National Conservation Council

General Meeting

(GAB 1038, 14 June 2017, 2pm)

AGENDA

1. Call to Order
   a. Attendees, Apologies, Quorum
   b. Declaration of Interests

2. Confirmation of Minutes
   a. 22 March 2017
   b. 3 May 2017

3. Matters Arising From Previous Meetings
   a. Green Iguanas
   b. Terrestrial Protected Areas – public lands
   c. EIAs

4. Committees

   a. DoE
      i. Alien & Invasive Species
      ii. WP-04: Consultation and Licencing reporting
   b. Climate Change Committee
   c. Public Education & Outreach Committee

5. New Matters
   a. Protected Areas
      i. WP-01 Brac and Barkers Nominations
      ii. Change of nomination schedule
   b. MRCU Oxitec
      i. WP-02 MRCU Oxitec Expansion Application
      ii. WP-03 DoE Screening Evaluation
   c. WP-05 Little Cayman Conservation Warden Confirmation

6. Next Meeting
   a. 28 September, 2017, 2-5pm < Location TBD>
   b. Issues expected <TBD>

7. Any Other Business

8. Adjournment

9. Attendance Appendix

Council Member	**XX YYY. 2015**	**Representation**
Christine Rose-Smyth		Chairperson; Bodden Town, Plants, Terrestrial & Marine Biodiversity
Davy Ebanks		West Bay and Marine Conservation
Lisa Hurlston-McKenzie		George Town and Sustainable Development & Climate Change
McFarlane Conolly		East End and Sustainable Development
		North Side
Wallace Platts		Sister Islands and Terrestrial Biodiversity
Christina Pineda		National Trust for the Cayman Islands
Patricia Bradley		Avifauna & Biodiversity
Fred Burton		Terrestrial Biodiversity
Adrian Estwick		Director of Agriculture
Haroon Pandohie		Director of Planning
Colleen Stoetzel		Planning Officer, representing the Director of Planning
Gina Ebanks-Petrie		Director of Environment
Timothy Austin		DoE Deputy, Research
John Bothwell		Secretary

Barkers National Park - Block 16A parcels 13 and 14

Noting that in 2016 the Council received nominations for protection of all of the Barkers Peninsula, and that a nomination for all the Crown lands in the Barkers Peninsula was approved by Cabinet on 17th May 2017; and noting that the Council has been advised that two parcels of land in the Barkers Peninsula identified as Block 16A, parcels 13 and 14 and vested in the Financial Secretary, were on 17th May 2017 approved by Cabinet to become vested in the Crown; and further noting that the Council has already established that the Barkers Peninsular meets multiple objectives and criteria set out on section 8 of the Law;

Council therefore resolves to advance a proposal to declare Block 16A parcels 13 and 14 Protected Areas under Section 7 of the Law and therefore instructs DoE to:

1. Publish notices of this proposal in accordance with section 9 (6) (a) of the Law
2. Notify all adjacent landowners in accordance with section 9 (6) (b) of the Law
3. Place a proposal to declare Block 16A parcels 13 and 14 Protected Areas under Section 7 of the Law, out to public consultation, with a view to potentially including these parcels as part of the Barkers National Park.

Bluff Cliffs nomination, Cayman Brac

Noting that the Council has now received an amended nomination for the Bluff Cliffs of Cayman Brac, from the Department of Environment which takes into account the Council's recommendations on the original nomination, and noting that the Council has already established that the Bluff Cliffs meet multiple objectives and criteria set out on section 8 of the Law;

Council therefore resolves to advance a proposal to declare the nominated sections of the Crown cliffs Protected Areas under Section 7 of the Law and therefore instructs DoE to:

1. Publish notices of this proposal in accordance with section 9 (6) (a) of the Law
2. Notify all adjacent landowners in accordance with section 9 (6) (b) of the Law
3. Place a proposal to declare these Bluff Cliffs Protected Areas under Section 7 of the Law, out to public consultation.

East Lighthouse Park nomination, Cayman Brac

Noting that in 2016 the Council received multiple nominations for protection of the eastern lighthouse area of Cayman Brac, and noting that the Council has already established that these nominations meet multiple objectives and criteria set out on section 8 of the Law; and noting that at this time the owners of Block 111A, parcel 11 within the nominated area have indicated interest in negotiating for sale to the Cayman Islands Government for the purposes of environmental protection;

Council therefore resolves to advance a proposal to purchase and declare Block 111A, parcel 11 a Protected Area under Section 7 of the Law and therefore instructs DoE to:

1. Formally write to the landowners in accordance with section 9 (7) of the Law
2. Publish notices of this proposal in accordance with section 9 (6) (a) of the Law
3. Notify all adjacent landowners in accordance with section 9 (6) (b) of the Law
4. Place a proposal to declare this parcel a Protected Area under Section 7 of the Law, out to public consultation.

Protected Area Nomination

Barkers National Park, West Bay

This nomination is made under Section 9 of the National Conservation Law, 2013
Nominator: **Save Cayman**

Date: 13/10/16

1. Description of the Area

This nomination covers Barkers National Park in its entirety, located on the northeast tip of West Bay. All block and parcel numbers starting from and eastwardly of Ristorante Pappagallo are being nominated; they include, but are not limited to the below:

Private: 8A 16(8REM2), 19, 20, 23, 24, 61, 62, 70, 71, 72, 76, 86, 154, 219

Private: 9A 76(9REM1), 95, 98, 99

Private: 16A 1-23, 28

Crown: 9A 240, 16A 16, 21, 24, 25, 27

The park's coastal and inland terrain is composed of wetland, dry forest, and beach/beach ridge. It is inhabited by a diversity of native fauna and flora, and is a peaceful area with virtually no development.

2. Reasons for Nomination

2.1) Designation of Barkers National Park as a protected area will secure an expanse of mangrove wetlands, a smaller area of dry forest, and beach/beach ridge. It will allow indigenous populations of plants, insects, crabs, wetland birds, bats and other fauna populations, including migratory birds, to continue to exist.

a. Significant populations of *Gecarcinus ruricola* (black land crab), *Gecarcinus lateralis* (red land crab), *Cardisoma guanhumi* (white land crab), and *Coenobita clypeatus* (soldier crab) are present in the area. Bat species, such as *Artibeus jamaicensis* (Jamaican fruit-eating bat) are also found here.

   Visiting scientists conducted a bat survey in April 2016 and a land crab survey May through August 2016 at Barkers National Park. This area was chosen as one of their field sites due to the high density of bats and land crabs found at this location. Visiting scientists will be repeating the land crab survey during the summer of 2017.

b. The larvae of the endemic and endangered *Brephidium exilis thompsoni* (Western pygmy blue butterfly) feeds on *Salicornia* (glasswort), a rare plant, only found in highly restricted areas on Grand Cayman. Both species can be found in Barkers National Park.

c. Coastal *Rhizophora mangle* (red mangrove) of Barkers act as a nursery for juvenile reef fish, conch, lobster, crab larvae and many other small invertebrates. Many of these marine creatures end up on the North Sound reefs, which tourists come here to see. Conserving these nurseries will inevitably benefit eco-tourism. The mangroves also aid in coastal protection to prevent erosion and sedimentation on the reefs.

2.2) This area is one of the last areas on the West Side of Grand Cayman that is undeveloped, allowing populations of native fauna and flora to exist.

a. It may be the very last accessible forested area on the West Side that is undisturbed by development which Caymanians, residents and visitors alike can use recreationally. This location is well known to be a peaceful, secluded area for nature lovers to explore, to hold a picnic or just relax.

b. Scientists often use Barkers as a field site to collect data due to its natural state. Being able to obtain information on the fauna and flora which exist on the West Side may provide a valuable comparison to what is found on the Eastern Side of the Island. These studies expand the DoE's database and provides them with the resources to make recommendations that are grounded in scientific study.

3. Conservation problems and protective measures required

The issue concerning Barkers National Park is that there are no protections in place safeguarding the critical habitats from development. It's ecological and social value holds great weight, but could potentially be a desirable location to construct condominiums or hotels.

The adverse effects of any development would displace and threaten the existence of many fauna and flora species, some endemic to Grand Cayman. In addition, locals and tourists would lose yet another public beach access or be highly restricted to use. Protection of the nominated land under the National Conservation Law (NCL) will preserve the aforementioned points.

4. Management considerations

The Barkers National Park project was taken on by the Office of the Premier and the Ministry of Tourism in 2011. This designation in itself acknowledges the ecological value of the area, but is does not establish any protections.

In addition, this area is currently divided into several block and parcel numbers; some are owned by the Crown and the rest are privately owned by developers such as Cayland Group Ltd. and DART.

Protected Area Nomination

It is noteworthy that the land should be protected as a whole, and where not possible, protection should encompass large expanses of adjacent land. If this is not taken into consideration where developments are proposed, habitats will become fragmented, adversely affecting the health, productivity and biodiversity the ecosystem.

It is being proposed that all block and parcel numbers of Barkers National Park are given proper protection, as a national park would suggest, under the NCL, to be managed by the Department of Environment.

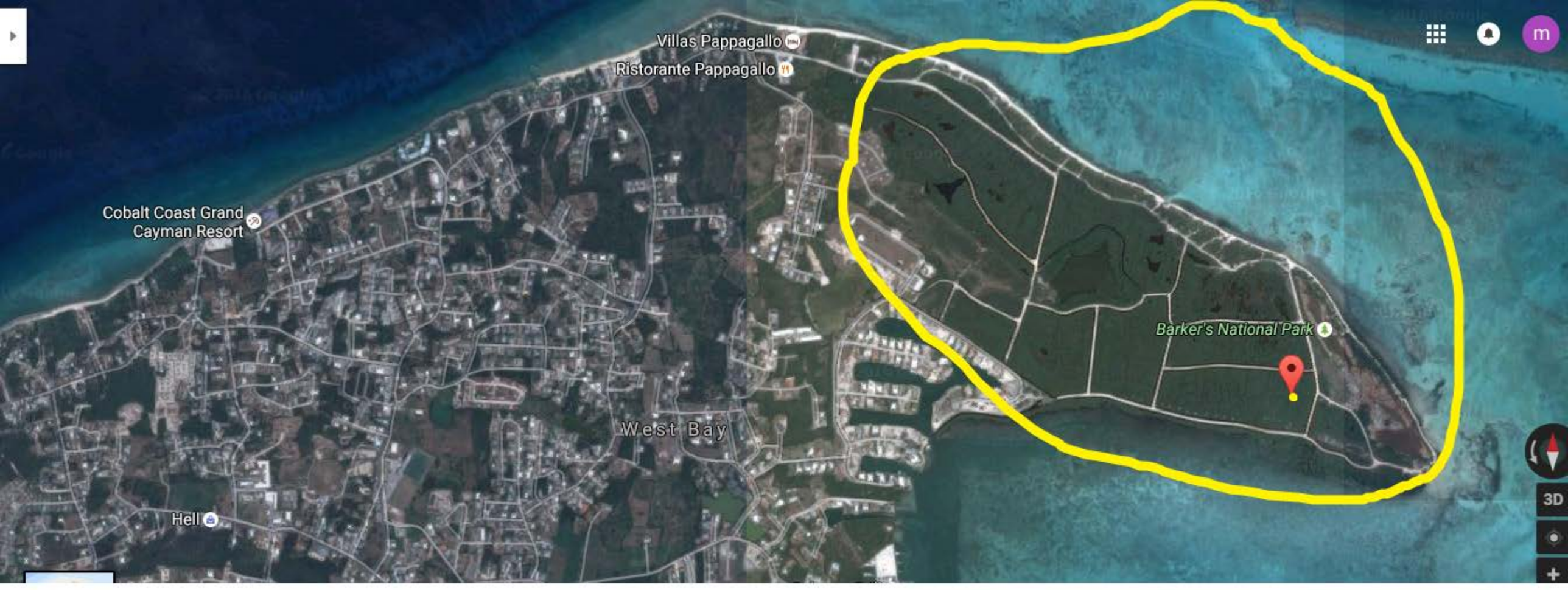
Thank you for taking the time to have this nomination considered. It is with great urgency that we send in this nomination for protection of a very special place. If there is any place left on the West Side to save, it is Barkers National Park.

Thank you,

[redacted]

Sincerely,

Save Cayman
SaveCayman.ky@gmail.com


Villas Pappagallo
Ristorante Pappagallo
Cobalt Coast Grand
Cayman Resort
West Bay
Barker's National Park
Hell
3D

Terrestrial Protected Area Nomination:

Barkers, Grand Cayman

Three nominations for protection of lands in the Barkers Peninsula have been considered by the National Conservation Council, which has resolved to accept the proposal that all the Crown lands and one parcel of privately owned land in the Barkers peninsula be made a Protected Area under Section 7 of the National Conservation Law. If Cabinet and the private landowner approve this proposal, the natural environment in these areas will be protected and managed according to a Management Plan to be developed under Section 10 of the Law.

Crown land in Barkers was predominantly acquired before the passage of the National Conservation Law, for the purposes of establishing the "Barkers National Park." With the NCL now in effect, the Barkers National Park concept can begin to be carried forward within a legal framework.

January 2017

Barkers has a long history of public recreational activity on privately owned land, and is valued as a recreational area by the residents of West Bay, and the island as a whole. In recent years commercial operations including horse riding tours and kite surf training have also been using the Barkers area. Easter camping in Barkers is a culturally important annual tradition.

Adjacent to a Marine Protected Area (Replenishment Zone), the Barkers beach ridge supports one of the last substantial coastal sand forests remaining in Grand Cayman. The high sand ridge supports a diversity of tree species including Ironwood, Bull Thatch, Broadleaf, Silver Thatch, Washwood and Mahogany, with an understory of Cocoplum and other shrubs. A *Cerion* land snail endemic to Grand Cayman is associated with the forest, while an endemic butterfly breeds in the glasswort flats where the beach ridge transitions to mangroves.

The interior of the Barkers Peninsula is wetland, supporting several distinct mangrove communities. Ponds including Sea Pond and Palmetto Pond are valuable for water birds and an endemic brackish water fish. The shallow water immediately off the south coast of the peninsula is an important shark nursery, and the mangroves release valuable nutrients into the North Sound ecosystem.

Protection of coastal forest and wetland in the Barkers peninsula, with a management plan developed in consultation with the community and all stakeholders, should allow for continued public enjoyment of the area without further degradation of the environment from unmanaged activity.

Protected Area Nomination

Barkers National Park

This nomination is made under Section 9 of the National Conservation Law, 2013

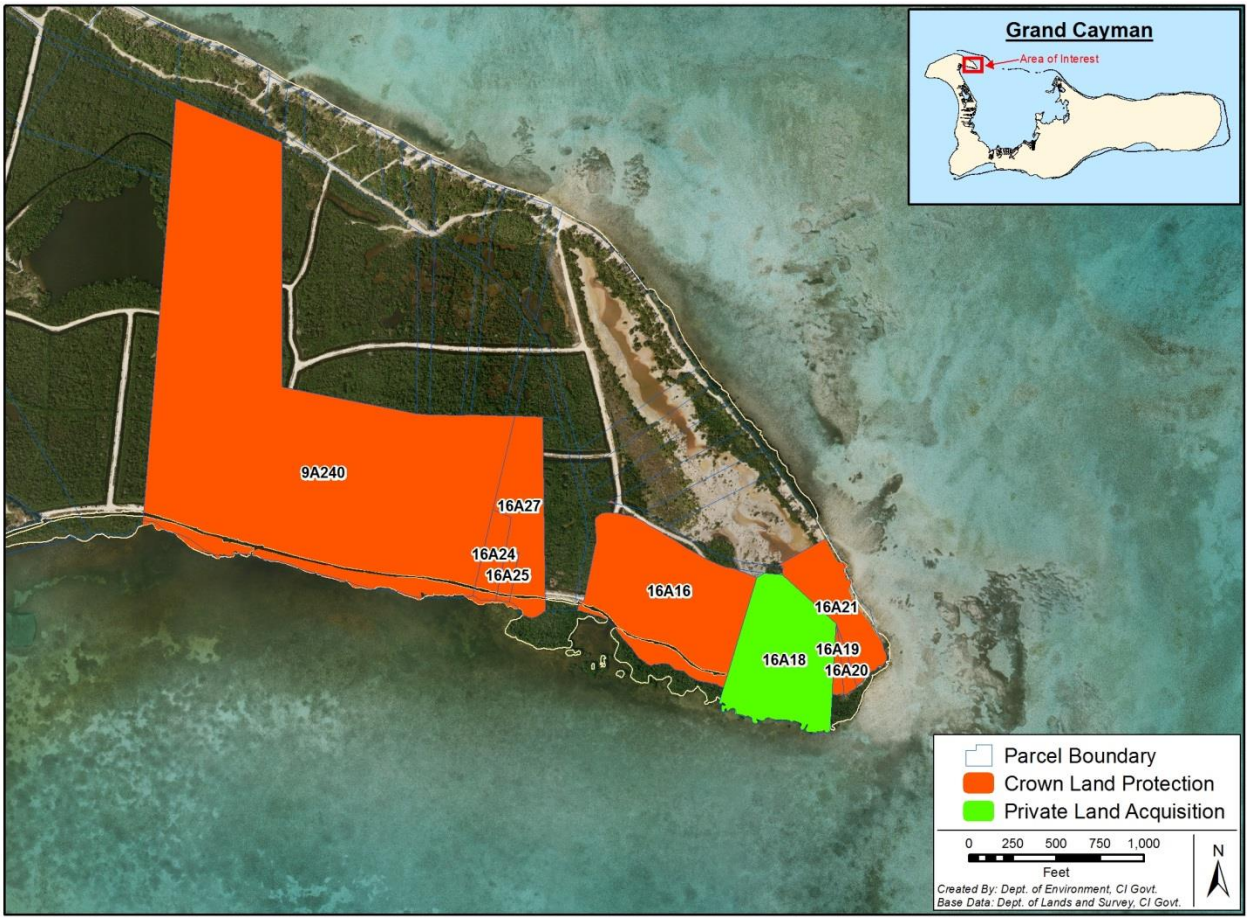
1. Description of the Area

The nomination covers Crown and privately owned parcels of land within the original conceptual area for the Barkers National Park. Most of the Crown parcels were purchased by the Government to begin establishing this National Park, but no legislative framework was in place at that time to formalize the Park's status. This nomination resumes the process and proposes these Crown parcels plus a single private purchase parcel, to consolidate and formalize protection of the mangrove wetlands bordering the southern aspect of the proposed Park and extending to the point of Barkers. The nominated area is defined in the map below, and comprises all or part of the following parcels as indicated:

Block	Parcel	Ownership	Portion of parcel
9A	240	Crown	whole
16A	24	Crown	whole
16A	25	Crown	whole
16A	27	Crown	whole
16A	16	Crown	whole
16A	18	Private	whole
16A	19	Crown	whole
16A	20	Crown	whole
16A	21	Crown	whole

The nomination also includes the mangroves growing immediately seaward of the parcel boundaries on the south side of the Barkers Peninsula, and the small associated mangrove islets that are not currently identified as mapped land parcels, as shown in the map below.

The nominated area is predominantly mangrove wetland, crossed by a number of MRCU dyke roads with associated canals. The mangrove communities are varied, and the nominated area includes part of Palmetto Pond, and part of Sea Pond. On the north aspect of Barkers Point the nominated area is adjacent to a Marine Parks Replenishment Zone.


