



Technical Progress Report

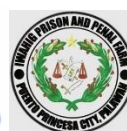
September - December 2016



Wildlife Reserves Singapore Group



Jewelmer



Peter and Indira Widmann

Katala Foundation, Inc.

Puerto Princesa, Palawan, Philippines

September 2017

TECHNICAL PROGRESS REPORT

COUNTRY: PHILIPPINES

PROJECT TITLE: PHILIPPINE COCKATOO CONSERVATION PROGRAMME
In-situ Conservation Project

PROJECT DURATION: September to December 2016

PROJECT SITES: Palawan, Philippines

PROJECT COOPERATORS:

Department of Environment and Natural Resources (DENR)
Municipal Government of Narra, Palawan, Philippines
Municipal Government of Dumarán, Palawan, Philippines
Municipal Government of Balabac, Philippines
Bgy. Pandanan Government, Balabac, Palawan, Philippines
Local Protected Area Management Committees (LPAMC)
Protected Area Management Board-RIWS (PAMB-RIWS)
Palawan Council for Sustainable Development Staff (PCSDS)
Jewelmer Corporation Inc.
Iwahig Prison and Penal Farm (IPPF)
Concerned agencies and authorities

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EXECUTIVE SUMMARY

Objective 1: Conservation of cockatoo population on Pandanan and Bugsuk Islands, Balabac

- Highest count for the roost count in Malinsuno was 183 cockatoos. Numbers declined early in the reporting period because of very strong winds affecting the roost site on Malinsuno and during the late part, since adult birds were already starting to occupy areas around their nest trees.
- Food supply was sufficient during the early weeks of the reporting period, with ten to fifteen fruit-providing tree species bearing fruits in September, thirteen species were available in October.
- All thirteen nest trees were monitored by the first week of October and ten already showed fresh marks of cockatoos, particularly bite marks around nest opening and fresh feces.
- Wardens replaced 14 of the Horseradish Tree cuttings which did not survive the earlier planting in this year's rainy season. Additional 15 cuttings of horseradish Tree and 32 cuttings of *Erythrina* were planted.
- No illegal activities were observed during patrols on Pandanan in September and December, however limited cutting of *Sonneratia* mangroves was observed in Buliluyan (southern tip of Palawan). In October apparently a small number of new settlers arrived in Sition Gabong on Pandanan. In November four newly cut trees were observed in vicinity of a cockatoo nest tree.
- A focus group discussion was conducted with thirty visitors during a Thanksgiving celebration in Bodis Area on Pandanan on November 21.
- Due to the security situation, visits to the project site by outside KFI staff are currently not possible.

Objective 2: Conservation of cockatoo population on Rasa Island, Narra

- Numbers of cockatoos in the traditional roost site kept dropping dramatically, as was already apparent in August 2016. So far six additional, but temporary roost sites have been found.
- Highest cumulative numbers of cockatoos on a site basis were increasing slightly within the reporting period from 89 in September to 109 in December.
- A large flock of ca. 110 birds was observed to forage in the *Sonneratia* area ca. 6-8 km SW of Rasa on October 3. Roosting was suspected.
- Since the year 2000, hatchlings are regularly sampled and screened for PBFD. All sampled birds tested negative over the years, until the results came back positive in 2016 for 14 out of 26 samples from Rasa Island, indicated by faint but distinct bands and ca. 700bp. Hatchlings from all other project site tested negative.
- In order to rule out false positives, we had five of the positive samples from Rasa DNA-sequenced. BLAST-matching did not indicate presence of the virus.
- Later in the year we then resampled all cockatoos we have in captivity, all samples were negative.
- However, three Budgerigar samples from birds purchased in Narra and Puerto Princesa tested positive which gives a clear indication of potentially high risk of infection of wild parrot populations in Palawan, should they get in contact with infected domesticated parrots.

- Flashing of newly discovered nest trees with painted iron sheets, and replacement of rusted ones commenced in October.
- Monitoring of claimed coconut areas did not indicate any expansion of areas or other illegal activities.
- On November 23, Scott Wilson and Dr. Simon Cowell visited the project site and had discussions with wildlife wardens and KFI staff in Panacan.
- Conservation education conducted in villages adjacent to Rasa reached more than 600 participants in this reporting period.
- As part of funding from Singapore Wildlife Reserves, visits to Rasa were organized for local stakeholders. On November 15, executives and lawmakers from the local government, members of the Rasa Island Wildlife Sanctuary Management Board, and other stakeholders took the opportunity to visit Rasa and to get updated on the conservation activities.
- For the time being we assume that our wild cockatoo populations are still PBFD-free, but there is a high risk of infection, since cockatoos regularly enter into residential areas, where potentially infected captive parrots are present.
- We started a dialogue with the local conservation and animal industry agencies, trying to find ways to restrict keeping of domestic parrots at least in the vicinity of remaining cockatoo populations.

Objective 3: Conservation of cockatoo population on Dumaran Island, Dumaran

- Numbers of cockatoos stayed stable on a relatively low level with between 15 and 17 birds counted throughout the reporting period.
- Seven birds intended for release were transferred from Katala Institute in Narra to the pre-release aviary in Omoi Cockatoo Reserve on Dumaran on August 30. Birds were given antibiotics mixed with 'Omnivore' feeding mix for one week and they settled in without major problems.
- Throughout the reporting period wardens patrolled the cockatoo reserve and monitored wildlife along the 29 biodiversity monitoring stations.
- No illegal activities were encountered in the Critical Habitat during patrolling throughout the reporting period.
- On October 19, the 25th Local Protected area Management Committee (LPAMC) Meeting was conducted in Dumaran Poblacion. The adoption of the declaration of the Critical Habitat by PCSD was endorsed by the LPAMC for adoption by the local council (SB) of Dumaran.
- In September, November and December a total of 8,228 native tree seedlings respectively were planted in the corridor.

Objective 4: Education and research at the Katala Institute

- Due to the initial result of PBFD-positive cockatoos on Rasa, all cockatoos under care in Katala Institute were re-sampled. All birds tested negative for the virus.
- Two cockatoos were rescued from Puerto Princesa suffering from slingshot or air gun wounds. One recovered, while the other one died.
- A training module for propagation of food- and nest-providing trees of cockatoos was finalized and field tested with staff of KFI within the current reporting period. Participants learn how to collect seeds, to prepare seed bags and to propagate trees with focus on food-providing plants for the cockatoo. During the reporting period,

eight groups comprising a total of 189 participants were instructed in these techniques, far surpassing the 60 anticipated participants in the project proposal.

- Pond dipping courses meanwhile continued, following the module developed during the previous reporting period.

Other highlights:

- One dead Dugong was recovered stranded from a beach opposite of Rasa Island on September 16. The animal had healed scar, possibly caused by boat propellers, but no recent external injuries.
- A pre-summit stakeholder consultation regarding Palawan Integrated Natural Resource Management Project has been participated on October 21. KFI has voiced out its concerns particularly the plans to construct 'ring roads' around remaining forest areas, and the classification of lowland forests as production forests.
- Siegfred H. Diaz retired from his service with KFI, after being with the organization from the very start and being a founding member of Katala Foundation.

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ACRONYMS

BMB	Biodiversity Management Bureau (formerly PAWB)
CE	Conservation Education
CENRO	Community Environment and Natural Resources Office(r)
DENR	Department of Environment and Natural Resources
ELAC	Environmental Legal Assistance Council
ENIPAS	Enhanced National Integrated Protected Area System
IUCN	International Union for the Conservation of Nature and Natural Resources
KEEC	Katala Environmental Education Center
KFI	Katala Foundation, Inc.
KI	Katala Institute
LGU	Local Government Unit
LPAMC	Local Protected Area and Management Committee
LPF	Loro Parque Fundación
MENRO	Municipal Environment and Natural Resources Officer/Office
MOA	Memorandum of Agreement
PA	Protected Area
PAMB	Protected Area Management Board
PASu	Protected Area Superintendent
PCCP	Philippine Cockatoo Conservation Program
PCSD(S)	Palawan Council for Sustainable Development (Staff)
PENRO	Provincial Environment and Natural Resources Office
PFTCP	Philippine Freshwater Turtle Conservation Program
PNP	Philippine National Police
PWRCC	Palawan Wildlife Rescue and Conservation Center
RA 9147	Republic Act 9147 otherwise known as the Wildlife Protection Act
RIWS	Rasa Island Wildlife Sanctuary
SDENRO	Special Deputy Environment and Natural Resources Officer
WPU	Western Philippines University
ZGAP	Zoologische Gesellschaft für Arten- und Populationsschutz

INTRODUCTION

The Philippine Cockatoo *Cacatua haematuropygia*

The Philippine Cockatoo or Red-vented Cockatoo *Cacatua haematuropygia* is restricted to lowland forest areas and mangroves in the Philippines. Formerly, it could be found all over the archipelago (Dickinson *et al.* 1991). Only in the last decades a rapid decline set in, which brought the species to the brink of extinction (e.g. Boussekey 2000a; Lambert 1994). The reasons for the decline of the populations are (e.g.; Collar *et al.* 1999; Lambert 1994; Widmann *et al.* 2001):

- Habitat destruction, particularly in respect of nesting and food providing trees.
- Persecution as crop pest.
- Poaching for pet trade.
- Potential diseases caused by the introduction of captive birds in the range of wild populations.
- Tropical storms and typhoons

Habitat destruction and poaching are the most important factors threatening the Philippine Cockatoo.

Since 1888 Katala Foundation Inc. (KFI) implements the PCCP in the Philippines. Comprehensive conservation projects in this phase are currently undertaken in three sites in Palawan (Fig. 1): Rasa Island (Narra), Dumarán Island (Dumarán), Pandanan and Bugsuk Islands (Balabac). The two former sites contain by now protected areas declared on municipal or higher levels, specifically established to include the cockatoo populations. The Pandanan site is predominantly owned by Jewellmer Corporation, with which KFI has a Memorandum of Agreement for the conservation of the species.

We estimate that between 640–1,120 Philippine Cockatoos exist in the wild (assuming few populations have been overlooked in recent surveys of historical locations, and 100-150 individuals survive in the Sulus, for which only incomplete information is available).

The single-most important Philippine Cockatoo population on Rasa is secured under presidential proclamation as “Rasa Island Wildlife Sanctuary” since February 2006, in addition to local legislations. Highest population count was 317 individuals in 2014. Pandanan, holds possibly the second-most important population with at least 220 birds (up from 80) individuals.

With these three project sites in Palawan, it is estimated that between a third to half of the remaining wild population is currently covered in PCCP projects. Cockatoo populations are stable or increasing in all sites, and improved legal conservation could be achieved (e.g. through creation of cockatoo reserves). However, law enforcement by state agencies remains weak and pressure on these areas is rather increasing (migrant influx to Palawan, mining, planned large-scale projects, like biofuel plantation or coal plant).

Warden schemes remain the single-most important tool to assure the short-term survival and recovery of the species, whereas lobbying, conservation education, habitat restoration and reintroduction, as well as provision of alternative livelihood options are important for the long-term improvement of the frame conditions for cockatoo conservation in the Philippines.

Objective of the Philippine Cockatoo Conservation Program

Conservation and restoration of the most viable subpopulations of the Philippine Cockatoo and their habitats, including associated flora and fauna under involvement of all key stakeholders, resulting in a down-listing of the species from 'Critical' to 'Endangered' through reversing its population decline and under consideration of the precautionary principle.

Program Strategy



The main strategy of the programme is to conserve *in-situ* the most important subpopulations of the Philippine cockatoo through adopting participative methods.

