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TARONGA
CONSERVATION
SOCIETY
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HUSBANDRY MANUAL: CHRISTMAS ISLAND FLYING-FOX (*PTEROPUS MELANOTUS* *NATALIS*)



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Only trained personnel with up-to-date rabies (Lyssavirus) vaccinations (titres to be checked every two years) should be allowed to work with flying-fox. Please refer to Department of Health guidelines for current health advice.

(Guidelines for the Public Health Management of Rabies and Other Lyssavirus (including Australian Bat Lyssavirus) Exposures and Infections http://www.health.wa.gov.au/CircularsNew/circular.cfm?Circ_ID=13128).

Cover Photo: Christmas Island flying fox covered in yellow pollen, Carol de Jong, Queensland Government Department of Agriculture and Fisheries

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1.0 Introduction

European collections currently house the most populous representation of *Pteropus* in captivity in a zoological setting. Australian zoological institutions hold relatively few flying-fox, and few collections are managed in mixed sex populations for breeding. With increasing understanding of the zoonotic diseases transmitted by pteropodids in Australia, captive management has become less appealing. Many of the Australian populations are being phased-out by natural attrition. However, with the increase in vulnerability of all Australian flying-fox populations, captive management may indeed be necessary for the survival of some species.

As the only remaining endemic mammal species on the remote Australian Territory of Christmas Island (Indian Ocean), the Christmas Island flying-fox (*Pteropus melanotus natalis*) is facing population pressure from several potential threatening processes. The species was listed as Critically Endangered in January 2014 under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act), heightening the need for active population management. Should critical trigger points for captive management be identified, it is in the national interest to have a captive management plan, and general husbandry manual in place so that actions to save the species can be implemented immediately.

There remains scant natural history data about the Christmas Island flying-fox, however, there is substantial effort underway to obtain this information and fill key knowledge gaps. The following husbandry manual for the captive management of the Christmas Island flying-fox, has been compiled by drawing together what we do know of the Christmas Island flying-fox's natural history, and where necessary, drawing links from other *Pteropus* species held in zoological institutions across the globe.

The purpose of this husbandry manual is to guide the captive management of Christmas Island flying-fox. The terms of reference of this manual do not include; the establishment of decision making trigger points for captive breeding, captive management aims and project goals, establishing management strategies for genetic management of the species, reintroduction strategies for returning animals to the wild, satisfying animal ethics requirement for the establishment of captive management, or a risk assessment for captive management. These parameters are discussed in a separate document, the Christmas Island flying-fox Captive Management Plan.

The Christmas Island Draft Biodiversity Conservation Plan (Director of National Parks, 2014) also complements these documents.

This document is dynamic and should be regularly updated as we discover more about this unique island species; thus, the contents are subject to change.

2.0 Conservation Status

The Christmas Island flying-fox (*Pteropus melanotus natalis*) is currently recognised as a subspecies of the Black-eared, or Blyth's, flying-fox (*Pteropus melanotus*); however there is a strong argument to have them listed as a distinct species.

The Christmas Island flying-fox is found only on Christmas Island, a 135 km² Australian Territory located in the Indian Ocean. They exist as a closed, single, interbreeding population.

The Christmas Island flying-fox is a small flying-fox (220 – 540g) with uniformly long, near black fur. Some animals may exhibit a faint reddish collar, or a light salting of white/grey hairs. It's small stature, and uniform fur gives the Christmas Island flying-fox a chubby appearance. There appears to be no sexual dimorphism, but males are easily recognised by external genitalia.

The Christmas Island flying-fox has undergone a sharp population decline since first studied by Tidemann in 1984. Tidemann (1985) estimated that the population of Christmas Island flying-fox at that time was around 6,000 individuals, with the population heavily skewed towards mature females (3:1). More recent studies have estimated the population to be closer to 1,300 individuals (Woinarski et al., 2012) and a population shift, or trapping bias, to favour males and sexually immature animals (Hall et al., 2014). Studies to confirm population demographics and reproductive parameters are ongoing.

