult as this required co-ordination between the Park and tourist industry. The Park has suffered severe administrative disruption over the last year due to political problems in Ecuador. We continue to look at ways to circumvent this and are currently hoping to install a small visitor exhibit at the lab to which tourist groups can be brought to see the activities. Information for tourists is available via the project website.

Dissemination of results and reporting

Two scientific papers have been submitted:

- Kilpatrick AM, P Daszak, SJ Goodman, H Rogg, LD Kramer, V Cedeño, and AA Cunningham. West Nile virus Threatens Galápagos through Tourism (Submitted to *Conservation Biology* (see Annex 5 for copy).
- Whiteman NK, SJ Goodman, BJ Sinclair, T Walsh, AA Cunningham, LD Kramer, and PG Parker. Redetection of the avian disease vector *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae) on the Galápagos Islands, Ecuador, after a 14-year interval. *IBIS*, in press (see Annex 7 for copy).

The project website is now online at http://www.ggepl.org.

A report of the West Nile Virus workshop has been produced and distributed to majorstakeholders in Galapagos (see Annex 6). It is also available from the website.

In June 2004 Dr. Goodman presented an invited talk on the project at an international symposium on Galapagos at Jersey Zoo organised by the Galapagos Conservation Trust UK. Dr. Cunningham and Dr. Goodman also gave presentations about the project work at the Institute of Zoology and at the University of Leeds. Dr. Cedeño has given numerous presentations in Galapagos to local stakeholders, most recently at the beginning of March at a reception at the laboratory.

We are discussing the possibility of the project featuring in a documentary to be made for The Discovery Channel by an independent film company (Richmond Productions). Some filming for this documentary took place in March 2005 during a research cruise for our sea lion work.

Half year report was submitted on schedule. It was decided to replace newsletters with information dissemination via the project website, as this was considered more efficient as it can be updated more regularly.

The project featured in articles written by Dr. Goodman and Dr. Cedeño in publications of the Galapagos Conservation Trust (“West Nile Virus, a new plague in

paradise?", Spring 2005 newsletter), the ZSL annual report, and the Ecuadorian Fundacyt magazine.

Additional Outputs and Activities

A number of additional activities have taken place. In February 2005 Dr. Cedeño visited the UK, as part of a visit to attend a UNESCO conference in France. Dr. Cedeño visited the Institute of Zoology and University of Leeds, where she gave presentations about the project work, and discussed establishing collaborations with UK universities to facilitate training of Ecuadorian graduate students on UK post-graduate courses in biological scieneces.

We have also established a collaboration with Dept of Ecology and Evolutionary Biology at the University of Yale, USA in order to workshops in Conservation Genetics. The first of these is scheduled to take place in July 2006. The Yale group have also agreed to return their archive of tortoise DNA samples to be permanently housed at the lab in Galapagos.

Links have been set up to send students from the University of Leeds Masters in Conservation Biology course to Galapagos to conduct research projects and help with training. The first student will visit Galapagos in July/August 2005.

We have been awarded a PhD studentship via the EU Marie Curie training scheme for a Belgian student to join the project in order to study Galapagos mosquitoes and their role as disease vectors beginning in October 2005.

Problems encountered

The year from April 2004 has been one of political upheaval in Ecuador, which has had knock on effects in Galapgos. There have direct conflicts between the Park and local fishermen, several changes of Park director as a result of orders from the Ecuadorian Ministry of Environment. This culminated in series of strikes by Park staff which halted all Park activities. In addition there has been disruption and reduction of funding to the Park from the central government which resulted in staff being laid off or not being paid for extensive periods. All this has resulted in severe disruption to administration at the Park. This has had little impact on activities the project can carry out autonomously, such as most of the lab operations. However, there has been disruption to activities we need to carry out with the Park. This has included administration of funds in Park accounts and some of the education activities involving tourists, guides and Park staff.

Project enhancement

Last year there was significant extra investment in the project by the University of Guayaquil. This year there have also been further enhancements by providing opportunities to involve UK students in the work, the addition of an element focusing mosquito ecology through the awarding of a Marie Curie PhD studentship, and development of a collaboration to hold a conservation genetics workshop next year.

Workplan for the next reporting period, April 2005-March 2006

Institutional Capacity Building and Training

- April; 2005 to March 2006 - on the job and guided self-teaching programmes countine.
- April 2005 & August 2005 - staff training by Simon Goodman
- August 2005 to October 2005 - Veterinary pathology residency training at the University of Saskatchewan for Marilyn Cruz.

- January-February 2006 - workshops in marine mammal pathology, wildlife anaesthesia and immunology (Dr. Karian Acedevo-Whitehouse (Institute of Zoology) and Dr. Ailsa Hall (Sea Mammal Research Unit, UK).
- February-March 2006 - staff training workshop by Andrew Cunningham.

Research and disease monitoring programme

- April 2005 to March 2006 – Sampling and testing programme continues (birds, tortoise, reptiles and mammals)
- April 2005 to March 2006 – Continued development and implementation of new diagnostic procedures at laboratory (PCR, serology and histology).
- April 2005 - Expedition to collect blood and clinical samples from tortoise populations on volcanoes on Isabella
- May 2005 to August 2006 – Further collection of blood samples from domestic dogs and cats to assess risk of pathogen transfer into Galapagos pinniped populations
- May 2005 - Expedition to collect samples from Galapagos petrels
- July 2005 - Expedition to collect blood and clinical samples from tortoise populations on San Christobal
- June-July 2005 - Assessment of tortoise gut nematode burdens
- September 2005 - further serology testing of carnivore blood samples
- October-November - 2005, sampling of migratory birds for WNV monitoring
- December 2005 - Expedition to collect blood and clinical samples from land iguana populations on volcanoes on Isabella
- December 2005 to April 2006 - continued mosquito sampling and monitoring, vector competence and genetic analysis of mosquitoes.
- Jan-Feb 2006 - Sampling expedition to collect pinniped blood and clinical samples
- April 2005 to March 2006 – opportunistic pathology analysis of other taxa

Education and conservation awareness

- April 2005 to March 2006 – School visit and teaching programme continues (monthly visits of high school children to laboratory or project staff to schools).
- April 2005 to March 2006 – Further development of educational materials (leaflets, posters and presentations) for tourists and local community. Possibility of development of visitor centre and education officer at laboratory to be explored.
- February 2005 – Local community workshop on disease threats to Galapagos fauna.
- April 2005 to March 2006 - Masters and undergraduate students continue with research projects in laboratory.

Dissemination of results and reporting

- April 2005-March 2006 – Project website continues to be updated and maintained.
- October 2005 – Half year report submitted
- February 2006 – Organisation of final project workshop on Galapagos disease threat management commences
- March 2006 – Submit abstracts to international conference for presentation of final project results
- March 2006 – Paper on giant tortoise health status and threats submitted.
- March 2006 – Broadcast media feature on project in production

5. Actions taken in response to previous reviews (if applicable)

The main points raised by reviewers to the previous report were:

i) *A request to provide more details on the specific diseases that are being investigated* – this has been addressed in this report, the specific diseases being investigated have been listed in the appropriate sections. (e.g. see section 4 and Annex 4).

ii) *A request to provide more details on sampling protocols* – these details are provided in this report (see section 4, and Annex 7).)

iii) *A suggestion that training in epidemiology is provided* - Project staff were included in the development of the risk analysis for West Nile Virus introduction. Further training in epidemiology is schedule to take place in 2005/2006. However, developing independent expertise in epidemiology at the laboratory is probably beyond the scope of this project as it would require intensive long term training for an individual such as would be obtained for a PhD. However, this would be an achievable goal to aim for in the longer term future.

6. Partnerships

Collaboration between ZSL, University of Leeds and the host country partners

Note Dr. Goodman moved to the School of Biology, University of Leeds in November 2004, and so now the University of Leeds is an additional UK project partner. The collaboration between UK partners and the host country partners has continued to be excellent despite the upheavals in the Park. Despite changes in Park director the project continues to have the strong support of the Park. As for last year the support of the Park is evidenced by continuing contributions of Park staff time, accommodation and logistical support. The University of Guayaquil has provided additional administrative support including use of bank accounts for in country project expenditure. Dr. Virna Cedeño continues to be a key facilitator and scientific contributor for the project.

Collaboration with other organisations

We have continued to work closely with other organisations in Galapagos as detailed in last years report. We have strengthened our key collaborations with The Charles Darwin Research Station; Dept. Biology, University of St. Louis, Missouri; St. Louis Zoo; WildAid; Consortium for Conservation Medicine and New York State Department of Health with whom we are running several joint projects.

In addition we have initiated new collaborations with groups of Dr. Geoff Powell and Gisella Caccone at the Dept. of Ecology and Evolution at Yale University (Tortoise health and Conservation Genetics workshop); Dr. Fritz Trillmich, Department of Animal Behaviour, University Bielefeld, Germany (Pinniped disease risks); Dr. David Aurioles, Autonomous National University Of Mexico (Pinniped disease risks) and Dr. Gabriele Gentile, University of Tor Vergata, Rome, Italy, (Health status of land and marine iguanas in Galapagos).

7. Impact and Sustainability

We continue to have good relations with all the major stakeholders with interests in disease threats to Galapagos biodiversity, and continue to receive political support for the project from the local Galapagos and central Ecuadorian governments through the efforts of Dr. Cedeño. Dr Cedeño talks regularly with the heads of these regional and national organisations to promote the project, and has produced written articles for the general public in Ecuador over the last year (see section 4 and table 1). A recognition of the importance of the project work by the main stakeholders is evidenced by the continuing support we have received despite the unstable political

situation. These links demonstrate a large professional and political desire to develop scientific capacity and biodiversity conservation in Ecuador.

With the aid of lobbying efforts colleagues in local stakeholder organisations (Galapagos National Park, SESA, Charles Darwin Foundation, WildAid and the Global Environment Facility Galapagos Project), our project has already had a direct influence on national government policy as evidenced by the new national legislation relating to the insecticide treatment of transport to Galapagos.

The Vice President which we met on our first visit to Ecuador in 2003 has now taken on the role of President, so there is awareness of our work at the highest possible level.

Public awareness and interest is still developing for our project. Dr. Cedeño has played a central role in this through her work promoting with the project local and national government bodies, and with our schools programme on Galapagos. Most School children on Santa Cruz have already visited the laboratory. Dissemination of project activities to the general public in Ecuador has begun through features in local broadcast and print media as already described. Our website will be available in Spanish in the near future.

An exit strategy that will ensure continuation of the project once Darwin funding ceases is already in place as the University of Guayaquil and Galapagos National Park Service have already committed to continued funding. The strength of this commitment is demonstrated by the additional investment provided last year. We are also pursuing new grant applications with UK and international funding bodies to ensure laboratory activities continue to grow and can be sustained at the highest possible capacity. We have already succeed in obtaining money for a new PhD studentship related to the project, and hope to obtain further funding over the next year. We are currently pursuing the possibility of establishing an endowment with donations from the USA and Europe, to provide additional long term income to the lab. One of the main factors in sustainability is that the project should leave a legacy of trained competent staff. We are well on the road to achieving this aim through the training programmes for project staff, and by contributing to the wider development of bioscience skills in Ecuador by including Ecuadorian undergraduate and graduate students in our programmes.

8. Post-Project Follow up Activities (max 300 words)

We believe that this project is a strong candidate for follow up funding. We have succeeded in establishing a new and extensive research capacity for the Galapagos National Park that has already delivered research outputs that have influenced management policies for the Galapagos. This facility is also in the process of becoming an international research centre for the archipelago. Follow on funding would help cement the critical management role of the laboratory and its staff in the management of the Park, and will continue to raise the international profile of the Darwin Initiative as more visiting scientists use the facility.

Follow up activities would comprise continued training for the project staff, and support for new staff members that would free up Marilyn Cruz, the project veterinarian, allowing her to undertake a PhD at a UK university (funding for this would be sought elsewhere). Further activities would be to maintain the monitoring and surveillance activities of the laboratory at the highest levels. This will be especially important over the next 5 years as the risk of disease introduction to Galapagos is growing. West Nile Virus poses a particularly serious risk, and it is vital that if it reaches Galapagos, this is recognised immediately so that appropriate measures can be taken.

Developing key staff such as Marilyn Cruz is very important to embedding the project as it will significantly contribute to their development as future conservation leaders and researchers, beyond what can be achieved in the scope of the 3 year project.

Having an Ecuadorian staff member with a PhD would cement the national and international credibility of the lab, and would allow them to apply for funding in their own right, promoting independence. We estimate these activities would require funding of approximate £30,000 per year.

As the description of the exit strategy above demonstrates, the main partners in the project, the University of Guayaquil and Galapagos National Park have a long term commitment to the project, and the project staff are developing the necessary skills to participate fully in the follow up activities.

9. Outputs, Outcomes and Dissemination

Outputs

The outputs are generally on track, notably we have been able to submit two scientific journal articles this year, at an early point than expected in the project. More journal articles are anticipated in the next year. We also produced a small mosquito identification guide which was not previously scheduled, as no other one was easily available. Progress on tourist education material has been slower than anticipated due to disruption at the Park, as outlined in section 4.

Dissemination in host country

The project has a high profile locally due the ongoing programme of High School visits to the laboratory and from project staff visiting local schools. These visit are aimed at educating school children about the contributions of science to their lives, including to conservation, and that this is an activity being carried out by Ecuadorians rather than people from more developed countries. In addition Dr. Cedeño maintains close relationships with all stakeholders in country. The project has been featured in Fundacyt magazine (the Ecuadorian National Academy of Sciences), and in the local press. These articles were targeted at moderately well educated readers, but who were unaware of conservation issues around the project. The project website is now accessible in Ecuador and will soon be available in Spanish.

The staff trained by the project will continue to educate Ecuadorian professionals and Galapagos residents in wildlife disease issues after the end of the project using funding already guaranteed by the Galapagos National Park Service and the University of Guayaquil (see original grant application).