Grand Cayman
Area of Interest
9A240
16A27
16A24
16A25
16A16
16A21
16A18
16A19
16A20
Parcel Boundary
Crown Land Protection
Private Land Acquisition
0 250 500 750 1,000
Feet
N
Created By: Dept. of Environment, CI Govt.
Base Data: Dept. of Lands and Survey, CI Govt.

2. Reasons for Nomination

Purposes and Objectives

2.1 Protection of these nominated lands will safeguard key habitats for resident and migratory water birds, and for endangered and endemic species as listed below under "species of special concern". NCL ref. 8 (1) (a)

2.2 Protection of these nominated lands will safeguard habitats which are representative of mangrove habitat along the western and southern shores of North Sound, which overall have suffered extensive loss. Protecting the Barkers mangrove interface with North Sound will help towards long term maintenance of the marine and terrestrial biodiversity that depends on this habitat. NCL ref. 8 (1) (b)

2.3 Protection of the nominated land will help maintain mangrove nutrient flows essential to the productivity of North Sound, which will benefit sustainable harvest of marine life for human recreation and consumption. NCL Ref. 8 (1) (c)

2.4 Protection of areas of special concern within these nominated lands may assist recovery of an endemic butterfly population whose area of special concern lies partly within the nominated area. NCL ref. 8 (1) (d)

2.5 Protection of these nominated lands will secure part of an area traditionally used by the public for a wide range of recreational activities, including camping, fishing, kite surfing, snorkeling, walking and running, horse riding and bird watching. It is also a significant nature tourism resource. NCL Ref. 8 (1) (e, f)

Criteria for Protection

2.6 The nominated lands are predominantly in a natural state, though fragmented by MRCU dyke roads and canals, and other un-surfaced tracks. NCL Ref. 8 (2) (a)

2.7 The nominated lands support a significant diversity of life, especially since they span a range of different habitats including both terrestrial and marine species. NCL Ref. 8 (2) (b)

2.8 The nominated lands are of ecological importance in that they support species of special concern, and because they provide a life-supporting role for marine life in North Sound. The mangroves also help to stabilize the shoreline during storms and hurricanes. NCL Ref. 8 (2) (c)

2.9 The nominated lands include species and habitats of scientific interest, notably related to the presence of the species of special concern listed below. NCL Ref. 8 (2) (e)

2.10 Management of the nominated lands is expected to be complex but feasible, and is outlined in "Management considerations" below. NCL Ref. 8 (2) (g)

2.11 The nominated lands offer a significant nature tourism opportunity, which is already being realized to a significant degree. The extent to which this can be further developed will depend on any cooperative agreements that may be made with the other main landowner in the area. NCL Ref. 8 (2) (h)

3. Species of special concern

The following table lists the species of concern known to depend at least in part on the nominated lands:

Common name	Scientific name	Description	NCL Schedule 1	Habitat use
Pygmy Blue Butterfly	*Brephidium exilis thompsoni*	Butterfly	Part 1. Extremely habitat specific	Depends on Saltwort marshes
Mosquitofish	*Limia caymanensis*	Brackish water fish	Part 2. Endemic	Former major population in Sea Pond
Mosquitofish	*Gambusia xanthosoma*	Brackish water fish	Part 2. Endemic	Restricted to Red Mangrove fringes of North Sound
White Land Crab	*Cardisoma guanhumi*	Crab	Part 2. In decline	Burrows in dyke road margins
Washwood	*Jacquinia keyensis*	Shrub	Part 2 In decline	Grows on beach ridge and dry cays
Glasswort	*Salicornia perennis*	Salt-tolerant succulent	Part 2	Open saline flats: host plant for Pygmy Blue

4. Conservation problems and special protective measures required

4.1 Invasive Common (Green) Iguanas, *Iguana iguana*, are present in the Barkers area as throughout Grand Cayman, and pose extremely serious threats to natural ecosystems. Control measures for this alien invasive species are outside the scope of this nomination.

4.2 MRCU dyke roads provide an easy access for ingress of other alien invasive species, including feral cats, free-roaming dogs, and a variety of invasive plants. No immediate solution is evident at this time.

4.3 *Casuarina equisetifolia*, the alien and invasive Australian Pine, has invaded some of the man-modified areas within the nominated lands. Local eradication will require short term removal followed by annual seedling removal for a number of years.

4.4 The Pygmy Blue Butterfly depends on a habitat which is scarce in the nominated area, and fragile to trampling by humans and horses. The distribution of the host plant must be mapped in detail, and measures taken to protect it from physical disturbance.

5. Management considerations

Management of the Barkers National Park as a holistic entity is liable to be complex because it involves a variety of stakeholders, including established nature tourism businesses, a majority landowner, the Mosquito Research and Control Unit, and currently un-managed use by the general public.

The extent to which the Department of Environment's original vision for the establishment and management of the Barkers National Park can be realized depends primarily on any cooperative agreements that may be possible with the private company which owns almost all the area of the proposed Park which is not Crown.

A Management Plan for the whole Barkers area will be the ideal, but if this proves unachievable, a less comprehensive Management Plan for the protected Crown Land will require DoE to develop a new but reduced concept for the National Park.

Protected Area Nomination

Bluff Cliffs, Cayman Brac

This nomination is made under Section 9 of the National Conservation Law, 2013

Nominator: **Cayman Islands Department of Environment**

1. Description of the Area

The nomination covers the vertical and near-vertical faces of the cliffs forming the edges of the sections of the Bluff on Cayman Brac, wherever they are adjacent to lands owned and protected by the National Trust for the Cayman Islands, and around the east end of the island where the cliffs provide one of the Cayman Islands' most important nesting sites for the Brown Booby. The cliffs are mapped in horizontal projection, and as nominated here they include all the adjacent vertical and overhanging cliff faces which cannot be represented in the horizontal parcel maps, and any caves opening to these cliff faces.

The nominated land parcels are as follows, and shown in maps below:

Block 94D, parcel 14 in part, to the extent where it is adjacent to Block 95C parcel 190.

Block 96D, parcel 18 in part, to the extent where it is adjacent to Block 97C parcel 52REM1.

Block 102A parcel 90 in part, to the extent where it is adjacent to Block 102A parcel 164.

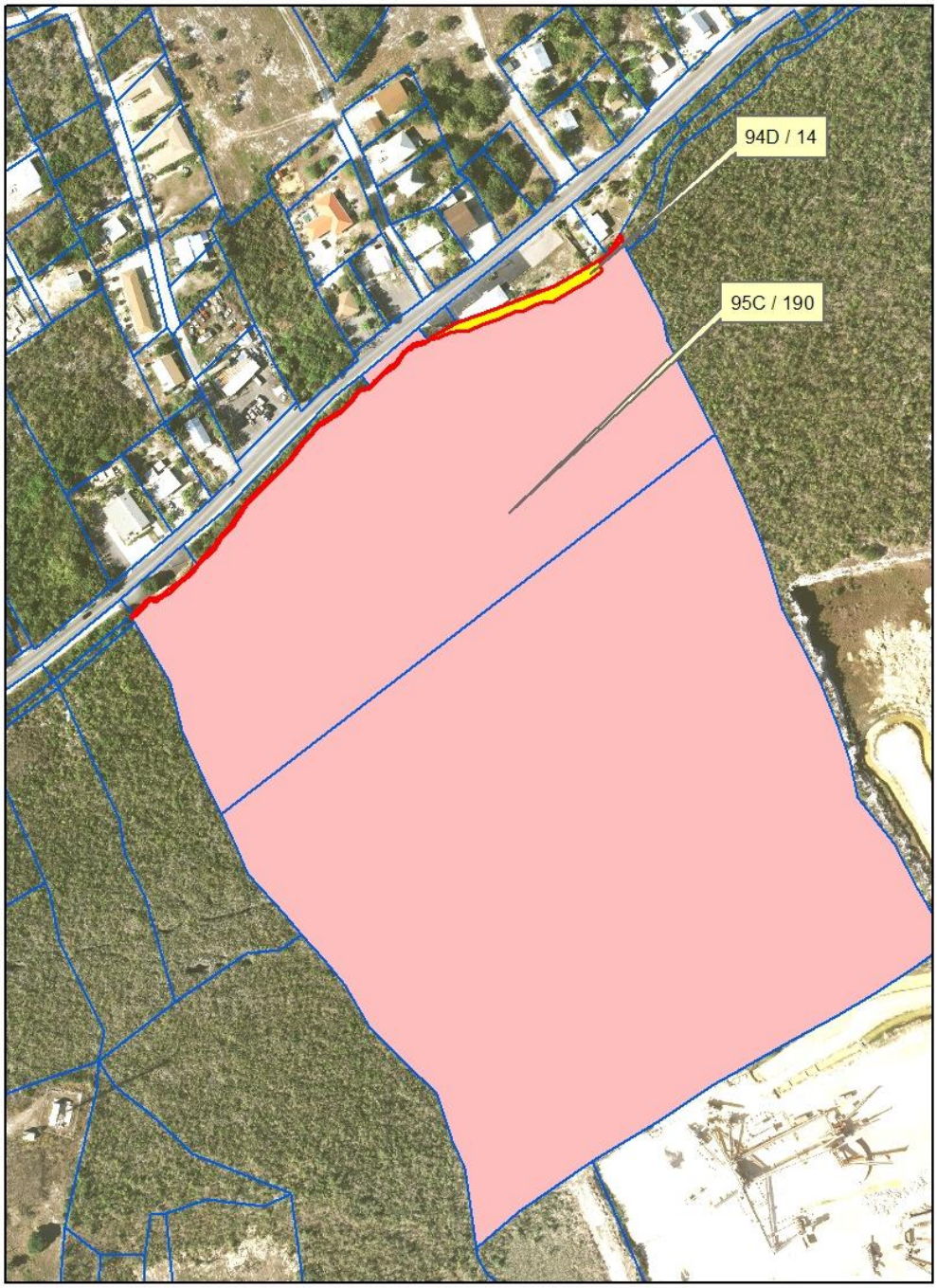
Block 103D parcel 61 in part, to the extent where it is adjacent to Block 104A parcel 65, Block 107A parcel 94, and Block 107A parcel 2.

Block 106E parcel 123 in part, to the extent where it is adjacent to Block 107A parcel 2.

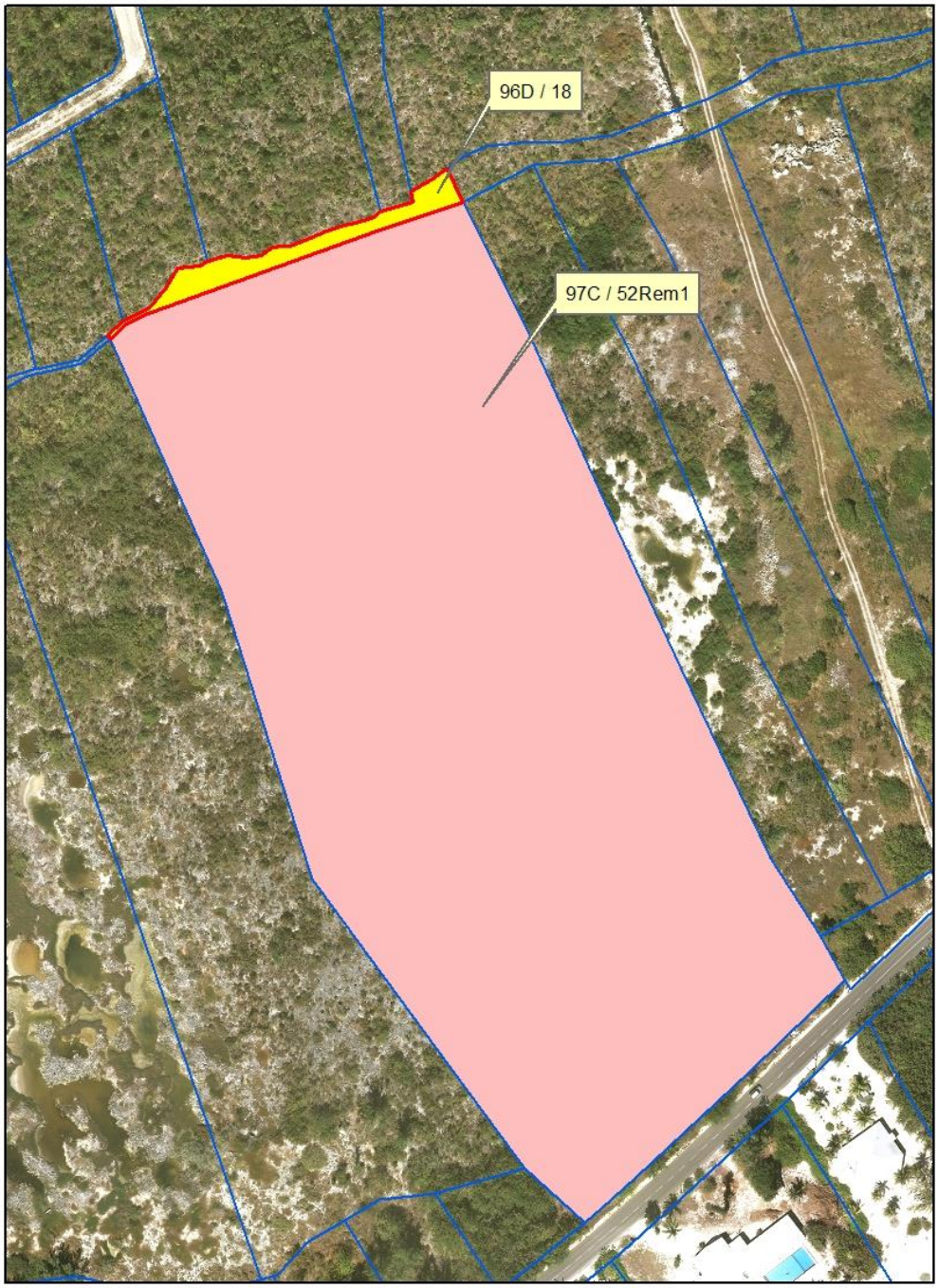
Block 107A parcel 47 in part, to the extent where it is adjacent to Block 104A parcel 23, Block 104A parcel 66, Block 107A parcel 95, and Block 107A parcel 2, and including those parts of the cliffs which are not represented in the horizontally projected parcel maps within this extent.

Block 111E parcel 220; Block 111A parcels 5, 70, and 71; Block 112A parcels 71 and 97.

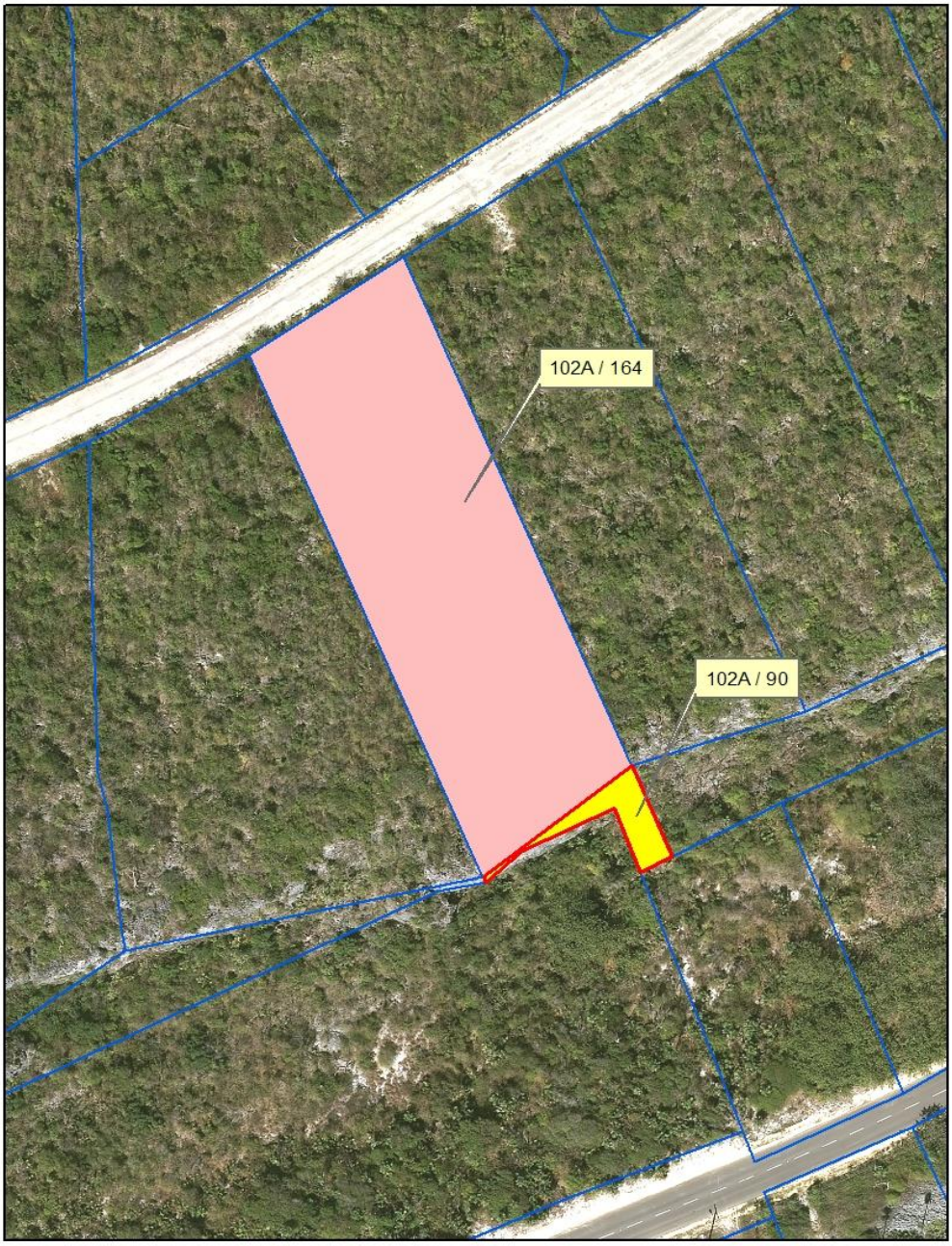
Block 112A parcel 62 in part, starting from its boundary with Block 112A97, and extending continuously to the point where it reaches the eastern boundary of Block 112A parcel 81.