Taxon (scientific name): *Pteropus melanotus natalis*

Common name: Christmas Island flying-fox

IUCN status: *Pteropus melanotus*, Vulnerable (with the stipulation that, should Christmas Island flying-fox be recognised as a distinct species, it would be classified as Critically Endangered)

EPBC Act 1999 listing: Critically Endangered (January 2014)

CITES: Appendix II (all flying-fox)

Action Plan for Australian Mammals: Critically Endangered

3.0 Housing

An enclosure must be constructed to ensure that flying-fox cannot escape under circumstances that can be reasonably foreseen and guarded against, including cyclones, which are regular occurrences on Christmas Island.

Some examples of exhibit design from other institutions are included in Appendix A.

3.1 Containment

Flying-fox are naturally highly inquisitive (Griffith, 2004). Enclosures should be made from smooth, non-porous, non-abrasive materials that are appropriately sealed and able to withstand a lot of hosing. Vinyl or polyethylene coated mesh is a preferred construction material, and Teflon sprayed or non-galvanised wire mesh is also suitable (Jackson, 2003). Galvanised wire should be avoided as it may cause rubbing injuries, or zinc toxicity if corroded surfaces are ingested (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995; Jackson, 2003; Olsson & Woods, 2008). Thin wire gauge, such as chicken wire (0.7 – 1.0 mm wire thickness), should be avoided to prevent damage to joints.

Thick gauge netting can be used to loosely line the roof and walls of the enclosure, providing multiple high points for animals to establish territories and foot friendly perches as animals navigate across the roof and roost. Care needs to be taken to ensure animals cannot become trapped between the roof and netting.



Photo (Griffith, 2004): Image showing netting secured with peaks and troughs to allow a varied topography, for individual animals to establish territories in a large group exhibit. Food buckets and whole apples can also be seen hanging from multiple points around the enclosure.

Although flying-fox may occasionally become entangled in mesh, installation of thick gauged wire and appropriate mesh size will minimise injuries. The Philadelphia Zoo recommends 25 x 13 mm vinyl coated wire mesh, piano wire, and rigid welded wire for use in exhibits housing *P. rodricensis* (Lengel & Oberlin, 2016). Jackson (2003) suggests *Pteropus* spp. can be maintained in enclosures with a 20 mm mesh size. Placement of landing furniture (such as ropes, branches, vines and/or hanging cloth) away from walls will provide alternative landing options and reduce the risk of injury from collision with rigid walls.

Enclosures should allow for natural ventilation and sufficient airflow, shade for all animals without creating overcrowding, and access to rain and direct sunlight.

The use of a double enclosure is recommended to reduce the possibility of escape, reduce contact and possible disease transmission between wild and captive flying-fox that may be attracted to the enclosure, and reduce the potential for unwanted mating between wild and captive flying-fox. The distance between enclosures should be at least 10 - 15 cm to minimise the risk of a flying-fox reaching through one enclosure and getting entangled in the second (Jackson, 2003). An ante-room will also prevent animals from escaping through open doors during daily husbandry and provide a space for personnel to store enclosure specific tools.

All doors should remain padlocked when not being serviced by staff to prevent accidental contact by unvaccinated staff or members of the public.

3.2 Shelter Requirements

While it is important to provide flying-fox with access to the natural elements, shaded areas must always be provided as protection from wind, rain and extreme weather events (including heat). This can be created by using clear plexiglass, plywood, or vegetation within the enclosure. Aluminium panelling should be avoided and this can absorb heat and cause overheating.

Heating is probably not required as animals will be kept in natural climactic conditions on Christmas Island, but cooling will be necessary as flying-fox are extremely susceptible to high temperatures. Installation of sprinklers or soaking hoses may be beneficial in this regard.

3.3 Substrate

Flooring material should be easy to clean and have good drainage. Flying-fox may occasionally descend to the ground, so non-abrasive substrates should be considered. Pest proofing is important to prevent predation, and the potential transfer of parasites and disease causing organisms. Smooth, slightly angled, concrete is recommended.

Natural substrates can be used for small groups of flying-fox but pest proofing, drainage, and ease of cleaning does need to be considered. Sand and sawdust is not recommended as it may be ingested.