Table 1. Project Outputs (According to Standard Output Measures)

Code No.	Quantity	Description
4A, 4B	4, 26	Student projects (up to 26 weeks in 2004/5,
4C, 4D	1, 26	see annex 8)
6A, 6B	10, >52	Haematology, Insect trapping/identification, marine mammal handling and pathology, veterinary pathology (see annex 3)
7	3	Briefing presentations for stakeholders on WNV risk assessment and Vaccination in wildlife conservation (English and Spanish), Information leaflet for tourists
8	23	UK project staff weeks, visits by Simon Goodman, Andrew Cunningham, Karina Acedevo-Whitehouse, and Mike Haart

9	2	WNV risk assessment guide lines (see annex 6) and vaccination policy document (produced in collaboration with WildAid).
10	1	Galapagos mosquito identification guide (see Annex 9)
11B	2	(see table 2)
14A	9	WNV risk assessment workshop, Vaccination workshop, local community presentation on function of lab by Virna Cedeno (March 2005), school visits (x6)
14B	4	Galapagos Conservation Trust conference (SG), Seminars at Institute of Zoology (SG, AC), Seminar University of Leeds (SG)
15A, 15B	1,1	Article in Fundacyt magazine, articles in Galapagos newspaper about opening of laboratory
15C	2	Articles in publications of the Galapagos Conservation Trust, and Zoological Society of London, Institute of Zoology.
16A, 16B, 16C	2, 200, 100	News updated regularly via project website
18A,B	1	Filming for documentary by Discovery Channel in March 2005, production yet to be completed.
20	£138,000	Estimated value of laboratory and equipment in Galapagos
21	1	Laboratory in Galapagos
23	$4000	Grant from Galapagos Conservation Trust

Table 2: Publications

Type * (e.g. journals, manual, CDs)	Detail (title, author, year)	Publishers (name, city)	Available from (e.g. contact address, website)	Cost £
Journal	**West Nile virus Threatens Galápagos through Tourism.** Kilpatrick AM, P Daszak, SJ Goodman, H Rogg, LD Kramer, V Cedeño, and AA Cunningham. (Submitted to *Conservation Biology*)	Blackwells, London	http://www.blackwell-synergy.com/loi/cbi or academic libraries	Depends on status
Journal	**Establishment of the avian disease vector *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae) on the Galápagos Islands, Ecuador.** Whiteman NK, SJ Goodman, BJ Sinclair, T Walsh, AA Cunningham, LD Kramer, and PG Parker. 2005, *IBIS*, in press.	Blackwells, London	http://www.blackwell-synergy.com/servlet/useragent?func=showIssues&code=ibi or academic libraries	Depends on status

Workshop proceedings	**Proceedings of the Galapagos West Nile Virus Workshop, Galapagos National Park Headquarters, Puerto Ayora, 29th April 2004** Anonymous, 2004	Self published	http://www.biology.leeds.ac.uk/ggepl/English/Education_Policy.htm#West%20Nile%20Virus%20policy%20development	-

10. Project Expenditure

Table 3: Project expenditure during the reporting period (Defra Financial Year 01 April to 31 March)

Item	Budget (please indicate which document you refer to if other than your project schedule)	Expenditure	Balance

Additional Income and support

Grant of $4000 from the Galapagos Conservation Trust, UK.

11. Monitoring, Evaluation and Lessons

The means of verification for indicators of project outputs are all tied to documentary evidence. Outputs related to research or management issues (e.g. papers or management plans) will receive peer review which indicate their quality against comparable international work. Further the success of outputs such as workshops can be assessed by the endorsement of workshop reports by participating international expert attendees. The success of policy level outputs are being assessed by getting commitments from senior managers and government officials to use material generated by project. These commitments will be documented in Memorandums of Understanding and workshop reports. The success of educational and awareness programmes can be assessed by the students who have participated in the project passing their courses and the willingness of the local community to implement policies discussed during workshops, as assessed by post-workshop follow-up discussions. These evaluation and monitoring schemes will be implemented as the project moves on to its main phase over the next year.

Such indicators show the project has been successful over the last year as evidenced by the acceptance of one scientific paper (another is under review), and the translation of recommendations from the West Nile Virus workshop into Ecuadorian law. Indicators of the final success of educational elements will come in the next year when students get the results of their degrees.

Political instability exists in Ecuador that may affect administrative approval of project activities. However, support for the project remains strong and no substantial problems are expected that would compromise implementation of the main activities. Our experience from the last year shows that these difficulties can usually be circumvented.

12. OPTIONAL: Outstanding achievements of your project during the reporting period (300-400 words maximum)

■ **I agree for ECTF and the Darwin Secretariat to publish the content of this section**

The project has made several notable achievements over the last year. Firstly we have established major new laboratory in Galapagos that is equipped to a very high standard. In fact it's probably one of the best facilities in Ecuador. The lab is starting to become a major focus for research by both national and international scientists working in Galapagos. The importance of this is was highlight in December 23rd issue of Nature 2005 (Vol 432 p 948), in an article describing the wish lists of leading scientists. Prof. Hunt Willard at Duke University, Durham NC, USA, wished for a "modern genetics laboratory in Galapagos".

Secondly we have submitted two scientific papers dealing with two important aspects of disease threats to Galapagos. In the first we confirm the establishment in Galapagos of breeding populations of *Culex quinquefasciatus*, an important mosquito vector for avian diseases. In the second we develop a risk analysis framework for the introduction of West Nile Virus to the archipelago. We show that transport of infectious mosquitoes on commercial airliners pose the greatest risk, rather migratory birds, as is the case in other parts of the Americas. These findings have already been used to change policy in Galapagos, and have resulted in changes being made to Ecuadorian law, requiring treatment of all craft travelling to Galapagos to prevent the introduction of live insects.

Annex 1: Report of progress and achievements against Logical Framework for Financial Year: 2003/2004

Project summary	Measurable Indicators	Progress and Achievements April 2004-Mar 2005	Actions required/planned for next period
Goal*:* To draw on expertise relevant to biodiversity from within the United Kingdom to work with local partners in countries rich in biodiversity but poor in resources to achieve • The conservation of biological diversity, • The sustainable use of its components, and • The fair and equitable sharing of the benefits arising out of the utilisation of genetic resources			
Purpose To establish the ability of researchers and managers in the Galapagos national park to determine the nature and prevalence of disease threats to endemic fauna (with a focus on birds) stemming from the introduction of novel pathogens and vectors, and to build a capacity for the continued monitoring of introduced diseases in these populations.	New knowledge on the nature and prevalence of diseases and their vectors for endemic and potential reservoir species. A conservation management plan for endemic species in relation to disease threats endorsed by the National Park authorities and Ecuadorian government. Increased understanding of disease threats to endemic wildlife among professional and local people.	We have succeeded in establishing the laboratory and staff training programmes required for the Institution infrastructure. The research programmes are starting to deliver the new knowledge (see publications), and research outputs have already been translated into changes to policy (changes in legislation to reduce risk of West Nile Virus introduction).	Project will continue to proceed as detailed in the original log-frame and implementation timetable
Outputs			
Identity and prevalence of key pathogens and vectors that threaten endemic species determined.	Findings endorsed by international conservation and scientific communities.	Pathogen sampling and testing programmes are running, and are already yielding important new information on pathogen prevalence and distribution in seal lions, tortoises and birds.	For 2005-2006 activities will focus on collecting the data required to identify prevalence of key pathogens and vectors through a programme of field surveys and laboratory analysis.

A management plan for endemic species in relation to disease threats.	Management plan peer reviewed and presented at international meeting on wildlife disease.	West Nile Virus risk mitigation document finished and passed to stakeholders resulting in policy changes. Manuscript sent for review.	For 2005-2006 activities will focus on collecting the data required to inform the management plan, and building further relationships with policy makers.
A wildlife disease lab and continuing monitoring programme with trained personnel established.	Laboratory operational and at least 2 staff trained in wildlife pathology continuing to monitor disease.	Laboratory delivered with staff in place, programmes operational.	Laboratory continues to run according to plan.
Educational events and materials (locals & tourists).	Participation of locals & tourists in events, material distributed.	Local education with schools and stakeholders (proceeding), but local political disruption has impeded tourist presentations. Tourist information leaflet available from website	Programmes continue according to plan. Investigate possibility of opening visitor centre at lab. Work with Park on establishing tourist presentations.
Media representation	Project featured in local and international media	Project has been featured in local and international media.	Media contacts now identified to permit further dissemination of project achievements.

Note: Please do NOT expand rows to include activities since their completion and outcomes should be reported under the column on progress and achievements at output and purpose levels

Annex 2: Original Logical Framework

Project summary	*Measurable indicators*	*Means of verification*	*Important assumptions*
Goal: To draw on expertise relevant to biodiversity from within the United Kingdom to work with local partners in countries rich in biodiversity but poor in resources to achieve • the conservation of biological diversity, • the sustainable use of its components, and • the fair and equitable sharing of the benefits arising out of the utilisation of genetic resources			
Purpose To establish the ability of researchers and managers in the Galapagos national park to determine the nature and prevalence of disease threats to endemic fauna (with a focus on birds) stemming from the introduction of novel pathogens and vectors, and to build a capacity for the continued monitoring of introduced diseases in these populations.	New knowledge on the nature and prevalence of diseases and their vectors for endemic and potential reservoir species. A conservation management plan for endemic species in relation to disease threats endorsed by the National Park authorities and Ecuadorian government. Increased understanding of disease threats to endemic wildlife among professional and local people.	Project reports, and workshop reports involving partner organisations, publications in peer reviewed journals. Management plan document and correspondence. Records of training workshops with professional workers, and educational programme with local people and tourists, including educational materials generated for both. Students trained under programme pass their courses.	Researchers and managers use project findings to help minimise disease impacts on endemic species. Disease monitoring programme receives continued funding to maintain its activities. Note continuing funding from Galapagos National Park Service and University of Guayaquil is already agreed.
Outputs Identity and prevalence of key pathogens and vectors that threaten endemic species determined. A management plan for endemic species in relation to disease threats. An wildlife disease lab and continuing monitoring programme with trained personnel established. Educational events and materials (locals & tourists). Media representation	Findings endorsed by international conservation and scientific communities. Management plan peer reviewed and presented at international meeting on wildlife disease. Laboratory operational and at least 2 staff trained in wildlife pathology continuing to monitor disease. Participation of locals & tourists in events, material distributed. Project featured in local media	Publication of results in peer reviewed international scientific journals. Management plan published and distributed. Copies sent to Darwin Initiative. Proceedings from meeting. Annual and field reports, peer reviewed papers, continued output of data supporting management programmes. Educational leaflets and posters, press releases, reports Articles & recordings	Laboratory and monitoring programme active after year 1. Monitoring programme generates data required for management plan. Links to educational organisations and media are established (agreements are in place to do this via the Galapagos National Park Service).
Activities	**Activity Milestones (Summary of Project Implementation Timetable)**		
Capacity building and training.	Yr1: Establish pathology laboratory and run training workshop, finalise project diagnostic protocols and sampling strategy; Yr2 and Yr3 Follow up training workshops, 2 in each year		
Research & Disease Monitoring	Yr1: Develop diagnostic procedures including genetic based testing, start screening of samples collected during monitoring program. Yr2 and Yr3: Continuation of screening, Workshops to discuss results. Scientific publications and management plan written in year 3.		
Education programme	Yr1: Work with local organisiations and schools to develop educational programme and materials to inform about conservation biology and disease threats, programmes for local people and tourists. Yr2: and Yr 3. Continue to run programmes		
Dissemination of results	In each year: Annual reports and news letters, establish and up date project website. Engage local and international media interest. Yr2 and Yr3: Presentation of results at international conferences, workshops, papers submitted to international peer reviewed journals by 1 year after end of project.		

Annex 3: Training events, methodology & topics

Training Activity	Type	No. people	Date & Duration	Topics
West Nile Virus introduction risk assessment	Workshop	12	April 2004 1 day	Development of risk analyses for disease introduction via natural and anthropogenic routes, mitigation strategies (See also Annex 6)
Use of vaccines in control of wildlife disease, role for vaccines in domestic animals in Galapagos conservation (organised with WildAid)	Workshop	15	August 2004 1 day	Utility of vaccines in animal disease, role for vaccine use in Galapagos, dangers of using vaccines in domestic and wildlife populations, vaccines for CDV
Pinniped pathology, handling and sampling (Marilyn Cruz only)	On the job training during research cruise	1	November-December 2004 10 days	Pinniped capture, handling, sampling, necropsy techniques and pathology.
Wildlife haematology	Workshop /on the job	5	February – March 2005 1 week workshop, on going on the job	Theory and practical aspects of haematology, clinical diagnosis using haematology, sampling and handling of blood, preparation of samples to assess haematological parameters, interpretation of haematological parameters
Veterinary Pathology (Marilyn Cruz only)	One to one instruction, on the job, and self teaching	1	May 2004-March 2005 11 months to date	Development of basic aspects of veterinary pathology, pathological diagnosis using gross analysis, histology, haematology, serology and molecular techniques
Molecular Biology	Taught MSc	14* (done through University of Guayaquil so not counted as direct output)	April 2004 – March 2005 1 year part-time (5 to 10 days per month)	Theoretical and practical molecular and cell biology, immunology.
Trapping, identification and handling of mosquitoes	One to one instruction and on the job training	3	April 2004 – March 2005 On going on the job training	Use of CDC-light traps and oviposition traps for mosquito capture, differentiation of Galapagos mosquito species and other dipterans, storage, DNA extraction and PCR from captured mosquitoes.

Annex 4. List of Pathogen PCR assays being applied in the laborartory

Pathogen	***Genera***	***Primers***	***Reference:***
Newcastle disease virus	*Avian*	*A-5'-TTGATGGCAGGCCTCTTGC- 3'* *B-5'-AGCGT(C/T)TCTGTCTCCT-3'* *C-5'-G(A/G)CG(A/T)CCCTGT(C/T)TCCC-3'*	Tiwari AK, Kataria RS, Nanthakumar T, et al. Differential detection of Newcastle disease virus strains by degenerate primers based RT-PCR COMPARATIVE IMMUNOLOGY MICROBIOLOGY AND INFECTIOUS DISEASES 27 (3): 163-169 MAY 2004
Paramyxovirus	*Avian*	*Unpc 333_ 350: 5' GCCCCAGTTCAACAAYAG 3'* *Unpe 594_ 611: 5' GCAGCAAGGTAGAGTCCA 3'* *Lnpk 955_ 972: 5' AGGCGCAAAGCTCATCTG 3'* *Lnph 1349_ 1366: 5' TTGCCACTGCTCTCATCA 3'*	Barbezange C, Jestin V Development of a RT-nested PCR test detecting pigeon Paramyxovirus-l directly from organs of infected animals JOURNAL OF VIROLOGICAL METHODS 106 (2): 197-207 DEC 2002
Infectious bronchitis virus (IBV)	*Avian*	*5' CAT AAC TAA CAT AAG GGC A 3'* *5' TGA AAA CTG AAC AAA AGA CA 3'*	Pang YS, Wang H, Girshick T, et al. Development and application of a multiplex polymerase chain reaction for avian respiratory agents AVIAN DISEASES 46 (3): 691-699 JUL-SEP 2002
Avian influenza virus (AIV)	*Avian*	*5' AGC AAA AGC AGG GGA TAC 3'* *5' GTC TGA AAC CAT ACC ATC C3'*	
Infectious laryngotracheitis virus (ILTV)	*Avian*	*5' ACG ATG ACT CCG ACT TTC-3'* *5' CGT TGG AGG TAG GTG GTA-3'*	
Newcastle disease virus (NDV)	*Avian*	*5' GGA GGA TGT TGG CAG CAT T-3'* *5' GTC AAC ATA TAC ACC TCA TC-3'*	
Mycoplasma gallisepticum *(MG)*	*Avian*	*5' GGA TCC CAT CTC GAC CAC GAG AAA A-3'* *5' CCT TCA ATC AGT GAG TAA CTG ATG A-3'*	
Synoviae *(MS)*	*Avian*	*5' GAA GCA AAT AGT GAT ATC A-3'* *5' GTC GTC TCG AAG TTA ACA A-3'*	