The general program strategies are:

- Management of local resources in the framework of Philippine law;
- Capacity-building for local decision-makers and key stakeholders to ensure sustainability of the conservation efforts; and,
- Ecosystemic conservation approach with the Philippine cockatoo as flagship species.

Figure 1. Map of the Philippines indicating sites of the Philippine Cockatoo Conservation Program: 1. Pandanan, Balabac; 2. Rasa Island, Narra; 3. Katala Institute, Narra; 4. Omoi and Manambaling Cockatoo Reserves, Dumarang; 5. Iwahig Prison and Penal Farm, Puerto Princesa; 6. Kangbangyo and Poneas Islands, Del Carmen. Black: project sites covered in this report; red: other PCCP sites

Deliverables

Objective 1: Conservation of cockatoo population on Pandanan and Bugsuk Islands, Balabac

- Warden scheme on Pandanan and Bugsuk Island continued and extended to adjacent mainland.
- Monitoring of cockatoo population and habitat on Pandanan and Bugsuk Island continued.
- Conservation education in Pandanan Island and adjacent mainland continued.
- Research on conservation-related aspects of cockatoo biology on Pandanan and Bugsuk continued, with focus on factors influencing breeding success and foraging ecology.

- Advocacy in respect to impacts and perpetrations in cockatoo habitats, particularly networking with local stakeholders, particularly Jewelmer Corporation, the largest private landowner, continued.

Objective 2: Conservation of cockatoo population on Rasa Island, Narra

- Warden and mainland volunteer scheme continued.
- Members of Protected Areas Management Board in the management of the Philippine Cockatoo and Rasa Island Wildlife Sanctuary capacitated and meetings facilitated.
- Conservation education for stakeholders continued.
- Research on conservation-related aspects of cockatoo biology on Rasa continued, with focus on factors influencing breeding success and foraging ecology.
- Advocacy in respect to impacts and perpetrations in cockatoo habitats continued.

Objective 3: Conservation of cockatoo population on Dumarán Island, Dumarán

- Warden scheme continued.
- Members of Local Protected Areas Management Committee in the management of the Philippine cockatoo, as well as Omoi and Manambaling Cockatoo Reserve assisted and capacitated.
- Research on conservation-related aspects of cockatoo biology on Dumarán continued, with focus on factors influencing breeding success and foraging ecology.
- Buffer zone restoration around existing cockatoo reserves continued.
- Creation of forest corridor connecting the two existing cockatoo reserves continued.
- Advocacy in respect to impacts and perpetrations in cockatoo habitats continued.

Objective 4: Education and research at the Katala Institute for Ecology and Biodiversity Conservation

- Captive management of Philippine Cockatoo and other highly threatened species continued through employment and training of zookeepers and volunteers.
- Landscaping with native species propagated in the Katala nursery continued.
- Educational trail, enclosures and visitors facilities upgraded.
- Proposal submission to other potential donors continued.

Description of Project Sites

Rasa Island, Narra, Palawan

Rasa is a small coral island of 8.34 km² land area situated in the Sulu Sea, just offshore of the Municipality of Narra, Palawan, Philippines (Fig. 2). About 1.75 km² are covered with coastal forest, mangrove (5.60 km²), cultivated areas (predominantly coconut; 0.39 km²), 0.60 km² are barren or sparsely vegetated sand and coral outcrops. In February 2006, the island became a Wildlife Sanctuary through Presidential Proclamation 1000 and since a Protected Area Management Board is functioning as management body for Rasa Island Wildlife Sanctuary (RIWS). In 2008, RIWS was chosen as Top 13 Bird Watching Sites in the Philippines by the Department of Tourism.

The island is the pilot site of the program since 1998. Due to intensive poaching, only 23-25 Philippine cockatoos were left on the island then. Key component of this project site is the warden scheme which involves patrolling and protection of the birds during and outside the breeding season. This scheme has proven to be efficient and lead to dramatic recovery of the Philippine Cockatoo population as of to date. As of 2014, 317 cockatoos have been simultaneously counted on roost sites on Rasa and adjacent mainland.

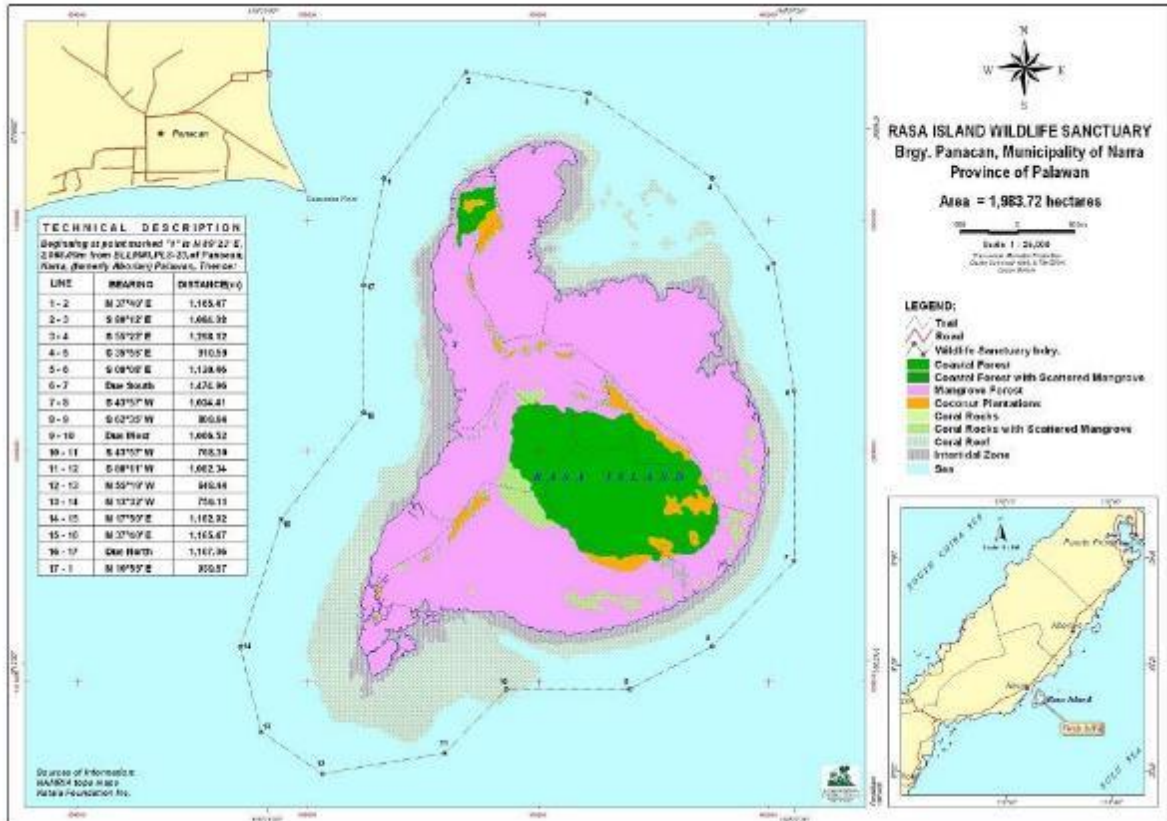


Figure 2. Vegetation cover of Rasa Island Wildlife Sanctuary, Palawan, Philippines

Rasa Island probably therefore holds the highest population density of Philippine Cockatoo that remains in the wild. The world population of Philippine cockatoo was estimated to range between 1,000 to 4,000 individuals (Lambert 1994). More recent estimates put the number of cockatoos remaining in the wild between 640 and 1,120. About 75% of this population is found in Palawan.

Not only Philippine Cockatoos live on the island, but a variety of other species, with an unusual high percentage of globally threatened and near-threatened taxa (IUCN 2015), considering the small size of Rasa. Noteworthy among the 112 recorded bird species are Red-headed Flameback *Chrysocolaptes erythrocephalus* (EN), Grey Imperial-pigeon *Ducula pickeringii* (VU) and Mantanani Scops-owl *Otus mantananensis* (NT).

Dumaran Island, Dumarang, Palawan

Dumaran is situated in north-eastern Palawan between 10°22' and 10°41'N and 119°28' and 119°55'E. Nine Barangays are situated on Palawan mainland, seven on western Dumaran

Island. The island is situated in the Sulu Sea and separated by a ca. seven km wide channel from the mainland.

PCCP currently manages three areas on the island: Omoi and Manambaling Cockatoo Reserves (Fig. 3) and the traditional roosting site in Lagan. A Local Protected Area Management Committee (LPAMC) functions as its management body. Both cockatoo reserves, a buffer zone and a corridor connecting both areas was declared as critical habitat, comprising 1,500 ha.

All natural terrestrial ecosystems in Dumarán are tree-dominated. On Dumarán Island only few small and isolated forest patches remain, none of them larger than 103 ha. The most abundant formation is evergreen and semi-evergreen lowland forest with Ipil *Intsia bijuga*, Amugis *Koordersiodendron pinnatum* being emergent tree species of commercial value.

Ornithological surveys conducted by Katala Foundation so far yielded 136 species from the island. A prominent species of conservation concern is the Philippine cockatoo, which can be found with viable population in the mangroves and forest remnants of Dumarán Island, but apparently not anymore on the mainland. The last remaining forest patches are therefore of global conservation concern. This notion is supported by the recent records of other globally threatened species, particularly the Palawan Forest Turtle *Siebenrockiella leytensis* (CR).

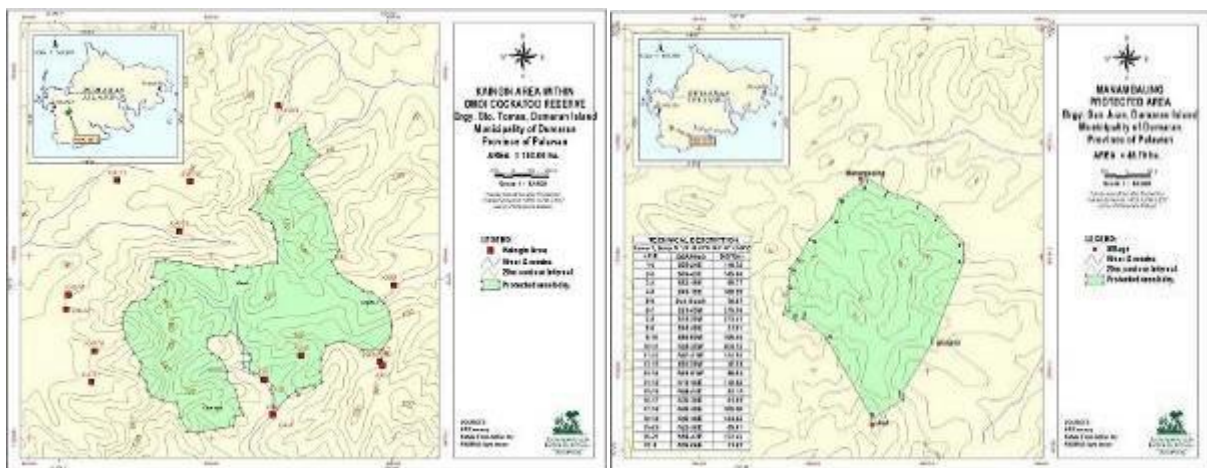


Figure 3. Omoi Cockatoo Reserve (left) and Manambaling Cockatoo Reserve (right) cover the last forest patches on Dumarán Island.

Other species of conservation concern are Palawan Hornbill *Anthraceroceros marchei* (VU), Blue-headed Racquet-tail (VU) and Palawan Pencil-tailed Tree-mouse *Chiropodomys calamanianensis* (DD).