3.4 Furnishings

While captive flying-fox do seem to prefer to hang from ceiling mesh, natural branches, vines, ropes, crawl ladders, and netting with a variety of diameters will also provide roosting opportunities and a means of preventing nail overgrowth. A network of furniture will be utilised to encourage natural behaviours including grooming, displaying, foraging, roosting, wing walking, and avoidance of aggression. As Christmas Island flying-fox have been observed actively foraging close to the ground, this mosaic of furniture should also extend to the floor. This will provide any animals that accidentally find themselves grounded with an avenue to climb back up into the 'canopy'.

Furniture with sharp or pointed edges should be avoided to prevent wing punctures and abrasion injuries. Care should be taken to ensure there are no narrow gaps that might damage wings or claws.

Visual barriers such as fabric hangings are recommended to reduce stress, by providing avenues of escape for individuals, and to create screens from visual stimuli. Visual barriers can also be utilised as a form of behavioural enrichment. Fabrics such as polar fleece, woollen blankets, sheepskin,

hessian sacks, and towels are recommended as they tend not to absorb fluids (urine, water) and/or are quick to dry (Griffith, 2004) but they may not be suitable in this environment. Alternatives such as brush barriers, or folds of soft mesh could be utilised but care must be taken to ensure that the materials used cannot cause injury to the animals. If fabrics are used as visual barriers, they must be checked regularly for frays, tears and holes that may entangle animals and cause injury.

Enclosure design should allow sufficient space for flying-fox to fly. In this regard, consideration must be given to the placement of exhibit furniture, feeding and water stations to allow clear flight paths.

Exhibit furniture may occasionally need to be replaced or rotated through the enclosure to provide behavioural enrichment. As flying-fox may be territorial, changes to the overall topography of permanent furniture should be carried out with this in mind. Additional furniture, such as branches, and ropes, etc., are important for behavioural welfare, however placement should be considered with regard to individual flying-fox ‘territories’ and regular flight paths.

Plexiglass sheets can be used to exclude flying-fox from any areas where they are not desired.

Prior to animals being placed into a newly constructed facility, a team of personnel should conduct an inspection to find any potentially dangerous items. If the floor is not solid concrete, a metal detector should be passed over the substrate to locate any pieces of metal that may have been deposited during construction. Walls and furniture should be checked thoroughly for sharp edges.

3.5 Minimal Acceptable & Optimal Size of Enclosures

Enclosures should be of sufficient size to allow for a broad spectrum of natural behaviours including flight, stretching, and static flight (See: Behaviour and Social Organisation). Without flight, wing muscles will atrophy and flying-fox will lose their ability to fly (Jackson, 2003). Naturally, the size of the enclosure will depend on the size of the colony to be housed.

To allow sustained flight, enclosures should be at least eight times the wingspan of the flying-fox (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995; Jackson, 2003; Olsson & Woods, 2008). The exact wing span of the adult Christmas Island flying-fox is unknown at this time, but estimated to be around 1 meter. Square enclosures are suitable, but other shapes may also facilitate sustained flight.

Captive flying-fox appear to be more comfortable in enclosures above human eye level (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995). Olsson and Woods (2008) suggest that enclosures should be at least 1.8 m high. This may pose problems for catching animals for husbandry and medical purposes, so a slightly sloping roof, or the use of an ante-room, may also be considered.

Flying-fox maintain a hierachal group structure. Individual territories are clearly defined and dominant animals generally prefer to roost at the highest point within the enclosure. By lining the roof of the enclosure with a scalloped net, a number of peaks will be created for individual animals to establish their territory thus reducing the potential for aggression over high vantage points (Griffith, 2004).

3.6 Capture & Handling Facilities

It may be beneficial to have a separate empty cage, or cages, for managing animals that need to be separated from the group for quarantine, isolation, or other purpose. It is not recommended that animals are housed alone for prolonged periods of time but should the need arise to separate an animal from the colony, a short-term non-flight enclosure of 1.5 times the wing span, and twice the body length of the flying-fox, or a restricted flight enclosure of four times the body length will allow for wing stretching and the animal to roost comfortably (Jackson, 2003; Griffith, 2004).