Avian pneumovirus APV-Col	*Avian*	*5' ACA CCT CCT ACA GTG CTA CTA GAG CAG C 3'* *5' ACT TCA GGA CAT ATC TCG TAC CCT GGT G 3'*	Ali A, Reynolds DL A reverse transcription-polymerase chain reaction assay for the detection of avian pneumovirus (Colorado strain) AVIAN DISEASES 43 (3): 600-603 JUL-SEP 1999
Pasteurella multocida	*Avian*	*PM23F1 5' GGC TGG GAA GCC AAA TCA AAG 3'* *PM23R2 5' CGA GGG ACT ACA ATT ACT GTA A 3'*	Miflin JK, Blackall PJ Development of a 23S rRNA-based PCR assay for the identification of Pasteurella multocida LETTERS IN APPLIED MICROBIOLOGY 33 (3): 216-221 SEP 2001
Chlamydia psittaci	*Avian*	*5 ' CAA ACT CAT CAG ACG AG 3'* *5'CTT CTT TAA GAG GTT TTA CCC3'*	McElnea CL, Cross GM Methods of detection of Chlamydia psittaci in domesticated and wild birds AUSTRALIAN VETERINARY JOURNAL 77 (8): 516-521 AUG 1999
Mycoplasma gallisepticum	*Avian*	*MG 1 5' GGA TCC CAT CTC GAC CAG GAG AAA A 3'* *MG 2 5' CTT TCA ATC AGT GAG TAA CTG ATG A 3'*	Wang H, Fadl AA, Khan MI Multiplex PCR for avian pathogenic mycoplasmas MOLECULAR AND CELLULAR PROBES 11 (3): 211-216 JUN 1997
M. synoviae	*Avian*	*MS 1 5' GAA GCA AAT AGT GAT ATC A 3'* *MS 2 5' GTC GTC TCG AAG TTA ACA A 3'*	
M. meleagridis	*Avian*	*MM1 5' GGA TCC TAA TAT TAA TTT AAA CAA ATT AAT GA 3'* *MM2 5' GAA TTC TTC TTT ATT ATT CAA AAG TAA AGT AC 3'*	
M. iowae	*Avian*	*MI1 5' GAA TTC TGA ATC TTC ATT TCT TAA A 3'* *MI2 5' CAG ATT CTT TAA TAA CTT ATG TAT C 3'*	
Mycoplasma agassizii sp.	*Chelonian*	*MAF 5´AGAGTTTGATCCTGGCTCAGGA-3´* *MAR 5´-TGCACCATCTGTCACTCTGTTAACCTC-3´*	*Brown MB, Brown DR, Klein PA, et al.* *Mycoplasma agassizii sp nov., isolated from the upper respiratory tract of the desert tortoise (Gopherus agassizii and the gopher tortoise (Gopherus polyphemus)* *INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY 51: 413-418 Part 2 MAR 2001*

Aspergillus sp.	*Degenerate*	*AFU 5S: AGG GCC AGC GAG TAC ATC ACC TTG* *AFU 5AS: GGG GRG TCG TTG CCA ACY CYC CTG A*	*Buchheidt D, Baust C, Skladny H, et al.* *Clinical evaluation of a polymerase chain reaction assay to detect Aspergillus species in bronchoalveolar lavage samples of neutropenic patients* *BRITISH JOURNAL OF HAEMATOLOGY 116 (4): 803-811 MAR 2002*
Aspergillus sp	*Degenerate*	*AFU 7S: CGG CCC TTA AAT AGC CCG* *AFU 7AS: GA CCG GGT TTG ACC AAC TTT*	
Aspergillus sp.	*Degenerate*	*Asp F 5' TTC GAG GCC CTG TAA TTG GA 3'* *Asp R 5' GTC CTA TTC CAT TAT TCC TAG 3'*	*Loeffler J, Kloepfer K, Hebart H, et al.* *Polymerase chain reaction detection of Aspergillus DNA in experimental models of invasive aspergillosis* *JOURNAL OF INFECTIOUS DISEASES 185 (8): 1203-1206 APR 15 2002*
Plasmodium *ribosomal sub-unit gene*	*Avian*	*P F 1 15'-CGACTTCTCCTTCCTTTAAAAGATAGG-3'* *P R 2 5'-GGATAACTACGGAAAAGCTGTAGC-3'* *P nF1 5'-TAACACAAGGAAGTTTAAGGC-3'* *P nR2 5'-TATTGATAAAGATTACCTA-3'*	*Miller, G.D., Hofkin, B.V., Snell, H., Hahn, A., and Miller, R.D.* *Avian malaria and Marek's disease: potential threats to Galapagos penguins Spheniscus mendiculus.* *MARINE ORNITHOLOGY 29(1): 43-46, 2001*
Marek disease virus	*Avian*	*MDV F 5'-GCAAGTCATTATGCGTGAC-3'* *MDV R 5'-TGTTTCCATTCTGTCTCCAAGA-3'*	
Herpesvirus	*Chelonian*	*HV F 5'-TGCACTTTGATGCGTGGGAT-3'* *HV R 5'-TTGATCGTATTCGAATGCCG-3'*	*Origgi FC, Romero CH, Bloom DC, et al.* *Experimental transmission of a herpesvirus in Greek tortoises (Testudo graeca)* *VETERINARY PATHOLOGY 41 (1): 50-61 JAN 2004*
Herpesvirus turtle	*Chelonian*	*U-73 5' AGG CGG GAA AGG ATT ATG TC 3'* *L-588 5' AGT TTG ATA GGG GAT TTG AA 3'* *U-289 5' GAT TTA CTG GCG TGG CTA TG 3'*	*Murakami M, Matsuba C, Une Y, et al.* *Development of species-specific PCR techniques for the detection of tortoise herpesvirus* *JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION 13 (6): 513-516 NOV 2001*

Haemoproteus	*Avian*	*HAEMF (5'-ATGGTGCTTTCGATATATGCATG-3')* *HAEMR2 (5'-GCATTATCTGGATGTGATAATGGT-3')*	*Bensch S, Stjernman M, Hasselquist D, et al.* *Host specificity in avian blood parasites: a study of Plasmodium and Haemoproteus mitochondrial DNA amplified from birds* *PROCEEDINGS OF THE ROYAL SOCIETY OF LONDON SERIES B-BIOLOGICAL SCIENCES 267 (1452): 1583-1589 AUG 7 2000*
Salmonella gallinarum	*Avian*	*SG1 5' TCA CGA CTT ACA TCC TAC 3'* *SG2 5' CTG CTA TAT CAG CAC AAC 3'*	*Myeong-kyu Park, Kyoung-seong Choi, Myeong-chul Kim and Joon-seok Chae* *Differential diagnosis of Salmonella gallinarum and S. pullorum using PCR-RFLP* *Journal of Veterinary Science 2(3) 213-9 December 2001*
Salmonella typhimurium	*Avian*	*SAL-1F, 5'-GTA GAA ATT CCC AGC GGG TAC TG-3'* *SAL-2R, 5'-GTA TCC ATC TAG CCA ACC ATT GC-3'* *SAL-3F, 5'-TTT GCG ACT ATC AGG TTA CCG TGG-3'* *SAL-4R, 5'-AGC CAA CCA TTG CTA AAT TGG CGC A-3'*	*Waage AS, Vardund T, Lund V, et al.* *Detection of low numbers of Salmonella in environmental water, sewage and food samples by a nested polymerase chain reaction assay* *JOURNAL OF APPLIED MICROBIOLOGY 87 (3): 418-428 SEP 1999*
Trematodes universal 18S	*Degenerate*	*Uni 18S F 5' GCT TGT CTC AGA GAT TAA GCC 3'* *Uni 18S R 5' ACG GAA ACC TTG TTA CGA C 3'*	*Dzikowski R, Levy MG, Poore MF, et al.* *Use of rDNA polymorphism for identification of heterophyidae infecting freshwater fishes* *DISEASES OF AQUATIC ORGANISMS 59 (1): 35-41 APR 21 2004*
Trematode 18S	*Degenerate*	*Het 18S F 5' TCA TAT GCT TGT CTC AGA 3'* *Het 18S R 5' ACG GAA ACC TTG TTA CGA 3'*	
Culex pipiens *complex and its sibling species*	*Mosquito (Identification of mosquito species)*	*ACEaus 5_-CTTGTGGTGATTTAGTTGTTCGG-3_* *ACEquin 5_-CCTTCTTGAATGGCTGTGGCA-3_* *ACEpall 5_-ATGGTGGAGACGCATGACG-3_* *ACEpip 5_-GGAAACAACGACGTATGTACT-3_* *ACEtorr 5_-TGCCTGTGCTACCAGTGATGTT-3_* *B1246s 5_-TGGAGCCTCCTCTTCACGG-3_*	*Smith JL and Fonseca DM* *Rapid Assays For Identification Of Members Of The* Culex (Culex) Pipiens *Complex, Their Hybrids, And Other Sibling Species(Diptera: Culicidae)* American Journal of Tropical Medicine and Hygine *70(4), 2004, pp. 339–345*

Leptospira	*Mammalian*	*S3a 5'-GCG GAT ATG GGA AGC TTA GAA ACT-3'* *S3b 5'-CCGAAA CTG TAG CCG AAG AAG AAA-3'* *S4a 5'-TCC TTT TGG CGA TTT AGC AGA A-3'* *S4b 5'-CGT GTC CGG AGT AGA AGT GAA TGT-3'*	*Lucchesi,PM, Parma,AE, and Arroyo,GH* *Serovar distribution of a DNA sequence involved in the antigenic relationship between Leptospira and equine cornea.* *BMC Microbioy 2: (1), 3, (2002).*
Canine Distemper Virus	*Mamalian*	P1 5′–ACA GGA TTG CTG AGG ACC TAT–3′ P2 5′–CAA GAT AAC CAT GTA CGG TGC–3′ (reverse)	*Frisk,AL, Konig,M, Moritz,A, and Baumgartner,W.* *Detection of Canine Distemper Virus Nucleoprotein RNA by Reverse Transcription-PCR Using Serum, Whole Blood, and Cerebrospinal Fluid from Dogs with Distemper.* *Journal of Clinical Microbiology, 37: (11), 3634–3643, (1999).*

Annex 5. Manuscript for WNV risk assessment, currently submitted to *Conservation Biology*

Predicting pathogen introduction: West Nile virus and Galápagos

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Word count (Abstract-References): 4601

Abstract

Emerging infectious diseases are a key threat to conservation and public health, yet predicting and preventing their emergence is notoriously difficult. Here, we present a quantitative risk assessment framework for the introduction of exotic pathogens to new locations. We use it to determine the most likely route of West Nile virus introduction into Galápagos and measures that can be taken to reduce the risk of introduction. The introduction of this highly pathogenic virus to this unique World Heritage Site could have devastating consequences, similar to those seen following introductions of pathogens into other endemic island faunas. Our model identifies the transport of mosquitoes on airplanes as the highest risk for WNV introduction, and shows that natural pathogen dissemination through avian migration is less likely than is currently assumed. Our risk assessment framework has broad applicability to other pathogens and other regions.

Keywords: Mosquito, disease, introduction, risk assessment, model

Introduction

Emerging infectious diseases (EIDs) are a key threat to conservation, as well as public health (Daszak et al. 2000; Meffe 1999). The majority of programs to deal with these threats involve surveillance, outbreak control, vaccine and drug development, and are by nature reactive, occurring after the introduction of diseases (Smolinski 2003). However a growing number of researchers have proposed new approaches to EIDs, based on forecasting outbreaks (Davis et al. 2004; Linthicum et al. 1999), predicting pathogen dynamics once an outbreak has occurred (Keeling et al. 2001), or predicting broad patterns in pathogen evolution or the underlying causes of emergence (Burke 1998; Moya et al. 2004; Taylor et al. 2001).

Here we present a predictive model for the introduction of a zoonotic vector-borne pathogen by considering each of the pathways by which a pathogen may be introduced to a new area and comparing the relative risk of each pathway. We illustrate our model by assessing the risk for introduction of an EID, West Nile virus, that is lethal to a wide range of species into an important World Heritage Site, the Galápagos islands. Below we illustrate how this model can easily be used for other pathogens and other locations.

WNV represents the most imminent threat to Galápagos' fauna. In only five years it has spread west across North America and south into the Caribbean and Central America (Marra et al. 2004). Its arrival in South America and Ecuador are likely imminent. In the new world WNV has shown low host specificity and high virulence in a wide range of vertebrate species (Marra et al. 2004). Although the susceptibility of endemic Galápagos avifauna is unknown (Wikelski et al. 2004) (Galápagos does not have any endemic corvids), their small populations and evolution in the absence of WNV and other blood-borne pathogens suggests they would be highly susceptible, as was the case with Hawaii's avifauna and avian malaria (Van Riper et al. 1986). Galápagos' unique reptile fauna, including land iguanas (Conolophus subcristatus and Conolophus pallidus) marine iguanas (Amblyrhynchus cristatus), lava lizards (seven species of Tropidurus), and giant tortoises (Geochelone elephantopus) may also be under threat because WNV has been shown to cause significant mortality in some reptiles (e.g., crocodilians (Miller et al. 2003; Steinman et al. 2003) but see (Klenk & Komar 2003)).

Galápagos has three mosquito vectors capable of transmitting WNV (Culex quinquefasciatus, Aedes aegypti, and Ochleratatus taeniorhynchus; (Komar 2003; Peck et al. 1998)) and many bird species that are closely related to known competent avian hosts (Komar et al. 2003) which are abundant throughout the areas where mosquitoes are known to be present (e.g., Puerto Ayora, Santa Cruz Island). As a result, the establishment of WNV in the Galápagos would be highly likely if the virus reached the islands. In order to reduce the probability of WNV introduction and its likely disastrous consequences, we performed a quantitative risk assessment of the pathways by which WNV could reach Galápagos at present and into the future.

Methods

We consider the risk of the introduction of a pathogen by six pathways: mosquitoes by 1) airplane, 2) wind, and 3) boat; 4) infected humans, 5) human transported birds or other vertebrates, and 6) migratory birds. Pathways 1-4 are modes of introduction for many vector-borne pathogens with vertebrate hosts and pathway 6 is applicable to pathogens that have migratory birds as the primary hosts (e.g., flaviviruses such as St. Louis Encephalitis virus).

For each pathway, we estimated the number of individuals arriving each year and the fraction likely to be infectious for the pathogen. We multiplied this by the duration of infectiousness to determine the number of infectious days for each pathway. We note that an infectious bird-day and an infectious mosquito-day are not necessarily equivalent. The

probability of pathogen introduction by an infected vector depends on that vector finding a susceptible host in an environment were the reproductive rate of the pathogen (R0) is greater than 1. Similarly, an infective host must be bitten by a competent vector in an environment where the reproductive rate of the pathogen (R_0) is greater than 1.

We used this modeling framework to consider the risk of WNV introduction into the Galapagos as follows.

Infected Human

Human WNV infections in immunocompetent individuals show low (<103PFU/ml) peak viremias (Biggerstaff & Petersen 2002) which are insufficient to infect mosquitoes (Sardelis et al. 2001). As a result, the risk from this pathway was estimated to be negligible. However, this may not be the case for other zoonotic pathogens.