Habitat degradation and destruction, rather than poaching, remain the biggest challenges for cockatoo conservation in Dumarán. In the current phase ca. five hectares of secondary forest and grassland were purchased with support of the Stadtholding Landau in the course of a carbon-mitigation project. These areas have been rehabilitated and integrated in the buffer zone of the Omoi Cockatoo Reserve.

The Critical Habitat established through PCSD Resolution No. 14-513 connects the two existing cockatoo reserves through a corridor and extends to include remaining forest fragments in the area. This is the first critical habitat established in the Province of Palawan.

Pandanan Island, Balabac

Pandanan Island in Bgy. Pandanan belongs to the north easternmost municipality of Balabac in Palawan (Fig. 4). Coastal forests are dense and stock on flat limestone originating from elevated coral reefs. Large trees in the coastal forest are mostly deciduous and widely spaced due to water stress during the dry season. The understory is very dense with abundant vines. Emergent trees comprise the genera *Dipterocarpus*, and *Ficus*. A narrow rim of beach forest with *Erythrina*, *Calophyllum* and *Barringtonia* is present. The dense coastal forest cover is as well protected because the large portion of the island is privately-owned and entries are monitored by private guards. Coconuts are the major crop grown in the coastal areas and shifting cultivation including lowland rice, corn, and root crops inside forested areas are common land use forms. Extensive mangroves are thriving.

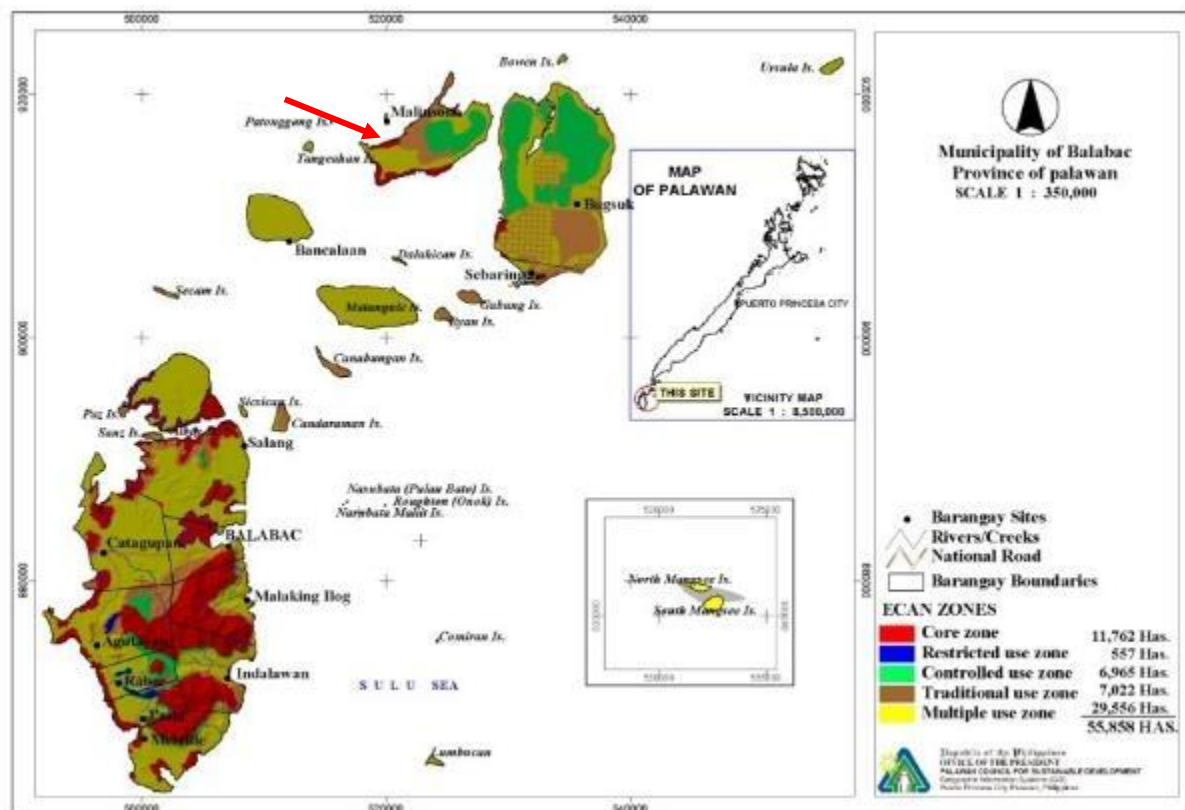


Figure 4. Location map of Pandanan Island indicated by red arrow (Map Source: PCSDS).

So far, 91 bird species have been recorded in Pandanan and adjacent Malinsuno. Among these are six globally threatened and six near-threatened species (IUCN 2015). Of outstanding conservation concern are particularly the larger tree cavity nesters, like Palawan Hornbill, all three parrot species of Palawan, Philippine Cockatoo, Blue-naped Parrot and Blue-headed Racquet-tail, and other conservation relevant species like Grey Imperial-pigeons and Mantanani Scops-owl (Widmann *et al.* 2008). The first and only record for the Philippines of a Fairy Pitta *Pitta nympha* comes from Malinsuno as a result of the conservation project.

The implementation of the warden scheme recruiting cockatoo poachers resulted in significant increases of the cockatoo population in the first years of project implementation, comparable to those of the early stages on Rasa Island. In recent surveys, roosting site is at a coconut plantation in Malinsuno Island just across Pandanan Island.

Methods

The Philippine Cockatoo Conservation Programme (PCCP) employs an ecosystemic and community-based approach to biodiversity conservation using the Philippine cockatoo as its flagship species. The main components of the program are nest protection or warden schemes; scientific researches on feeding, biology and ecology of the Philippine Cockatoo and other threatened species; identification, protection and management of key conservation sites; conservation education; habitat restoration; and capacity building. Researches on the Philippine Cockatoo include distributional surveys, rescue of individual birds, reintroduction and translocation assessments, and captive management for conservation education and conservation breeding for later reintroduction.

Information on the biology and ecology of the cockatoo is gathered mainly through direct observation. On Rasa, movements of the cockatoos can be best observed from a boat, from beaches or coral outcrops. Very dense vegetation on the island considerably hampers visibility on transect walks or point counts. On Dumaran and Pandanan movements are observed through wardens monitoring and patrols at protected areas and roost sites.

Monitoring of the population trend on Rasa, Dumaran and Pandanan is done through counting individuals at a traditional roost site. A traditional roost site is situated in a mangrove area on Rasa and can be observed from a boat while in Dumaran a privately-owned coconut plantation serves as the roost site bordering close to a mangrove area. On Balabac, at least two roosting sites are presently monitored; one in Malinsuno Island and the other on Pandanan Island. Counts are conducted monthly either before sunset on Rasa and Balabac islands and daily on Dumaran. Counts are also conducted during dawn before birds leave the roost site. Whenever possible, counts on Rasa are conducted under similar weather and light conditions.

The core component in all project sites is the warden scheme, employing former poachers as wildlife wardens. Wardens inspect and verify existing and potential nest trees starting end of September. During the breeding season, the nest trees are under permanent surveillance. Trees are climbed and nest holes controlled every ten days during that time. For safety reasons, dead or damaged trees are not climbed. Nest trees are characterized through species identification, tree height, diameter at breast height (DBH), height of nest hole, exposition of nest hole, diameter of hole, and diameter at base and depth of cavity. The geographic location of each nest tree is taken with the help of a GPS and marked in a map.

Presence or absence and condition of adult birds, eggs, nestlings or nest predators are noted. Nestlings are weighed with Pesola spring balances/and or electronic balance and banded with aluminum rings bearing the inscription of the Department of Environment and Natural Resources (DENR), the number and year (e.g. DENR 0001-15).

Volunteers are detailed in monitoring stations at the mainland coasts of Narra within and outside the breeding season. These volunteers record all sightings of cockatoos and other significant wildlife in the area of assignment.

Surveys to find remnant cockatoo populations are based on historical sources or recent information. To initially narrow down the searches, non-formal interviews with key informants (poachers, other forest users, barangay officials, school teachers) are conducted. Surveys aim to identify remnant cockatoo populations or areas which are suitable for translocation.

Herbarium collections are made of key plants in cockatoo habitats, particularly food-providing plants, and nest and roost trees. The physical structures of cockatoo breeding habitats are characterized through forest profiles. Phenological information on fruiting and flowering of food-providing trees are systematically collected on Rasa and Dumaran.

Larger-scale restoration of lowland forest habitat is currently done in Dumaran. Particularly nest- and food-providing plants for cockatoos are systematically tested for their suitability for reforestation. MS Access and excel programs are used for analysis.

Composition of remaining bird communities in project and survey sites is assessed using MacKinnon-Lists and, occasionally, mist-netting. Composition of mammal, reptile and amphibian communities in project sites is assessed through direct observations, mist- and harp-netting, live-trapping (Sherman type and locally-made cage type) and pitfall trapping.

To identify potential cooperators for the projects, livelihood needs, and capacities, stakeholder and SWOT analyses are employed. Participatory planning is done through goal-oriented project planning methodology. Alternative livelihood is provided for key-stakeholders of the cockatoo and the PAs, based on the needs assessments.

Conservation education activities employ the PRIDE approach which uses marketing methodologies to galvanize community support for conservation. The approach conducts pre and post project surveys to assess changes in levels of knowledge, awareness and behavior among target audience by using control groups. Survey Pro is used for analysis on changes over time. Proven marketing vehicles like billboards, posters, fact sheets, puppet shows, school and community visits, festivals and media participation are used to deliver relevant and compelling conservation messages.

Relevant trainings and seminars are conducted to help capacitate local partners in conservation. Cross visits to Rasa and other project sites are encouraged to facilitate exchange of experiences, lessons learned and good practices to boost morale of local partners and reinforce knowledge.

Please refer to each output for particular methodologies used in achieving results.

Results and Progress

Objective 1: Conservation of cockatoo population on Pandanan and Bugsuk Islands, Balabac

Research on conservation-related aspects of cockatoo biology on Pandanan and Bugsuk continued, with focus on factors influencing breeding success and foraging ecology

Roosting

Highest count for the roost count in Malinsuno was 183 cockatoos. Numbers declined early in the reporting period because of very strong winds affecting the roost site on Malinsuno and during the late part, since adult birds were already starting to occupy areas around their nest trees. A temporary roost site on Pandanan was occupied which also was in closer proximity to fruiting *Sonneratia*.