For transporting animals between enclosures, or for short distances, a standard wire cat or dog hospital cage fitted with a secure perch, a cover to limit visual stimuli, and newspaper over the base to catch waste, is suitable for pteropodids. These small transport cages must be at least 2 times the body length in height and wide enough for the flying-fox to extend and flap both wings (Olsson & Woods, 2008).

A capture ante-room, or a section of the enclosure which can be closed off, where food is offered regularly without negative stimulation, is useful so that catch-up can take place without creating additional stress from herding animals or chasing them with a net. Other reports suggest that animals can easily be captured from any location in the enclosure by utilising long-pole catching nets. Anxious flying-fox will generally stretch out both wings in a defensive stance, so catching nets need to be wider than wingspan and holding areas at least 1.5 times the wingspan to avoid wing damage (Griffith, 2004).

3.7 Temperature, Humidity, and Ventilation Requirements

Christmas Island has a tropical equatorial climate with temperatures ranging from 22 – 28°C, a distinct wet (November – June) and dry (July – October) season, and year-round high levels of humidity. Cyclones and other extreme natural events known to occur on Christmas Island could have catastrophic effects on animals held in captivity. As flying-fox will be kept in open air enclosures, natural conditions will be maintained but consideration should be given to the management of issues arising from extraordinary climactic events.

If heat lamps are used, they should be properly housed in exclusion cages or, ideally, outside of the main enclosure to prevent animals from getting too close to the hot lamps or surfaces.

Flying-fox are very sensitive to high temperatures. In a captive environment, it is recommended that sprinklers or mister hoses are used when temperatures exceed 26°C for prolonged periods to maintain optimum humidity and provide cool refuges. Cooling systems should be left on constantly when temperatures exceed 38°C (Griffith, 2004). Symptoms of chronically low humidity include dry skin and/or wing membranes, and/or cracked nails (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995). Flying-fox should not remain wet at all times though, and access to dry sunny areas where they can dry-off are required.

Effective ventilation, airflow, and husbandry are necessary to avoid the build-up of unpleasant odours in enclosures housing large colonies. If housed indoors, the suggested rate of air change for larger colonies is 6 – 10 exchanges per hour with fresh air (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995).

3.8 Lighting

Open air enclosures will be subject to natural light cycles. Shaded areas must always be provided for resting and roosting, however access to full sunlight is also necessary to maintain health.

If indoors, light should be provided to replicate natural photoperiods and UV-B lights are needed for flying-fox to synthesise Vitamin D (Stringer, et al., 2016).

There are no special light fixtures needed but all lights, especially those that generate heat, should be shielded from flying-fox to reduce the possibility of burns.

Flying-fox can see most colours, including red (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995).

3.9 Utilities

There are no special concerns or requirements for sewage disposal however recommendations can vary by region and managers should be aware of local regulations. Consideration should be given to disposal of uneaten food as this will attract pests, and possibly wild flying-fox. Wild flying-fox feeding from discarded food items will be more susceptible to predation, and disease. Water from hosed surfaces should therefore be collected into a central drainage area fitted with a sump basket or filter that can be emptied daily to collect waste that is not captured by raking or sweeping. Waste, including food waste, should be disposed of in a secure location away from enclosures to minimise pest activity around the enclosure, and prevent wild flying-fox from accessing food waste and coming into contact with other waste products.

3.10 Water Sources

Specific water requirements are unknown for flying-fox but water should be offered at all times even though some species may rarely be seen drinking. While some species may drink from bowls, others may prefer watering devices, and several sources of water should be offered (bowls, drippers, misters, sprinklers), especially in hot weather.

As with food, multiple water sources should be located around the enclosure. Water should be changed daily as it may be soiled by food, spat, urine, and faeces. The likelihood of soiling water sources may be reduced by placing water under smooth surfaces where flying-fox are unable to hang. Water drippers should be checked daily to ensure the mechanism does not become clogged or caked with food.

Drowning in open water features has been reported from some institutions, but water features have also worked successfully with no associated deaths in others (Lengel & Oberlin, 2016).