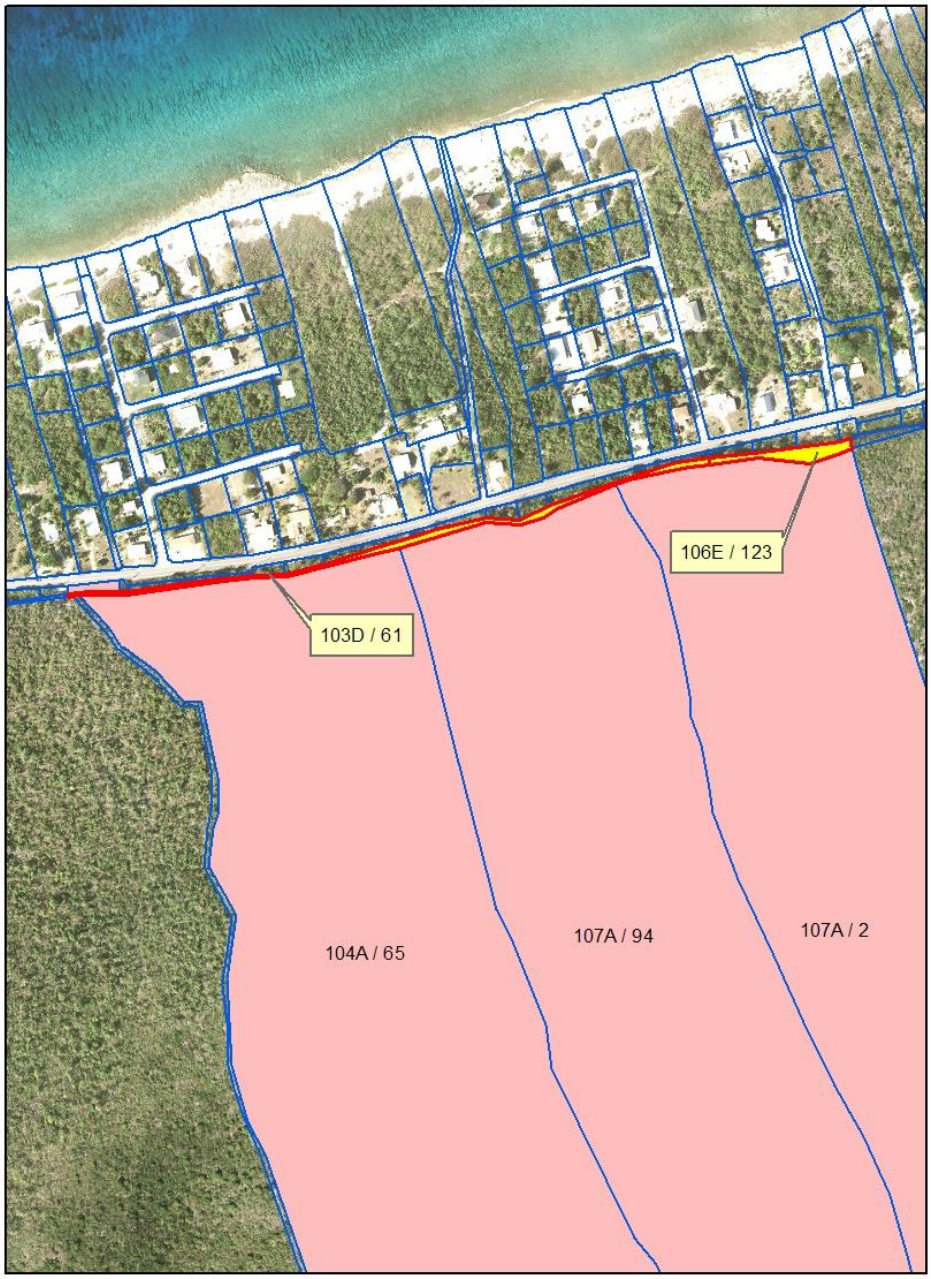
"The Splits" (National Trust)



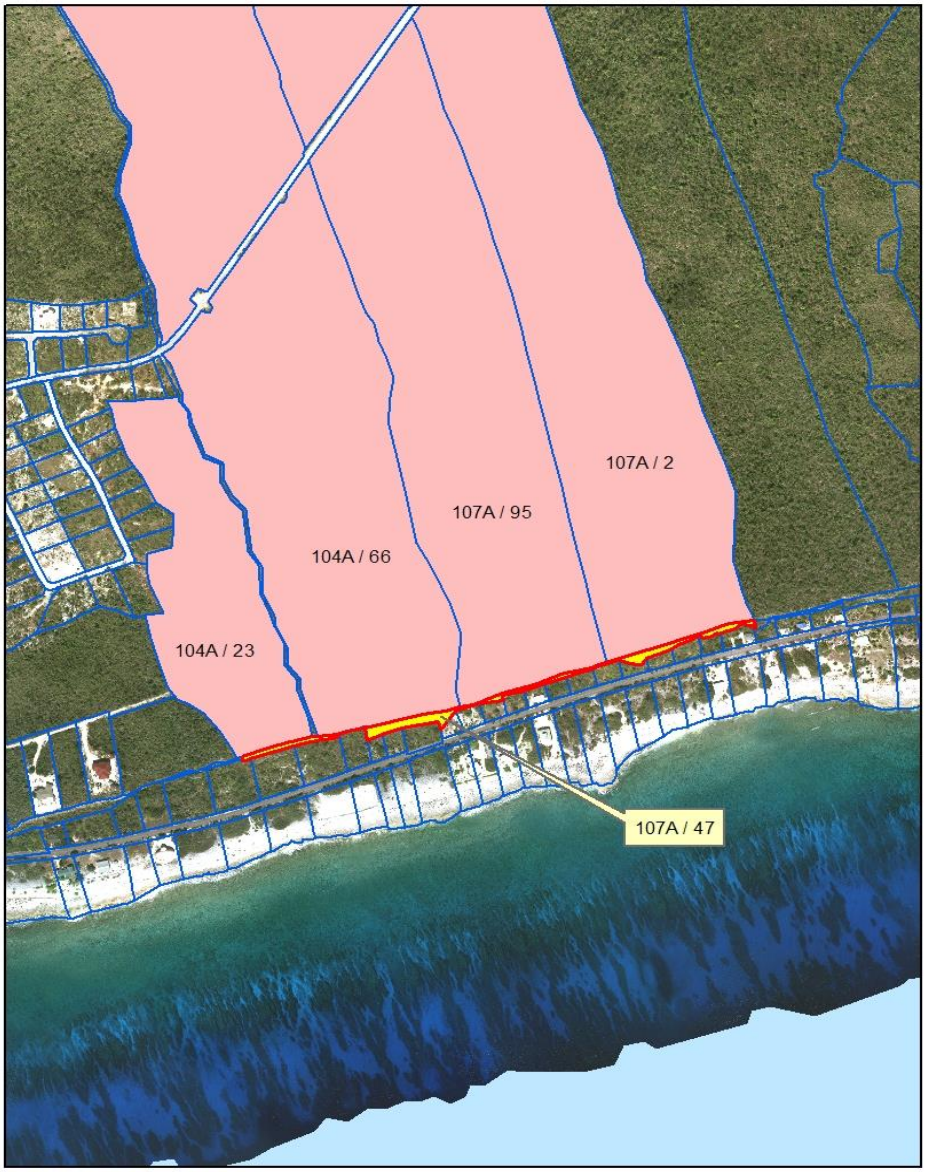
South Side Seasonal Wetland (National Trust)



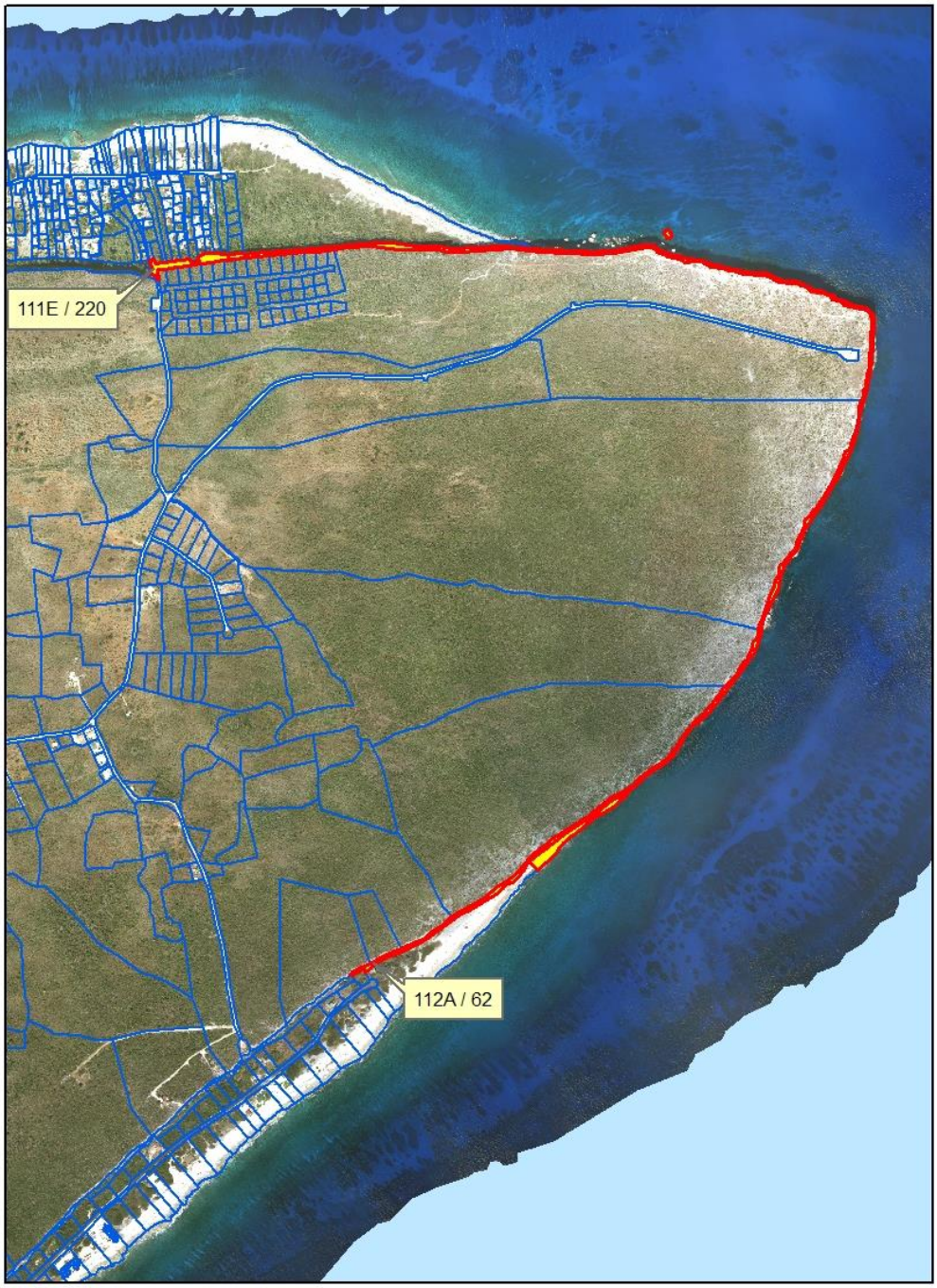
“Iguana Overlook” (National Trust)



Brac Parrot Reserve, north parcels (National Trust)



Brac Parrot Reserve, south parcels (National Trust)



East End Cliffs

2. Reasons for Nomination

Purposes and Objectives

2.1 Protection of the Bluff cliffs will safeguard key habitats for a range of endangered, threatened, endemic and migrant species, as described below under "species of special concern". NCL Ref. 8 (1) (a)

2.2 The Bluff cliff habitats of Cayman Brac are unique within the Cayman Islands, and their protection will support long term maintenance of the biodiversity that depends on them. NCL Ref.8 (1) (b)

2.3 Subject to additional species conservation measures, protection of the Bluff cliffs will be essential to recovery of seabird populations that nest on them. NCL Ref. 8 (1) (d)

2.4 Protection of the Bluff cliffs will secure their value as a cultural icon of the island with celebrated aesthetic value, in addition to their outstanding ecological value, and their value for paleontological and geological studies. NCL Ref. 8 (1) (e)

2.5 Protection of the Bluff cliffs will enable sustainable management of recreational activities such as rock climbing and caving, and will continue to contribute to the enjoyment of residents, visitors, photographers and artists who appreciate their natural grandeur. NCL Ref. 8 (1) (f)

Criteria for Protection

2.6 The Bluff cliffs are predominantly in a natural state. Significant modifications associated with existing road and footpath accesses are excluded from the nominated parcels, and other modifications are extremely localised. NCL Ref. 8 (2) (a)

2.7 Although sparsely vegetated over much of their extent, the Bluff cliffs support a significant diversity of life, including a high incidence of species of special concern. NCL Ref. 8 (2) (b)

2.8 The Bluff cliffs provide a suite of specialised ecological niches that are rare or absent elsewhere in the Cayman Islands, which supports the biodiversity which occurs there. NCL Ref. 8 (2) (c)

2.9 The caves in the Bluff Cliffs qualify as areas of special concern to cave-dwelling bats, and with appropriate management may facilitate regeneration of bat species which have declined. NCL Ref. 8 (2) (d)

2.10 The layers of rocks exposed by the tilted bluff edges include the only exposed face of the Brac Formation, whose type section is on the north coast cliff base near Little Cayman

Brac. The junction between the Brac Formation and the Bluff Formation rocks which lie above them, can be seen on the eastern cliff faces. Caves include some extensive caverns with flowstones which have already been investigated to shed preliminary light on ancient climates. Fossil assemblages in the cave sediments tell of unique mammals which are now extinct and other historic faunal changes. The geological and biological riches of the Bluff cliffs continue to attract international interest from scientists and conservation workers. NCL Ref. 8 (2) (e)

2.11 Management of the Bluff cliffs is feasible, though it will involve some challenges. See "Management considerations" below for further details. NCL Ref. 8 (2) (g)

2.12 The Bluff cliffs already serve as a nature tourism attraction for Cayman Brac, and their protection will maintain this tourism asset. The cliffs and trails that climb them attract hikers, photographers, artists, cavers and rock climbers. NCL Ref. 8 (2) (h)

3. Species of special concern

The following table lists the species of concern known to depend at least in part on the Bluff cliffs:

Common name	Scientific name	Description	NCL Schedule 1	Cliff use
No local name. Genus known as "Crown-beards" in USA	*Verbesina caymanensis*	Small flowering shrub	Part 1, unique to Cayman Brac. Critically Endangered	Grows on the shaded cliff faces over Spot Bay village to Channel Land
No local name	*Consolea millspaughii* var. *caymanensis*	Cactus	Part 1.Critically Endangered	Grows above, on and below cliffs on the south side
Sister Isles Rock Iguana	*Cyclura nubila caymanensis*	Rock Iguana	Part 1, unique to Cayman Brac and Little Cayman. Critically Endangered	Basks on cliffs and uses them to travel between coast and bluff top
Waterhouse's Leaf-nosed Bat	*Macrotus waterhousii minor*	Insectivorous bat	Part 1	Roosts in caves
Jamaican Fruit-eating Bat	*Artibeus jamaicensis*	Fruit eating bat	Part 1	Roosts in caves
Buffy Flower Bat	*Erophylla sezekorni*	Pollen and nectar eating bat	Part 1, rare in the Cayman Islands	Roots in caves
Barn Owl	*Tyto alba*	Owl	Part 1	Dens in caves

Brown Booby	*Sula leucogaster*	Seabird	Part 1	Nests on and near cliffs
Boatswain Bird	*Phaethon lepturus*	Seabird	Part 1. Declining population	Nests in cliff holes
Merlin	*Falco columbarius*	Migrant raptor	Part 1	Soars on updrafts from cliffs
Killyhawk or Peregrine	*Falco spaverius*	Migrant raptor	Part 1	Soars on updrafts from cliffs

4. Conservation problems and special protective measures required

4.1 Many bats are critically dependent on the caves they roost in, and are often intolerant of human disturbance. Frequent visitation can therefore lead to desertion of caves by bats which may struggle to find alternative dwelling space. The Buffy Flower Bat is especially vulnerable to disturbance. Opening of some of the more accessible caves in the Bluff cliffs to tourism has already resulted in a significant reduction of viable bat habitat. Management of the Bluff cliffs to allow the remaining bat populations to be maintained will require caves important to bats to be identified, and for some to be closed to public entry.

4.2 Fossil assemblages in cave floor sediments are of interest to casual visitors as well as paleontologists, and their indiscriminate removal or disturbance compromises the record the cave sediment may offer to careful scientific investigation. As with 4.1 above, caves with undisturbed fossiliferous sediments should be identified and protected from disturbance.

4.3 Stalactites in caves can be fragile and may be broken by clumsy explorers or taken purposely for private collections as 'souvenirs'. A combination of education and selective access controls may be required to limit such damage.

4.4 Road construction creating new accesses from coastal platforms to the Bluff top, inevitably involve destruction of segments of the Bluff cliff. Protection of the Bluff Cliffs will enable NCC to guide NRA / PWD to avoid unnecessary damage to key sites for endangered species.

4.5 Maintenance of the path from Spot Bay village up past Peter's Cave to the Lighthouse trail, has resulted in localized loss of the critically endangered flowering shrub *Verbesina caymanensis*, which is unique to Cayman Brac any only found on this north-facing section of the Bluff cliffs. Invasive Shamrock (*Tecoma stans*) and Leaf-of-Life *(Bryophyllum pinnatum)* plants are displacing the *Verbesina*. Education of trail maintenance crews and their supervisors, combined with occasional reminder visits and monitoring, are likely to be the only practical means to reverse this localized damage.

4.6 The Bluff cliffs are only mapped in horizontal projection, and the Crown ownership is based on that. The mapping of the upper cliff edge in the Lands & Survey digital maps is inaccurate in several areas, as evidenced by aerial photography. Sections of cliff that are vertical or overhanging are simply invisible in horizontal projection, and so do not appear on the parcel maps. Caves are not mapped, even where they reach far beneath privately owned land, and situations may arise where openings to a cave could come both from the land above and from the Bluff cliff face. These mapping limitations could lead to challenges over the true boundaries of the protected area in vertical as well as horizontal planes, and over cave ownership. Any improvement to the protected area definition will require assistance from Lands & Survey and may need a definition of the Protected Area which goes beyond the block and parcel maps alone.

4.7 Buildings constructed close to the Bluff edge, and utility wires strung from coastal platform to bluff top, detract from the natural visual appeal of the Bluff cliffs and pose a hazard to young seabirds learning to fly. Light from buildings close the bluff edge is also likely to interfere with nesting Brown Boobies. Protection of the Bluff cliffs will raise awareness and sensitivity to the natural landscape when regulatory agencies are considering proposals to develop land adjacent to the Bluff cliffs.

5. Management considerations

The key tourist features of the Bluff cliffs (mostly caves and trails) are already under management by District Administration. Rock climbing and caving are currently almost unregulated.

A Management Plan developed in consultation with all stakeholders could realistically meet the aspirations of almost all users in a sustainable manner, and could be enforced cooperatively by on-island agencies with only a light oversight and monitoring obligation on DoE.

Ref. No.	A	S	I	P				Office use

CAYMAN ISLANDS GOVERNMENT

National Conservation Council

Department of Environment
DATE STAMP
and
SIGNATURE
of Issuing Officer
required here for validation

Cayman Islands Environmental Centre . 580 North Sound Road .
PO BOX 10202GT . George Town . KY1-1106 . CAYMAN ISLANDS
TEL: (345) 949 8469 . FAX: (345) 949 4020 . www.DoE.ky

IMPORT &/OR RELEASE OF ALIEN OR GENETICALLY ALTERED SPECIES

Once date-stamped and signed by the by the Department of Environment on behalf of the National Conservation Council, this permission constitutes

1) Advice to the Department of Agriculture on the application to import alien or genetically altered species;

2) Permission or denial to release alien or genetically altered species.