Mosquito

We conservatively estimated the rate of mosquitoes reaching Galápagos by wind as less than one per thousand years. Only one species of mosquito, Oc. taeniorhynchus, appears to have colonized Galápagos unaided (Hardy 1960) in the four million years that suitable habitat has been available. As a result, our estimate assumes that ~4000 mosquitoes reached Galapagos by wind in the last four million years and resulted in a single species establishment. Increasing the colonization rate by five orders of magnitude (10^5) would not affect our conclusion that this pathway represents a relatively low risk for WNV introduction compared to mosquitoes on airplanes (see Table 1 below). Although long distance wind-aided flights have been documented for Culicoides (biting midges) and Simuliids (blackflies), fewer have been documented for mosquitoes (Lounibos 2002). However, once established in the archipelago, wind transport may be an important pathway for transporting WNV between islands.

Two large-scale studies of inadvertent mosquito transport on commercial airplanes landing in Australia and Japan (Russell et al. 1984; Takahashi 1984) found that on average 0.9 and 2.2 live mosquitoes were transported on each flight, respectively, and that 95% of the mosquitoes were Culex pipiens or Cx. quinquefasciatus (the latter was introduced to Galápagos during the 20th century; (Peck et al. 1998)). Another large-scale study found 4 live Culex mosquitoes in 11,265 shipping containers (0.00036 live Culex/container; 95%CI: $9.1x10^{-5}$ – $9.1x10^{-4}$) on boats arriving in New Zealand (MAF 2003).

The shipment of tires is also known to present a risk for the introduction of mosquitoes, especially in the larval stage (Lounibos 2002). Vertical transmission of WNV has been documented in Cx. quinquefasciatus (minimum filial infection rate 3.0/1000 (95% CI: 0.00037-0.0099; (Goddard et al. 2003)) and Cx. pipiens (1.8/1000 (95% CI: 0.00067-0.0040); (Dohm et al. 2002b)) However, no published data exists on the number of mosquitoes transported on a per tire (or per ship) basis. Consequently, we determined the number of larvae per tire such that the risk from this pathway was equivalent to that for airplane transported adult mosquitoes.

We estimated the fraction of mosquitoes that would be WNV-infectious, once WNV reaches Ecuador, as the product of the fraction of infected Cx. quinquefasciatus mosquitoes that are able to transmit the virus with a bite, (0.22 95%CI: 0.064 – 0.48; (Sardelis et al. 2001)), and the WNV minimum infection rate (MIR = 1000 x # WNV positive pools/# individuals tested). We used an estimate for the MIR of mosquitoes based on data from 2232 pools of Cx. quinquefasciatus trapped in California between July and September, 2004 (MIR = 9.8±SE 0.7 or 0.98% of mosquitoes tested (Kramer 2005)). This value is intermediate between the mosquito WNV prevalence during epidemics in New York for Cx. pipiens (MIR = 3.5; (Bernard et al. 2001)) and Colorado for Cx. tarsalis (MIR = 50; (Pape 2004)). We used data for Cx. quinquefasciatus because it is present in Galapagos and Guayaquil, Ecuador, where all flights to Galapagos currently originate or pass through before landing in Galapagos. Finally, we

conservatively estimated that mosquitoes would be WNV-infectious for approximately 10-20 days, based on an average lifespan of 30-60 days for Cx. quinquefasciatus in the lab (Oda et al. 2002) and 7-14 days needed for viral development within the mosquito (Dohm et al. 2002a).

Migratory Birds

Because of uncertainties about the ability of WNV-infected birds to migrate successfully we made assumptions to maximize the risk from this pathway. We estimated the fraction of migrating birds that would be viremic (have WNV in their bloodstream) from a four year study of migrating birds in the Eastern United States (15 of 12,000 birds (0.00125; 95% CI: 0.0007 – 0.0021; R. McLean pers. comm.). We assumed it required only a single day for migration to Galápagos from the area where the migrating bird became infected with WNV and that 100% of viremic birds would survive the migration. We calculated the infectiousness of a bird to a mosquito using the viremia-infectiousness relationship for Cx. quinquefasciatus (see below) which is thought to have a more restricted range in Galápagos than the less competent vector Oc. taeniorhynchus (Turell et al. 2001). Finally, we assumed that all migrants came from areas where WNV was fully established, despite the fact that it has yet to become established in parts of northwest North America (CDC 2003) where many Galápagos migrants from or pass through after breeding.

We estimated the number of days that each migratory bird landing in Galápagos would be infectious as:

$$\frac{\varphi}{n}\sum_{i=1}^{n}\sum_{j=i}^{n}\left(\int_{5}^{15.3} I_m(v)N(v_i,\sigma)dv + \int_{15.3}^{\infty} N(v_i,\sigma)dv\right)$$

where φ is the fraction of birds that are viremic with WNV, and the summation is over the viremic period n (in days) for that species (Komar et al. 2003). The terms in parentheses represent the integral of the probability distribution of an animal's viremia on day i assuming a normal distribution, $N(v_i,\sigma)$, after log-transformation with mean $\log_{10}$(viremia), v_i, and variance, σ^2 multiplied by the probability of a bite leading to a disseminated infection in a mosquito, I_m, given the host's viremia, v. The first summation and 1/n terms account for the possibility that infectious migratory birds may arrive in Barbados on any of the n days that they are viremic. The second summation calculates the number of infectious days for the remaining j to n days in the viremic period.

Over 95% of the birds that migrate to Galápagos are shorebirds in the family Charadriidae (Castro & Phillips 1997). As a result, we used data from experimental infection of Killdeers (Charadrius vociferous; in the order Charadriiformes and family Charadriidae) to estimate WNV viremia parameters; v_i = 6.2,7.5,8.1,4.9, 2.6 on days 1-5 post-infection; (Komar et al. 2003), σ^2 = 1.90, n = 18 bird-days; Komar unpublished data). The probability, I_m, was based on a vector competency study of Cx. quinquefasciatus (Sardelis et al. 2001):

$I_m = 0.097*\log_{10}(v) - 0.48$; ($I_m$ = 0 for $\log_{10}(v) < 5.0$, and I_m = 1 for $\log_{10}(v) > 15.3$). This viremia-infectivity relationship was based on viremias ranging from $10^5 – 10^7$ PFU/ml which are slightly lower than the range of mean viremias that we used in the calculation, $10^{4.9} – 10^{8.1}$, necessitating an extrapolation of the fitted line.

Human-transported host vertebrates

Current regulations ban the import of live animals into Galápagos except for day-old chickens which are shipped in mosquito-proof containers. However, some illegal transport of domestic animals occurs and it is possible that the containers for the day-old chickens could be broken. We cannot accurately estimate the risk from the transport of these animals because both of these events are unquantifiable without additional information. However, we assessed the risk from day old chickens using by calculating the frequency of that would need to be transported for this

pathway to present a risk equal to that of migratory birds. We estimated the mean and variance for the WNV viremia of day-old chickens using data from experimental infections (Turell et al. 2001).

Sensitivity Analysis

As in other prospective analyses, many of the parameter estimates in our model are approximate or derived from work in other locations. We addressed the uncertainty inherent in our analyses in three ways. First, we used a range of values for parameter estimates which we were unable to estimate directly. Second, we incorporated error in the parameter estimates into a confidence interval (CI) for the estimate of I_d. We calculated the upper and lower bounds of the confidence interval of I_d by using the high and low values of the parameters for which we used a range and the upper and lower limits of the 95% confidence intervals for parameters where we could estimate the error. Third, we performed a sensitivity analysis on all parameters in the models by considering the change in the estimated risk (in infectious days) from a 25% percent change in each parameter estimate.

Results

Infectious mosquitoes transported on airplanes carrying tourists represent the highest risk of WNV reaching Galápagos by a vector pathway (Table 1). Our assessment predicts that 9.6 (Confidence Interval (CI): 0.9-45) WNV-infectious mosquitoes will arrive in Galápagos each year after WNV is established in Ecuador, representing approximately 95.7 (CI: 9-448) infectious mosquito-days. In order for larvae in tires to present an equal risk, an average of 72.5 larvae would need to be present in each tire, which seems unlikely. Other mechanisms of WNV introduction by an infected vector pose a risk at least an order of magnitude lower than that due to airplane-transported mosquitoes (Table 1).

For hosts, we estimated that approximately 15.6 (CI: 8.8-25.8) viremic migratory birds will arrive in Galapagos each year, representing 5.5 (CI: 3.1-9.0) infectious bird-days (Table 1). The importation of day-old chicks would carry a similar risk of WNV introduction if approximately 5.1 in 1000 chicks were accidentally infected with WNV en route to Galapagos. Finally, infected humans do not present a substantial risk for introducing WNV to Galápagos because the viremia or concentration of virus in WNV-infected humans is generally insufficient to infect mosquitoes.

Although it is difficult to accurately compare the relative risk of host and vector pathways, the mean number of WNV-infectious days from mosquitoes on airplanes was estimated to be more than 17 times higher than migratory birds. As a result, the risk from mosquitoes on airplanes is likely to be higher than from migratory, in part because we made assumptions to maximize the risk from the migratory bird pathway, and in part because the risk of WNV introduction by a mosquito on an airplane is likely to rise significantly in the future (see below).

Except for the infectious period of migratory birds, our sensitivity analysis revealed simple linear scaling, so that the risk from each pathway increased (or decreased) 25% for each 25% increase (or decrease) in each of the parameter estimates. This is due to the simplicity of the risk calculations; they are simply products of the parameter estimates for each component of the equations for these pathways. In contrast, the risk increased/decreased by the following amounts for a 25% increase/decrease in the other parameters for the migratory bird pathway: 81%/63% (mean host viremia); 3%/3% (variance in host viremia); 90%/70% (slope of mosquito infectivity-viremia relationship); -48%/-67% (y-intercept of mosquito infectivity-viremia relationship). This analysis suggests that mean host viremia and mosquito infectivity-viremia relationships are key components in determining the risk from this pathway.

Discussion

Our analysis is the first published example of a predictive model for the introduction of a pathogen that allows for assessment and mitigation of the risk of introduction. Our work builds on the risk assessment models for invasive species that have been used by many countries to reduce the introduction of pest species (Simberloff 2005; Wearing et al. 2001; Work et al. 2005; Yamamura & Katsumata 1999). Our case study on WNV and Galapagos suggests that mosquitoes transported on airplanes represents the dominant pathway for introduction. Whether this will be the most important pathway for the introduction of WNV to other locations, or for the movement of other pathogens between two areas, depends on the rate of movement of vectors, hosts, and the epidemiology of the pathogen between the two locations.

Importantly, the framework we have outlined here can be used to determine the key pathways for pathogen introduction in other mainland-island systems, as well as for the movement of pathogens between continents. Similarly, it can be adapted to model a broad range of vector-borne pathogens (e.g., airport malaria; (Gratz et al. 2000; Karch et al. 2001), avian flu, etc.), as long as data are available to estimate the flow of humans, transport, goods, and mobile animals, and the epidemiology of the pathogen.

Modeling pathogen introductions as a predictive approach necessarily involves assumptions and analyses based on incomplete data. In our illustrated case study we made three key assumptions: 1) WNV would eventually reach Ecuador and become established in local mosquito populations, 2) WNV-infectious birds would continue to migrate and would survive the overseas trip to Galápagos, and 3) the presence of WNV-infectious mosquitoes and birds could lead to the establishment of WNV in Galápagos. We believe that uncertainties underlying these assumptions should not prevent analyses of the type we have performed here. In addition, our approach can be used to identify high risk pathways that merit new research to refine risk estimates. However, the rate of spread of WNV across North America suggests that its movement into Ecuador is likely occur before sufficient data could be collected to address all uncertanties. As a result, we suggest that implementation of measures to reduce the risk of pathogen introduction from the pathways we have identified should be performed concurrent with research to refine risk assessments.

The most effective short term action to reduce the risk of WNV introduction would be implementation of existing requirements that all airplanes landing in Galápagos be chemically treated to kill incoming insects. Previous research has shown that residual disinsection (using an insecticide coating on the interiors of planes) is much more effective than fog fumigants (Naumann & McLachlan 1999). In addition, because 82% of mosquitoes on airplanes were found in cargo holds (Takahashi 1984) the use of insecticides only in the cargo holds would have a substantial impact. Unfortunately, the risk of WNV introduction by mosquitoes on airplanes is likely to grow in the immediate future. The number of tourists visiting Galápagos has shown steady increase from 40,746 in 1991 to 90,533 in 2003 representing a mean annual growth rate of 6.9% (Galapagos National Park Service 2004). There is severe political pressure within Ecuador to expand the number of tourists visiting Galápagos and air travel to Galápagos is almost entirely related to ecotourism. In addition, plans for tourism expansion include opening up the Galápagos to direct international flights, and developing an additional airport on Santa Cruz Island where mosquito populations are larger (UCPPAPG 2001). As ecotourism grows the threat of WNV-infectious mosquitoes arriving on airplanes will increase and likely make the risk from other pathways negligible by comparison.

In this study we have demonstrated that predictive approaches to disease emergence are possible, and can be used to identify strategies to prevent, rather than react to conservation crises. In addition, as intervention strategies are implemented or new information becomes available our model framework allows for continuous reassessment. As a result, it fits well with

adaptive management strategies (Salafsky et al. 2002). We believe that taking a proactive approach to pathogen introduction may offer insight into how to stem the wave of emerging diseases linked with globalization of our planet.

Acknowledgements

We thank the Galápagos National Park and the Charles Darwin Foundation for assistance for the 2004 Galápagos WNV Workshop, Patty Parker, David Wiedenfield, B. Sinclair, and members of the conference for helpful comments; and N. Komar for providing unpublished viremia data. Funding was provided by a core grant to the Consortium for Conservation Medicine from the V. Kann Rasmussen Foundation, NIAID-NIH contract #NO1-AI-25490 to LDK, and by a UK government Darwin Initiative grant #162-12-017 to SG and AC.

Literature Cited

Bernard, K. A., J. G. Maffei, S. A. Jones, E. B. Kauffman, G. D. Ebel, A. P. Dupuis, K. A. Ngo, D. C. Nicholas, D. M. Young, P. Y. Shi, V. L. Kulasekera, M. Eidson, D. J. White, W. B. Stone, and L. D. Kramer. 2001. West Nile virus infection in birds and mosquitoes, New York State, 2000. Emerging Infectious Diseases **7**:679-685.

Biggerstaff, B., and L. Petersen. 2002. Estimated risk of West Nile virus transmission through blood transfusion during an epidemic in Queens, New York City. Transfusion **42**:1019-1026.

Burke, D. S. 1998. The evolvability of emerging viruses. Pages 1-12 in C. R. Horsburgh, editor. Pathology of emerging infections. American Society for Microbiology, Washington D.C.

Castro, I., and A. Phillips 1997. Guide to birds of the Galapagos Islands. Princeton, NJ: Princeton University Press. Princeton University Press., Princeton, NJ.

CDC. 2003. West Nile virus http://www.cdc.gov/ncidod/dvbid/westnile/index.htm. Accessed on 12/8/2003.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Wildlife ecology - Emerging infectious diseases of wildlife - Threats to biodiversity and human health. Science **287**:443-449.