4.0 Management

4.1 Personal Protective Equipment

Only trained personnel with protective rabies (Lyssavirus) titres (to be checked every two years) should be allowed to work with flying-fox. Please refer to Department of Health guidelines for current health advice (<http://www.health.gov.au/internet/main/publishing.nsf/Content/ohp-rabies-consumer-info.htm>). Australian Bat Lyssavirus (ABLV) infection is very similar to classical rabies virus and human diploid cell rabies vaccinations are used to prevent ABLV infection in people.

All personnel working around flying-fox must wear personal protective equipment (PPE), including:

- Hat
- Eye protection
- Long-sleeve shirt – rolled down and buttoned at wrist. Hexarmour sleeve (gauntlet) should also be worn when handling conscious flying-fox to protect the arms from stray claws which can catch between sleeves and gloves.
- Gloves - Thick welding or deer skin gloves with nitrile or latex gloves underneath to capture/handle conscious animals. Double layer of nitrile gloves when handling fully restrained/anaesthetised animals.
- Long pants
- Covered footwear
- Surgical mask or face shield should be worn if exposure to bodily fluids or injury from a conscious flying-fox is likely.

4.2 Record Keeping

All flying-fox should have an individual husbandry and medical record, and an individual physical/visual identifier that relates back to this record. An individual medical record for each flying-fox (e.g. Appendix B) and a daily husbandry record (diary) (e.g. Appendix C) for each group are recommended.

Each record should clearly communicate:

- Birth date or date entering captive population, parentage (if known) or origin
- Sex
- Individuals unique identifier (thumb band combination, PIT tag number, etc.) or changes to identification (e.g. thumb band replacement)
- Weights and measurements (regular weights should be noted)
- Date of introduction to the enclosure, or movement to new enclosure (e.g. for breeding)
- Behavioural notes (e.g. medical, aggression, breeding)
- Breeding data (pairings, observed mating, pregnancies)
- Changes in diet (i.e. noted not to eat, additional or unusual food items, food preferences)
- Veterinary information (injuries, unusual faecal production, physical exams, medications administered, test results)
- Other notes that may be relevant to husbandry (including unusual weather events, pest species found in enclosure)

All neonatal deaths, stillborn, and aborted foetuses should be recorded as these data are important in determining population demographics and individual reproductive health parameters.

Any neonate that requires hand-rearing should also have a similar record which includes birth date and reason for hand-rearing, weights, growth, feeding requirements, behaviour, weaning, and movement to inclusion in the larger colony (e.g. Appendix D).

4.3 Individual Identification Methods

There is no sexual dimorphism in Christmas Island flying-fox. Identification of males and females is easily achieved by observing the external genitalia. While some organisations report being able to tell individuals apart by physical and behavioural attributes, we recommend permanent identification for all captive Christmas Island flying-fox.

Each identification method has its own series of advantages and disadvantages, and each method tends to be species specific. Popular identification methods for pteropodids are listed below:

- Passive Integrated Transponders (PIT tags, or microchips) – Permanent. Subcutaneous placement of tags on the dorsal midline between scapulae where it will not interfere with movement.
 - o Advantages: relative permanence, few health risks.
 - o Disadvantages: animal must be in hand to be able to read tag as identification is not outwardly visible (though scanning an animal as it hangs should be possible in most instances). Trovan microchips are widely used throughout Australian facilities, as well as for domestic animals, and would be suitable for use with Christmas Island flying-fox (<http://www.microchips.com.au/>).
- Thumb bands¹ – Combinations of bands can be read from the distal end of the thumb towards the wing. Coloured bands are placed loosely enough to turn easily and not cut off circulation, but are not so loose that they are easily removed. Aluminium bands are not recommended as they can become compressed and cause injury. Plastic bands are not recommended as flying-fox can easily chew them off. Coloured powder-coated stainless steel, and uniquely numbered stainless steel bands are available and recommended for identification of flying-fox.
 - o Advantages: Easy visual identification of coloured bands, many colour combinations available.
 - o Disadvantage: Animals may be able to remove plastic bands, numbered steel bands are not easily readable from a distance, and bands may get caught and be associated with thumb injuries.
- Forearm band – Extreme care must be taken if using forearm bands as they can cause injury to muscles and tendons by restricting circulation to the propatagium if bent. Forearm bands have been reported to cause a number of injuries and affect survival rates in wild chiropterans (Jackson, 2003).
- Stainless steel beaded necklace
 - o Advantages: Easy identification of coloured necklaces from a distance, many colour combinations available.