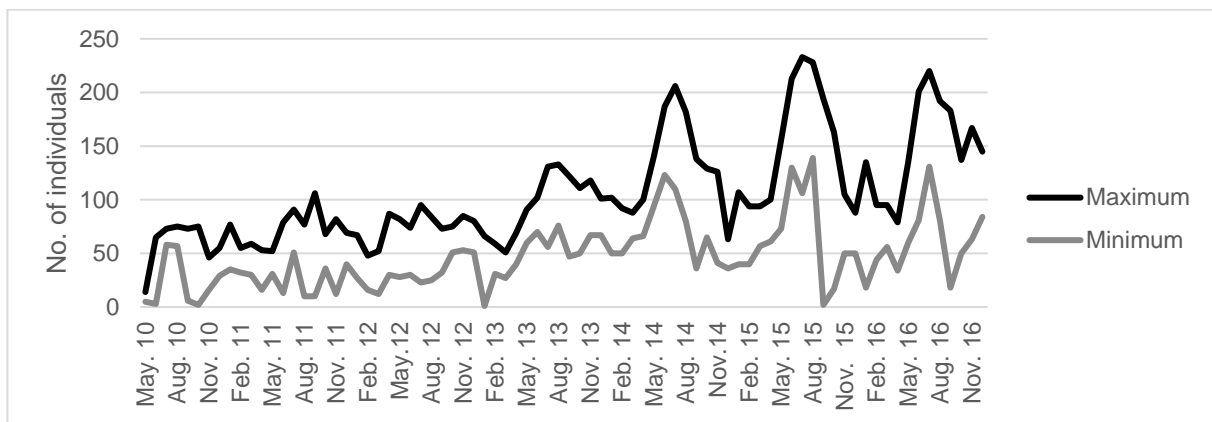


Figure 5. Minimum and maximum numbers of cockatoos roosting on Malinsuno, Balabac, by month

Foraging

Food supply was sufficient during the early weeks of the reporting period, with ten to fifteen fruit-providing tree species bearing fruits in September, thirteen species were available in October. In the same month flocks up to 50 cockatoos were observed foraging in *Sonneratia* stands on the southern tip of Palawan. Herbarium specimens of food-providing trees were collected throughout November.

Breeding

All thirteen nest trees were monitored by the first week of October and ten already showed fresh marks of cockatoos, particularly bite marks around nest opening and fresh feces. Cockatoos were observed around three nests in early November. In December already nine trees showed signs of occupation.

Warden scheme continued

Wardens replaced 14 of the Horseradish Tree cuttings which did not survive the earlier planting in this year's rainy season. Additional 15 cuttings of horseradish Tree and 32 cuttings of *Erythrina* were planted and the area was weeded in October. Also in October, 72 *Rhizophora* seedlings were planted experimentally near the temporary cockatoo roost site in Dalahican on Pandanan Island.

In October 1,276 raptors were recorded migrating southwards in large groups, probably to avoid the super typhoon 'Lawin'. Of these were 1,250 Grey-faced Buzzards *Butastur indicus*

and 26 Ospreys. The large majority was counted on October 24, when winds changed direction from SW to N and therefore facilitating easier migration.

No illegal activities were observed during patrols on Pandanan in September, however limited cutting of *Sonneratia* mangroves was observed in Buliluyan (southern tip of Palawan) done by new settlers to make way for stilt houses. In October apparently, a small number of new settlers arrived in Sitio Gabong on Pandanan.

In November four newly cut trees were observed in vicinity of a cockatoo nest tree. Only few parts of the lumber could be utilized by the timber poachers, since all trees had more or less rotten cores.

No illegal activities were recorded in December.



Figure 6 Planting of *Erythrina* cuttings, a very important but now quite rare food-providing tree for cockatoos (Photos: R. Antonio)

Conservation education

A focus group discussion was conducted with thirty visitors during a Thanksgiving celebration in Bodis Area on Pandanan on November 21. Participants were concerned about apparently increasing illegal logging activities.

Constraints and measures taken

- Due to the security situation, visits to the project site by outside KFI staff are currently not possible. Site coordinator and wardens have to work independently on site, and Rene is reporting once a month to the Katala office in Puerto Princesa.

Objective 2: Conservation of cockatoo population on Rasa Island, Narra

Research on conservation-related aspects of cockatoo biology on Rasa continued, with focus on factors influencing breeding success and foraging ecology

Roosting

Numbers of cockatoos in the traditional roost site kept dropping dramatically, as was already apparent in August 2016. So far six additional, but temporary roost sites have been found (two on Rasa, one on a nearby small island and the remaining on the mainland), but synchronized counts are virtually impossible due to unpredictability of birds and lack of manpower. This year's offspring seem not to use the traditional roost site anymore. Also for

the first time since we started monitoring, a single temporary undisclosed roost site held more cockatoos than the two sites on Rasa combined. It remains to be seen, if the traditional roost site will be abandoned completely after more than 19 years of monitoring, or if bird numbers there will increase again.

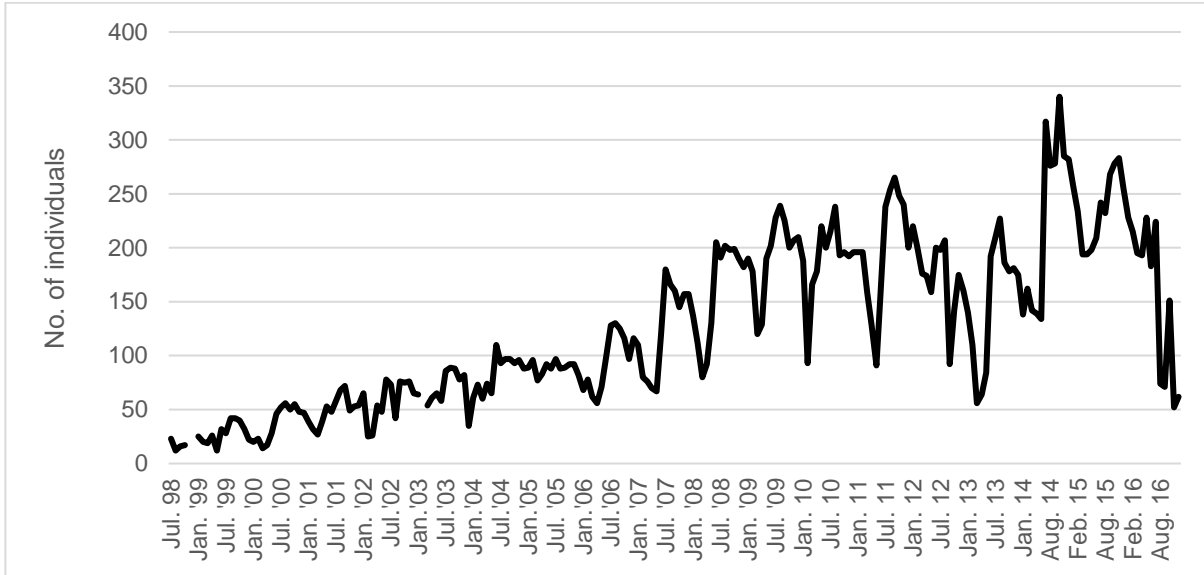


Figure 7. Numbers of cockatoos on simultaneous counts in traditional and transient roost sites in Rasa and adjacent mainland.



Figure 8. Two of the temporary roost sites on mainland opposite of Rasa Island (Photos: A. Agullo)

Foraging

Highest cumulative numbers of cockatoos on a site basis were increasing slightly within the reporting period from 89 in September to 109 in December. Some high numbers (80-90 birds) were also encountered on a newly established roost site in the centre of Panacan, so that it is not always possible to distinguish between foraging and roosting birds. Overall there is an upward trend of foraging birds on the mainland, regarding flock size and number of sites visited.

The most important foraging site remains in Panacan in Marcelo area, where most Horseradish trees were planted and are permanently maintained. A Municipal Resolution is

in place which requires residents with suitable home lots to plant these trees for own consumption and to provide foraging grounds for cockatoos.

A large flock of ca. 110 birds was observed to forage in the *Sonneratia* area ca. 6-8 km SW of Rasa on October 3. Roosting was suspected since birds were observed leaving a coconut area before 7.00 a.m., but search for a roost site in the area did not yield positive results.

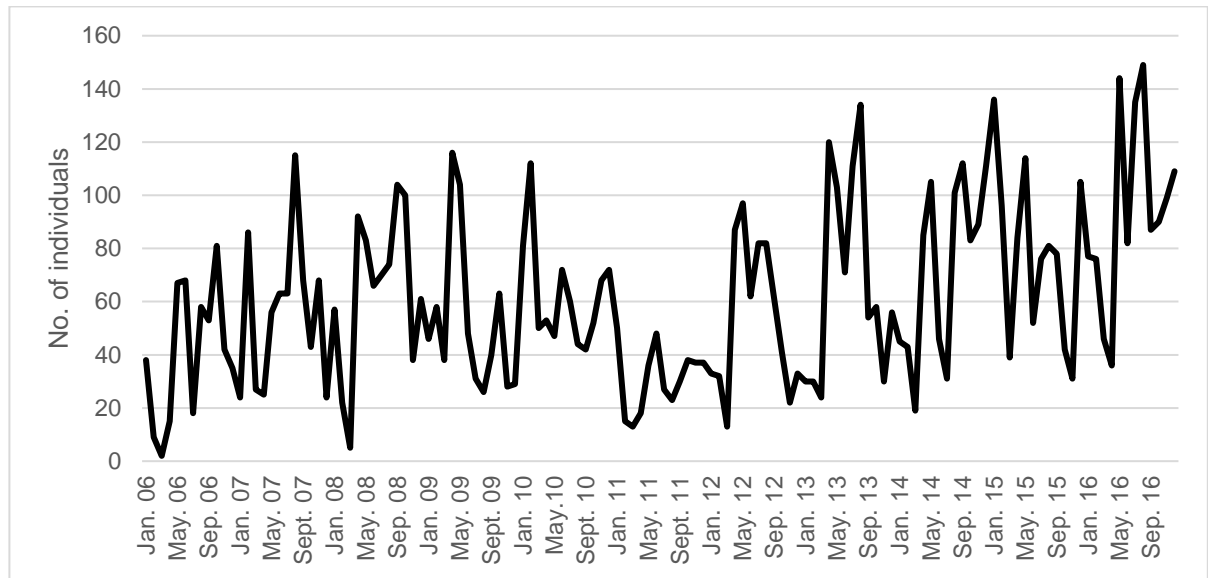


Figure 9. Highest cumulative daily number for each month of cockatoos transferring from Rasa to mainland

Psittacine Beak and Feather Disease Screening

Since the year 2000, hatchlings are regularly sampled and screened for PBFD within each breeding season in all project sites. Initially tests were done in the UK in a commercial lab, but in the past years this is done together with the DNA Barcoding Lab, University of the Philippines Diliman. All birds tested negative over the years, until the results came back positive in 2016 for 14 out of 26 samples from Rasa Island, indicated by faint but distinct bands and ca. 700bp. Hatchlings from all other project site tested negative. Polymerase chain reaction was used to amplify DNA, and test for the virus was done with a commercial test kit (PureLink® Genomic DNA Mini Kit, Invitrogen, USA).

In order to rule out false positives, we had five of the positive samples from Rasa DNA-sequenced through University of the Philippines and FirstBase Asia in Malaysia. BLAST-matching did not indicate presence of the virus. Later in the year we then resampled all cockatoos we have in captivity, some of which were intended for translocation, as well as three samples from domestic budgerigars. All samples of our cockatoos were negative. However, all three budgerigar samples tested positive which gives a clear indication of potentially high risk of infection of wild parrot populations in Palawan, should they get in contact with infected domesticated parrots. A survey of kept pet birds in Panacan revealed that at least one household has Budgerigars which are kept in an outside aviary. The location is well within cockatoo foraging areas.