Davis, S., M. Begon, L. De Bruyn, V. S. Ageyev, N. L. Klassovskiy, S. B. Pole, H. Viljugrein, N. C. Stenseth, and H. Leirs. 2004. Predictive thresholds for plague in Kazakhstan. Science **304**:736-738.

Dohm, D. J., M. L. O'Guinn, and M. J. Turell. 2002a. Effect of environmental temperature on the ability of Culex pipiens (Diptera : Culicidae) to transmit West Nile virus. Journal of Medical Entomology **39**:221-225.

Dohm, D. J., M. R. Sardelis, and M. J. Turell. 2002b. Experimental vertical transmission of West Nile virus by Culex pipiens (Diptera: Culicidae). Journal of Medical Entomology **39**.

Galapagos National Park Service. 2004. Progreso de Uso Publico (Internal Report), Puerto Ayora, Santa Cruz, Galapagos, Ecuador.

Goddard, L. B., A. E. Roth, W. K. Reisen, and T. W. Scott. 2003. Vertical Transmission of West Nile Virus by Three California Culex (Diptera: Culicidae) Species. Journal of Medical Entomology **40**:743-746.

Gratz, N. G., R. Steffen, and W. Cocksedge. 2000. Why aircraft disinsection? Bulletin of the World Health Organization **78**:995-1004.

Hardy, D. E. 1960. Insects of Hawaii; Volume 10. Diptera. University of Hawaii Press, Honolulu, HI.

Karch, S., M. F. Dellile, P. Guillet, and J. Mouchet. 2001. African malaria vectors in European aircraft. Lancet **357**:235-235.

Keeling, M. J., M. E. J. Woolhouse, D. J. Shaw, L. Matthews, M. Chase-Topping, D. T. Haydon, S. J. Cornell, J. Kappey, J. Wilesmith, and B. T. Grenfell. 2001. Dynamics of the 2001 UK foot and mouth epidemic: Stochastic dispersal in a heterogeneous landscape. Science **294**:813-817.

Klenk, K., and N. Komar. 2003. Poor replication of West Nile virus (New York 1999 strain) in three reptilian and one amphibian species. Am. J. Trop. Med. Hyg. **69**:260-262.

Komar, N. 2003. West Nile virus: epidemiology and ecology in North America. Adv. Vir. Res. **61**:185-234.

Komar, N., S. Langevin, S. Hinten, N. Nemeth, E. Edwards, D. Hettler, B. Davis, R. Bowen, and M. Bunning. 2003. Experimental infection of north American birds with the New York 1999 strain of West Nile virus. Emerging Infectious Diseases **9**:311-322.

Kramer, V. 2005. Summary of West Nile virus activity, California. National conference on West Nile virus in the United States, San Jose, CA.

Linthicum, K. J., A. Anyamba, C. J. Tucker, P. W. Kelley, M. F. Myers, and C. J. Peters. 1999. Climate and satellite indicators to forecast Rift Valley fever epidemics in Kenya. Science **285**:397-400.

Lounibos, L. P. 2002. Invasions by insect vectors of human disease. Annual Review of Entomology **47**:233-266.

MAF. 2003. Sea Container Review. New Zealand Ministry of Agriculture and Forestry, Wellington, NZ.

Marra, P. P., S. Griffing, C. Caffrey, A. M. Kilpatrick, R. McLean, C. Brand, E. Saito, A. P. Dupuis, L. D. Kramer, and R. Novak. 2004. West Nile virus and wildlife. Bioscience **54**:393-402.

Meffe, G. K. 1999. Conservation medicine. Conservation Biology **13**:953-954.

Miller, D., M. Mauel, C. Baldwin, G. Burtle, D. Ingram, M. Hines, and K. Frazier. 2003. West Nile virus in farmed alligators. Emerging Infectious Diseases **9**:794-799.

Moya, A., E. C. Holmes, and F. Gonzalez-Candelas. 2004. The population genetics and evolutionary epidemiology of RNA viruses. Nature Reviews Microbiology **2**:279-288.

Naumann, I. D., and K. McLachlan. 1999. Aircraft disinsection. Australian Quarantine and Inspection Service, Canberra, Australia.

Oda, T., Y. Eshita, K. Uchida, M. Mine, K. Kurokawa, Y. Ogawa, K. Kato, and H. Tahara. 2002. Reproductive activity and survival of Culex pipiens pallens and Culex quinquefasciatus (Diptera: Culicidae) in Japan at high temperature. Journal of Medical Entomology **39**:185-190.

Pape, J. 2004. The Human and Governmental Costs of West Nile Virus. Protecting Hawaii and the Pacific from West Nile Virus Conference, Honolulu, HI.

Peck, S. B., J. Heraty, B. Landry, and B. J. Sinclair. 1998. Introduced insect fauna of an oceanic archipelago: the Galápagos Islands, Ecuador. American Entomologist **44**:218-227.

Russell, R., N. Rajapaksa, P. Whelan, and W. Langsford. 1984. Mosquito and other insect introductions to Australia aboard international aircraft and the monitoring of disinfection

procedures. Pages 109-141 in M. Laird, editor. Commerce and the spread of pests and disease vectors. Praeger, New York.

Salafsky, N., R. Margoluis, K. H. Redford, and J. G. Robinson. 2002. Improving the practice of conservation: a conceptual framework and research agenda for conservation science. Conservation Biology **16**:1469-1479.

Sardelis, M. R., M. J. Turell, D. J. Dohm, and M. L. O'Guinn. 2001. Vector competence of selected North American Culex and Coquillettidia mosquitoes for West Nile virus. Emerging Infectious Diseases **7**:1018-1022.

Simberloff, D. 2005. The politics of assessing risk for biological invasions: the USA as a case study. Trends in Ecology & Evolution **20**:216-222.

Smolinski, M. S., M. A. Hamburg, J. Lederberg 2003. Microbial Threats to Health: Emergence, Detection, and Response. The National Academies Press, Washington D.C.

Steinman, A., C. Banet-Noach, S. Tal, O. Levi, L. Simanov, S. Perk, M. Malkinson, and N. Shpigel. 2003. West Nile virus infection in crocodiles. Emerging Infectious Diseases **9**:887-889.

Takahashi, S. 1984. Survey on accidental introductions of insects entering Japan via aircraft. Pages 65-79 in M. Laird, editor. Commerce and the spread of pests and disease vectors. Praeger, New York.

Taylor, L. H., S. M. Latham, and M. E. J. Woolhouse. 2001. Risk factors for human disease emergence. Philosophical Transactions of the Royal Society of London, B. **356**:983-989.

Turell, M. J., M. L. O'Guinn, D. J. Dohm, and J. W. Jones. 2001. Vector competence of North American mosquitoes (Diptera : Culicidae) for West Nile virus. Journal of Medical Entomology **38**:130-134.

UCPPAPG. 2001. Union de cooperatives de produccion pesquera artesanales de la provincial de Galapagos, Guayaquil.

Van Riper, C., S. G. Van Riper, L. M. Goff, and M. Laird. 1986. The epizootiology and ecological significance of malaria in Hawaiian land birds. Ecological Monographs **56**:327-344.

Wearing, C. H., J. D. Hansen, C. Whyte, C. E. Miller, and J. Brown. 2001. The potential for spread of codling moth (Lepidoptera : Tortricidae) via commercial sweet cherry fruit: a critical review and risk assessment. Crop Protection **20**:465-488.

Wiedenfield, D. 2004. Migratory birds and potentially susceptible avian species in Galapagos. Evaluating the threat posed by West Nile Virus to Galapagos Fauna, Galapagos, Ecuador.

Wikelski, M., J. Foufopoulos, H. Vargas, and H. Snell. 2004. Galápagos Birds and Diseases: Invasive Pathogens as Threats for Island Species. Ecology and Society **9**:5.

Work, T. T., D. G. McCullough, J. F. Cavey, and R. Komsa. 2005. Arrival rate of nonindigenous insect species into the United States through foreign trade. Biological Invasions **7**:323-332.

Yamamura, K., and H. Katsumata. 1999. Estimation of the probability of insect pest introduction through imported commodities. Researches on Population Ecology **41**:275-282.

Table 1. Estimated number of WNV infectious mosquito- or host-days per year. Parameter estimates in columns 2 and 3 are mean values or the middle of the range used. Column 4 gives the mean and confidence interval using the ranges and error estimates as described in the Methods.

Pathway	Number arriving in Galápagos/yr	Infectiousness x duration	Infectious host or mosquito-days
Mosquito by			
1) Wind:	$<10^{-3}$	(0.0098)(0.22)(15)	$<1x10^{-6}$
2) Air:	(1910[a])(1.55)	(0.0098)(0.22)(15)	95.7 (9.2-448)
3a) Sea (adults):	(9750[a])(0.0005)	(0.0098)(0.22)(15)	0.14 (0.002-0.87)
3b) Sea (larvae in tires):	(1250 tires)(72.5*)	(0.0098)(0.22)(15)(0.0024)	See text
4) Human	90,533[b]	0	0
5) Human transported vertebrate	Day old chickens: 12,000[a]	(0.0051**/5)(0.36)	See text
6) Migratory bird	Shorebirds ~ 12,500[c]	(0.00125/5)(1.75)	5.5 (3.1-9.0)

Notes: *Value estimated so that risk from this pathway equaled adult mosquitoes on airplanes pathway; see text; ** Value estimated so that risk from this pathway equaled migratory bird pathway; see text; [a] (Galapagos National Park Service 2004); [b] Galapagos Department of Transportation & Commerce, 2003; [c] (Wiedenfield 2004)

Annex 6. West Nile Virus Workshop Minutes

Summary of policy recommendations to reduce the risk of West Nile Virus Introduction in to Galapagos

(From the Proceedings of the Galapagos West Nile Virus Workshop, Galapagos National Park Headquarters, Puerto Ayora, 29th April 2004)

WNV is projected to reach Ecuador by 2008. When WNV reaches Ecuador there is a high probability of it's introduction in to Galapagos unless rigorous control measures are implemented prior to the arrival of the disease. If WNV is introduced in to Galapagos it is likely to cause catastrophic mortality of endemic birds, reptiles and mammals, leading to irreparable ecological and economic damage to the archipelago. WNV can cause disease and death in humans, thus further threatening livelihoods on Galapagos. Disease introduction is most likely to occur through the human transport of infectious mosquitoes, particularly via inadvertent transport in airplanes. Transport of mosquitoes by boat or of infected vertebrate hosts are also significant risks for WNV introduction. To minimise these risks, the following control measures are required.

1. Aircraft pose the highest risk, so the most critical control measure is to enforce the insecticide treatment of transport to Galapagos to prevent the incidental transport of mosquitoes. Provision in law already exits for this.
    - 'residual disinsection' (a residual insecticide applied to the interior surface of aircraft) methods must be employed, as traditional fog fumigation has been shown to be ineffective in aircraft cabins.
    - No aircraft should be allowed to fly to Galapagos unless they have an up to date residual disinsection treatment, including private and military aircraft.
    - There should be no direct flights other than from mainland Ecuador, as direct flights from areas that are already affected by WNV (e.g. the continental USA) greatly increase the risk of WNV introduction.
2. All sea transport, including cargo ships and private boats should be quarantined until inspected and fumigated at a central port.
    - Cargo, such as tyres and machinery, must be stored and packed to minimise the collection of rainwater that acts as breeding sites for mosquitoes or otherwise enhances mosquito survival.
3. Transport of vertebrate WNV hosts to Galapagos must be conducted under the strictest quarantine conditions.
    - Before WNV reaches Ecuador, the current transport of chicks to Galapagos should be revaluated as this poses another significant introduction risk. At the least, chicks on the mainland must be hatched and reared in mosquito free conditions and kept in mosquito proof containers during transport to the islands.
4. Early surveillance for WNV should be initiated at major ports of travel to and from Galapagos (e.g Baltra, Guayaquil).

While some financial commitment is required to implement these policy measures, these costs are insignificant compared to the ecological and economic losses that would be experienced should WNV become established in Galapagos.

Proceedings of the Galapagos West Nile Virus Workshop, Galapagos National Park Headquarters, Puerto Ayora, 29th April 2004

This workshop was convened as part of the "Building capacity and determining disease threats to endemic Galapagos fauna" project; a partnership between the Institute of Zoology (Zoological Society of London), the University of Guayaquil and the Galapagos National Park Service (GNPS), funded by the UK Government's Darwin Initiative programme.

The purpose of the workshop was to gather together experts in West Nile virus (WNV) biology and epidemiology, experts in Galapagos fauna and potential WNV vectors in Galapagos, and experts in disease threats to wildlife conservation to examine the likely threat of WNV to Galapagos fauna and to examine possible preventative and responsive measures to this threat.

The workshop was opened by Edwin Nuala, Director, GNPS who welcomed all the participants from Ecuador and from overseas. He noted the timeliness of the workshop and the importance of addressing disease threats to Galapagos fauna. The support of the GNPS was promised to assist with the areas of work raised as important in the workshop output.

Overview of West Nile Virus (WNV) and its Spread Across the Americas
(led by Laura Kramer)

- WNV is a positive sense RNA virus: i.e. the viral RNA is infectious
- WNV is part of the Japanese Encephalitis serogroup of flaviviruses; other members of this subgroup include: Saint Louise Encephalitis (SLE) virus, Japanese Encephalitis (JE) virus, MVE and Kunjin (KUN) virus. KUN virus is a sub-type of WNV.
- Plaque reduction neutralization tests are required for the confirmation of the presence of antibodies specific to WNV because of the high degree of cross-reactivity among flaviviruses..
- The USA strain of WNV can kill some types of bird (e.g. corvids, gulls, house finches, and many more), horses, and selected other animals (e.g., farmed alligators; squirrels) , but a vaccine currently is available for equines. Humans are generally at low risk of severe disease unless very young or old or immunocompromised.
- As the virus has spread across the USA (1999-2004), nucleotide sequence analysis of virus isolated from crows (without cell culture passage) indicates that there is evidence of minimal, but directed, genetic change in the envelope region of the viral genome, , although it is still highly conserved.

Phylogenetic tree analysis based on genome sequence data of WNV shows that there are two distinct lineages (Lanciotti et al. 2002):

- Lineage 1, causes disease in humans and is spreading across Europe and the Americas.
- Lineage 2, found only in sub-Saharan Africa, where it does not cause disease in humans.
- Until recently, most research on WNV had been done on the Egyptian Strain (e.g. 101), but, although this virus is in lineage 1, it is quite different to the strain introduced to the Americas.

Spread of WNV through the Americas (from CDC documentation of human and avian infections)

- First reported in New York in August 1999.