¹ The Australian Bird and Bat Banding Scheme (ABBBS) regulate all banding in Australia, and banding can only be carried out by licensed personnel.

- Disadvantages: Colours fade, and necklaces may break, may become abrasive, cause food to cake around the necklace, or harbour ectoparasites. Flying-fox may be able to remove necklaces. They may foster opinions of negative welfare.
- Nail polish
 - Advantages: Can be seen from a distance, multiple colour combinations available, unlikely to be removed by the animal.
 - Disadvantages: Need to restrain animal while paint dries, will require reapplication on a monthly basis (or more).
- Ear notching – ear notching can be a valuable form of visual identification, but may be difficult to see from a distance.
- Ear tags – not recommended as tags can cake with food, tear out leaving disfigurement, be difficult to read from a distance, and may not be available in an appropriate size for this species.
- Tattoos and punch-marking (patagium or ear) – not recommended in black animals, identification may not be permanent, and animals need to be in hand to identify the tattoo.
- Body piercing – Techniques for installing body piercing hardware (coloured barbells) for permanent identification of flying-fox are similar to those for implanting PIT tags (discussed above) but the animal should be chemically restrained for this procedure. Barbells should be installed between the scapulae, parallel to the length of the body, under sterile surgical conditions (Barnard & Abram, 2010). Plastic or metal, straight or curved barbells are available. Plastic curved barbells are recommended (Barnard & Abram, 2010).
 - Advantages: Permanent, different colour combinations available, visible identification, can be removed if animals are to be released.
 - Disadvantages: May not be visible in long fur of Christmas Island flying-fox, may migrate out of skin over time, can only be placed in fully grown adult animals, installation by a veterinarian required.

Regardless of the method of identification chosen, identification should be checked regularly to ensure that they are still functional (i.e. band combinations should be checked against records regularly, and animals should be scanned to check the functionality of PIT tags on a regular basis). We recommend a combination of permanent identification (PIT tags) and a non-permanent visual identifier.

4.4 Hygiene and Cleaning

Good exhibit hygiene is essential for maintaining flying-fox and personnel health. Daily husbandry should allow time to observe and monitor individual animals to ensure they have no obvious injuries or abnormal behaviours. Regular removal of faeces, urine, discarded fruit and spat will ensure odours and pests are kept to a minimum, and reduce the risk of fungal and bacterial growth on exhibit surfaces and furniture.

Due to the warm, humid environment, uneaten food will spoil quickly and therefore must be removed on a daily basis. Flying-fox should be given their main feed late in the day as flying-fox are most active at night, while routine cleaning should be carried out in the mornings. Water should also be supplied daily with regular cleaning of water dishes/dispensers to avoid a build-up of algae and food contamination.

Enclosure floors should be swept to remove large pieces of solid waste and foliage, and then hosed to remove small particles, and urine.

Exhibit furniture and walls should be cleaned as necessary with a paint scraper or stiff bristled brush to remove build-up of food and faeces. 1% bleach or a sodium bicarbonate solution may safely be used, but must be rinsed off thoroughly before being allowed to dry (Jackson, 2003).

Fabric hangings should be washed in hot water as required, but replacement hangings should be fitted immediately and care should be taken while removing the hangings to ensure none of the flying-fox are startled or using them at the time.

4.5 Feeding Schedule

Flying-fox typically consume a lot of food relative to body size as it is processed quickly through their gastrointestinal system (Olsson & Woods, 2008).

Once daily feeding is sufficient for most species, and should take place late in the day as flying-fox are typically active and feeding at night. Christmas Island flying-fox differ from many other flying-fox species however as they are typically also active during part of the day. Enrichment, including food, should be offered earlier in the day to coincide with these active periods, to maintain normal dietary patterns, and provide mental stimulation for captive flying-fox.