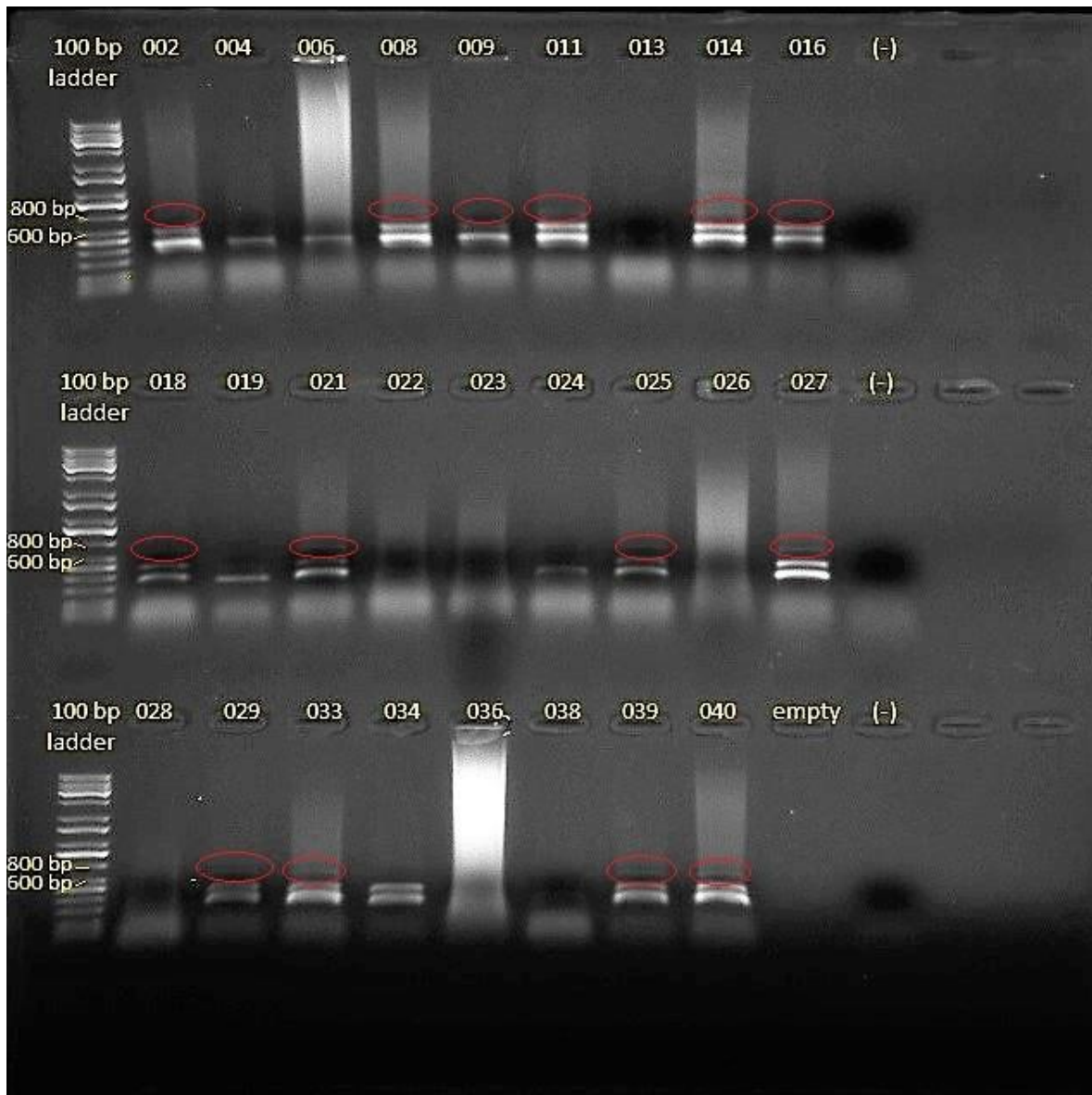


Figure 10. Positive samples from Rasa Island indicated by faint yet distinct band (red) at ca. 700 bp.

Warden and mainland volunteer scheme continued

Checking of nest trees on Rasa resumed by the last week of September. Flashing of newly discovered nest trees with painted iron sheets, and replacement of rusted ones commenced in October.

Monitoring of claimed coconut areas did not indicate any expansion of areas or other illegal activities. Phenology of vegetation on Rasa was recorded throughout the reporting period. Nest characterization was conducted for newly discovered nest sites.

Repair of the collapsed boardwalk was discussed with DENR Park superintendent; DENR was taking the lead in this activity. In October, wardens also assisted DENR personnel in marine assessment of corals and seagrasses around Rasa.



Figure 11. Painting of metal sheets for nest tree flashing (left); service boat repairs (right; Photos: S. Diaz)

Wardens and volunteer fisherfolks continue to provide data on captured commercial fish and sea cucumbers with indications of capturing efforts and market prices.

Whenever time permitted, wardens assisted in tree nursery work and related activities in Katala Institute.

On November 23, Scott Wilson and Dr. Simon Cowell visited the project site and had discussions with wildlife wardens and KFI staff in Panacan.



Figure 12. Dr. Simon Cowell and Mr. Scott Wilson from Chester Zoo were able to approach feeding cockatoos in Panacan opposite of Rasa Island (left), and during a meeting with wildlife wardens, mainland volunteers and KFI staff at the exit point to Rasa (right; Photo: P. Widmann)

Conservation education activities for stakeholders

On September 13 an education campaign was conducted in Caguisan Malinau 2 involving ca. 80 and 120 participants respectively. Other one was conducted in Poblacion, which reached 112 participants, in Antipuluan Elementary School on September 19 and 20 (with 400 participants), as well as in Calategas National Highschool on September 30. Topics covered included conservation activities of KFI and impacts of climate change.

On October 21, KFI participated in Narra's annual Palay Festival. One of the highlights was the participation of the cockatoo mascot during the parade.

From November 6-8, a capacity building workshop was conducted in Narra by DENR in cooperation with KFI for the members of the Protected Area Management Board (PAMB) of Rasa Island Wildlife Sanctuary (RIWS). Topics included pertinent laws and regulations in relation to protected area management, manual of operation for RIWS and a continuation of the revision of the management plan for the area.



Figure 13. On November 15 members of the PAMB and other stakeholders were informed on the result of the valuation study of Rasa and on the result of other research activities (Photos: KFI)



Figure 14. Conservation education activities conducted by Fred and Anna (Photos: KFI)

A METT-assessment for Rasa Island with involved stakeholders was conducted from November 10 to 13. This activity is part of the GIZ-funded project to enhance management for Rasa, and will provide data on performance during the implementation period, measured against the baseline collected before its start.

In discussions with locals from Narra, we got the impression that many of them never had an opportunity to visit the protected area at their doorstep. As part of funding from Singapore Wildlife Reserves, visits to Rasa were organized for local stakeholders. Two basic itineraries for Rasa Island were prepared which take into account time of the day (since foraging

cockatoos can only be observed in the morning; roosting is in the evening), and depending on weather and tides (roost site can only be visited in clear weather conditions, visit of interior of Rasa only possible during high tide). The package consists of three components: orientation and cockatoo observation from Palawan mainland, boat trip to Rasa (tidal flat, coral reef, seagrass bed and mangroves, cockatoo roost site), and Rasa interior (wildlife warden's camp, birdwatch tower, coastal forest).

On November 15, executives and lawmakers from the local government, members of the Rasa Island Wildlife Sanctuary Management Board, and other stakeholders took the opportunity to visit Rasa and to get updated on the conservation activities. Other groups involving teachers and students, fisher folks, and others proceeded and followed this activity.



Figure 15. Local officials from villages adjacent to Rasa were invited for a guided tour through the protected area of Rasa. For some this was the first time to visit the island (Photos: KFI)

Advocacy in respect to impacts and perpetrations in cockatoo habitats continued

Indira is member of the ECAN-Board in Narra, which assesses environmental impacts on proposed projects and gives recommendations to PCSD. She is occasionally represented by Anna in these meetings.

Constraints and measures taken

- For the time being we assume that our wild cockatoo populations are still PBFD-free, but there is a high risk of infection, since cockatoos regularly enter into residential areas, where potentially infected captive parrots are present. Cockatoos in captivity seem to be heavily affected by PBFD, and carrier of the virus without symptoms seem to be very rare. We have not yet observed cockatoos in the wild which show similar symptoms as infected birds in captivity.
- We started a dialogue with the local conservation and animal industry agencies, trying to find ways to restrict keeping of domestic parrots at least in the vicinity of remaining cockatoo populations. We also want to examine a bigger sample of domestic parrots, also including other species which are commonly kept, including lovebirds, cockatiels and blue-naped parrots. Finally, we want to improve our sampling protocol, so we can avoid false positives (and false negatives as well).

Objective 3: Conservation of cockatoo population on Dumaran Island, Dumaran

Research on conservation-related aspects of cockatoo biology on Dumaran continued, with focus on factors influencing breeding success and foraging ecology

Roosting

Numbers of cockatoos stayed stable on a relatively low level with between 15 and 17 birds counted throughout the reporting period. Released birds continue to stay apart from the roosting site, but still join the wild flock for foraging.

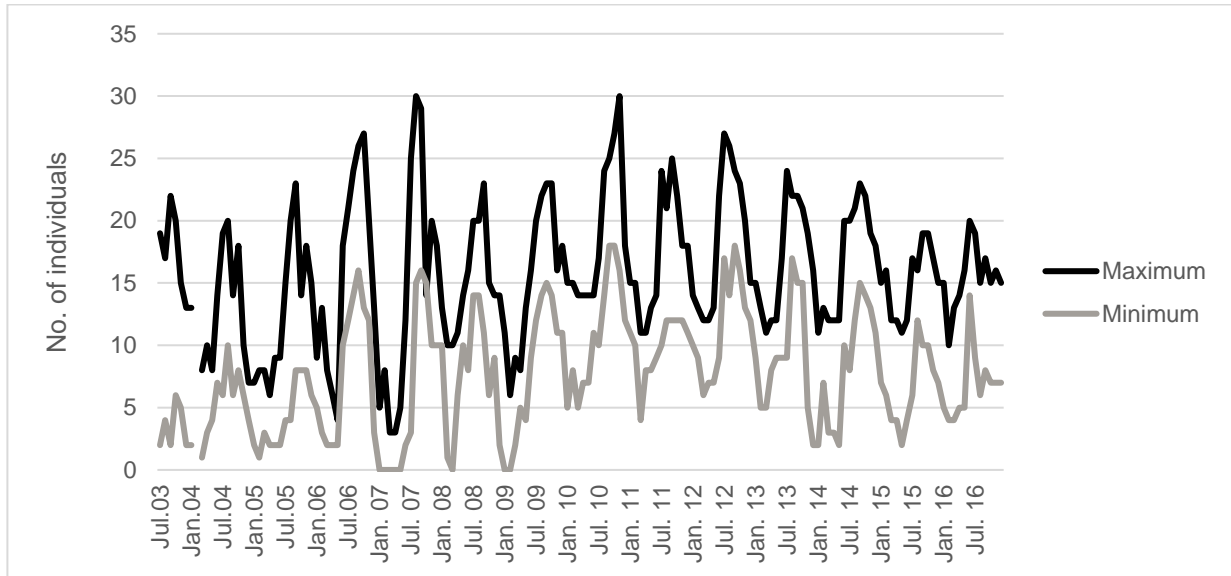


Figure 16. Minimum and maximum numbers of cockatoos counted on the traditional roost site in Lagan, Dumaran per month

Translocated cockatoos

Seven birds intended for release were transferred from Katala Institute in Narra to the pre-release aviary in Omoi Cockatoo Reserve on Dumaran on August 30. Birds were given antibiotics mixed with 'Omnivore' feeding mix for one week and they settled in without major problems. Starting mid of September, birds were fed with naturally occurring food items, during which their weight dipped for a couple of days, but was recovered in less than a week. They were already weaned to these foods in Katala Institute, before being brought to Dumaran, so this change did not pose any difficulties. Angel, our zookeeper from Narra, stayed for nine days on Dumaran to instruct wardens in proper bird handling and monitoring.