- Subsequent years report focus in the SE states in addition to a continued focus in NE states, (although a lack of monitoring in intervening coastal states may account for lack of cases reported there)
- 2002 saw an explosion in reported human cases of WNV disease (from approx. 29 human cases in 2001 to approx. 4,156 in 2002, and approx. 14,000 horses died of WNV in 2002) as the virus spread from eastern foci through to most of Eastern and Central USA (a lot of human cases were in the Chicago area).
- 2002 was the first year a human case was reported on the West coast (in California) – the person, who worked for FedEx, had not travelled and no infected mosquitoes or birds were found in the area – this person was possibly infected by a single infected mosquito which had been shipped with a FedEx parcel?
- 2003 reported human cases of virus have shifted Westward to new locations (note that human cases follow bird cases reported in previous year), incl. southern California
- Horse cases were 14,000 in 2002; 4,000 in 2003 (probably fewer in 2003 since many were vaccinated by then)
- WNV arrived in Mexico in 2002 and subsequently has spread throughout the Mexican states
- Also reported in birds on the island of Jamaica (where it appears to have become established in resident birds); recently also spread to El Salvador and Guadeloupe. Infections (Ab + ve) now have been reported in Puerto Rico in resident and migratory bird species. There is also an unconfirmed report of a seropositive flamingo in Chile.

Wildlife Infections of WNV - Birds

- Birds show high morbidity, mortality, with viral shedding from cloacal and oral cavities (can have high concentrations of virus shed from the oral cavity).
- Clinical signs of infected birds include weakness, recumbency and ataxia.
- WNV causes meningoencephalitis and necrotizing myocarditis.
- 15,000 dead birds were surveyed 2000-03 in US. 12,000 were passeriformes, and 30% were WNV +ve; of these 45% were corvids
- Of top 10 WNV positive bird species in US, 5 were corvid species
- A total of 225 species of birds have now been documented as being WNV positive in the USA.
- Migratory birds as well as residents are infected in the USA
- Crows infected with 10pfu all die; have not defined an LD_{50} (ie are very, very sensitive). Note that infected crows display titres of up to 10^{14} pfu/ml in their blood (so are highly infectious)
- It has been shown that crows can start to die as early as day 4 post-infection, but more usually by day 6 or day 7. All infected crows are dead by day 10.
- Crow to crow transmission has been observed – possibly an important route of transmission for gregarious species and for birds on the nest
- Infection can also occur via eating infected carrion (demonstrated by feeding infected mice to crows).
- Morbidity and mortality experimentally determined for 3 species:

  Doves: 18% morbidity, 0% mortality

  Sparrows: 19% morbidity, 19% mortality

  Crows: 100% morbidity, 100% mortality

- There is a high variance in the WNV titres reached in the blood of infected sparrows. The mean titer is approx. 10^8 pfu, sufficiently high to be infectious to mosquitoes.

- Viral RNA can persist in random tissues (e.g. spleen, heart, spinal cord) of infected birds (eg. sparrows and pigeons) for at least up to 27 weeks, but no infectious virus has been recovered from these RNA-positive tissues.
- Sparrows and pigeons can maintain protective Ab titres for a long time (for at least 28 weeks post-infection).

Wildlife Infections of WNV - Other vertebrate orders

- Mammals can be infected by WNV, the most commonly affected being horses, squirrels, dogs, cats, and a range of other species, including bats and marine mammals (e.g. seals). Zoo surveys have shown that a large range of species can be infected and seroconvert, although the range of species that show evidence of disease or mortality is not known.
- Reptiles have also been shown to be Ab positive and are symptomatic (alligator, 1 turtle and 1 crocodile monitor with symptoms)
- In 2003, huge death rates in farmed alligators in FL observed: 10-50% mortality

Risk Analysis of WNV Reaching Galapagos
(led by Marm Kilpatrick)

Both known vectors and known vertebrate hosts of WNV are present on the Galapagos Islands, therefore only the virus is required for it to threaten the Galapagos fauna. If it reaches the archipelago, there is plenty of opportunity for it to become established and for it to be spread widely throughout the islands. Therefore, an analysis of the likelihood of the virus reaching the islands is required in order to estimate the degree of threat posed by WNV. In order to conduct such an analysis, and with a lack of certain data on WNV ecology, several important assumptions were made, based on work conducted on WNV in the USA and elsewhere.

Important assumptions for risk analysis:

- WNV is established at sources for potential introduction (mainland Ecuador for human transport, sites from which birds migrate)
- Mosquitoes will be the primary vectors of WNV in the Galapagos.
- A minimum viraemic titre of 10^4pfu is required for infection to be passed on to a biting mosquito (although it is unknown if this is the case for mosquitoes on Galapagos).
- Only 20% of infected mosquitoes will successfully transmit WNV (also unknown if this is the case for mosquitoes on Galapagos).

Five potential routes of introduction have been identified:

1) Introduction by infected human
2) Wind blown mosquitoes
3) Mosquitoes transported by humans (sea or air)
4) Human transported animals
5) Infected migratory birds

1. Infected humans

- Viraemic titres in humans probably only reach ~2.1 $\log_{10}$, therefore infected humans would not be as source of virus transmission if bitten by mosquitoes. (Titres may be higher in immunocompromised humans, however, and a small risk may be posed by such people.)

2. Wind blown mosquitoes

- Based on other studies, such as the introduction of Japanese Encephalitis to Papua New Guinea presumably via wind-borne mosquitoes, the maximum range is about 155km. Thus wind blown mosquitoes are unlikely to arrive on Galapagos from mainland. This is also borne out by the presence of only one endemic species of mosquito on Galapagos (Ochleratatus taeniorhynchus), suggesting that colonisation from the mainland is a rare event. Wind dispersal of Oc. taeniorhynchus (including Oc. taeniorhynchus eggs) from the mainland might pose a very minor risk, but the rate of WNV infection via vertical transmission is low.

3. (i) Mosquitoes transported by humans – boats

- Highest risk for mosquito introduction is via tyres or machinery (including cars), where water collects and in which larvae can survive. For adults, closed spaces (e.g. containers, cabins, possibly cars) are most important for their incidental transportation via shipping.
- A study in New Zealand showed that 7 of 10,000 containers had mosquitoes
- Given 10,000 tons of goods shipped to Galapagos pa; a 0.2% infection rate in progeny and 0.22 transmission rate - predict 0.002 to 0.21 infected adults imported by boat each year.

3. (ii) Mosquitoes transported by humans – aeroplanes

- Large studies have been carried out of mosquitoes arriving in planes to Japan and Australia (note, that many planes were also spray fumigated). They showed an average of 1-2.2 mosquitoes arriving per plane
- Most mosquitoes were transported in the cargo hold and not in the passenger cabin.
- There are 1,910 flights to Galapagos p.a. and assuming a similar infection rate as above this leads to an estimated 1.3-13 live, infectious mosquitoes introduced by aeroplane every year (note, this translates to 26-260 "infectious mosquito days", assuming an adult mosquito lives for 30 days and becomes infectious on day 10 as an adult).
- Spray fumigation did not have a significant impact on the numbers of mosquitoes successfully transported by aeroplanes.

4. Animals transported by humans

- Assume that current quarantine laws are continued and are 100% effective (i.e. there are no illegal or incidental introductions of animals).
- 10-15,000 day old chicks are transported to Galapagos annually. Adult chickens are not a threat as they have a very low viraemia and are not killed by WNV. Vertical transmission in hens is highly unlikely (as infected embryos die). Young chickens (up to about 1 week old), however, are highly susceptible to infection and produce a very high viraemia.

5. Migratory Birds

- Assuming a worst case scenario of 60,000 migrants per year- (probably an overestimate – see David Wiedenfeld's presentation below in which it is estimated that only 15,000 migrants come near-shore or on-shore).

- Assuming migration from a WNV infected area, a day for migration (and all migrants survive), the fraction of viraemic birds is 0.00046. Translates to 1.25 infectious bird days per year.

CONCLUSION: plane flights pose high risk, with secondary risks through cargo arriving by ship and bird migration.

WNV Threats to Galapagos Fauna
(led by David Wiedenfeld)

Should WNV reach the Galapagos, the species potentially at risk can be classified into five groups:

1. Species present in low numbers and with restricted distributions, e.g. Galapagos hawk, flightless cormorant, lava gull
2. Non-endemic species which are known to be susceptible to WNV infection in other countries, e.g. pelican, heron, flamingo, yellow warbler, short-eared owl, plus chickens, dogs, horses
3. Migratory species in which WNV has been detected elsewhere, e.g. grebe, turnstone, purple martin, swallow
4. Endemic species closely related to animals known to be susceptible to WNV infection elsewhere, e.g. penguins, cormorants, hawks, mockingbirds, finches. (It is highly likely that these species will also be susceptible to infection.)
5. Other members of orders known to be susceptible to WNV elsewhere (i.e. reptiles) and which may also be susceptible to infection, e.g. tortoises, iguanas, lava lizards.

Survey of migrant numbers

- 25,000 regular migrants come to Galapagos, but 15,000 are phalaropes and only come within 250m of shore (so probably don't get bitten because mosquitoes don't fly over water much and flight over the ocean would be desiccating. Also, it is doubtful that they would need to fly offshore to feed as hosts on land are unlikely to be limiting.)
- A few migrants occur in low numbers and there are also a few species that exchange with the continent
- Overall estimate is that 12,500 migratory birds will be near mosquitoes.

Mosquitoes on the Galapagos
(led by Helmuth Rogg)

Three species of mosquito are known to occur on the Galapagos Islands:

- Oc. taeniorhynchus, which is endemic and is widely distributed throughout the archipelago.
- Culex quinquefasciatus, which is introduced and which has only been reported from Puerto Ayora, Santa Cruz and San Cristobal, where it is much wetter and where there is an airport.
- Aedes aegypti, which is introduced and which is limited to the inhabited areas of Santa Cruz. (Note – this species of mosquito is specific to humans and doesn't bite other animals, therefore it is of low risk for WNV transmission.)

Other mosquito species that are at risk of introduction to the Galapagos are:

- Aedes albopictus, the introduction of which could be particularly problematical as it feeds on both humans and other animals, is a known WNV transmitter and is extant in Central and South America.
- Anopheles, which is unlikely to establish in Galapagos as it needs swamps.

Note there is no correlation between Aedes aegypti numbers and rainfall, but there is a positive correlation with temperature.

- Permanent Dengue control program here in Puerto Ayora via the use of "Abate" (an organochlorine pesticide) in water.

Note: By law, aeroplanes and boats arriving in Galapagos must be sprayed with insecticide, BUT this law is not being enforced

Non-mosquito vectors of WNV may also be present on Galapagos, such as the blackfly (which is present in high numbers on San Cristobal. The competence of these other potential vectors is unknown.

Pathology and Training for WNV on Galapagos
(led by Nicole Gottdenker)

- WNV causes lesions and neurological signs, but other diseases can cause similar lesions; furthermore some other viruses (e.g. Eastern Equine Encephalitis, Western Equine Encephalitis, Venezuelan Equine Encephalitis) can also cross-react serologically with WNV.
- Specific diagnostics for WNV include immunohistochemistry (IHC), in situ hybridization, virus isolation (the gold standard), RT-PCR, RT and sequencing, Taqman, EM, ELISA and serology
- Several of these techniques e.g. PCR, IHC will be utilised at the new Galapagos Epidemiology and Pathology Laboratory
- Proper equipment for the handling of potentially infected animals, tissues and carcasses (e.g. Microbiological Safety Cabinet) will also be in place at the new laboratory.
- Proper education and training for personnel who may come into contact with WNV-infected animals will need to be implemented.

Discussion Session/ Summary West Nile Virus Workshop

Timescale of Introduction
It was agreed that the most important factor threatening introduction would be the arrival of WNV in mainland Ecuador. Although the establishment of WNV in western North America could be an important stage before introduction to Ecuador is likely, there was agreement that this stage would not be a necessity: Ecuadorian migrants also come from the eastern USA and Central America), so WNV could arrive in Ecuador via this route. An unconfirmed report of

WNV in Chilean flamingo raised the possibility that WNV may already be in mainland South America.

Extrapolating from the spread of WNV through North America and the Caribbean, WNV will almost certainly be established in Ecuador within 5 years (possibly arriving in 2 or sooner), after which its arrival in Galapagos will be inevitable unless preventative measures are taken.

Reducing the risk of WNV Introduction

The main risk is from infected mosquitoes arriving in aeroplanes. This threat will be high once WNV become established in Ecuador as (almost) all air travel to the Galapagos comes from mainland Ecuador. The transport of infected mosquitoes in cargo shipped to the Galapagos is a second significant risk and infected migratory birds pose a third significant risk. Risks posed by imported domestic animals (especially day-old chicks) were also recognized.

Currently, all aircraft arriving in Galapagos should be fumigated with insecticide, but this is not being carried out. Elsewhere, fumigation has been shown to be pretty ineffective in preventing the import of live mosquitoes, whereas coating the inside of aircraft with an insecticidal residue has been shown to be highly effective. Efforts should, therefore, be made to bring about enforcement of the "disinsection" law, but with the use of an effective means of killing on-board mosquitoes. This will have additional benefits of reducing the risks of importing other (alien) insects to the islands.

It was suggested that the addition of a small $ charge to the cost of (tourist) ticket would be enough to cover the costs of aircraft disinsection. It was recognised, however, that this additional charge could be politically difficult.

Fortunately, both the Ecuadorian aviation authority and the Port Authority in Guayaquil are in favour of enforcement of the disinsection law, so we are hopeful that we may be pushing against an open door. It was recommended that SICGAL and SESA should be approached to take this plan forward.

One area of particular concern that was raised was the proposed changes to the regulation of air travel to the Galapagos and to the numbers of tourists visiting the islands. Currently, there are political demands on the table requesting a de-regulation of flights to the islands, with an increase in numbers of flights from the mainland and the introduction of flights directly to the Galapagos from other countries (e.g. USA, Panama). The latter would greatly increase the risk of WNV introduction to the islands whether or not the virus had reached mainland Ecuador as infected mosquitoes could be transported directly from countries where the virus is already endemic. Strict enforcement of the ban on introduced animals and of the most effective methods of disinsection would be required to minimize this risk.

An increase in the number and size of tourist boats is also being demanded, which would also increase the numbers of flights to the islands (as all tourists arrive by plane and then transfer to boats in the Galapagos). In order to accommodate the projected increase in air travel, some people are calling for the development of an airport on Santa Cruz (close to Puerto Ayora) to replace the airport on Baltra. The siting of Baltra for the main airport has turned out to be propitious as far as WNV introduction goes because (i) it is arid, thus the population of mosquitoes there is not high, and (ii) it is leaward to Santa Cruz, thus prevailing winds will tend to blow vectors away from Santa Cruz and out to sea. Should it be introduced, the chances of WNV (or any other introduced arthropod-borne disease) becoming established would be much greater if the airport was on Santa Cruz rather than on Baltra (especially if it was near Puerto Ayora). It is likely that only luck has prevented matters on San Cristobal (where the airport is close to town) from being much worse than they have been so far. It was pointed out, however,

that the authorities should not be complacent about this and that even Baltra could provide highly suitable mosquito habitat during El Nino years.

It was noted that WNV is a zoonosis and has killed a large number of people (mainly elderly) in the USA. If WNV was to reach the Galapagos, associated increased morbidity and mortality would have a major impact on healthcare costs for the islanders. Also, should a tourist die from WNV contracted on the islands, the negative impact on the tourist industry is likely to be significant.