4.6 Capture, Handling & Restraint

Only trained and vaccinated personnel with adequate rabies neutralizing antibody titres should handle flying-fox. Capture should only be undertaken when necessary, for transport, routine health checks, veterinary procedures, or enclosure maintenance (if necessary). At least 2 people should be present when catching is needed, and a catching plan with risk assessment and clear roles and responsibilities should be implemented before entering the enclosure. Capture methods may vary depending on the design of the enclosure and the number of flying-fox to be captured.

There are two factors that need to be considered when capturing and handling flying-fox:

1. Animal Safety

- Wing bones are delicate and can be easily broken during capture.
- Never hold a flying-fox by its wing tips or thumbs.
- Use a net that is wider than the animal's wingspan.
- In smaller enclosures, a large towel can be used to engulf the flying-fox as it hangs, wrapping the entire body in the towel with the wings neatly tucked in to the body.
- Never pull down on hanging flying-fox to remove them from roosts. Take care to firmly restrain animals while gently unhooking nails from perches. Do not hyperextend the joints of the digits.
- Gripping the animal by the back of the neck may result in increased aggression. Flying-fox also remain calmer when they have something to grip with their feet. Thus, reasonable restraint may be achieved by allowing the feet to grip a glove or towel while firmly (but not tightly) restraining the wings in their



naturally tucked position (Griffith, 2004). In this way, the neck can be gently restrained but the animal will feel less inclined to struggle and bite. (Photo: Andrew Breed – physical restraint of an adult spectacled flying-fox (Olsson & Woods, 2008)).

- Hold restrained animals head down, or horizontal. Not head up.
- Food traps can be used to lure flying-fox to a smaller enclosure that can be closed off when necessary, or to a specified area of the enclosure that makes capture easier. Animals should be fed in this area regularly, without negative interactions, so that likelihood of success is increased.
- Guiding poles with a cloth screen at the top can be used to move or heard flying-fox around the enclosure, without having to catch them up. They may also be used to guide flying-fox down the walls of the enclosure to make capture easier if roofing is too high to comfortably reach animals.

2. Personnel Safety

- Bites, scratches, and bodily fluids are all modes of zoonotic disease transfer.
- Two people should always be present when catching up animals. Both should have sufficient PPE and training and be fully aware of first aid protocols.
- In many states, animals that bite or scratch someone are required to be euthanased for Lyssavirus testing. Current state health protocols should be followed (http://www.health.wa.gov.au/CircularsNew/circular.cfm?Circ_ID=13128).
- Wear long sleaved welding gloves or gauntlets when handling flying-fox to reduce the risk of scratches. An extended wing has a long reach so long-sleeve shirts buttoned at the wrists should also be worn. Thick gloves will reduce dexterity and extreme care should be taken not to apply excessive pressure to the flying-fox. It can also be difficult to manipulate the animal and unhook claws while wearing thick gloves so patience should be adopted and activities not rushed. It may be useful for one person to restrain the flying-fox, while the second person untangles or unhooks the flying-fox. Clear communication needs to be used here so that both people are aware of the position of the head of the animal, and teams should work together to ensure the flying-fox is comfortably restrained.
- Other personal protective clothing that should be worn when handling flying-fox includes enclosed shoes, long pants, and nitrile gloves. Surgical masks and safety goggles may also be appropriate when trying to handle animals overhead, or when handling sick animals.
- Flying-fox are best restrained by wrapping them with a soft, thick towel while they hang to restrain the wings and cover the head. Grip the head under the mandible with the thumb and middle finger and place the index finger over the cranium. Be sure not to grip too tightly and compress the throat. Use the other hand or the other handler to gently unhook the claws and restrain the feet.
- Flying-fox can be placed into a secured soft cloth bag for weighing.
- If bitten, it may be difficult to dislodge the flying-fox. Blow gently on the animal's face until it releases its mouth. Do not pull the hand away or attempt to pry the mouth off as this may cause the bat to increase jaw pressure, and it may also injure the animal.
- An 'ABLV Exposure Kit' including povidone-solution and Melolin plus Fixomull for bandaging the wound should be on hand whenever catching-up animals (Healesville Sanctuary SOP guidelines). Current first aid response for an exposure to ABLV is included in Appendix E.