Panels of the aviary facing the trail and camp site were covered with bamboo mats to reduce human contact. However, entry in the aviary was still necessary for cleaning, replacement of twigs with natural fruits and monitoring of the birds (particularly weighing). Wardens were instructed to fill in monitoring sheets in regular intervals which contained information of bird behaviour during timed observation periods, food acceptance and intake, but also presence of potential predators near the release site.

One bird was injured, possibly due to attack by a rat while in the isolation cage. Wounds on leg and base of beak turned out to be superficial and healed well after being disinfected regularly.

Four feeding stations were installed within 200 m radius of the aviary. Two isolation cages were prepared, in case birds needed to be separated.

By November a plywood silhouette of a White-bellied Sea-eagle was installed on a zipline system passing over the aviary. Birds showed signs of panic after the silhouette passed over, indicating that their anti-predator response was sufficient. The silhouette was removed after this exposure.



Figure 17. Collection of natural food items in large quantities for cockatoos before release is time-consuming for wildlife wardens (left); Cockatoo inside aviary feeding on *Terminalia* fruit (Photos: S. Diaz)



Figure 18. Birds were regularly weighed inside the aviary (left); cockatoo with fresh foliage and wild fruits inside pre-release aviary (right; Photo: S. Diaz)

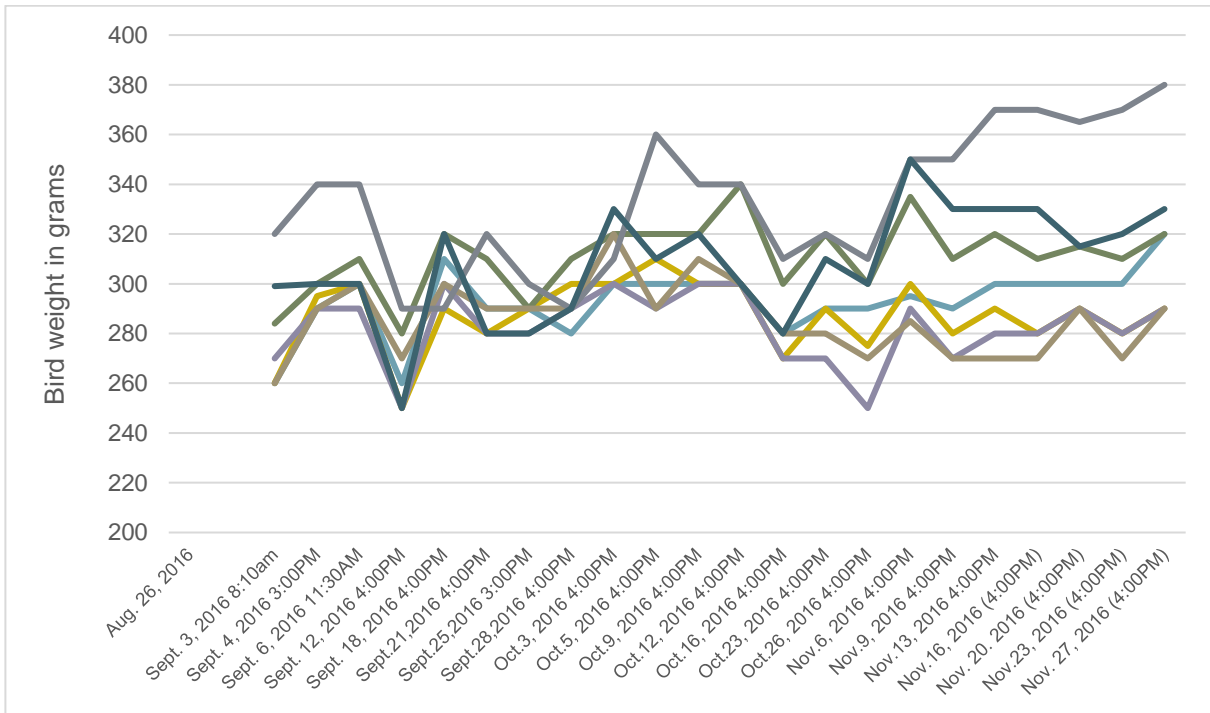


Figure 19. Weight development of seven cockatoos in pre-release aviary Omoi Cockatoo Reserve, Dumaran

Warden scheme continued

Throughout the reporting period wardens patrolled the cockatoo reserve and monitored wildlife along the 29 biodiversity monitoring stations. Target species, including all three species of parrots, Palawan Hornbill and two species of woodpeckers were regularly encountered.

No illegal activities were encountered in the Critical Habitat during patrolling throughout the reporting period. However, cutting of mangroves was reported from the coastal areas of Dumaran Poblacion.

Capacity Building for stakeholders

On October 19, the 25th Local Protected area Management Committee (LPAMC) Meeting was conducted in Dumaran Poblacion. The adoption of the declaration of the Critical Habitat by PCSD was endorsed by the LPAMC for adoption by the local council (SB) of Dumaran. Next step would be the demarcation of the area and preparation of and implementation of a management plan.

A total of 26 families were identified to have claims within this Critical Habitat. Many of these benefitted from payment for native tree seedling production and payment for assisted natural regeneration efforts.

Buffer zone restoration around existing cockatoo reserves and creation of forest corridor connecting the two existing cockatoo reserves continued

In September, November and December a total of 8,228 native tree seedlings respectively were planted in the corridor. Inventory by end the reporting period in the nursery was 9,896

seedlings, comprising more than 40 species. Due to sufficient rain fall, survival rate of seedlings exceeded 80%.

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Constraints and measures taken

- Only five food-providing tree species in Omoi Cockatoo Reserve were bearing fruit. Natural food items for birds in pre-release aviary had to be brought in from other areas, to not deplete sources in Omoi. Food also was temporarily supplemented by chicken conditioner.

Objective 4: Education and research at the Katala Institute

Captive management of Philippine Cockatoo and other highly threatened species continued through employment and training of zookeepers and volunteers

Due to the initial result of PBFD-positive cockatoos on Rasa, all cockatoos under care in Katala Institute were re-sampled. Less than 0,5 ml of full blood was taken from the brachial veins and sent for screening. All birds tested negative for the virus.

On October 28 cockatoo rescued WESCOM, large blood clot on left wing, probably due to a slingshot or airgun injury. The bird was treated twice with antibiotics. Feeding response was weak. Although the bird initially gained some weight, it died on December 23.

Another cockatoo which was rescued on August 22 from Puerto Princesa with a leg wound probably caused by an airgun recovered completely after being treated with antibiotics. By early September the bird could be placed in a flight aviary and was accepting wild food items without difficulty.

On November 17, the facility was inspected by staff from the Biodiversity Management Bureau from Quezon City, accompanied by local DENR personnel. Objectives of Katala Institute were explained by Anna and Angel.



Figure 20. After being bled for PBFD-testing, birds are given a treat and monitored (Photos: P. Widmann)

Educational trail, enclosures and visitors facilities upgraded

On October 22, the facilities of KI were introduced to a group of 30 travel agents from Luzon, who intended to increase their offers with some ecotourism components. The tour was completed with a visit to Rasa Island. In November ca 100 municipal councilors all over Palawan visited KI and was introduced to the facility. This was organized by the Palawan League of Councilors.

A training module for propagation of food- and nest-providing trees of cockatoos was finalized and field tested with staff of KFI within the current reporting period. Participants learn how to collect seeds, to prepare seed bags and to propagate trees with focus on food-providing plants for the cockatoo. They were taught in how to gather data on performance of plants during the seedling stage and after planting it. Finally, they were instructed on how to collect and share data on foraging cockatoos in their respective areas as part of the already ongoing citizen science project in Narra.

During the reporting period, eight groups comprising a total of 189 participants were instructed in these techniques, far surpassing the 60 anticipated participants in the project proposal. Groups consisted of village officials, blue and green guards (wildlife wardens deputized by DENR for coastal and forest resource protection), and students of Narra and the neighboring municipality of Aborlan. Average increase in knowledge and attitude after the training was 17 percentage points, indicating that there is still room for improvement on how the course is communicated.

Pond dipping courses meanwhile continued, following the module developed during the previous reporting period. Both components are funded by WRS in the course of KFI's citizen's science project.

As part of the training course, 49 seedlings have been planted on the facility grounds. At the end of the reporting period 2,303 seedlings in 20 species were present in the nursery.



Figure 21. Participants filling in the pre-training questionnaire (left; Photo: J. Nunez) and learning how to measure tree seedlings (right: Photo: P. Widmann)



Figure 22. Introduction to the KI in pavilion (left); group of municipal councilors all over the province after visit (right; Photos: P. Widmann)

Constraints and measures taken

- The three cockatoos destined for Jurong Bird Park are still in quarantine due to ongoing incidents of Newcastle disease on Luzon. The facility is therefore not available for newly rescued birds, and these have to be accommodated in caretaker house.

Other highlights

Other reported wildlife within the reporting period:

Dugong (*Dugong dugon*) (IUCN: Vulnerable)

One dead individual was recovered stranded from a beach opposite of Rasa Island on September 16. The animal had healed scar, possibly caused by boat propellers, but no recent external injuries. The carcass was buried nearby and bone will be recovered later for exhibition purposes.



Figure 23.; Stranded dead Dugong (left); healed scars on back (right; Photos: S. Diaz)

Red-footed Booby *Sula sula* (IUCN: Least Concern)

One was recorded as captured bird on September 5 on Malinsuno, Balabac. The species is very rarely recorded near mainland Palawan.



Figure 24.; A captive Red-footed booby on Malinsuno, Balabac (left; Photo: R. Antonio), a pair of Malaysian Plovers on Arena Island near Rasa (right; Photo: P. Widmann)

Malaysian Plover *Charadrius peronii* (IUCN: Near-threatened)

A pair was observed on November 23 on Arena Island, Narra.

Green Turtle *Chelonia mydas* (IUCN: Endangered)

One individual which got entangled in a fish trap was released in December near Pandanan.

Cooperation and advocacy affecting all project sites

- On September 15 we attended the 3rd PCSD Research Symposium. Three papers were presented by Mary (MCN), Josh (JN) and Rene (RA) respectively on Philippine Cockatoo, Balabac Mouse-deer and Calamian Deer.
 - On September 20 Peter met with staff from the new USAID-funded Protect Wildlife Project during a validation workshop by USAID. Rasa Island was identified as a site for cooperation, initially ranching of sea cucumbers could be explored.
- A pre-summit stakeholder consultation regarding Palawan Integrated Natural Resource Management Project has been participated on October 21. KFI has voiced out its concerns particularly the plans to construct 'ring roads' around remaining forest areas, and the classification of lowland forests as production forests. Despite substantial input from stakeholders during this workshop, the plan as presented did not incorporate the vast majority of recommendations as a result of this workshop.

Personnel and equipment status

- Siegfred H. Diaz retired from his service with KFI, after being with the organization from the very start and being a founding member of Katala Foundation. He will be replaced by Anna Rose L. Agullo.
- The old (spare) boat hull of the Pandanan project site was repaired and painted in early September.
- The roof of the field house in Malinsuno was repaired with new nipa shingles.
- A newly acquired boat hull for Rasa Island project site through GIZ-funding was painted, the old service boat was refitted.

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Annex 1

Sex Determination and Detection of the Psittacine Beak and Feather Disease (PBFD) From Feather Samples of *Cacatua haematuropygia* from Dumaran, Iwahig, Pandanan, and Rasa Island, Philippines, 2016

This report includes the results for detecting Psittacine Beak and Feather Disease (PBFD) from DNA extracted from feather samples of *Cacatua haematuropygia* (Philippine Cockatoo). In a study by Ypelaar et al., (1999), DNA samples were used and subjected to Polymerase Chain Reaction (PCR) to determine the presence of the PBFD viral DNA.