Large numbers of day-old (or two-to-three-day old) chicks are imported to the Galapagos for rearing as broilers (there being no commercial laying enterprise on the islands). This provoked much discussion and concern, as young chicks are susceptible to WNV, developing viraemia high enough for virus transmission via mosquitoes. It was thought that the import of large numbers of young chicks could pose a conduit for WNV introduction to Galapagos if/when the virus becomes established on mainland Ecuador. Suggestions to mitigate this included hatching and packing birds in mosquito-proof caging; banning the import of chicks if/when WNV reaches the mainland, and banning the import in favour of establishing egg production on the islands. (The latter might be favourably received by locals as it would provide employment and help the local economy, but the supply of fresh water may be a limiting factor.) It was suggested that a working group with SESA should be set up to look into this further, perhaps with a cost-benefit analysis being conducted on the local production of chicks.

<u>Minimising the risk of WNV becoming established</u>

It was recognized that, should WNV be introduced to the islands, the only effective way of preventing it from becoming established would be via a mosquito control programme. In addition to having a major mosquito control programme on stand-by to be implemented very rapidly after WNV introduction, the continued control of mosquito populations at the most likely portals of WNV entry should be conducted. Such portals of entry are the towns (where cargo is imported; where there is a high throughput of people and their belongings; and where the highest species complement and populations of mosquitoes are found), the airports and the cargo port on Baltra. Such control is important at all times from now on, but will be particularly important once WNV reaches mainland Ecuador and especially so during El Nino years.

<u>Surveillance: Which species, who, when, where and how much will it cost?</u>

The group identified surveillance for WNV as a high priority and there was much discussion as to how this could best be done. One way would be to regularly trap and test mosquitoes around the most likely entry points (see above), but the percentage of positive mosquitoes can be very small (and almost undetectable) while the disease is just getting established in a new location as the prevalence is low. (During the midst of an epidemic, however, the % of mosquitoes infected is usually 0.3-5%, which is easy to pick up when testing pools of 50 mosquitoes.) Therefore, testing of birds and possibly other vertebrates was considered to be important. Again, the sample size required for testing if the virus causes no mortality needs to be very high. Testing of birds and other wildlife was, therefore, considered important for detecting the extent of spread of WNV, but not a useful tool for early warning of WNV introduction. Testing should be done on purposefully-obtained samples and also on any samples obtained opportunistically from any species) from other studies. Such testing could be carried out in the new pathology laboratory using standard protocols, and this would also allow rapid feedback of the results.

The best way to do conduct surveillance for early warning of WNV introduction would be via the regular testing of sentinel species (and preferably the use of a sentinel species that is highly susceptible to WNV, in which the infection causes rapid onset mortality). Horses were

considered likely to be the best sentinel species. These could be bled regularly for WNV serology. Also, in the event of mortality, a local mosquito control programme could be initiated while awaiting necropsy results. Such a response plan would be an integrated part of the surveillance strategy as it would be crucial for control to be instigated as rapidly as possible after WNV introduction. It was suggested that sentinels could be held at the main ports on Baltra, Santa Cruz and San Cristobal, and possibly also at Guayaquil.

Funding Plans

- revisit Princeton proposal for baseline
- note possible tourism risk – selling points for CDC and human oriented company grants such as (OFF-makers J&J)
- various private US foundations
- companies (e.g. J&J), tourists and tour companies
- new proposal to Darwin Initiative
- GEF?
- SESA, SICGAL and GNPS, health and agriculture depts. should lead (but note they have no funds)
- estimated cost for entomological monitoring alone may be ~ $50K (HR)

May be better to fundraise for endowment fund rather than grants (but potential for political fighting over who controls it?)

Other notes:

1) costs may be lowered by collaborations, free testing e.g. CDC, St. Luis, Cornell, NYDoH
2) Sell a well packaged plan: since risk is high, prevention and surveillance is a cheaper long term solution

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Annex 7: Manuscript on the establishment of Culex quinquefasciatus

Establishment of the avian disease vector *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae) on the Galápagos Islands, Ecuador

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Word Count: 2229 (title page and text)

Avian disease has been implicated as a major factor in decline of the endemic Hawaiian avifauna (Warner 1968; van Riper et al. 1986, 2002; Atkinson et al. 2000; Yorinks & Atkinson 2000). The introduction into Hawaii of avian pox (Avipoxvirus spp.), avian malaria (Plasmodium relictum) and a suitable vector, the Southern house mosquito (Culex quinquefasciatus Say 1823; Hardy 1960), are thought to be the mechanisms driving this decline (van Riper & Scott 2001, van Riper et al. 2002). Culex quinquefasciatus is a cyclopropagative vector (in which the pathogen undergoes further development and multiplication) for avian malaria, and a mechanical vector (in which the pathogen is carried on or in mouthparts, legs, etc., but does not undergo further development or multiplication), for avian pox in Hawaii. The endemic birds of Hawaii are more susceptible than are introduced birds, to both of these pathogens (van Riper et al. 2002, Atkinson et al. 2000, Yorinks & Atkinson 2000).

In contrast, the avifauna of the Galápagos Islands is largely intact (due to relatively recent human colonization; Snell et al. 2002), yet is highly endemic (84% of land birds are unique; Tye et al. 2002). Several endemic bird populations are in decline (Snell et al. 2002), although none are extinct archipelago-wide. For example, the Galápagos Hawk (Buteo galapagoensis Gould 1837) has been extirpated on three human-inhabited islands (de Vries 1975), while breeding populations still reside on eight islands. Invasive organisms and disease agents, including viruses such as West Nile Virus (WNV), now pose the greatest threat to the continued persistence of Galápagos' unique birds (Wikelski et al. 2004, Thiel et al. 2005). We report here the establishment in the Galápagos Islands of the avian disease vector C. quinquefasciatus, first reported from the archipelago in 1989 (Peck et al. 1998), and documented now as part of a larger survey of avian disease and their vectors in the archipelago begun in 2001. We also report the date 1985 as the first collection of this mosquito in the archipelago, earlier than was published previously (1989). The implications of the establishment of this insect in the Galápagos Islands, specifically the threat it poses to avian health, are discussed.

METHODS

Adult mosquitoes were sampled during a total of nine trapping attempts using U.S. Centers for Disease Control & Prevention miniature ultraviolet light traps on Isla Santa Cruz in the Galápagos Islands (Archipelago de Colón), Ecuador, in July and August, 2003 (purchased from BioQuip Products, Rancho Dominguez, CA, U.S.A). Light traps were turned on approximately one hour before dusk (~5 pm local time) and turned off from 1 to 5 hours after dawn (~7am-11am). Culicids were then separated from other insect taxa and stored in 95% ethanol for identification. Label information from specimens collected prior to this study was obtained from vouchers housed at the Canadian National Collection of Insects in Ottawa, Canada. All 2003 collections were made in and around the coastal town of Puerto Ayora, Isla Santa Cruz, which lies within the Arid Zone (with focused sampling at the Charles Darwin Research Station; 0° 44' 20" S latitude, 90° 18' 25" W longitude; 6 m) and within the town of Bellavista, which lies within the upper Transition Zone (0° 42' S latitude, 90° 22' W longitude; 194 m). Bellavista, Isla Santa Cruz annually receives more rainfall and is cooler in temperature than Puerto Ayora, Isla Santa Cruz (Snell & Rea 1999).

Oviposition traps were made from 5 litre 'pitcher' style plastic water containers by cutting away the neck and front walls of the vessel to half height. The containers were filled with ~1.5 litres of fresh, potable water and a handful of dry straw and placed in partially shaded locations around the Galápagos National Park Service Headquarters in Puerto Ayora, Isla Santa Cruz. Two traps were set on consecutive days from 28 April -14 May 2004. Traps were checked daily and the number of eggs counted. Egg rafts were removed to separate hatching containers and allowed to complete the development cycle, after which a selection of adults was collected for identification. Identifications of culicid specimens were made using a species-diagnostic molecular analysis of the internal transcribed spacers (ITS1 and ITS2) of the nuclear ribosomal gene array (Crabtree et al. 1995), conducted at the Arbovirus Laboratories, Wadsworth Center, NY, U.S.A.

RESULTS

Eleven adult individuals of the Southern house mosquito (C. quinquefasciatus) were collected from two traps placed at two locations (one trap within the Arid Zone and one trap within the upper Transition Zone) on Isla Santa Cruz in August 2003 (Table 1). One of the traps (placed in Bellavista) that produced two Southern house mosquitoes also produced 11 individuals of the black salt marsh mosquito (Ochlerotatus taeniorhynchus (Wiedemann 1821)). Seven traps placed in other areas, including near the Charles Darwin Research Station, produced 155 O. taeniorhynchus individuals and no Culex individuals. Thus, 11 Southern house mosquito and 166 black salt marsh mosquito individuals were collected from the nine trapping attempts. Voucher specimens of both species have been placed at the Zoologisches Forschungsinstitut und Museum Alexander Koenig, Adenauerallee 160, D-53113 Bonn, Germany. Reexamination of museum label data from C. quinquefasciatus collected in the Galápagos Islands prior to this study indicate that the date of first record of occurrence in the Galápagos was not 1989 as reported by Peck et al. (1998), but rather 1985.

A total of 27 egg rafts were laid in oviposition traps between 28 April and 14 May 2004. Adults reared from these eggs rafts were subsequently confirmed as C. quinquefasciatus using the molecular analysis described above.

DISCUSSION

The establishment of C. quinquefasciatus on the Galápagos Islands after its first detection two decades ago, in 1985, is troubling from an avian conservation perspective. This species is capable of biting humans or migrating birds and transmitting exotic disease agents, such as WNV (Turell et al. 2001). West Nile Virus is present within other island systems in the New World tropics and it may be simply a matter of time before it enters the Galápagos ecosystem (Dupuis et al. 2003). This mosquito is also a mechanical vector for Avipoxvirus, now present in both domesticated and wild birds in the Galápagos (Thiel et al. 2005), and thus its presence may exacerbate the spread of pox within and between islands. If Plasmodium relictum or another avian malaria species ever enters the Galápagos, C. quinquefasciatus can serve as a competent vector. This combination of events would likely be devastating to the local bird community.

Interestingly, the first 2003 C. quinquefasciatus collection locality on Isla Santa Cruz was in a small town (Bellavista), and only 5 km from the first collection locality (in 1985) on Isla Santa Cruz, at the Media Luna. However, these two sites, though geographically proximate, are separated by ~400 m in elevation. Bellavista is an agricultural settlement located ~8 km inland, situated in the more mesic highlands of the upper Transition Zone. The 1985 sampling locality (the Media Luna) remains uninhabited and is in the mesic Miconia Zone. The second 2003 collection location on Santa Cruz was located within the Arid Zone but a trap was intentionally placed near a laundry room of a private residence, where mosquitoes had been observed previously. Culex quinquefasciatus also readily oviposited in fresh water traps on Santa Cruz. Thus, C. quinquefasciatus has now been reported from three altitudinal zones within Isla Santa Cruz and from the Arid Zone within Isla San Cristóbal. Since breeding by C.

quinquefasciatus could be limited by the presence of fresh water (it is a fresh water obligate; Patrick & Bradley 2000) its distribution in the Galápagos is probably most common near human habitations where fresh water can be found. However, C. quinquefasciatus is likely to increase its range within the Arid Zone during the wet season. Furthermore, the absence of C. quinquefasciatus from the majority of light traps may be due to the fact that we sampled during the dry season and not the wet season. Nonetheless, this species was present within both the Arid and Transition Zones during the dry season, which underscores the potential for C. quinquefasciatus to invade coastal areas of other islands, particularly during the wet season and during El Niño Southern Oscillation events. Simple control measures, such as reducing the availability of human-made oviposition sites (e.g., used tires, open containers) may reduce the local abundance and the eventual spread of these obligate freshwater breeding mosquitoes in the archipelago. Other control measures, such as the use of the biological control agent Bacillus sphaericus, which is toxic to C. quinquefasciatus (Regis et al. 2000), could be implemented. However, resistance to the 'Bin toxin' has been observed (Oliveira et al. 2004). The toxin produced by Bacillus thuringiensis israelensis (Bti), the effects of which are also relatively specific to larval dipterans, would be preferable since mosquitoes do not develop resistance to it. However, non-target taxa, particularly other insects within the dipteran suborder Nematocera, such as chironomid midges, may be negatively affected by its application (Hershey et al. 1998).

Peck et al. (1998) speculated that C. quinquefasciatus arrived in the archipelago as larvae in water. However, local air travel now occurs among three islands within the archipelago (Islas Isabela, Santa Cruz, San Cristóbal) and between two islands and the mainland, including the city of Guayaquil, Ecuador, situated in the humid tropical lowlands. As Peck et al. (1998) noted, 11,448 insect specimens were collected from aircraft in Hawaii (Dethier 1948, see also Lounibos 2002). This route of dispersal is likely to ensure the presence of such invasive pests in Galápagos, and new mosquito-borne diseases are likely to be introduced unless control measures are implemented for aircraft flying into the archipelago (Kilpatrick et al. unpublished results). Tour operators, tourists, residents, and scientists on inter-island boat trips should be vigilant in ensuring that they are not transporting these mosquitoes. An educational campaign should be instituted to alert communities on the Galápagos to eliminate standing water. Nonetheless, C. quinquefasciatus now appears to be established on Isla Santa Cruz and is quite likely still present on Isla San Cristóbal, where it was collected in 1989. It seems probable that this species is also present on Islas Isabela and Floreana, the only other islands inhabited by humans in the archipelago, but further sampling is needed to confirm this.

The black salt marsh mosquito (O. taeniorhynchus) is present on all main islands within the Galápagos and has been known since first record in the late 1890s (Linsley & Usinger 1966). This species breeds in brackish water and is regarded as less threatening as a vector of avian disease agents. However, it should not be ignored as a threat, because, although it may prefer feeding upon mammals, individuals also feed on birds (Edman 1971). Ochlerotatus taeniorhynchus individuals have been observed feeding on endemic birds within the Galápagos and locally high mosquito population densities have

led to cases of nest desertion by endemic birds (Anderson & Fortner 1988). Moreover, individuals of O. taeniorhynchus have tested positive for WNV elsewhere (Hribar et al. 2003), and individuals are capable of transmitting WNV (Turell et al. 2001). This insect is also likely to serve as a mechanical vector of Avipoxvirus among birds in the Galápagos Islands (Thiel et al. 2005).

Data on host preferences (by genetically characterizing the identity of mosquito blood meals; Ngo & Kramer 2003), distribution, and intra- and inter-island movement of these mosquitoes (e.g., population genetics), and how each of these interacts with seasonality, are needed to more fully understand the threat posed by these vectors to the unique Galápagos avifauna.