APPLICANT NAME	Dr. William Petrie
POSITION	Director
INSTITUTION	Mosquito Research & Control Unit
ADDRESS	Marco Giglioli Centre 99 Red Gate Road PO Box 486 Grand Cayman KY1-1106 Cayman Islands
PHONE	949-2557
EMAIL	William.Petrie@gov.ky
Intended Date Of Importation	Beginning in March 2017

Please submit application electronically, in MS Word form, via email to DoE@gov.ky.

Under the National Conservation Law and the Animals and Plants Laws the import and release of alien or genetically altered species without permission of the Department of Agriculture and the National Conservation Council respectively is illegal.

This Permit serves as

1) **Advice to the Department of Agriculture on the application to import alien or genetically altered species;**
2) **Permission or denial to release alien or genetically altered species.**

1. SPECIES & QUANTITIES TO BE IMPORTED

row	Species Common Name	*Species Scientific Name*	Quantity
1	Yellow fever mosquito	*Aedes aegypti (OX513A)*	Up to 2 Kg (eggs) / month
2			
3			
Add rows as necessary, or attach list separately.			

For those species which are IUCN listed certification of captive breeding or sustainable harvest should be provided along with application to import.

2. PURPOSE OF IMPORT

	Pet		**Breeding**	
X	**Display**		**Farming or other consumption**	
	Human Interaction		**Retail**	
X	**Other:** *Aedes aegypti* population control			
Tick as appropriate. Fill in if other purposes.				

3. LOCATION WHERE SPECIES WILL BE KEPT

OX513A eggs will be shipped from Oxitec Limited in the UK in regular shipments to support MRCU program requirements in the Cayman Islands. Following clearance and release of the shipments at Cayman customs, eggs will be transported and stored at the central MRCU compounds, 99 Red Gate Road, George Town, Grand Cayman until additional insectary capacity is established. Eggs may be processed through to adult stages at existing facilities located at MRCU compounds, or further distributed to a satellite production insectary(s) located within the Cayman Islands.

As a continuation to the OX513A *Aedes aegypti* control program underway in West Bay under existing permission from the Cayman Department of the Environment (DoE), the programmatic use of OX513A is proposed island wide for Grand Cayman, and beyond in other areas of the Cayman islands as emerging *Aedes aegypti* threats are identified by the MRCU. A staged expansion of the

Under the National Conservation Law and the Animals and Plants Laws the import and release of alien or genetically altered species without permission of the Department of Agriculture and the National Conservation Council respectively is illegal.

program across Grand Cayman, including the expansion of OX513A production capacity is foreseen.

Current program activities are described in Standard Operating Procedures (SOPs), which will be adapted as needed as operational scale increases and additional production is required to support program activities.

Community engagement activities may entail the use of male OX513A transported to various sites in mesh cages for display purposes whereby male OX513A are not intentionally released. Periodic entry of a human volunteer hand may take place through a mesh entry sleeve to demonstrate the non-biting nature of male OX513A, and the mesh entry sleeve is otherwise kept knotted. At the end of their use, the cages containing OX513A males are placed at -15 degrees Celsius or colder for more than 12 hours prior to disposal of the insect material.

A general description of the activities being proposed/requested/permitted.

4. BIOSECURITY MEASURES

Biosafety Standard Operating Procedures (SOP) are in place and staff will be trained to ensure conformance with these standards.

The production facilities will incorporate certain elements of Arthropod Containment Level 2 (ACL-2) standards appropriate to the chosen facility design for the specific stages of program expansion. Mosquito life stages not used for the release or other procedures are frozen below -15°C for more than 12 hours and disposed of.

Additional Biosecurity measures unique to the OX513A strain have been described in permission issued by the Department of the Environment (DoE) June 8th, 2016. There has been no additional information since that time which warrants increased biosecurity measures for the OX513A program.

Indicate whether biosecurity measures (anti-escape & species/people security) are in place or intended.

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5. Health & Safety Considerations / Measures Taken

Health and safety considerations have been described in permission issued by the Department of the Environment (DoE) June 8th, 2016. There has been no additional information which increases the health and safety risk profile of the OX513A program since that time.

6. Location Where Species Will Be Released

Releases are planned to expand over the entire island of Grand Cayman by 2019 through a staged roll-out of the program over an approximately 18-24 month period once additional production insectary capacity is established. Additionally, in response to surveillance and monitoring, other areas in the Cayman Islands such as Cayman Brac would be prioritised for treatment if the MRCU deems the OX513A program would be best suited for deployment in those areas.

The timing of OX513A releases will be in response to the local population levels of *Aedes aegypti,* which will vary according to seasonality, and response to the OX513A releases and other controls in the context of Integrated Vector Management (IVM).

Once suppression targets are achieved in a treatment area, release rates may be adjusted to a lower level maintenance phase release rate.

Standard Operating Procedures (SOPs) establish record keeping requirements whereby release points are recorded and retained on file, and as the program expands, release points are mapped and planned in advance. MRCU will provide notification to the DoE in advance of expansion activities, concurrent with community engagement activities in proposed areas for program expansion.

Descriptions have been given in the permission issued by the Department of the Environment (DoE) June 8th, 2016 under the headings *Releases, Monitoring, Fluorescent Marker, Other Measures,* and *Dispersal.* The descriptions provided therein remain valid, with the exception that the untreated comparator site referred to will eventually be subject to treatment consistent with the goal of island wide suppression of *Aedes aegypti.*

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A general description of the activities being proposed/requested/permitted.

7. Other Relevant Information to be Considered

The permission issued by the Department of the Environment (DoE) June 8th, 2016 outlines other relevant information to be considered at the time when the application was made. The information therein remains relevant and no new information which substantively changes the environmental or human and animal risk profile has been identified.

The use of *Aedes aegypti* OX513A as a control for local *Aedes aegypti* has been under evaluation by the MRCU in Grand Cayman through various stages from early field trials in 2009-2010, to the current early stage operational project which began releases in July 2016. The MRCU is now well positioned to oversee, in collaboration with Oxitec Ltd, the programmatic deployment of the OX513A *Aedes aegypti* as required across the Cayman Islands.

By signing my initials to each line, I, **The Applicant,** confirm that I have read, understand, and agree to the following:		**Applicant Initials**
1.	I will conduct my actions as described in this document, and will abide by the terms set forth in this agreement. Imported species will be used only for the reasons listed above. Any change of use of these species should be reviewed by the Department of Environment on behalf of the National Conservation Council, and the Department of Agriculture.	**WP**
2.	I will get the permission of and abide by all requirements of the Department of Agriculture before importing any alien species.	**WP**
3.	I will follow all procedures required by the Cayman Islands Government Department of Environment, as set out in this permission.	**WP**
4.	There will be no unpermitted release of live organisms or their offspring (if incidental reproduction occurs) or viable material, e.g., seeds, cuttings, etc., into any uncontrolled environment, either natural or artificial, of the Cayman Islands.	**WP**
5.	To avert possible introduction of exotic parasites or diseases, as well as offspring, there will be no discharge of effluents, disposal of waste, transport or housing media or other materials associated with the alien species or disposal of diseased or dead organisms or parts thereof except via lawful municipal waste discharge or disposal methods, i.e., sewage or garbage disposal.	**WP**
6.	These conditions follow the specimens imported and applicant should inform recipients of specimens (offspring, retail sale, etc.) of the above stipulations.	**WP**
7.	I understand that previous import permission for a listed or related species does not mean continuing permission to import nor does it mean that future permission to import will necessarily be granted.	**WP**

Under the National Conservation Law and the Animals and Plants Laws the import and release of alien or genetically altered species without permission of the Department of Agriculture and the National Conservation Council respectively is illegal.

I, **The Applicant,** undersigned, confirm that I have read, understand, and agree to abide by the permissions, conditions or denials outlined in this document.

SIGNATURE OF APPLICANT	**DATE**

FOR OFFICIAL USE ONLY:

IMPORT PERMISSION GRANTED	**YES / PARTIAL / NO**
RELEASE PERMISSION GRANTED	**YES / PARTIAL / NO**
REQUIREMENTS, RESTRICTIONS or REASONS FOR DENIAL	
NAME OF ISSUING OFFICER	**DATE**
SIGNATURE OF ISSUNG OFFICER	**DATE STAMP**
ON BEHALF OF THE NATIONAL CONSERVATION COUNCIL **Gina Ebanks-Petrie,** **Director, Department of Environment**	

SPECIES RECOMMENDATIONS

row	***Species Scientific Name***	**Quantity**	**Import**	**Release**
1				
2				
3				

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Interim report MRCU - February 2017
Friendly *Aedes aegypti* project in West Bay

Introduction

A nine month collaborative project has been established between the Mosquito Research and Control Unit (MRCU) and Oxitec to deliver a pilot operational program of Oxitec's innovative control system for *Aedes aegypti*, by sustained release of the OX513A *Ae. aegypti* males (also known as Friendly *Ae. aegypti*). The objective of the project is to demonstrate the efficacy of the technology in an operational setting as a precursor for full integration into the MRCU operations across the Cayman Islands. The current project is due to finish in April 2017, and this report provides interim results.

Oxitec's approach is based on a genetically modified strain of *Ae. aegypti* (OX513A) developed in 2002, which has two additional genes; a self-limiting gene, and a fluorescent marker. When OX513A male mosquitoes, that cannot bite and spread diseases, are released they seek out and mate with wild females. All the offspring fathered by the OX513A males inherit the two additional genes. The self-limiting gene causes descendants to die before adulthood; hence repeated releases of Oxitec males cause reductions in the successive generations. The fluorescent marker enables dynamic adaptive management of the release rate to ensure suppression of the local *Ae. aegypti* population.

Oxitec delivers this solution in conjunction with local teams and adapted to local circumstances including the use of complementary vector control tools such as breeding-site reduction, public education and chemical insecticides. Projects begin with a period of community engagement and infestation monitoring before starting releases of Friendly *Ae. aegypti*. The location of release points and number of Friendly *Ae. aegypti* released are adapted in response to the local infestation as informed by weekly monitoring.

In the Cayman Islands, the Friendly *Aedes aegypti* project was announced on May 5th 2016 followed by an extensive program of community engagement activities that ensured the majority support of Caymanians. The first release was conducted on July 28th 2016 at the Public Health Clinic of West Bay in presence of Timothy McLaughlin, the Public Health surveillance officer. The production and releases were ramped up and OX513A males started to be distributed in the treatment area mid-August. In parallel, the monitoring in the treatment and comparator areas, started in 2015, continued in order to evaluate the suppression of the local *Ae. aegypti* population and enable dynamic adaptive management of the programme.

Community engagement

The Friendly *Aedes aegypti* project started with an intense community engagement program utilising a wide variety of methods adapted to the local conditions and culture. Communication strategy was developed in collaboration and with support from public services. Both the Mosquito Research and Control Unit and the Public Health Department provided resources to help spread information and answer questions about the project.

A number of activities were conducted including door-to-door visits in the release area, information booths and distribution of leaflets in several locations on Grand Cayman, a public meeting, radio and television talk shows as well as public service messages in newspapers and on radio.

A public opinion survey conducted in July 2016 revealed that 80% of the population of Grand Cayman had heard about the project and that 69% supported the Friendly *Aedes aegypti* project, validating the strategy followed. Activities to reach out to the community have continued since the beginning of the releases and will continue throughout the project.

Releases

The first release of OX513A *Ae. aegypti* males was conducted on July 28th 2016. The production was ramped up to operational schedule, three batches per week, from the beginning of August 2016. Consequently, full operational releases effectively started in mid-August with average level of circa 300,000 OX513A males per week (from weeks 33-2016 to 43-2016, Figure 1A). There was a temporary drop in target production during weeks 44-2016 and 45-2016 (Figure 1A). This was due to disruption in transportation of egg shipments from the UK due to Hurricane Matthew.

Subsequently the release rate increased by approximately 33% from circa 300,000 to 400,000 OX513A males per week. Increase in production from initial rates was in response to the high mosquito population, due to seasonality, and the corresponding below target percentage of fluorescent larvae recovered from ovitraps. The percentage of fluorescent larvae is a proxy for the fraction of wild females mated with OX513A males; a target of over 50% is recommended for operational deployment. The production was increased by moving the maturation and storage of the adults into a nearby facility and increasing production space.

The quality of the males released was confirmed by the quality control processes conducted in the production facility ensuring that the OX513A males were in optimal conditions to find and mate with the wild *Ae. aegypti* females.

Monitoring

Regular weekly ovitrap surveillance was initiated before releases began and has been maintained to assess local population infestation in the release and the comparator areas. Ovitraps mimic natural breeding sites of Aedine mosquitoes and are used to collect eggs laid by females. They provide a sensitive measure of both presence (Ovitrap Index: proportion of positive traps) and abundance (Average number of eggs per trap) of the local population of *Ae. aegypti*.

Within the release area, ovitraps can additionally be used to assess mating fraction (proportion of the wild females mated with OX513A males) by detecting the fluorescent marker in the larvae hatched from the eggs collected. This key metric allows dynamic adaptive management of the releases to respond to the local population density thereby ensuring efficiency. For operational use, we target over 50 % mating fraction for rapid suppression.

Through regular monitoring of the population in both the treatment and comparator areas, a sharp seasonal increase in *Ae. aegypti* population in early June 2016 was detected and maintained until November 2016 at the end of the rainy season when the population decreased in both areas. Average number of eggs per trap was approximately 5 fold higher in the high season compared to the preceding

low season (mean of 7 and 7 eggs/trap in low season vs. 33 and 36 eggs/trap high season for release and comparator areas respectively). Infestation levels in the release and comparator areas remained comparable through pre-release baseline monitoring into high season when releases were initiated.

Mating Fraction was assessed in the release area from initiation of releases with fluorescent larvae being detected in the ovitraps in August. Due to the pre-mated females present before the start of the releases - female *Ae. aegypti* mate only once in a lifetime of 2 to 4 weeks - it is expected that the fluorescence will increase in the 4 weeks of initial releases before stabilising. Although increasing from 5% in August/September to 28% in October/November, we did not go beyond the 50% fluorescent rate target (Figure 1A). From mid-November onwards there was a sharp increase in mating fraction, averaging 95% (standard error of the mean [sem]: 3.4%) from week 47-2016 to 03-2017 well above the 50% target. This corresponded with both, an increase in release rate by approximately 33% and the start of the dry season resulting in seasonal drop in population of *Ae. aegypti*. Both these factors likely contributed to higher numbers of released OX513A males compared to local male counterpart.

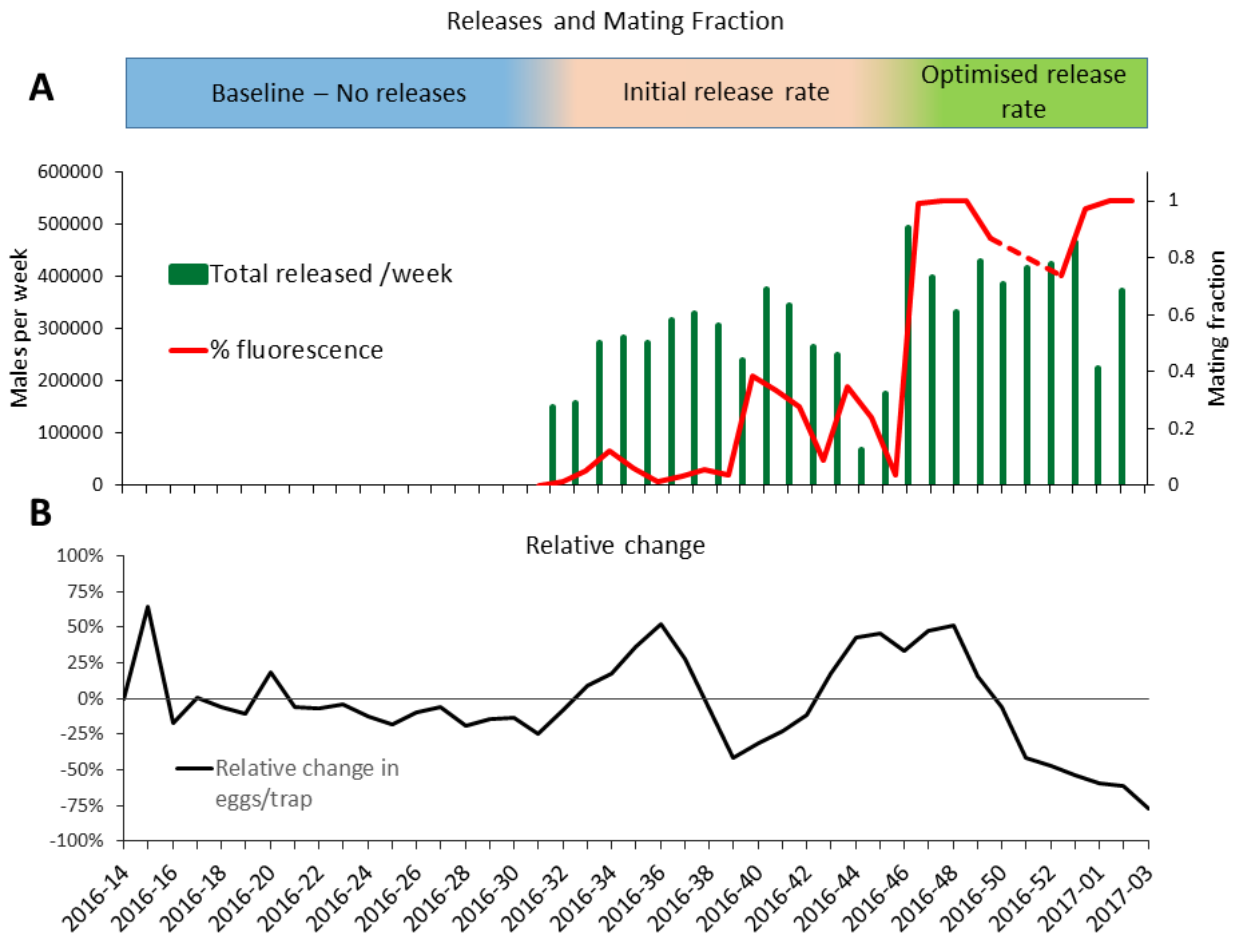

Figure 1: (**A**) Number OX513A males released per week and Mating Fraction (Percentage of marked fluorescent larvae) observed in the release area of West Bay. The dotted line indicates two missing data points; (**B**) Relative change in population in the release area relative to the comparator area. A negative value indicates suppression in the release area.

Adaptive Management

In accordance with adaptive management approach to Oxitec's control strategy, a two-fold strategy was adopted to achieve the 50% fluorescence target, when this was not initially attained under the challenging conditions of seasonally driven high infestation rates; an increase in release rates, coupled with insecticide applications in an integrated vector control approach.

Increased release rate

The production unit was expanded in order to allow a 33% increase in total production. In order to do this the maturation and storage of the adults was transferred to a nearby facility increasing overall production space.

Traditional control

During the rainy season, *Ae. aegypti* population levels are high and an integrated vector management (IVM) approach is desirable to achieve suppression. MRCU typically implements multiple methods to tackle *Ae. aegypti* populations including; house surveys, thermal-fogging and aerial pesticide applications particularly in response to dengue, chikungunya or Zika virus cases reports. In response to the below-target mating fraction and high mosquito numbers, an IVM consisting of aerial applied larvicide and adulticide sprays was initiated. Both release and comparator areas would be exposed to the same larvicide and adulticide applications to ensure adequate assessment of the impact of OX513A releases (Table 1).