DNA samples were also used to determine the sex of each individual. For birds, males have sex chromosomes ZZ and females have the sex chromosome ZW. This study made use of primers designed by Fridolfsson and Ellegren (1999), which could amplify specific regions within in the Z and W chromosome.

Objectives

This study was done to determine the presence of PBFD virus and the sexes of each individual using the DNA extracted from the feather samples of *C. haematuropygia*.

Methods

DNA extraction

DNA were extracted and isolated from 48 feather samples of *C. haematuropygia* from four different islands using PureLink ® Genomic DNA Mini Kit (Invitrogen, USA) using the protocol provided by the kit's manual. Table 1 provides a summary of the samples.

Table 1. Forty-eight feather samples from four different sites were received by the lab.
Feather samples were stored in -85°C to prevent degradation.

Common/ Scientific Name	Description of parts/ Leg band number	Quantity
Philippine cockatoo/ <i>Cacatua haematuropygia</i>	Dumaran Island DENR – 051 -16 (B1), 053 -16 (B1), 054 -16 (B1), 055 -16 (B1), 099 -16 (B1)	5 feather samples
Philippine cockatoo/ <i>Cacatua haematuropygia</i>	Iwahig Island DENR - 049 -16 (#27), 050 -16 (#27), 096 -16 (#25), 097 -16 (#25), 100 -16 (#23), 101 -16 (#23), 102 -16 (#23)	7 feather samples
Philippine cockatoo/ <i>Cacatua haematuropygia</i>	Pandanan Island DENR – 062 -16, 063 -16, 064 -16, 066 -16, 067 -16, 071 -16, 073 -16, 076 -16, 078 -16, 081 – 16	10 feather samples
Philippine cockatoo/ <i>Cacatua haematuropygia</i>	Rasa Island DENR – 002-16 (#3), 004-16 (#4), 006 -16 (#85), 008-16 (#23), 009-16 (#90), 011-16 (#72), 013-16 (#44), 014-16 (#09), 018-16 (#46), 019-16 (#29), 021-16 (#27), 022-16 (#18), 023-16, (#02) 24-16 (#80), 025-16 (#05), 026-16 (#89), 027-16 (#87), 028-16 (#73), 029-16 (#28), 033-16 (#38), 034-16 (#88), 036-16 (#83), 038-16 (#79), 039-16 (#78), 040-16 (#91)	26 feather samples

	Total	48
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PCR amplification of viral DNA

Primers (Primer 2 and Primer 4) designed by Ypelaar et al., (1999) were used to detect the presence of the Beak and Feather Disease Virus. A PCR mix of 25 µl was prepared for each sample. PCR mix was prepared with the following components: 11.625 µl of double distilled water, 5 µl myTaq reaction buffer, 1.25 µl of Primer 2, 1.25 µl Primer 4, 1.25 µl of dimethyl sulfoxide (DMSO), 0.5 µl of 25.0 mM MgCl₂, and 4 µl of DNA sample. PCR was done in a thermal cycler with conditions following the protocol used by Ypelaar et al., (1999) with some modifications. The condition had an initial denaturation temperature of 96°C for 5 minutes, followed by 32 cycles of 96°C denaturation step for 30 seconds, 45°C annealing for 30 seconds, and 72°C extension for 90 seconds. A final extension of 72°C for 5 minutes was added at the end of the cycles.

PCR amplification for sex discrimination

The same volumes of PCR components as that of the previous protocol were used, except that 9.625 of double distilled water and 6 µl of DNA sample were used. Primers 2718R and 2550F designed by Fridolfson & Ellegren (1999), were used to amplify regions from the Z and W chromosomes. PCR condition that was used were based from the protocol 2 of Vucicevic et al., (2013), with some modifications. The conditions consisted of an initial denaturation at 95°C for 4 minutes, followed by 35 cycles of 95°C denaturation step for 30 seconds, 45°C annealing for 30 seconds, and 72°C extension for 45 seconds. A final extension step at 72°C for 4 minutes was added at the end of the cycles.

Agarose Gel Electrophoresis and Band Visualization

PCR products were run in prepared 1% agarose gels. EtBr – UV method was used to visualize the results. For PBFD, the presence of a band at around 700 bp would indicate the presence of the viral DNA from the sample.

For sex discrimination, females may have up to two bands, one at around 700 bp (representing the amplified region from the Z chromosome) and another at the 500 bp (from the W chromosome). Males will only have one band (from the Z chromosome) at around 700 bp.

Results

Samples from the Dumarán, Iwahig, (Figure 1) and Pandanan Island (Figure 2) did not have distinct bands for PBFD virus, indicating negative results. From the samples from Rasa Island, 14 out of the 26 samples yielded faint but distinct bands at around 700 bp (Figure 3).

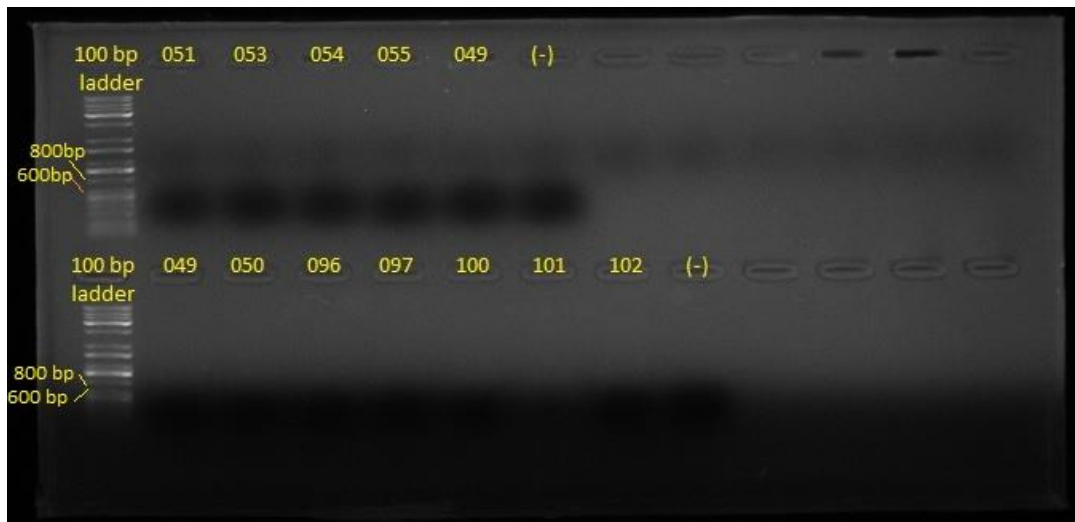


Figure 1. Samples from Dumarán and Iwahig Islands with no distinct bands for PBFD.

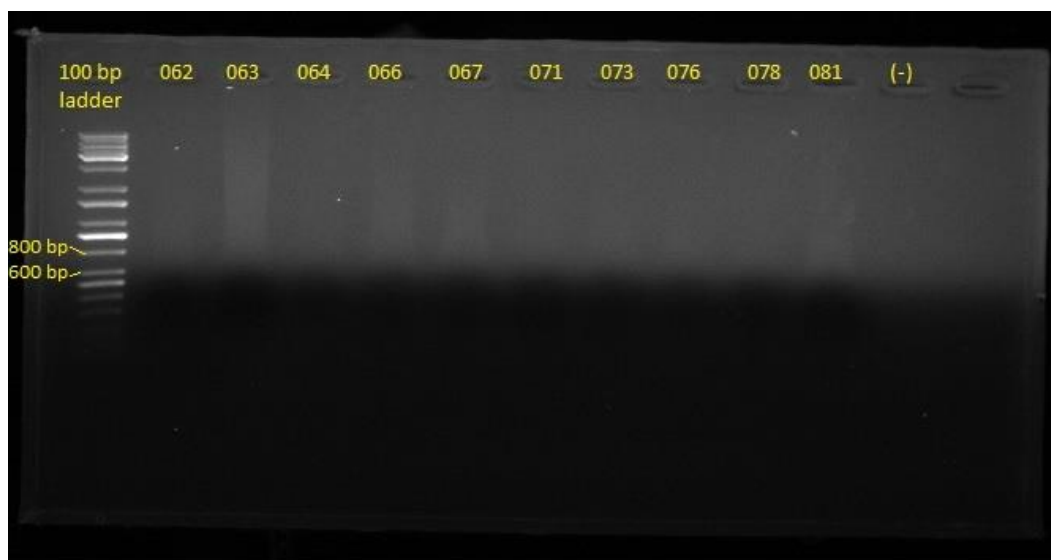


Figure 2. Samples from Iwahig Island with no distinct bands for PBFD.

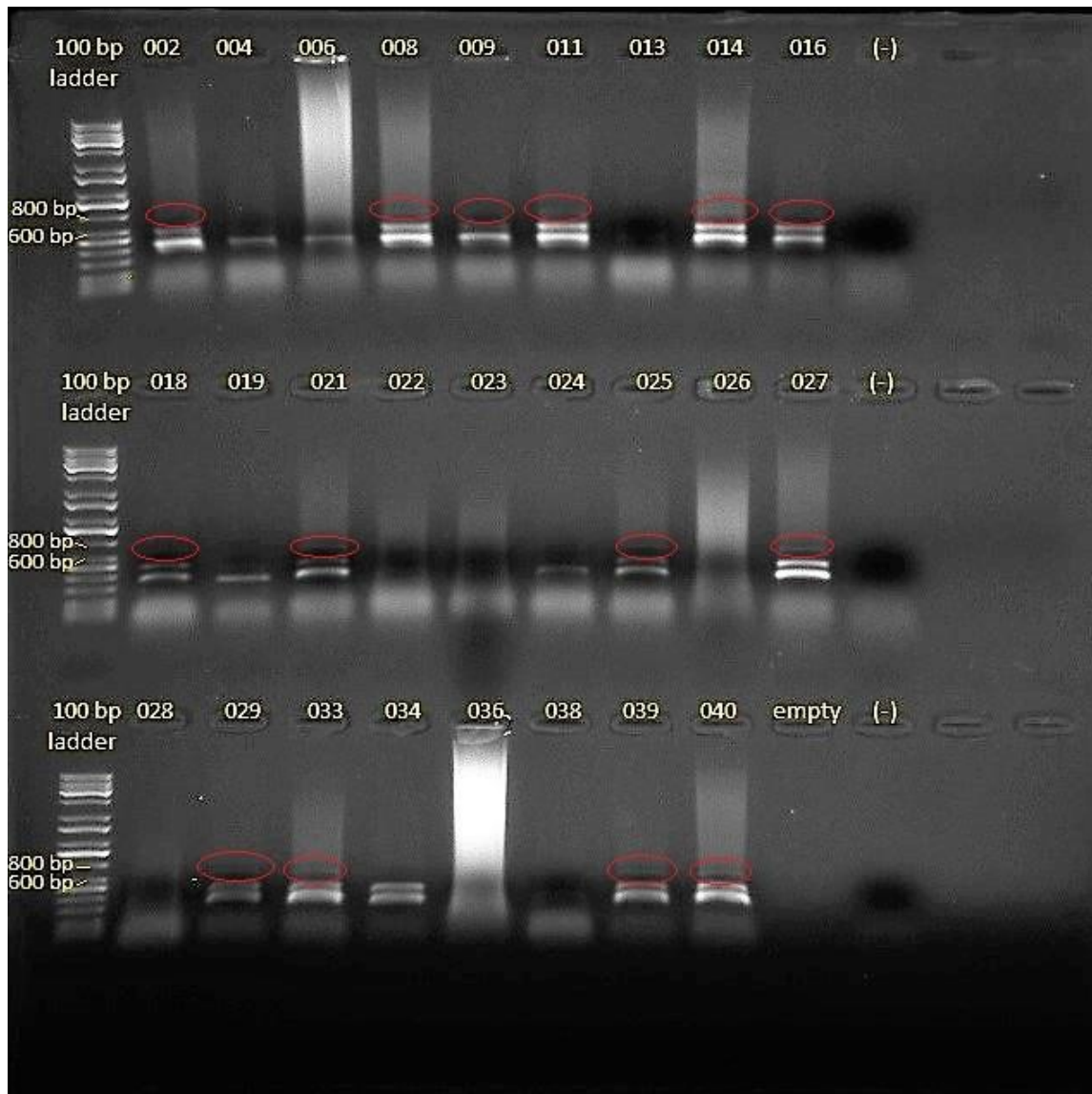


Figure 3. Samples from Rasa Island. Fourteen out of the 26 samples yielded a faint yet distinct band (encircled in red) at around 700 bp.