ACKNOWLEDGEMENTS

NKW and PGP were supported by an International Dissertation Enhancement Grant from the U.S. National Science Foundation (INT-030759), a Trans World Airlines scholarship in environmental studies, grants from the Field Research for Conservation Program of the Saint Louis Zoo, International Center for Tropical Ecology, Sigma Xi, and funds from the E. Desmond Lee and Family Fund's Collaborative Vision in Zoological Studies from UM-St. Louis and the Saint Louis Zoo. SJG and AAC were supported by a U.K. government Darwin Initiative Grant (no. 162-12-017, "Building capacity and determining disease threats to endemic Galápagos fauna"). We gratefully acknowledge the logistical support and research permits provided by the Servicio Parqué Nacional de Galápagos and the Estación Científica Charles Darwin, Isla Santa Cruz, Galápagos, Ecuador, with special thanks to Poly Robayo, Howard Snell, Hernan Vargas and David Wiedenfeld. TAME provided discounted roundtrip air-travel within Ecuador. Dr. Stewart Peck provided helpful comments on a previous version of this manuscript.

REFERENCES

Anderson, D.A. & Fortner, S. 1988. Waved albatross egg neglect and associated mosquito ectoparasitism. Condor **90:** 27–29.

Atkinson, C.T., Dusek, R.J., Woods, K.L. & Iko, W.M. 2000. Pathogenicity of avian malaria in experimentally-infected Hawaii Amakihi. J. Wildl. Dis. **36:**197–204.

Crabtree, M.B., Savage, H.M. & Miller, B.R. 1995. Development of a species-diagnostic polymerase chain reaction assay for the identification of Culex vectors of St. Louis encephalitis virus based on interspecies sequence variation in ribosomal DNA spacers. Am. J. Trop. Med. Hyg. **53:**105–109.

de Vries, Tj. 1975. The breeding biology of the Galápagos Hawk, Buteo galapagoensis. Le Gerfaut **65:** 29–57.

Dethier, V.G. 1945. The transport of insects in aircraft. J. Econ. Entomol. **38:** 528–531.

Dupuis, A.P., Marra II, P.P & Kramer, L.D. 2003. Serologic evidence of West Nile virus transmission, Jamaica, West Indies. (Dispatches). Emerg. Infect. Dis. 9: 860–863.

Edman, J.D. 1971. Host-feeding patterns of Florida mosquitoes. I. Aedes, Anopheles, Coquillettidia, Mansonia, and Psorophora: J. Med. Entomol. **8:** 687–695.

Hardy, D.E. 1960. Insects of Hawaii, vol. 10. Honolulu, Hawaii: University of Hawaii Press.

Hershey, A.E., Lima, A.R., Niemi, G.J. & Regal, R.R. 1998. Effects of Bacillus thuringiensis israelensis (Bti) and methoprene on nontarget macroinvertebrates in Minnesota wetlands. Ecol. Appl. **8:** 41–61.

Hribar, L.J., Vlach, J.J., Demay, D.J., Stark, L.M., Stoner, R.L., Godsey, M.S., Burkhalter, K.L., Spoto, M.C., James, S.S., Smith, J.M. & Fussell, E.M. 2003. Mosquitoes infected with West Nile Virus in the Florida Keys, Monroe County, Florida, USA. J. Med. Entomol. **40:** 361–363.

Linsely, E.G. & Usinger, R.L. 1966. Insects of the Galápagos Islands. Proc. Cal. Acad. Sci. **33:** 113–116.

Lounibos, L.P. 2002. Invasions by insect vectors of human disease. Ann. Rev. Entomol. **47:** 233–266.

Ngo, K.A. & Kramer, L.D. 2003. Identification of mosquito bloodmeals using polymerase chain reaction (PCR) with order-specific primers. J. Med. Entomol. **40:** 215–222.

Oliveira, C.M.F., Siva-Filha, M.H., Nielsen-Leroux, C., Pei, G., Yuan, Z. & Regis, L. 2004. Inheritance and mechanism of resistance to Bacillus sphaericus in Culex quinquefasciatus (Diptera: Culicidae) from China and Brazil. J. Med. Entomol. **41:** 58–64.

Patrick, M.L. & Bradley, T.J. 2000. The physiology of salinity tolerance in two species of Culex mosquitoes: the role of compatible solutes. J. Exp. Biol. **203:** 821–830.

Peck, S. B., Heraty, J., Landry, B. & Sinclair, B.J. 1998. Introduced insect fauna of an oceanic archipelago: The Galápagos Islands, Ecuador. Am. Entomol. **44:** 218–237.

Regis, L., Oliveira, M.H., Silva-Filha, S.B., Maciel, A. & Furtado, A.F. 2000. Efficacy of Bacillus sphaericus in control of the filariasis vector Culex quinquefasciatus in an urban area of Olinda, Brazil. Trans. Roy. Soc. Trop. Med. Hyg. **94:** 488–492.

Snell, H. L. & Rea, S. 1999. The 1997-1998 El Niño in Galápagos: Can 34 years of

data estimate 120 years of pattern? Noticias de Galápagos **60:** 11–20.

Snell, H. L., Tye, A., Causton, C.E. & Bensted-Smith, R. 2002. Current status of and threats to the terrestrial biodiversity of Galápagos. In Bensted-Smith, R. (ed) A Biodiversity Vision for the Galápagos Islands: 30–47. Puerto Ayora, Galápagos: Charles Darwin Foundation & World Wildlife Fund.

Thiel, T., Whiteman, N.K., Tirapé, A., Baquero, M.I., Cedeño, V., Walsh, T., Uzcátegui, G.J. & Parker, P.G. 2005. Characterization of canarypox-like viruses infecting endemic birds in the Galápagos Islands. J. Wild. Dis. **In press.**

Turell, M.J., O'Guinn, M.L., Dohm, D.J. & Jones, J.W. 2001. Vector competence of North American Mosquitoes (Diptera: Culicidae) for West Nile Virus. J. Med. Entomol. **38:** 130–134.

Tye, A. Snell, H.L., Peck, S.B. & Adsersen, H. 2002. Outstanding terrestrial features of the Galápagos archipelago. In Bensted-Smith, R. (ed) A Biodiversity Vision for the Galápagos Islands: 12–23. Puerto Ayora, Galápagos: Charles Darwin Foundation & World Wildlife Fund.

Van Riper III, C. & Scott, J.M. 2001. Limiting factors affecting Hawaiian native birds. Studies in Avian Biology **22**: 221–233.

Van Riper III, C., Van Riper, S.G., Goff, M.L. & Laird, M. 1986. The epizootiology and ecological significance of malaria in Hawaiian land birds. Ecological Monographs **56**: 327–344.

Van Riper III, C., Van Riper, S.G. & Hansen, W.R. 2002. Epizootiology and effect of avian pox on Hawaiian forest birds. Auk **119**: 949–942.

Warner, R.E. 1968. The role of introduced diseases in the extinction of the endemic Hawaiian avifauna. Condor **70**: 101–120.

Wikelski, M., Foufopoulos, J., Vargas, H. & Snell, H. 2004. Galápagos Birds and Diseases: Invasive Pathogens as Threats for Island Species. Ecology and Society **9**: article 5 [online: URL: http://www.ecologyandsociety.org/vol9/iss1/art5].

Yorinks, N. & Atkinson, C.T. 2000. Effects of malaria on activity budgets of experimentally infected juvenile Apapane (Himatione sanguinea). Auk **117**: 731–738.

Table 1. Collection records of the Southern house mosquito (Diptera: Culicidae) Culex quinquefasciatus and the black salt marsh mosquito (Ochlerotatus taeniorhynchus)) from the Galápagos Islands, Ecuador.

Species	Date	Island	Location	Abundance	
Culex quinquefasciatus	14.V. –13.VII.1985	Santa Cruz*	4 Km N Bella Vista, Media Luna, 620 m	4	*Same collection data reported previously (Peck et al. 1998).
	10.II.1989*	San Cristóbal*	Puerto Baquerizo, hotel light, swarming	9	
	01.VIII.2003	Santa Cruz	Town of Bellavista	2	
	03.VIII.2003	Santa Cruz	Near laundry room of private residence in Puerto Ayora	9	
				2003 Total: 11	
Ochlerotatus taeniorhynchus	16-17.VII.2003	Santa Cruz	Charles Darwin Research Station, ~1 km E Puerto Ayora, 6 m (CDRS), near scientists' dormitories)	2	
	17.VII.2003	Santa Cruz	Same data	1	
	20.VII.2003	Santa Cruz	CDRS (near Iguana rearing pens)	4	
	20.VII.2003	Santa Cruz	CDRS (near scientists' dormitories)	10	
	23.VII.2003	Santa Cruz	CDRS (near Iguana rearing pens)	36	
	28.VII.2003	Santa Cruz	CDRS (near scientists' dormitories)	91	
	01.VIII.2003	Santa Cruz	Town of Bellavista, 194 m (collected in same light trap as C. quinquefasciatus collected on this date).	11	
	03.VIII.2003	Santa Cruz	CDRS (outside of Ornithology Laboratory).	11	
				2003 Total: 166	

Annex 8: Details of students undertaking projects in the laboratory

Name	Degree	University	Project
Ms Pamela Martinez	BSc	University of Quito	Assesment of pathogens affecting Galapagos tortoises
Ms Karina Salinas	BSc	University of Quito	Development of molecular biology assays in Galapagos
Mr. Pablo Izquierdo	BSc	University of Quito	Micropropagation of endangered plants in Galapagos
Ms Ruth Llumiquinga	BSc	University of Quito	Micropropagation of endangered plants in Galapagos
Ms Patricia Jaramillo	MSc	University of Guayaquil	Development of AFLP markers Galapagos Calandrina populations

Annex 9: Galapagos mosquito guide

Slide 1

Reconocimiento de Mosquitos en las Islas Galapagos

• Son tres especies de mosquitos conocidos en Galapagos:

- Aedes aegyptii (introducido)
- Ochlerotatus taeniorhyncus (endemico)
- Culex quinquefasciatus (introducido)

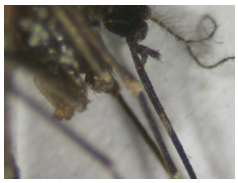
Objetivos:

• Como distinguir entre los géneros y las especies conocidas.
• Como reconocer y diferenciar especies de Diptera que se paracen a los mosquitos.
• Reconocer posibles otros géneros importantes de mosquitos no reportados.

LEPGG

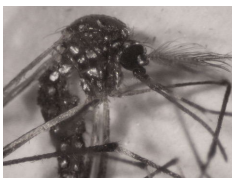
Slide 2

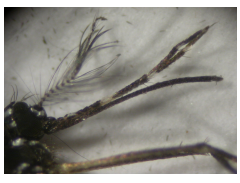
Diferencias entre macho y hembra de mosquitos



Hembra

Las palps son más cortas en relación al probóscide, y la antena es fina.





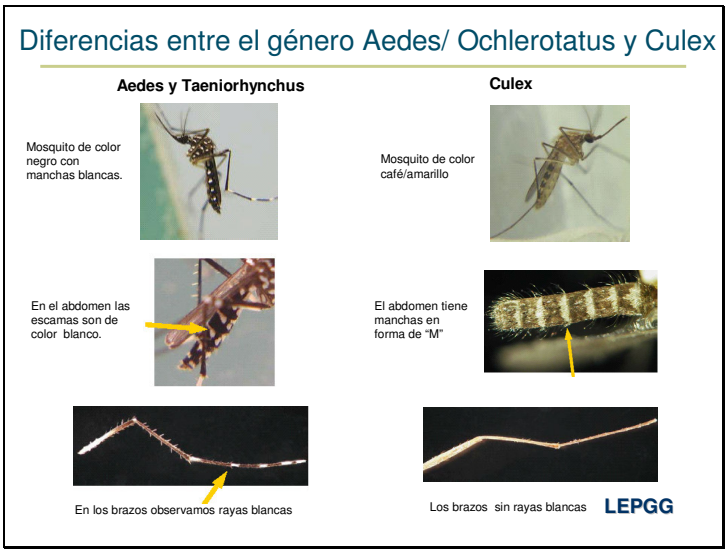
Macho

Las palps son más largas que el probóscide, y la antena es muy espesa.

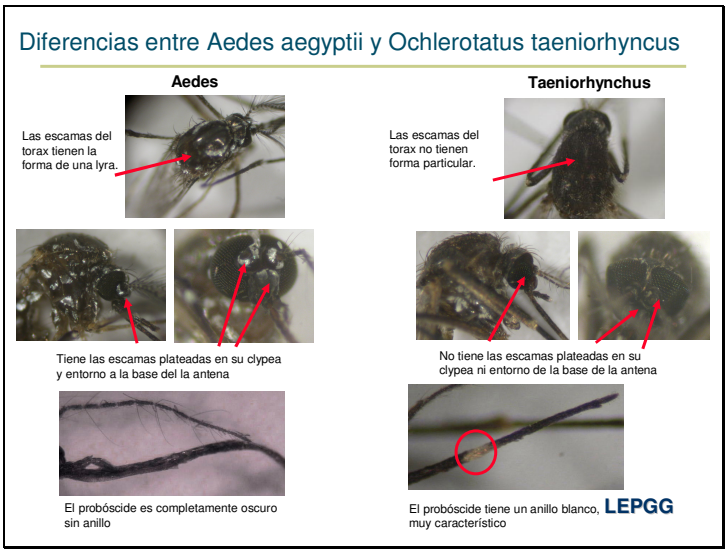
En las 3 especies conocidas en Galápagos, las hembras tienen las palps más cortas que el proboscide - en la género Anopheles (no conocida aqui) son más largas (diferencia muy importante).

LEPGG

Slide 3

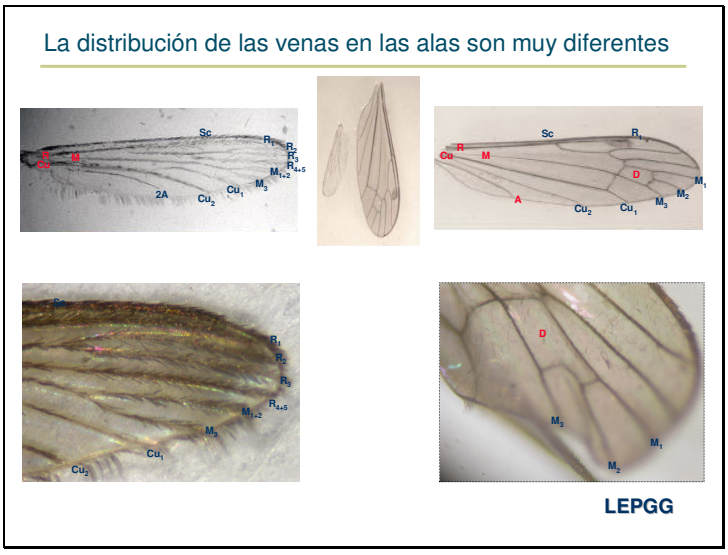


Slide 4



Slide 5

Slide 6



Annex 10 Other supporting material available on request

1. Policy maker briefing slide presentation for West Nile Virus risk assessment
2. Policy maker briefing slide presentation for vaccination policy
3. Vaccination briefing document
4. Galapagos lab tourist information leaflet (also available from website *http://www.biology.leeds.ac.uk/ggepl/)*
5. Galapagos Conservation Trust spring 2005 news letter article "West Nile Virus, a new plague in paradise?"
6. Numerous pictures of the lab and project activities