- Aerial larvicide was applied on October 20th 2016 in both areas; a second application was planned the following week for optimal impact on the population of *Ae. aegypti* but could not be conducted due to strong winds. Strong winds persisted throughout November and prevented a second application.
- Biweekly aerial application of adulticides were also planned. However, the high winds also prevented these scheduled applications of pesticide at the optimal time (1h before sunset) to target *Ae. aegypti* during November 2016. Six applications (during sunset) targeted at *Aedes taeniorhynchus*, the black salt marsh mosquito, were conducted over both areas between October 29th 2016 and November 16th 2016 (this was part of the *Ae. taenyorhyncus* standard MRCU control).

Although larvicide and adulticides could not be applied in optimal conditions, it is likely that they contributed in the decrease of the *Ae. aegypti* population in both areas in November 2016 together with the start of the dry season (lower rainfalls, high winds and decrease in temperature).

Table 1. Larvicide and Adulticide applications in the release and comparator areas conducted in 2016. The time of application of the adulticide is optimized for the species targeted as they have different activity periods. An application outside the peak activity period has a reduced effect on the species.

Date	Treatment	Formulation	Target species
October 20, 2016	Larvicide	*Bacillus thuringiensis* (*Bti*)	*Aedes aegypti*
October 29, 2016	Adulticide	Chlorpyrifos (Mosquito Mist 2)	*Aedes taeniorhynchus*
October 31, 2016	Adulticide	Chlorpyrifos (Mosquito Mist 2)	*Aedes taeniorhynchus*
November 5, 2016	Adulticide	Chlorpyrifos (Mosquito Mist 2)	*Aedes taeniorhynchus*
November 6, 2016	Adulticide	Chlorpyrifos (Mosquito Mist 2)	*Aedes taeniorhynchus*
November 11, 2016	Adulticide	Chlorpyrifos (Mosquito Mist 2)	*Aedes taeniorhynchus*
November 16, 2016	Adulticide	Chlorpyrifos (Mosquito Mist 2)	*Aedes taeniorhynchus*

Population suppression

Aedes aegypti populations are subjected to substantial variations depending on meteorological conditions (temperature, humidity and rainfall). In Cayman, this is manifest by higher mosquito population in the wet hotter season (May to November: avg. 28.3C, 130mm rainfall monthly) and lower in the colder dryer season (December to April: avg. 25.5C, 60mm rainfall monthly).

Consequently, it makes the assessment of the local population in terms of absolute number difficult to interpret over prolonged periods and seasons. The impact of the Oxitec programme is therefore assessed in terms of change in the population of the release area relative to a comparator site. This approach accounts for underlying seasonally driven effect and any impact of conventional control that were applied to both sites. The ongoing change in the population of the release site relative to the comparator site is presented in Figure 1B as 4 week rolling average values.

Until week 51-2016, the *Ae. aegypti* populations naturally fluctuated in both areas but remained comparatively stable (relative change average 4% (sem: 4.5%) through the low and high seasons, even though populations had increased by a factor of 5 in the high season (7 and 7 eggs/trap in low season vs. 33 and 36 eggs/trap in the high season for the release and comparator areas respectively). This indicates that populations in both areas are similar throughout seasons and subjected to similar weather conditions.

Following the high mating fraction from week 47-2016, steady and sustained *Ae. aegypti* population suppression was observed from week 51-2016 onwards (Figure 1A). The one to two month lag between sustained high mating fraction and impact on population is typical of the technology as the generation time for *Ae. aegypti* is 2 to 4 weeks.

Comparing the eggs/trap ratios between the two areas over 2016 and 2017 dry seasons gives a 79% suppression in the release area (Figure 2, 0.97 vs. 0.21). This figure highlights the importance of monitoring a comparator area as the natural level of the *Ae. aegypti* population during the 4 weeks considered in 2017 in the comparator area (5.2 +/- 0.6; week 53-2016 to 03-2017) is lower than the 12 weeks average in 2016 (7.3 +/- 1.2; week 12-2016 to 23-2016) likely due to meteorological conditions and traditional control methods. This level of suppression is to be expected at this stage of the programme and confirms that the programme is well on track.

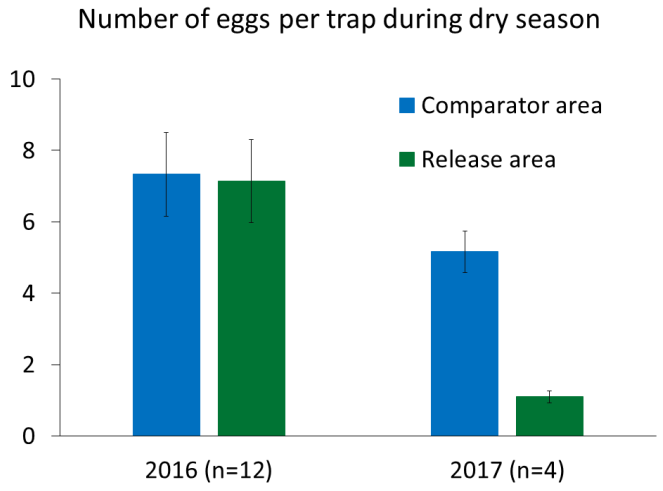

Figure 2: Average number of eggs collected in ovitraps in the release and comparator areas during the dry seasons of 2016 (before OX513A releases) and 2017 (after OX513A releases). A 79% suppression of the *Ae. aegypti* population was observed between the two periods.

Conclusion

The sustained high mating fraction over the 50 % target during the last 2 months is a clear indication that the released males are mating a large majority of the wild females and overwhelming the wild males in the release area. The impact on the wild population is already visible and we are now observing a 79% suppression of the *Ae. aegypti* population in the area. The monitoring of the release and comparator areas has enabled to take into account seasonal variations to obtain an unbiased estimation of the suppression measured in the release area regardless of the natural decrease due to the start of the dry season.

This outcome is fully within expectations according to previous field suppression observed in Cayman Islands, Brazil and Panama with substantial suppression observed within 4 to 6 months following start of releases at an adequate rate.

The adaptive management of the releases thanks to the fluorescent marker has enabled the programme to fit the local *Ae. aegypti* population requirements for suppression and the integration of traditional control methods. Integrated vector management using larvicides and adulticides, though difficult to evaluate, could have played a role in the rapid decrease of the populations in both areas in November 2016. The exact synergy between Oxitec and other control methods used by MRCU could be further evaluated in the future to optimise the impact on the wild population and reduce the cost of *Ae. aegypti* control.

The continuation of the releases and maintenance of a high mating fraction are expected to strengthen the suppression of the *Ae. aegypti* population in the release area in the coming months.

OX513A *Aedes aegypti* control programme in the Cayman Islands

Expansion phase

For submission to the National Conservation Council, Cayman Islands -January 2017

Introduction

The Cayman Islands Mosquito Research and Control Unit (MRCU) has a longstanding collaborative relationship with UK based Oxitec Ltd. in the deployment of the OX513A technology as a tool to supress *Aedes aegypti,* the mosquito that spreads Zika, dengue and chikungunya. Following successful experimental releases and suppression trials in 2009-2010 under permit from the Cayman Departments of Environment (DoE) and Agriculture (DoA), and the recent successful establishment of a small scale operational program in 2016 under DoE and DoA permission as approved by the National Conservation Council (NCC), the MRCU is seeking to expand the operational use of the OX513A tool in response to the most pressing MRCU operational needs across the Cayman Islands. The current production facility located in the MRCU compound has been servicing a populated area of approximately 2000 residents in West Bay and serves to establish the OX513A program as a valuable operational tool for the MRCU in its *Aedes aegypti* control efforts. Operational scale-up in Grand Cayman is proposed see the staged expansion of OX513A use across the entire island once additional production capacity is established. Scenarios for production scale up and program expansion are outlined within this document. Additionally, as *Aedes aegypti* control endpoints are realised in the currently permitted release area, additional production capacity of the currently installed Mobile Rearing Unit (MRU) can be utilized in response to the highest priority emerging threats posed by *Aedes aegypti* as identified by the MRCU. Currently there are two scenarios under consideration to ensure established OX513A production capacity is best utilized in 2017 while additional production capacity is being established. Beyond the currently permitted area in West Bay, scenarios for a modest expansion of the current treatment area in West Bay are under consideration, as well as using OX513A on the island of Cayman Brac in response to the recent detection of an established population of *Aedes aegypti* on that island. The identification of *Aedes aegypti* in Cayman Brac is illustrative of the need for flexibility in deploying the OX513A technology within the territory of the Cayman Islands as the MRCU undertake surveillance of *Aedes aegypti* populations and identify where the OX513A technology is best suited.

This document provides a general description of the programmatic use of OX513A and the operational approach to a staged expansion of the program across Grand Cayman and the Cayman Islands as needed, including the expansion of mosquito rearing facilities and expected production and release targets. The document is laid out in the following sections:

A. General programme description- Adaptive management of OX513A releases

B. Staged expansion of the OX513A programme

C. Compliance oversight and reporting

A DoE permit application is supplied as Appendix 6 which requests approval for the importation of a set maximum number of OX513A eggs/month for program deployment as needed within the Cayman Islands accounting for eventual production scale-up. As appropriate, the permit application includes reference only to the previous permit application to avoid redundancy as

key information on the OX513A technology has previously been reviewed by the NCC under the current project approval.

A. General programme description- Adaptive management of OX513A releases

In a globally or regionally centralised egg production facility (e.g. currently UK based) OX513A eggs are continually produced from a cycling colony of homozygous OX513A and subject to regular quality control checks as established in Standard Operating Procedures (SOPs). OX513A eggs are shipped in regular shipments throughout the course of the program to the facility near the release site where they are reared through to pupae, sex sorted to select male pupae, the males are matured to adults for release. Sexually mature OX513A males are released from predefined release points generally following the road patterns in the release area to ensure even coverage of the area.

OX513A can be deployed both to supress a local *Aedes aegypti* population, as well as maintain suppression and prevent population resurgence in an area with low levels of *Aedes aegypti*. Under the adaptive management model, release rates are dynamically adjusted in proportion to the local *Aedes aegypti* population as it responds to suppression through the release of OX513A self-limiting males, as well as fluctuations in response to seasonality and/or other controls used in the context of a broader Integrated Vector Management (IVM) approach.

The OX513A programme can be divided into three sequential phases:

1. *Preparation Phase:*

   This Preparation Phase is used to conduct baseline mosquito population measures, establish production and distribution capacity, and finalise a release plan for the release area, including initial application rates and locations. The OX513A program is compatible with conventional control programs and exploiting synergy in an integrated approach with local vector control activities is evaluated in the preparation phase in addition to collating historical climatic, and vector surveillance and control data. Although the chosen number of OX513A males for releases is relative to the estimated size of the target *Aedes aegypti* population, wild populations of *Aedes aegyp*ti are most closely associated to human populations and therefore the release rate is often described as 'number of OX513A males per person'. It is during the preparation phase that community engagement activities are started.

2. *Suppression Phase:*

   The Suppression Phase is when the *Aedes aegypti* population is initially brought under control. Ideally, this can be timed to take advantage of seasonally low pest pressures or as a follow-on from existing vector management activities.

Following the Preparation Phase, the initial releases of OX513A males take place in a systematic manner from pre-determined georeferenced release points at regular time intervals, for even and consistent coverage of the treatment area. Release points are generally spaced no more than 100m apart, and releases occur 3 to 7 times per week at a constant release rate. The initial release rate is a function of the human population and the estimated wild *Aedes aegypti* infestation level in the treatment area at start of releases. The rate of release is adapted on an ongoing basis as informed by data collected throughout the release, and is generally reduced as control of the wild *Aedes aegypti* population is achieved.

3. *Maintenance Phase:*

   Once the *Aedes aegypti* population has been reduced to target levels the programme enters the Maintenance Phase, designed to prevent resurgence of the wild *Aedes aegypti* population. A range of approaches can be adopted and customised to individual control programmes. Re-infestation in this context may be caused by the immigration of wild *Aedes aegypti* into the programme area, perhaps as eggs or adults inadvertently moved by humans. It may also relate to the size of the egg bank (eggs laid at an earlier period remaining in the environment), though the viability of such eggs is expected to decline over time such that this is expected to be a source of re-infestation for a limited period only. This approach can be applied to contiguous sub-areas of the program as they become well controlled, even while other areas remain in the Intervention Phase. In the event that effective elimination of *Aedes aegypti* is achieved in Grand Cayman, the Maintenance phase focuses on monitoring activities island wide, and potential ongoing releases in ports of entry, or other areas identified by MRCU, which are areas of high risk for re-infestation.

Ongoing monitoring of the wild population pre, during and post release is undertaken using egg surveillance (ovitrapping). Supplementary adult trapping may be deployed during critical phases of program. Identification, detection, and trapping methodologies are all well defined in SOPs and are used to inform the adaptive management of OX513A releases.

B. Staged expansion of the OX513A programme

The current *Aedes aegypti* control programme is being conducted under permit from the Cayman Department of the Environment (DoE) and importation of *Aedes aegypti* strain OX513A eggs permitted by the Cayman Department of Agriculture (DoA). The coverage area of the current project as described in the current DoE permit is the "South Part of West Bay" in an area which comprises about 1800 inhabitants over 300 acres (it may be more accurate however to describe it as the West part of West Bay district- as represented in Figure 1). The current activities represent the first stage of an *Aedes aegypti* control program which is anticipated to roll-out over the entire island of Grand Cayman over an 18-24 month period once additional rearing capacity is established, and subject to final Government approval.

Currently permitted activities:

- The current DoE permit serves to permit the release of *Aedes aegypti* OX513A in the current program area and is valid from June 8th, 2016 to June 30th 2017 (Appendix 1).
- The current DoA permit for importation was issued for a variance in OX513A egg importation amount and is valid from November 10th, 2016 to June 10th, 2017 (Appendix 2). Importation of OX513A eggs prior to November 10th, 2016 was under DoA permit issued June 20th, 2016 (Appendix 3).
- The DoE additionally issued, November 10th, 2016, approval for a variance in described activities, whereby OX513A pupae contained within sealed release devices are permitted to be housed in the Mosquito Research and Control Unit (MRCU) insectary (Appendix 4).

As a prerequisite to the permits issued for the variance application for the DoA and DoE permits issued November 10th, 2016, an interim project report was supplied to the National Conservation Council (NCC) (Appendix 5) and a site visit was conducted October 12, 2016. A minimum of one additional report under the current permit is to be provided by July 2017.

This section presents a proposed staged approach for the continuation of program activities under the OX513A *Aedes aegypti* control programme beyond the inhabited areas described in the current DoE permit. Operational expansion of the *Aedes aegypti* control program across Grand Cayman is proposed, and additionally, in response to recent surveillance activities, the potential for an OX513A control program to be deployed in Cayman Brac in 2017 is under evaluation by the MRCU. Delivery of the OX513A *Aedes aegypti* control program is to be undertaken via a partnership between Oxitec and the MRCU. Oxitec will continue with maintenance activities until the program has been implemented Island-wide in Grand Cayman, which is anticipated to be completed by mid-late 2019. Once suppression targets have been achieved island wide, ongoing maintenance is foreseen to be delivered by the MRCU through targeted releases. *Aedes aegypti* OX513A is seen as a tool in an Integrated Vector Management (IVM) approach for *Aedes aegypti* management and as such, the MRCU will be working with Oxitec to establish parameters for OX513A program delivery in the IVM context moving forward.

Stage 1 below describes proposed activities to be undertaken beginning in 2017, and Stage 2 below describes proposed scale up of activities into 2018 and beyond.

Stage 1 – Targeted use of existing production capacity beginning in 2017

Options are under consideration which aim to continue using the existing facilities at full OX513A production capacity as control targets are achieved in the existing treatment area.

<u>Option a)</u> The existing production facility could support a modest expansion into adjacent areas in West Bay in 2017 as part of an island wide roll-out. Targeted releases at lower levels could continue in the existing West Bay treatment area as the program moves into an operational maintenance phase in that area, intended to maintain suppression of the local *Aedes aegypti* population.

Option b) Once control targets are achieved in the current West Bay site, or concurrent with maintenance phase activities at that site, the existing OX513A production capacity could be used to target an emerging threat of *Aedes aegypti* in Cayman Brac. Ongoing surveillance of the local *Aedes aegypti* in Cayman Brac will inform OX513A program deployment options in that area.

Option c) Targeted releases at lower levels could continue in the existing West Bay treatment area as the program moves into an operational maintenance phase in that area, intended to maintain suppression of the local *Aedes aegypti* population.

Having the flexibility in regulatory permitting in response to the *Aedes aegypti* population dynamics will ensure the MRCU can best deliver control activities with the OX513A program.

Facilities:

As a continuation of the currently permitted activities, the mobile production facility situated in MRCU compounds at 99 Red Gate Road, and additionally the insectary subsequently approved under the application for variance to the permit are proposed to be collectively used the deliver the proposed Stage 1 activities beyond the current permit. The facilities have been previously described in the DoE permit (Appendix 1) and in the application for variance (Appendix 4). Additionally, a site visit was conducted by several NCC members on October 12, 2016 as part of the variance application process.

Production:

As part of the Quality Management System (QMS) implemented in the OX513A *Aedes aegypti* control prorgamme, Standard Operating Procedures (SOPs) are in place to ensure oversight on production through all stages of the program from the receipt of OX513A eggs from the UK to the release of OX513A males in the treatment area. The SOPs also serve to ensure traceability and chain of custody for all OX513A material handled in the course of program delivery. Records maintained as part of the QMS also ensure appropriate documentation of activities to demonstrate regulatory compliance for the current permitted activities, and will be adapted as production practices and facilities scale up to meet program delivery targets proposed activities. QMS records will remain an integral means to oversee and demonstrate regulatory compliance.

The anticipated production from the existing facilities at maximum capacity is not expected to exceed 700,000 OX513A adult males/week

Proposed treatment areas- options under consideration:

The current treatment area is represented in Figure 1, and release points have been documented to date and retained. Under the current project, a control area has been monitored to provide a comparator (see Figure 1).

As suppression targets are achieved in the current treatment area, OX513A male release numbers are aimed to be reduced based on the adaptive management design of the program, and the area will be considered to move into a maintenance phase at a reduced release rate.

Production capacity from the existing mobile rearing unit could then be used to treat the most at risk areas, depending on the status of the local *Aedes aegypti* population at the time.

One option currently under consideration is to expand current operations to areas immediately adjacent to the initial treatment area, and continue expansion as the original treatment area moves entirely into maintenance phase. Figure 1 provides an example of the scale of expansion which could be supported by the current production capacity in Stage 1. A second option for Stage 1 expansion is illustrated in Figure 2, whereby the populated areas of Cayman Brac undergo OX513A releases. Treatment of this area would be possible as one operation covering all populated areas, and due to the isolation and limited immigration potential is an ideal area for OX513A program deployment. The exact boundaries of the specific treatment areas and timing is dependent on the measurement of key parameters at the time, Figures 1 and 2 are intended only to provide a sense of scale, and area for potential expansion sites, specific boundaries are to be determined as Stage 1 expansion progresses as informed by adaptive management and ongoing data collection. Other viable options may arise in response to *Aedes aegypti* surveillance activities.