These 14 samples were from the individuals with tag numbers 002, 008, 009, 011, 014, 016, 018, 021, 025, 027, 029, 033, 039, and 040. Some of the samples had distinct bands below the 600 bp mark, but this might be because of non-specific annealing of primers.

From Dumarán Island, all the sexes were successfully determined (Figure 4). Three were female (051, 053, 055) and two were male (054 and 099).

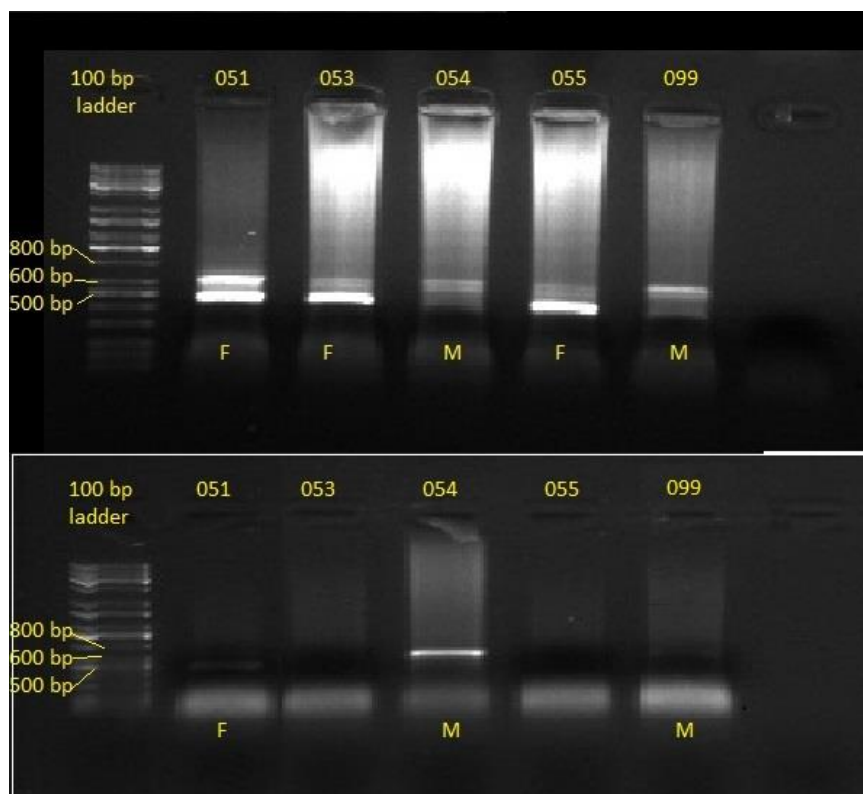


Figure 4. Discrimination of sexes for Dumaran Island samples. Test was done twice to validate the sexes of each individual.

From Iwahig Island, the sex of six out of seven samples were successfully identified (Figure 5.). Four were females (050, 096, 100, 101), while two were males (97 and 102). No bands appeared for sample 049.

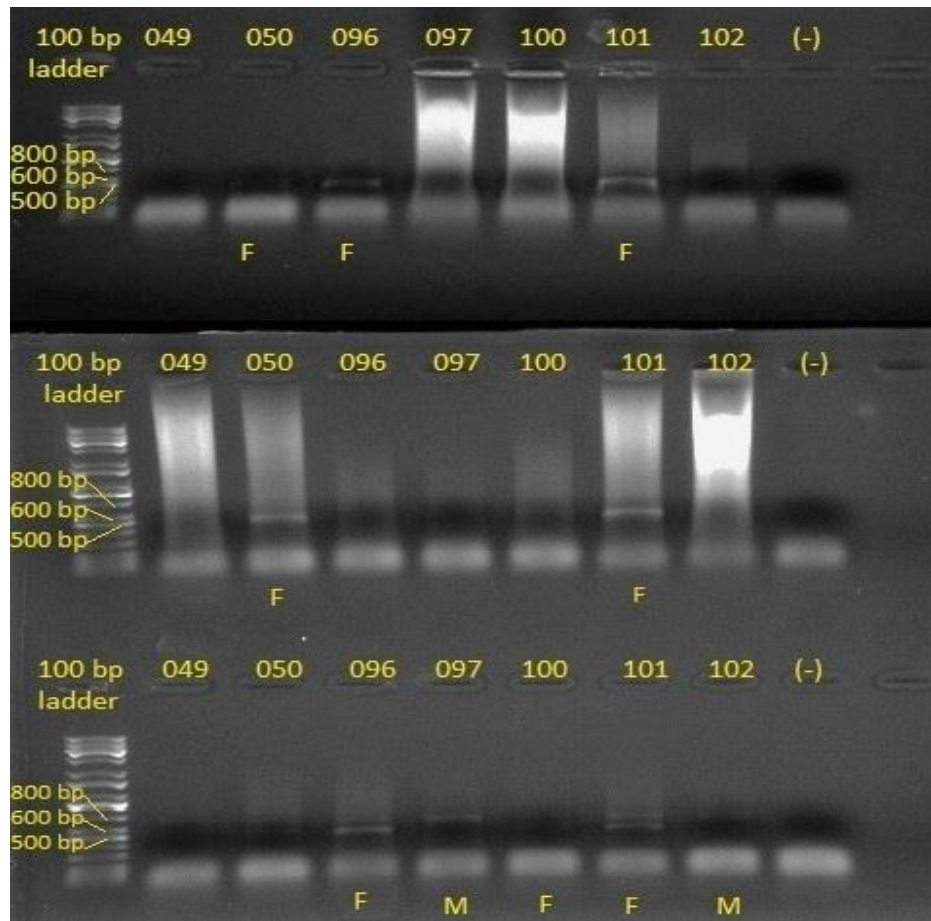


Figure 5. Discrimination of sexes for Iwahig Island samples. Test was done thrice to validate the sexes of each individual and such that all individuals' sexes were determined. However, sample 049 still did not manifest any bands.

For Pandanan Island, nine out of 10 sampled had their sex determined (Figure 6.). Six were females (064, 066, and 071) while six were males (062, 062, 076, 078, and 081). Only sample 067 did not have any bands.

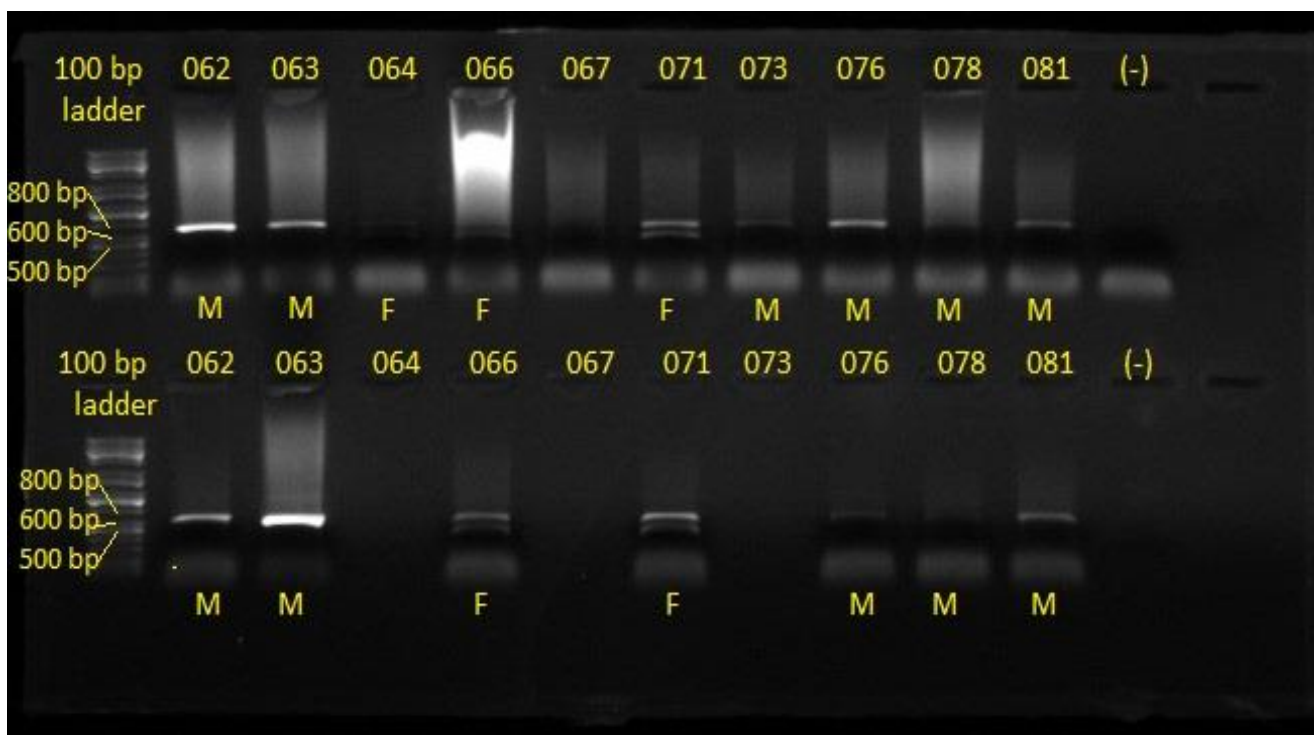


Figure 6. Discrimination of sexes for Pandanan Island samples. After two tests, sample 067 still did not produce any bands.

Rasa island had 14 out of 26 successfully determined of their sex (Figure 7). Eleven were identified as female (002, 006, 008, 009, 011, 014, 016, 021, 025, 033, and 039) and three were identified as males (027, 036, and 040).



Figure 7. Sex discrimination for samples from Rasa Island. Twelve samples did not have any resulting band (004, 009, 013, 018, 019, 022, 023, 024, 028, 029, 034, and 038) for DNA sexing.

Separate modified repeat test (DNA concentration was modified by either getting aliquots and diluting the samples or increasing the added DNA sample to the PCR mix, where a maximum of 8 µL was reached) was done for the remaining samples without bands, but no bands were produced from the test.

Conclusions and Recommendations

PBDF viral DNA were amplified from 14 samples from Rasa Island, but more repeat tests are required to validate these results. It is also recommended to isolate the resulting bands and have them sequenced so they can be compared with Beak and Feather Disease Virus DNA available on online databases.

For DNA sexing, sex chromosome markers were not amplified in 14 out of the 48 samples. Re-extraction of DNA from the remaining stored feather samples will be necessary for these samples.

References

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