At the time of operational expansion, more precise identification of program boundaries will be made and communicated to the National Conservation Council via the Department of the Environment as a matter of routine reporting. At that time, community engagement consistent with past program activities will be continued and focused on the expansion area in advance of operational expansion. Community engagement activities may entail the use of male OX513A transported to various sites in mesh cages for use in demonstration activities as appropriate (Figure 4). During community engagement demonstrations, OX513A males are not intentionally released from cages, but are used to demonstrate that male mosquitoes do not bite when a human hand enters the cage through a mesh sleeve, which is otherwise kept knotted to prevent release. At the end of their use, the cages containing OX513A males are placed at -15 degrees Celsius or colder for more than 12 hours prior to disposal of the insect material.

Beyond 2017, under an operational programme, the current facility is anticipated to be in constant production as part of the staged island wide coverage of Grand Cayman. Section B outlines how additional OX513A production capacity is proposed to be added in Stage 2 of island wide expansion.

Figure 1. Potential extent of area for 2017 expansion in West Bay.

Figure 2. Potential extent of area for 2017 expansion in Cayman Brac

Stage 2 - Operational program scale up with expanded production capacity.

Stage 2 operational expansion proposes to roll out the OX513A *Aedes aegypti* program across inhabited areas of Grand Cayman over an 18-24 month period to achieve island wide control of *Aedes aegypti*. A formal agreement between Oxitec Cayman limited and the Government of the Cayman Islands is anticipated, to establish the main program activities and responsibilities for each party. An overview of the Stage 2 facility requirements, production activities, and the treatment area are provided below independent of the individual responsibilities of each party which will be established through contractual arrangements.

The Stage 2 program objectives are as follows:

- Establishment of additional rearing capacity for of OX513A on Grand Cayman.
- Significant suppression of the local population of *Aedes aegypti* throughout the island of Grand Cayman within 24 months of a date to be established in the final Heads of Agreement with MRCU.

Note: Effective elimination of *Aedes aegypti* from the island of Grand Cayman is a potential outcome, but not defined as a specific goal, although this may require a longer time-period than the initial 24-month duration of the Stage 2-Operations Phase.

Once island wide suppression targets are achieved, depending on the scenario to be agreed in the future, the existing facility is proposed to be under the operation of the MRCU, to deliver maintenance releases of OX513A, and in the treatment of any localised "hot-spots" and "entry-ports" of *Aedes aegypti* re-infestation.

Key elements of Stage 2 are presented below.

Facilities

The rearing facilities used in Stage 2 may be based either on a modular system using multiple adapted shipping containers, the numbers of which can be increased based on production needs, or the adaptation of a suitable permanent building structure, or a combination of the two. A final decision will be taken based on the most optimal production solution in light of any constraints identified in the planning process.

Oxitec mobile insectaries such as the existing mobile rearing unit (MRU) in place in the MRCU compound, 99 Red Gate Road are designed to Cayman Islands Building Codes and are based on standard 40-foot shipping containers structurally modified and fitted out under factory conditions. The laboratories are internally insulated and contain basic services such as water supply/extract, air conditioning and lighting.

The MRU currently in use in Grand Cayman is comprised of a single modified 40-foot shipping container. This MRU design can accommodate egg hatching, larval rearing and adult eclosion activities for male OX513A, as was done early in the current project, or be used solely for egg hatching and larval rearing, with adult male OX513A eclosion taking place in a separate insectary

building or adapted shipping container, as is now undertaken following the approval of a variance application for this activity. The Stage 2 operational expansion in Grand Cayman may be supported by the installation of additional production capacity based on a similar modular insectary design for all production activities whereby 40-foot shipping containers are either stand alone, or integrated together with appropriate allowance for passage of persons and services between. As with the current MRU design in use, appropriate ramp access as well as relevant occupational health and safety elements will be incorporated to ensure that the required Certificate of Occupancy can be issued by Cayman Islands Department of Planning.

Modular insectaries could be installed in a suitable location to be agreed contractually with the Cayman government at a future date. Installation will require a stable ground surface with adequate access to utilities and waste water services. Integral to the modular design is that the production modules can be removed once suppression targets have been achieved island wide and the program enters island wide maintenance phase. Rearing of OX513A to support maintenance phase activities beyond the Stage 2 expansion can be supplied from the existing mobile unit and MRCU insectary currently situated at 99 Red Gate Road.

Depending on the site availability, a production insectary based on a conventional building structure may be purpose built, or modifications may be made to an existing building as required in a suitable location with adequate access to utilities and water and waste water supply. The leasing of an empty warehouse and retrofitting for the anticipated duration of the project for example would suit this purpose.

Production

As described in Stage 1, Standard Operating Procedures (SOPs) are in place to ensure oversight on production through all stages of the program from the receipt of OX513A eggs from the distribution facility (e.g. currently the UK) to the release of OX513A males in the treatment area. In the context of the Stage 2 expansion, specific SOPs will be developed to adapt to the scale up of production as part of the Quality Management System (QMS).

The anticipated maximum production capacity required is not anticipated to exceed 10 million OX513A males/week under the most optimal production scenario during the 18-24 month program Stage 2 period.

Treatment area

Figure 3 is a conceptual representation of potential expansion staging through from initial Stage 1 expansion beginning 2017, through to Stage 2a and 2b operational expansion through 2018-2019, with Grand Cayman brought into an ongoing operational maintenance phase beyond late 2019. As with Stage 1 expansion, specific boundaries are to be determined as informed by adaptive management and ongoing data collection. Less densely populated areas towards the eastern side of the island would be spot treated in inhabited areas only, thus most of the interior of the eastern side of the island would not be subject to release.

Designated animal sanctuaries under the Cayman National Conservation Law

The areas of Meagre Bay Pond and Colliers Bay Pond are designated Animal Sanctuaries under Part 3, and described in Schedule 4 of the National Conservation Law (2014). For permits authorising activities in a protected area, such as designated animal sanctuaries, the Council must be satisfied that the activity is compatible with the relevant management plan for the protected area. Given that the neither Meagre Bay Pond, nor Colliers Bay Pond represent typical *Aedes aegypti* habitat, OX513A releases in those areas are not anticipated, and excluding the areas will not impact program delivery. Any inhabited areas adjacent to the protected areas, such as the cluster of houses to the southwest of Meagre Bay pond, which may partially fall within the legal boundaries of the protected area as described in Schedule 4 of the National Conservation Law, may be treated with alternative measures as part of the overall Integrated Vector Management (IVM) activities undertaken by the MRCU. Due to the small size and ease of treating these potential areas with alternative measures, this approach should simplify the administration and permitting of the island wide proposal for the OX513A program, as such an approximately 500 metre buffer around the protected areas will not be subject to release.

Figure 3. Conceptual representation of potential expansion staging for Island wide treatment of Grand Cayman. Protected animal reserves and 500 surrounding buffer zone are indicated in red.

Figure 4. Mesh cages to be used in community engagement activities using OX513A

C. Compliance oversight and reporting

C.1 – Compliance oversight

Continuity in record keeping serves as the principal means to demonstrate and ensure compliance with conditions under current Department of the Environment (DoE) and Department of Agriculture (DoA) permissions with consistency and transparency.

The Cayman Islands DoE and DoA have both issued permits under their respective legal authorities to allow the OX513A *Aedes aegypti* control program to proceed in Grand Cayman. November 10th 2016, variances on total import volumes, and the location of rearing activities were granted. Project permits have a duration to June 2017 and specific conditions are defined in each (Appendices 1-4). Activities to date have been conducted in compliance with all permit conditions, and records are maintained as part of the operational standard for the delivery of the OX513A *Aedes aegypti* control program.

Documentation requirements such as Standard Operating Procedures (SOP's) are integral to program administration and records maintained as part of the Quality Management System (QMS) can serve to demonstrate compliance oversight. The principles of traceability and chain of custody for all OX513A material handled in the course of program delivery will remain integral

to the maintenance and development of new SOPs under all production scenarios moving forward. An analysis of record keeping requirements under an expanded OX513A *Aedes aegypti* control program will be undertaken to ensure that compliance with any new permit conditions may be demonstrated to the DoE, DoA or the NCC as needed.

C.2 - Reporting

A report is required to be provided by July 30th, 2017 under the existing DoE permit, with an additional project conclusion report at a later date if necessary.

It is proposed that beyond the reporting July 30th, 2017, as program moves into Stage 1 and Stage 2 operational expansion, the MRCU and Oxitec provide an annual report to include but not limited to, a technical summary report including treated areas, a report of community engagement activities, a regulatory compliance report against permit conditions.

Attachments:

Appendix 1- 2016 NCC release permit – signed

Appendix 2 -2016 MRCU import permit - 1kg Additional

Appendix 3-2016- MRCU Import Permit

Appendix 4- 2016 MRCU-Oxitec Variance Nov 2016

Appendix 5 - Interim report MRCU & Oxitec - Oct 2016

Appendix 6- Cayman Islands - Alien Species Application Permit

Appendix 1

Ref. No.	A	S	I	P				Office use

CAYMAN ISLANDS GOVERNMENT

DEPARTMENT OF ENVIRONMENT
Department of Environment
DATE STAMP
JUN 08 2016
SIGNATURE
of Authorising Officer
required here for validation
CAYMAN ISLANDS

Cayman Islands, Environmental Centre . 580 North Sound Road . PO BOX 10202GT . George Town . KY1-1001
CAYMAN ISLANDS
TEL: (345) 949 8469 . FAX: (345) 949 4020 . www.DoE.ky

PERMISSION TO IMPORT & RELEASE ALIEN OR GENETICALLY ALTERED SPECIES

Once date-stamped by the Department of Environment, this permission constitutes an agreement between the following Parties:

Department of Environment

(on behalf of the National Conservation Council)

and

The Applicant

The Applicant undertakes to import alien species as follows:

APPLICANT NAME	Dr. William Petrie
POSITION	Director
INSTITUTION	Mosquito Research and Control Unit
ADDRESS	Marco Giglioli Centre 99 Red Gate Road P.O. Box 486 Grand Cayman KY1-1106 Cayman Islands
PHONE	949 2557
EMAIL	William.Petrie@gov.ky
Intended Date Of Importation	June 2016

All particulars in the attached documents make up part of this Permit.

Importation requires a separate Permission by the Department of Agriculture.

Under the National Conservation Law and the Animals and Plants Laws the import and release of alien or genetically altered species without permission of the Department of Agriculture and the National Conservation Council respectively is illegal.

This Permit serves as

1) **Advice to the Department of Agriculture on the application to import alien or genetically altered species;**
2) **Permission or denial to release alien or genetically altered species.**

1. SPECIES & QUANTITIES TO BE IMPORTED

row	Species Common Name	*Species Scientific Name*	Quantity
1	Yellow fever mosquito	*Aedes aegypti (OX513A)*	1.650 Kg (eggs)
Add rows as necessary, or attach list separately.			

For those species which are IUCN listed certification of captive breeding or sustainable harvest should be provided along with application to import.

2. PURPOSE OF IMPORT

	Pet		**Breeding**	
	Display		**Farming or other consumption**	
	Human Interaction		**Retail**	
X	**Other: Mosquito population control**			
Tick as appropriate. Fill in if other purposes.				

3. LOCATION WHERE SPECIES WILL BE KEPT

The eggs would be sent from Oxitec Limited in UK approximately once a month and then hatched and processed in a dedicated ACL-2 production facility situated in MRCU compounds, 99 Red Gate Road, George Town, Grand Cayman. Up to 600,000 Oxitec males (strain OX513A) would be produced every week for up to a nine month period.

Rearing and Sorting

Eggs would be vacuum hatched and larvae distributed into trays. Over the following 7 to 9 days, the larvae would be fed fish food until pupation. After pupation, larvae and pupae would be separated using special mechanical and/or chemical processes. Pupae would then be separated by sex on the basis of size using specialised equipment (males are smaller than females) and aliquoted in release devices. Male pupae (a sub-sample) are therefore manually screened for the presence of females and re-sorted if the female threshold criteria is not met (see section 5). Male adults would be provided with sugar solution until they reach sexual maturity and then released within the following seven days.

A general description of the activities being proposed/requested/permitted.

Under the National Conservation Law and the Animals and Plants Laws the import and release of alien or genetically altered species without permission of the Department of Agriculture and the National Conservation Council respectively is illegal.

4. BIOSECURITY MEASURES

Biosafety Standard Operating Procedures (SOP) are in place and staff will be trained to ensure conformance with these standards.

The production facility is built to Arthropod Containment Level 2 (ACL-2) standards to minimise unintentional escapees from the unit. A double entry door will secure access, a sieve on the sink drains will prevent any biological material from escaping. Mosquito life stages not used for the release or other procedures are frozen at -15°C or colder for more than 12 hours and disposed of.

The inserted trait is self-limiting in the environment, whereby released mosquitoes will die in the environment and the transgene will rapidly disappear following cessation of releases (26). *Aedes aegypti* are not native to the Cayman Islands and have previously been eradicated on the islands. The strain is fully susceptible to insecticides used in mosquito control programs.

Indicate whether biosecurity measures (anti-escape & species/people security) are in place or intended.

5. HEALTH & SAFETY CONSIDERATIONS / MEASURES TAKEN

Tetracyclines in the environment:

Tetracyclines are an antidote to the self-limiting technology, whereby if transgenic larvae are being reared in the presence of tetracycline they will survive to adulthood. An estimated threshold level of 0.01 to 0.1 μg/ml of tetracyclines is required to see an effect on survival of the mosquitoes in the laboratory. The literature suggests that there is an average concentration of 0.00066 μg/ml in the environment, well below the threshold required for survival of OX513A mosquitoes.

Further reading on Tetracyclines in the environment:

Curtis Z, Matzen K, Neira Oviedo M, Nimmo D, Gray P, Winskill P, Locatelli MAF, Jardim WF, Warner S, Alphey L, Beech C. 2015. Assessment of the impact of potential tetracycline exposure on the phenotype of Aedes aegypti OX513A: implications for field use. PLOS Neglected Tropical Diseases. 9(8): e0003999

Duff, B. (2005). Presence of tetracycline antibiotics in surface water. A study of the presence/absence of tetracycline in the Racoon river watershed, Des Moines water works laboratory.

Gulkowska A, He Y, So MK, Yeung LW, Leung HW, Giesy JP, et al. The occurrence of selected antibiotics in Hong Kong coastal waters. Mar Pollut Bull. 2007; 54(8):1287–93. doi: 10.1016/j.marpolbul. 2007.04.008 PMID: 17553528.

Le-Minh N, Khan SJ, Drewes JE, Stuetz RM. Fate of antibiotics during municipal water recycling treatment processes. Water research. 2010; 44(15):4295–323. Epub 2010/07/14. doi: 10.1016/j.watres. 2010.06.020 PMID: 20619433.

Locatelli MA, Sodre FF, Jardim WF. Determination of antibiotics in Brazilian surface waters using liquid chromatography-electrospray tandem mass spectrometry. Archives of environmental contamination and toxicology. 2011; 60(3):385–93. Epub 2010/06/11. doi: 10.1007/s00244-010-9550-1 PMID: 20535610.

McQuillan D, Hopkins S, H., Champman T, Sherrell K, Mills D. Drug Residues in Ambient Water: Initial Surveillance in New Mexico, USA. 7th Annual New Mexico Environmental Health Conference; October 28–30 2002; Albuquerque, New Mexico2002.

Effect on Human Health:

In order to present a risk to human health, the expressed proteins tTAV and DsRed2 would have to (a) be expressed in salivary glands, (b) be secreted into the saliva, and (c) be toxic or otherwise hazardous to humans if injected in relevant quantities. Of these, (a) and (b) relate to potential exposure, while (c) relates to the hazard incurred by the gene products. Addressing (c) first, Oxitec has commissioned extensive analysis on the safety of the expressed gene products using internationally recognized criteria, and it has been determined that there are no toxicological or allergenicity concerns from the gene products (tTAV and DsRed2) expressed in the OX513A *Ae. aegypti*. In addition tTAV expression is reported in a wide variety of organisms [2, 3] and a feeding study using OX513A [4] showed no adverse effects. Should a person be bitten by a OX513A female, the risk to human health is thus minimal as bites from Oxitec females are no different from wild females apart from the fact that inadvertently released females will be disease free.

Male mosquitoes do not bite or transmit diseases. Though the aim is to only release males which do not bite, there is a small chance that females will be released which come through the mechanical sorting process. The sorting is performed using a proprietary piece of equipment known as a wire sorter. This equipment sorts male and female pupae by size, males are smaller and can pass through the wire grid whereas females are larger and cannot. Quality assurance procedures are in place during the sorting and in practice, efficiencies of sorting result in very low numbers of females (0.07% in Cayman (1) and 0.02% in Brazil (27). Therefore although there is the potential for females to be released, these are unlikely to be fit and survive well in the environment and are unlikely to survive long enough in the environment to acquire and transmit diseases.

In the laboratory, under optimal conditions, the survival of functional adults is 5% or less. Evidence suggests that survival is much lower than this in the field; monitoring of ovitraps in Cayman in 2010

(1) failed to detect any evidence of the transgene in eggs from the field two weeks after releases had stopped, and similar results were seen in Panama (26). Additional studies investigating the characteristics of these low numbers of heterozygous survivors have also been carried out. When reared through immature stages at temperatures other than the ideal 27°C, the number of surviving adults is further reduced.

Vectorial competence is the intrinsic ability for the mosquito to become infected and subsequently transmit the pathogen [5]. Laboratory studies detected no significant differences in infection rates between OX513A strain and the comparative wild type strain for dengue serotypes 1-3 and chikungunya. The only significant difference detected was that the comparator strain females had a higher infection rate for dengue 4 than OX513A. Overall the impact of the transgene insertion had no effect on dengue or chikungunya susceptibility of the OX513A strain in this study.

6. Location Where Species Will Be Released

The releases are planned to be conducted in the South part of West Bay. The area comprises about 1,800 inhabitants over 300 acres. West Bay is one of the hotspots for *Aedes aegypti* and transmission of mosquito borne viruses and thus a suitable place to evaluate OX513A mosquitoes.

Releases

3 to 7 times a week, the release devices would be placed in large containers and brought to the treated area in West Bay where they would be released following a predetermined path enabling an even distribution of the Oxitec males in the area.

Monitoring

- Ovitraps (which mimic natural oviposition sites in which females lay eggs)
- BG- Sentinel adult traps (Biogents) chemically attract all mosquito species towards them and the mosquitoes are subsequently trapped.
- Both of these recapture methods will be used in and around the release area; and an equivalent untreated control site. This is specialised monitoring which is conducted alongside routine island-wide MRCU monitoring and control activities for all mosquito species.
- Ovitraps will be serviced weekly, BG Sentinels weekly or daily at different stages of the experiment and the mosquitoes collected. Eggs from the ovitraps will be hatched and the resulting larvae analysed for paternity and/or species. The GM mosquitoes have an internal fluorescent marker (DsRed2) and the recaptured mosquitoes are examined by microscopy- allowing the MRCU to track gene flow through the wild population, and the GM mosquito dispersal throughout the study site and beyond.

Fluorescent marker

The introduced DsRed2 protein is a fluorescent marker that allows the OX513A to have a fluorescent phenotype when excited by illuminations of a specific wavelength under a special microscope. Expression of this non-toxic protein will facilitate the detection of the OX513A monitoring

Other measures

Existing mosquito controls will continue to be used if necessary in both treatment and control sites, although will need co-ordination with release dates.

Dispersal

The dispersal of OX513A adults by spontaneous flight, has been shown to be less than 100 meters [11] and the average distance travelled by wild adults is well-known to be less than 200 meters [11-25]. Therefore the risk of dispersal of adults into other countries is extremely low, especially from an island. Adults could move by passive transport further and so can eggs (in cars, plant pots, plastic containers etc.); however any offspring that inherit the OX513A gene would have a large selective disadvantage (a maximum of 5% survive) and would be rapidly lost from the recipient population. In addition, control of the *Aedes aegypti* population where the OX513A males are released (previously shown to take about 6 months to get over 80% reductions) would significantly reduce the risk of egg dispersal as far less eggs would be produced.

A general description of the activities being proposed/requested/permitted.

7. OTHER RELEVANT INFORMATION TO BE CONSIDERED

The mosquito-borne diseases dengue, chikungunya and Zika are transmitted primarily by the *Aedes aegypti* mosquitoes. These diseases generally cause symptoms such as fever, headache, vomiting, diarrhoea, rash and joint pain; in severe and complicated cases chronic arthritis, haemorrhagic fever, dengue shock syndrome, Guillain-barré syndrome and microcephaly.

It is not possible to prevent the introduction of such diseases to the Cayman Islands; hence MRCU currently conducts adulticiding and larviciding of all mosquito species across the islands to prevent transmission. However, insecticide resistance is increasing. It is conventionally accepted that novel strategies will have to be employed alongside traditional methods to control mosquito-borne diseases. Scientists at Oxitec (www.oxitec.com), a UK based biotechnology firm, have inserted a self-limiting gene into a strain of *Aedes aegypti* (OX513A). The technology works by releasing males, as they do not bite or transmit diseases, in the environment. These genetically modified males compete with wild

males for females. When the wild females are inseminated by OX513A males (females typically mate once in a lifetime) their progeny receive the self-limiting gene and die before reaching adulthood. With sustained release of the OX513A mosquitoes over a period of time, the population of *Aedes aegypti* in the area is anticipated to be significantly suppressed. In studies in Brazil (27), Cayman Islands (1) and Panama (26) local populations of *Aedes aegypti* have been suppressed by over 90%. Suppression of *Aedes aegypti* to this extent could greatly reduce the risk of transmission of dengue, chikungunya and Zika transmission in the Cayman Islands.

Aedes aegypti is not native to the Cayman Islands, it is an introduced peri-domestic species closely associated with human habitations. Breeding is tied to artificial water containers, such as potted plant holders, water tanks, tires, discarded plastic and metal containers as well as ephemeral containers, such as puddles. Once eclosed the adult *Ae. aegypti* mosquitoes live in and around houses where females have easy access to the blood meal necessary for egg development. As such suppression of the local *Ae. aegypti* population is thus not expected to alter population dynamics of non-target organisms as it is not a keystone species in the local food chain, nor does it perform services like pollinating flowers. Reducing the *Ae. aegypti* population is thus unlikely to have a negative impact on the environment.

The inserted trait is self-limiting in the environment, whereby released mosquitoes will die in the environment and the transgene will rapidly disappear following cessation of releases (26).

Animals that eat self-limiting *Aedes aegypti* will be exposed to nutritional elements- protein, fat, sugar and others- as they would be from eating any mosquito: they cannot take up genes from this route, and the introduced genes are non-toxic and non-allergenic and thus do not impact organisms which eat them. The effect of the self-limiting gene on the offspring of OX513A is through causing subtle adjustments to their cells in a way that is non-toxic to organisms that eat those cells.

Vertical gene transfer refers to the ability of genes to move via sexual reproduction, to a closely related species or wild relative. *Ae. aegypti* mating is extremely species-specific. There have been reports of mating with the related species *Ae. albopictus* in both the laboratory and the field [6, 7], but even these matings, if successful, appear to fail to produce embryos capable of developing to adulthood. Forced laboratory matings have been conducted with OX513A and *Ae. albopictus* [8]; none of the eggs produced were fertile. Consequently vertical gene transfer to closely related mosquito species is both theoretically and practically possible but is likely to be an extremely rare event, and when these matings occur naturally or are forced to occur, as in close contact in a laboratory

situation, fertile offspring are not produced. The strain OX513A contains no toxic proteins or DNA that would confer a selective advantage to an organism; in fact the contrary is true as they confer a strong selective disadvantage. Consequently any hypothetically transferred genetic material would be rapidly lost from the recipient population. Reviews of the fate of DNA from transgenic organisms ingested by animals conclude there are no safety concerns that could be identified [9, 10]. Studies have been conducted on non-target organisms consuming OX513A at 100% of diet and no adverse effects were noted.

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By signing my initials to each line, I, **The Applicant,** confirm that I have read, understand, and agree to the following:		**Applicant Initials**
1.	I will conduct my actions as described in this document, and will abide by the terms set forth in this agreement. Imported species will be used only for the reasons listed above. Any change of use of these species should be reviewed by the Department of Environment on behalf of the National Conservation Council, and the Department of Agriculture.	**WP**
2.	I will get the permission of and abide by all requirements of the Department of Agriculture before importing any alien species.	**WP**
3.	I will follow all procedures required by the Cayman Islands Government Department of Environment, as set out in this permission.	**WP**
4.	There will be no unpermitted release of live organisms or their offspring (if incidental reproduction occurs) or viable material, e.g., seeds, cuttings, etc., into any uncontrolled environment, either natural or artificial, of the Cayman Islands.	**WP**
5.	To avert possible introduction of exotic parasites or diseases, as well as offspring, there will be no discharge of effluents, disposal of waste, transport or housing media or other materials associated with the alien species or disposal of diseased or dead organisms or parts thereof except via lawful municipal waste discharge or disposal methods, i.e., sewage or garbage disposal.	**WP**
6.	These conditions follow the specimens imported and applicant should inform recipients of specimens (offspring, retail sale, etc.) of the above stipulations.	**WP**
7.	I understand that previous import permission for a listed or related species does not mean continuing permission to import nor does it mean that future permission to import will necessarily be granted.	**WP**

Under the National Conservation Law and the Animals and Plants Laws the import and release of alien or genetically altered species without permission of the Department of Agriculture and the National Conservation Council respectively is illegal.

I, **The Applicant,** undersigned, confirm that I have read, understand, and agree to abide by the agreement outlined in this document.	
William D. Petrie	9 June, 2016
SIGNATURE OF APPLICANT	**DATE**

FOR OFFICIAL USE ONLY:	
PERMISSION GRANTED	**YES / ~~NO~~**
PROCEDURES REQUIRED / RESTRICTIONS ON IMPORTATION / REASON FOR DENIAL	
i) No more than 22 million adult male *A. aegypti* may be released in the course of the project. ii) Un-used mosquitoes (eggs, larvae, adults) must be destroyed. (Frozen at -15 degrees Celsius or colder for more than 12 hours.) iii) Importation and release shall be as in this document and shall conclude by June 30 2017. iv) A copy of the Oxitec trade and business licence and a copy of the Certificate of Occupancy issued by the Department of Planning for the ACL-2 egg rearing are required and shall be supplied to the National Conservation Council to be retained with a copy of this Permit by the Department of Environment. v) MRCU continue public outreach throughout the study period. vi) MRCU report to the Council of progress as at June 30, 2016 and at the conclusion of the project (for the Council Annual Report).	
NAME OF PERMIT ISSUER John Bothwell	**DATE** 8 June 2016
SIGNATURE OF PERMIT ISSUER	**DATE STAMP** DEPARTMENT OF ENVIRONMENT JUN 08 2016 CAYMAN ISLANDS

SPECIES RECOMMENDATIONS			
row	***Species Scientific Name***	**Quantity**	**Importation & Release**
1	*Aedes aegypti (OX513A)*	1.650 Kg (eggs)	Approved

Under the National Conservation Law and the Animals and Plants Laws the import and release of alien or genetically altered species without permission of the Department of Agriculture and the National Conservation Council respectively is illegal.

Appendix 2

Please address all correspondence to:
Director of Agriculture

Ph.: (345) 947-3090
Fax: (345) 947-6501
Email: directoragriculture@gov.ky

CAYMAN ISLANDS
DEPARTMENTOFAGRICULTURE
working together, growing together

P. O. Box 459
#181 Lottery Rd
Bodden Town
Grand Cayman, KY1-1106
CAYMAN ISLANDS

***REF**: OF-17 Living Organisms/Aedes aegypti - MRCU 10.11.2016.Variance*
OUTPUT #:3100

Permit for the Importation of Living Organisms

Date of Issue	10th November 2016
Permit Type	Multiple Entry /Multiple use
Expiration Date	10th June 2017
Scientific name of Living Organism	Aedes aegypti strain OX513A
Common Name	Dengue/ Yellow Fever Mosquito
Life Stage	Eggs
Total Quantity	Up to an additional 1kg to the 150 million (1.64kg) in multiple monthly shipments starting June 2016 for a period not exceeding 12 months.
Consignor (Sender)	Oxitec Limited 71 Innovation Drive Milton Park Abingdon, Oxfordshire, OX14-4RQ United Kingdom Tel: +44 1235 832393 Fax: +44 1235 861138
Consignee (Receiver)	Mosquito Research & Control Unit (MRCU) The Marco Giglioli Building 99 Red Gate Road, Georgetown Grand Cayman, Cayman Islands Tel: 345-949-2557 Fax: 345-949-8912

Permission is granted for the importation of the above described organisms.

These insects may be imported in any number of separate shipments to make up, but not exceed, the total number.

A schedule shall be maintained by MRCU, describing, the total weight of eggs, and the approximate number of eggs imported.

Each shipment shall be accompanied by a signed letter from Oxitec Limited which:

1. Describes the contents, the total weight of eggs, and the approximate number of eggs being imported, and
2. Attests to the disease freedom of the mosquitoes in the shipment.

Signed:

Dr. Kanyuira Gikonyo, DVM
Senior Veterinary Officer
For Director of Agriculture

DEPARTMENT OF AGRICULTURE
NOV 10 2016
CAYMAN ISLANDS

Date and Official Stamp:

Attachment: Approval from National Conservation Council

Appendix 3

Please Address all Correspondence
To: Director of Agriculture
Tel: (345) 947-3090
Fax: (345) 947-6501

P. O. Box 459
KY1-1106
Grand Cayman
Cayman Islands

Cayman Islands

DEPARTMENT OF AGRICULTURE

***REF**: OF-17 Living Organisms/Aedes aegypti - MRCU 20.06.2016*
OUTPUT #:3100

Permit for the Importation of Living Organisms

Date of Issue	20th June 2016
Permit Type	Multiple Entry /Multiple use
Expiration Date	30th June 2017
Scientific name of Living Organism	Aedes aegypti strain OX513A
Common Name	Dengue/ Yellow Fever Mosquito
Life Stage	Eggs
Total Quantity	150 million (1.64kg) in multiple monthly shipments starting June 2016 for a period not exceeding 12 months.
Consignor (Sender)	Oxitec Limited 71 Innovation Drive Milton Park Abingdon, Oxfordshire, OX14-4RQ United Kingdom Tel: +44 1235 832393 Fax: +44 1235 861138
Consignee (Receiver)	Mosquito Research & Control Unit (MRCU) The Marco Giglioli Building 99 Red Gate Road, Georgetown Grand Cayman, Cayman Islands Tel: 345-949-2557 Fax: 345-949-8912

DEPARTMENT OF AGRICULTURE
JUN 20 2016
CAYMAN ISLANDS

MRCU 20.06.2016

Permission is granted for the importation of the above described organisms.

These insects may be imported in any number of separate shipments to make up, but not exceed, the total number.

A schedule shall be maintained by MRCU, describing, the total weight of eggs, and the approximate number of eggs imported.

Each shipment shall be accompanied by a signed schedule from Oxitec Limited which:

1. Describes the contents, the total weight of eggs, and the approximate number of eggs being imported, and

2. Attests to the disease freedom of the mosquitoes in the shipment.

Signed:

DEPARTMENT OF AGRICULTURE
JUN 20 2016
CAYMAN ISLANDS

Dr. Kanyuira Gikonyo, DVM
Senior Veterinary Officer
For Director of Agriculture

Date and Official Stamp:

Attachment: Approval from National Conservation Council

Appendix 4

Ref. No.	A	S	I	P				Office use

CAYMAN ISLANDS GOVERNMENT

National Conservation Council

DEPARTMENT OF ENVIRONMENT
Department of Environment
NOV 10 2016
CAYMAN ISLANDS

Cayman Islands Environmental Centre . 580 North Sound Road .
PO BOX 10202GT . George Town . KY1-1106 . CAYMAN ISLANDS
TEL: (345) 949 8469 . FAX: (345) 949 4020 . www.DoE.ky

PERMISSION TO IMPORT & RELEASE ALIEN OR GENETICALLY ALTERED SPECIES

Once date-stamped and signed by the by the Department of Environment on behalf of the National Conservation Council, this permission constitutes

1) Advice to the Department of Agriculture on the application to import alien or genetically altered species;

2) Permission or denial to release alien or genetically altered species.

APPLICANT NAME	Dr. William Petrie
POSITION	Director
INSTITUTION	Mosquito Research and Control Unit
ADDRESS	Marco Giglioli Centre 99 Red Gate Road PO Box 486, KY1-1106 Grand Cayman Cayman Islands
PHONE	949 2557
EMAIL	William.Petrie@gov.ky
Intended Date Of Importation	November 2016 – June 2017

Please submit application electronically, in MS Word form, via email to DoE@gov.ky.

1. SPECIES & QUANTITIES TO BE IMPORTED

row	Species Common Name	*Species Scientific Name*	Quantity
1	Yellow Fever Mosquito	*Aedes aegypti (OX513A)*	See Below
2			
Add rows as necessary, or attach list separately.			

For those species which are IUCN listed certification of captive breeding or sustainable harvest should be provided along with application to import.

Under the National Conservation Law and the Animals and Plants Laws the import and release of alien or genetically altered species without permission of the Department of Agriculture and the National Conservation Council respectively is illegal.

2. PURPOSE OF IMPORT

	Pet		**Breeding**	
	Display		**Farming or other consumption**	
	Human Interaction		**Retail**	
X	**Other: Mosquito Population Control**			

Tick as appropriate. Fill in if other purposes.

3. VARIATION OF ADVICE & PERMISSION

A) Import of up to an additional 1 kilogram of eggs.

B) Housing of OX513A pupae contained within sealed release devices in the MRCU insectary, in addition to the housing of OX513A life stages in the Oxitec dedicated facility at the MRCU compound, prior to release.

4. NOTES

1. All other aspects of the existing permission and advice remain. This includes
   a. the maximum release limit of 22 million adult male *A. aegypti*; and
   b. the destruction of all unused mosquitoes (eggs, larvae, adults). (Frozen at -15 degrees Celsius or colder for more than 12 hours.)
   c. Project Report to Council by 30 July 2017, with additional Project Conclusion Report at a later date if necessary.
2. As the use of two insectaries will increase movement of mosquitoes clear notices should be posted on outer insectary doors that only one door (outer or inner) should be open at a time. (In order to reduce the chance of accidental release.)

	4. GENERAL REQUIREMENTS
1.	All actions will be conducted as described in this permit, and will abide by the terms set forth in this and existing permits. Imported species will be used only for the reasons listed above. Any change of use of these species should be reviewed by the Department of Environment on behalf of the National Conservation Council, and the Department of Agriculture.
2.	Permission of the Department of Agriculture is required before importing any alien species.
4.	There will be no unpermitted release of live organisms or their offspring (if incidental reproduction occurs) or viable material, e.g., seeds, cuttings, etc., into any uncontrolled environment, either natural or artificial, of the Cayman Islands.
5.	To avert possible introduction of exotic parasites or diseases, as well as offspring, there will be no discharge of effluents, disposal of waste, transport or housing media or other materials associated with the alien species or disposal of diseased or dead organisms or parts thereof except via lawful municipal waste discharge or disposal methods, i.e., sewage or garbage disposal.
6.	These conditions follow the specimens imported and permittee should inform recipients of specimens (offspring, retail sale, etc.) of all stipulations.
7.	Previous import permission for a listed or related species does not mean continuing permission to import nor does it mean that future permission to import will necessarily be granted. Nor that release permissions will be granted.

FOR OFFICIAL USE ONLY:	**Expiration Date**	**30 June 2017**
IMPORT ASSENT GRANTED	**YES / ~~PARTIAL / NO~~**	
RELEASE PERMISSION GRANTED	**YES / ~~PARTIAL / NO~~**	
REQUIREMENTS, RESTRICTIONS or REASONS FOR DENIAL A) All existing requirements and restrictions continue in force. Including maximum release limit, destruction of unused biological material, full reporting at end of project. B) Notice of proper airlock door use at insectary entries.		
NAME OF PERMIT ISSUER Gina Ebanks-Petrie	**DATE** 10 November 2016	
Gina Ebanks-Petrie	**DATE STAMP**	

DEPARTMENT OF ENVIRONMENT
NOV 10 2016
CAYMAN ISLANDS

SPECIES RECOMMENDATIONS

row	*Species Scientific Name*	Quantity	Import	Release
1	*Aedes aegypti (OX513A)*	*1kg*	Assented	Approved
2				
3				

Under the National Conservation Law and the Animals and Plants Laws the import and release of alien or genetically altered species without permission of the Department of Agriculture and the National Conservation Council respectively is illegal.

Appendix 5

Interim report MRCU/Oxitec - October 18 2016
Friendly *Aedes aegypti* project in West Bay

Introduction

The project was announced on May 5th 2016 and was followed by a variety of community engagement activities that ensured the majority support of the Caymanians. The first release was conducted on July 28th in the Public Health Clinic of West Bay in presence of the Timothy McLaughlin, the Public Health surveillance officer. The production and releases were ramped up and OX513A males started to be distributed in the treatment area mid-August. In parallel, the monitoring in the treatment and comparator areas, started in 2015, continued in order to evaluate the suppression of the *Aedes aegypti* and enable dynamic adaptive management of the programme.

Community engagement

Every Friendly *Aedes aegypti* project starts with an initial intense community engagement period adapted to the local conditions and culture, generally in collaboration with public services. In Cayman Islands, the Mosquito Research and Control Unit and the Public Health Department provided resources to help spread information and reply to questions about the project.

A number of activities were conducted including door-to-door visits in the release area, information booths and distribution of leaflets in several locations on Grand Cayman, a public meeting, radio and television talk shows as well as public service messages in the newspaper and on the radio.

A public opinion survey conducted in July revealed that 80% of the population of Grand Cayman had heard about the project and that 69% supported the Friendly *Aedes aegypti* project, validating the strategy followed. Activities to reach out to the community have continued since the beginning of the releases and will continue throughout the project.

Releases

The first release of OX513A males was conducted on July 28th 2016. The production was ramped up to operational schedule, three batches per week, from the beginning of August 2016. Consequently, full operational releases effectively started in mid-August at the average level of circa 300,000 OX513A males per week (from week 33 to 41, figure 1). The quality of the males released was confirmed by the quality control processes conducted in the production facility ensuring that the OX513A males were in optimal conditions to find and mate with the wild *Aedes aegypti* females.

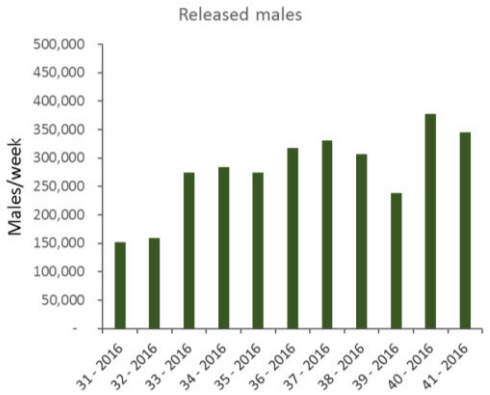

Figure 1: Number OX513A males released per week in the treatment area of West Bay.

Monitoring

Monitoring in the treatment and comparator areas started in 2015. Ovitraps that mimic the breeding sites and are used to collect eggs laid by the females, are used to follow both presence (Ovitrap Index: proportion of positive traps) and abundance (average number of eggs per trap) of the local population of *Aedes aegypti*. Through regular monitoring of the population in both the treatment and control areas, a sharp seasonal population increase of *Aedes aegypti* in early June 2016 was detected (Figure 2, after Week 23 - 2016) that has been maintained since that date although there have been some natural variations in the wild population.

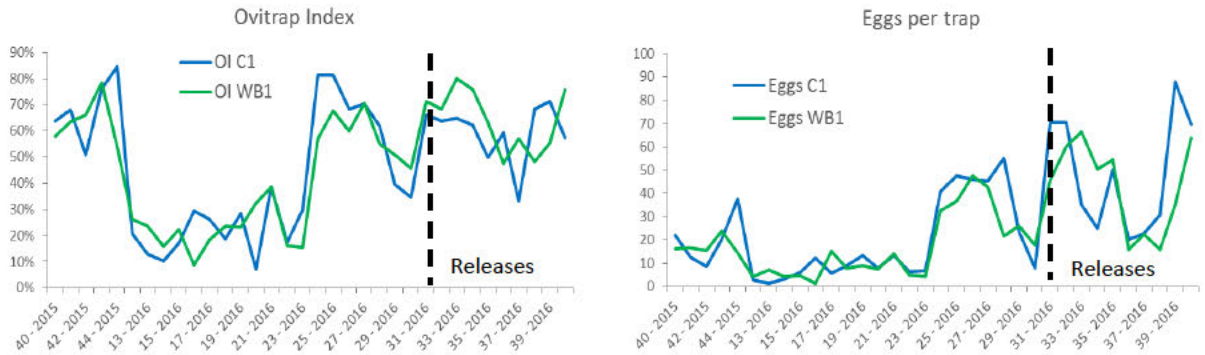

Figure 2. *Aedes aegypti* wild population monitoring results. (A) Ovitrap index: proportion of collected ovitraps that were positive for *Aedes aegypti.* (B) Eggs per trap: average number of eggs per ovitrap collected. The dotted line shows the start of the releases. C1: Comparator area; WB1: Treatment area.

Within the treated area, ovitraps can additionally be used to assess mating fraction (proportion of the wild females that are mated with OX513A males) by detecting the fluorescent marker in the larvae hatched from the eggs collected. This is a key metric that allows dynamic adaptive management of the releases to respond to the local population density thereby ensuring efficiency. For operational use, we target >50 % mating fraction for rapid suppression.

Following the first releases, fluorescent larvae were detected in the ovitraps in August. Due to the pre-mated females present before the start of the releases - females *Aedes aegypti* mate only once in a lifetime of 2 to 4 weeks - it is expected that the fluorescence will increase in the 4 weeks of initial releases before stabilising. The fluorescence observed in the ovitraps stayed below 10% until a sharp increase above 30% in October 2016 (figure 3). The absence of major rains in West Bay in September is likely to have caused a decrease in the wild population and resulted in an increase in the mating fraction as more OX513A males were able to compete for females. Though encouraging, the recent increase in mating fraction remains below our 50% target.

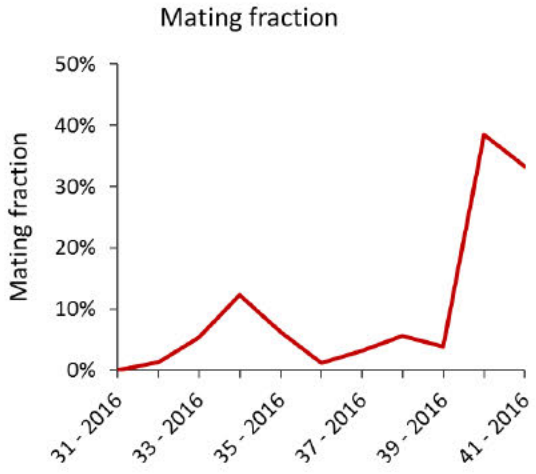

Figure 3: Mating fraction in the treatment area.

Conclusion/Recommendation

A key attribute of Oxitec technology is the fluorescent marker that facilitates adaptive release rates tailored to local population. Following 8 weeks of releases, production has been successfully established and sustained releases of 300,000 males/week have been conducted. Field collected samples show the released males are successfully mating with wild Aedes aegypti, but that the mating fraction is currently falling short of the targeted 50%. Population monitoring has shown high local population, expected at this time of year, and this is the most likely cause for not yet hitting 50% mating fraction. Routine quality assessment has shown no issues with male quality.

The optimal start for such a program is during the dry season when *Aedes aegypti* population is low, requiring lower release rates of OX513A males. Once control is achieved, it is possible to maintain and prevent seasonal increase with low release numbers. Indeed, this was the original plan for this project, but due to various administrative and judicial delays the releases were not initiated before the rains and corresponding increase in local mosquito population.

In order to achieve suppression of the population of *Aedes aegypti* with the shortest delay, an increase of the production levels is planned by using the entire existing mobile production facility described in the initial permit application for larval rearing and pupae sorting. The sorted males will be transferred to a separate insectarium built to ACL-2 standards where, using the existing processes, they will be put into release pots and fed until ready for release. This planned change should facilitate increasing the release rate above 500,000 OX513A males per week.

At the same time, the steering committee has decided that it may conduct targeted larvicide and adulticide treatments in both treatment and comparator areas before the end of the rainy season in November and/or December to bring the wild population down and hence boost the mating success of the release OX513A males. Using chemicals in synergy with the releases of OX513A males has always been envisaged as an appropriate use of the Oxitec solution within Integrated Vector Management strategy, consistent with recommendation from the World Health Organization for *Aedes aegypti* control. The adulticide applications which may be used would not have a residual effect and will be timed to minimise their impact on the released males.

Given the level of the wild population in the area at the moment, these two adjustments to the program, increased releases and chemical applications, are expected to speed up the suppression of the *Aedes aegypti* population.

MRCU / Oxitec application for operational deployment of genetically altered *Aedes aegypti* (OX513A) throughout the Cayman Islands.

Department of Environment screening evaluation

The National Conservation Council has received an application for a permit to release a genetically altered organism, from the Mosquito Research and Control Unit (Cayman Islands Government) and Oxitec (Cayman) Ltd.

The applicants propose to commence operational deployment of OX513A *Aedes aegypti* male releases as a tool to suppress the invasive wild population of *Aedes aegypti*, which currently poses an arbovirus disease risk to the human population on Grand Cayman and Cayman Brac. The application is made under section 35(2) of the National Conservation Law 2013, which states "*A person who wishes to introduce or release in any part of the Islands a live or viable specimen of an alien or genetically altered species shall apply to the Council for a permit to do so.*"

The purpose of this screening evaluation is to assist the Department of Environment and the National Conservation Council in determining whether the proposed activities pose any potential risk to the natural environment, or pose other risks identified in the Guidance Note, and if so what level of risk assessment will be necessary to inform the Council's decision on this application.

This taxon concerned is considered "alien" under Guidance Note criteria, which state: *Genetically altered organisms, selectively bred organisms and artificial hybrids are all by definition considered to be alien.*

Background:

This application for operational deployment follows a series of experimental trial releases of this same mosquito strain in Grand Cayman. The first two of these, in East End in 2009 and 2010, pre-dated the passage of the National Conservation Law and so were not subject to the current permitting process. Oxitec conducted its own risk assessment[1] in support of its request to the Cayman Islands Government to conduct these trials.

In June 2016 the Council issued a permit to MRCU & Oxitec to release OX513A males in an area of West Bay, Grand Cayman for a third trial which compared *Aedes aeqypti* population trends in the OX513A treated area with a matched control area also in West Bay. This trial was ongoing at the date the Council received the current application.

The current application is for periodic imports of eggs followed by release of OX513A males limited only by a maximum of 2Kg of eggs imported per month. This application is open-ended both in time and geographically within the Cayman Islands, and so should be considered as a permit request for operational deployment of this technology into the indefinite future.

Risk assessments carried out independently of Oxitec have been conducted[2] in response to trials of the OX513A *Aedes aegypti* strain proposed in Brazil, Malaysia and the USA. While these cannot entirely substitute for a risk assessment for the Cayman Islands specifically, there are many commonalities in the receiving environments and the genetically altered mosquito strain is identical in all cases. These pre-existing risk assessments are therefore an important resource directly relevant to this screening assessment.

Oxitec has also submitted a new risk assessment[3] for the current application, meeting the requirements of the NCL Guidance Note. Since this is an output from the applicant, it cannot be considered to be strictly independent, and so its conclusions and any content that is not narrowly factual should be considered carefully in that light.

Information required:

The Guidance Note requires the following information to be submitted by the applicants:

a) The full scientific name(s) of the specimen(s) proposed to be released.
b) The numbers and sex ratio of the specimen(s) proposed to be released.
c) The location(s) and date(s) of proposed releases.
d) The purpose of the proposed release.
e) The existing range of this taxon in the wild (including both its native range and its introduced range if any).
f) If the applicant is a business, a copy of the applicant's current trade and business license is required.
g) the relevant technical and scientific details as laid out in Paragraph 9 of Annex III of the Cartagena Protocol on Biosafety, under the Convention on Biological Diversity.
h) a comprehensive statement of the benefits intended to result from the release.
i) a complete description of facilities and procedures for acquiring and handling the specimens before release, including biosecurity standards and procedures, including procedures for disposal of any substances that may affect the expression of altered gene or genes.
j) copies of any prior risk assessments that have already been carried out on the genetically altered taxon proposed for release.
k) copies of any scientific papers relating to risks and benefits associated with the genetically altered taxon proposed for release.

As of 12th April 2017, the applicant has supplied all the required information.

Risk pathways and screening evaluation:

For the purposes of this screening evaluation, the following list of pathways by which the taxon may possibly become invasive and/or harmful has been compiled from prior risk assessments, from

considerations of public claims made by opponents of this technology, from information emanating from the current trial and previous trials, from literature provided by the applicants, and by Cayman Islands specific scenario explorations in discussion among scientific staff at the Department.

The pathways listed here are specific to this application and so do not consider alternative technologies. Pathways involving human health concerns have not yet been locally assessed at the professional epidemiological or medical level, though such expertise is reflected in prior risk assessments from other countries.

1. Local accumulation of Tetracycline (especially from wastewater disposal at the Oxitec facilities) allows larvae arising from escaped or released OX513A males mating with wild (and/or escaped OX513A) females to survive to adulthood, and so start a localized reproducing population of this GM strain. This population would then be subject to very strong selection for any mutation that confers reduced dominance or resistance to the lethal effect.
    a. Work has been done by the applicant to assess concentration of tetracyclines needed to suppress the activity of the lethal gene, and this is too high to be at all probable in the environment generally. The only possible site(s) of concern in the Cayman Islands would be direct accumulation of discharged water used to rear OX513A larvae. Any extant or future risk could therefore be managed.
    b. *Aedes aegypti* is characteristically a breeder in clean rainwater accumulated in peri-domestic containers, and in the Cayman Islands is not typically found breeding in septic tanks or wastewater treatment facilities.
    c. Wastewater disposal at the MRCU Oxitec facility is currently routed to a septic tank shared with the entire MRCU facility. The septic tank water has been analyzed by the Water Authority and is evidently unsuitable for *Aedes aegypti* breeding. A larger, new facility proposed by Oxitec will have its rearing water disposal separated from septic tank uses: no statement has been made as to how this tetracycline-treated waste water will be treated or disposed of.

    LIKELIHOOD: Unlikely
    CONSEQUENCES: Minor

2. The small percentage of OX513A females that do get accidentally released, carry and transmit diseases capable of affecting wildlife or humans, originating from infected blood used in the OX513A mosquito rearing process.
    a. Management of this risk would be through conditions that restrict Oxitec to use of synthetic blood. This is a speculative future risk, and probability at present is zero.

    LIKELIHOOD: Highly unlikely
    CONSEQUENCES: Minor

3. On Cayman Brac, native *Aedes mediovittatus* (which is not particularly closely related to *Aedes aegypti* but is also a competent vector of Dengue virus) hybridizes with released and/or escaped OX513A mosquitoes, and the hybridization process causes the lethal trait to become recessive or inactive. A hybrid population then rapidly evolves and becomes invasive, and could potentially be an efficient new vector of arboviruses.
    a. *A. aegypti* and *A. albopictus* can hybridize under certain (rare) conditions, but evidence questions whether such hybrids are viable. These two species are both in the subgenus *Stegomyia*. In contrast, *A. mediovittatus* is in the sub-genus *Gymnometopa*. Hybridization should therefore be considered even less likely, but we should not regard it as completely impossible. Tests in captivity could clarify whether or not any risk exists for this eventuality.

    LIKELIHOOD: Highly unlikely
    CONSEQUENCES: Intermediate

4. Niche vacation by species-specific reduction of *Aedes aegypti* may allow an expansion of invasive *Aedes albopictus*
    a. Trials to date have not shown this to occur. Monitoring would allow adaptive response if necessary.

    LIKELIHOOD: Unlikely
    CONSEQUENCES: Minor

5. Proteins arising from the modified genome could be toxic or provoke allergic reactions when mosquitoes bite, or are eaten.
    a. Other independent risk assessments note this has been tested for extensively, and can now be ruled out as a serious concern.

    LIKELIHOOD: Highly unlikely
    CONSEQUENCES: Marginal

6. Horizontal gene transfer carries the modified gene(s) into other organisms with unpredictable consequences
    a. Other independent Risk Assessments point out that natural horizontal gene transfer often occurs between prokaryotes, but natural horizontal gene transfer between eukaryotes, and between eukaryotes and prokaryotes, is practically unknown. In eukaryotes, genetic material is contained within intracellular membranes (nucleus, mitochondria). In prokaryotes the genetic material is free in the cell. It is therefore extremely unlikely that GM components could transfer from the mosquito (which is a eukaryote) to e.g. the bacteria in the mosquito larva's gut (prokaryotes).

LIKELIHOOD: Highly unlikely
CONSEQUENCES: Intermediate

7. Interbreeding between the strain of *Aedes aegypti* used in OX513A and the resident invasive strain, results in the resident population becoming a more efficient disease vector
    a. For this to occur, the lethal trait in OX513A (which is dominant) would have to be somehow disabled in some proportion of the offspring. The fluorescent marker would show the incursion of the GM components into the wild population, through detection in monitoring larvae. In all trials to date there has been no sign of this happening. Monitoring for this, or evolution of resistance (e.g. as in 1 above) should be a condition of approval.

LIKELIHOOD: Highly unlikely
CONSEQUENCES: Intermediate

8. Bacteria that become resistant to Tetracycline in the rearing facility get introduced into the natural environment, and spread
    a. The batch processing does not lend itself to long term, successive generations of bacteria evolving in the facility.
    b. The bacteria that occur in larval rearing water are unlikely to be pathogens of concern to humans or domestic animals, and so the consequence of this may be marginal, and such resistance would not be likely to be adaptively advantageous in the general environment.
    c. Only if such resistant bacteria then passed their resistance to a pathogenic prokaryote (by horizontal gene transfer) could an actual risk arise – this would seem to be an extremely improbably scenario.

LIKELIHOOD: Highly unlikely
CONSEQUENCES: Minor

Risk Screening Summary:

In the Department's opinion, no identified pathways carry a combination of risk and likelihood sufficient to justify an independent risk assessment under the NCL Guidance Note.

Recommendation:

The Department recommends:

1) That the Council be guided primarily by the independent risk assessments referenced below, recognizing these are independent of the applicant and have considerable relevance to the current application.
2) That the Council make conditions should a Permit be granted:
    a. that a plan for disposal of waste water from all current and future rearing facilities for OX513A in the Cayman Islands is submitted and implemented to the satisfaction of the Water Authority and the Department of Environment, such that no potential exists or can be created for OX513 *Aedes aegypti* to breed in wastewater containing tetracyclines from these facilities.
    b. that before any OX513A *Aedes aegypti* are transported to or released on Cayman Brac or Little Cayman, tests are conducted by the applicant to establish whether OX513A *Aedes aegypti* are at all capable of hybridizing with *Aedes mediovittatus*, and that expansion of OX313A *Aedes aegypti* releases to the Sister Isles be subject to separate consultation with the NCC after completion of these tests
    c. that OX513A *Aedes aegypti* eggs transported to the Cayman Islands are produced from females whose blood meals are from synthetic blood only
    d. that active monitoring for persistence or spread of OX513A *Aedes aegypti* is continued on a permanent, ongoing basis in and around all release areas and around rearing facilities, and that any anomalous occurrences are reported to the Council immediately and investigated by the applicant as a matter of urgency
    e. that before decision by the Council, the views of the Chief Medical Officer be sought on this application
3) Subject to these conditions and the Chief Medical Officer's views, the Department is of the opinion that given the substantial body of risk assessments that have already been conducted internationally, sufficient information is available to allow the Council to issue a permit if so minded, with precautionary conditions as above, including a requirement for ongoing monitoring.

Prior Risk Assessments referenced above:

1. Risk analysis – OX513A *Aedes aegypti* mosquito for potential release on the Cayman Islands (Grand Cayman). Oxitec, October 2009.
2. Environmental Assessment for Investigational Use of *Aedes aegypti* OX513A. Centre for Veterinary Medicine, US Food and Drug Administration, April 2016.

   Risk Assessment of *Aedes aegypti* strain OX513A. National Technical Biosafety Commission, Brasilia, Brazil. April 2014.

   Risk Assessment Report of the Genetic Modification Advisory Committee (GMAC) for an application to conduct a limited mark-release-capture of *Aedes aegypti* (L.) wild type and OX513A strains. Malaysian Department of Biosafety. September, 2010.

3. OX513A Environmental Risk Assessment – Cayman Islands. Oxitec, April 2017.

WP-05: Little Cayman Conservation Warden

The Department of Environment (DoE) on Little Cayman was appointed a Conservation Warden by the National Conservation Council on 24 August 2016 as the officer there, while not being a full Conservation Officer, is required to carry out routine enforcement checks as part of their job. Due to a change in staff the DoE is requesting the National Conservation Council to immediately designate, under section 48(1), Michael Guderian (Operations Manager, Sister Islands) as a Conservation Warden and grant him, under section 48(4), all powers and immunities of a Conservation Officer appointed under this Law, other than the power of arrest. Michael Guderian was formerly a Fisheries Officer under the Marine Conservation Law and will receive training regarding the applicable sections of the National Conservation Law and his powers and immunities as described under Section 48 (4-5).