Release from restraint is as important as the catching technique. Flying-fox, still wrapped in a towel or restrained in the glove, may be allowed or assisted in gripping a branch or overhead wire before the gloves or towel are gently lifted away. Never drop the flying-fox quickly as this may result in injury. A flying-fox restrained in a cloth bag may be released by orientating the opening of the bag to the base of a vertical branch and allowing the flying-fox to crawl out of the opening. Be sure to place the bag away from your face and body as this may be the first structure the flying-fox recognises and they may try to climb up it.

Capture, handling, and restraint can be very stressful and handling time should be kept to a minimum. Offering the flying-fox fruit juice from a syringe after release can assist in recovery from stress due to physical restraint.

Veterinary advice should be sought for information on chemical restraint.

Observe the animal post-release to ensure it is able to move and fly normally.

4.7 Transporting Procedures

If animals are to be transported by air, there are strict regulations enforced by the International Air Transport Association (IATA) that must be followed (<http://www.iata.org/publications/Pages/live-animals.aspx>).

If bringing animals into mainland Australia, appropriate permissions will need to be gained from the Australian Department of Agriculture and Water Resources, and the Department of the Environment. Individual state legislation will also need to be followed.

For routine transport of animals for husbandry and management purposes on Christmas Island, flying-fox can be transported for short periods in suitably sized pet carriers fitted with a perch for roosting (see section 3.6), or suspended in secure soft cloth bags. Injured animals should be wrapped securely in a soft cloth or towel to prevent further injury while being transported; however, care should be given to ensure animals do not overheat. Cloth bags should be turned inside out so that digits do not become entrapped in loose threads, seams or salvages.

As multiple animals may injure each other while moving around inside a transport box, individual transport is essential. Transport of nursing females should be avoided, but if necessary the dam and offspring should be transported together.

Transport cages should be designed in such a way that handlers do not need to insert fingers into the box/cage to move it. Towels should be used to block external stimuli to keep the animal calm. Lining the sides of the lower inner 2/3 of the crate will provide security while allowing airflow through the top. Liners should be made of a soft towel or fabric with no frayed edges or holes that could entangle digits.

Animals in transport boxes should not be left unattended. We recommend that transport is not carried out during the hottest part of the day, and that transport occurs during the early part of the day when flying-fox are more passive. Animals held in transport cages for prolonged periods should be offered water and food as appropriate. Animals accustomed to using a water dripper may be offered water or fruit juice in this manner, otherwise spill proof containers should be used.

4.8 Pest Control

While it has proven difficult to construct completely pest proof enclosures on Christmas Island, it is important that best efforts are made to exclude pests where possible. Cats, rats, geckos, giant centipedes, yellow crazy ants, snails and slugs are all pests that pose potential health threats to captive flying-fox.

Good hygiene and cleaning enclosures and the area immediately outside of enclosures will assist in keeping enclosures relatively pest free. All cracks and holes should be sealed as quickly as possible, a plexiglass skirting around the base of enclosures and exclusion wire or additional barriers are also useful.

Glue boards, snap traps, rodenticides, and snail baits must all be shielded from flying-fox as they will frequently descend to the floor of the enclosure and investigate unusual items.

4.9 Sanitation

Cleaning and maintenance must be carried out on a daily basis. Appropriate design, building materials, and cleaning products should facilitate this and make cleaning minimally intensive for personnel. Cleaning products should be carefully considered so as exposure to vapours and contact with disinfectants will not be harmful to the flying-fox. Allow adequate contact time for disinfectants and ensure objects are rinsed thoroughly.

5.0 Behaviour & Social Organisation

The behavioural and social organisation of the Christmas Island flying-fox is largely unknown and is currently being studied. Much of the information listed below has been determined for other wild and captive pteropodid species.

The Christmas Island flying-fox does show some general behavioural differences to mainland pteropodids presumably due to, until recently, their predator free environment. This includes diurnal activity and foraging, foraging low to the ground, generally passive nature, lack of vocalisation when being handled, and delayed avoidance to approach.

Some common flying fox behaviours are listed below:

Autogrooming: licking or nibbling own fur or scratching with one hind foot. Forelimbs are not used in grooming.

Allogrooming: grooming another individual. While seen quite frequently among all sex/age classes, adult males tend to reserve this behaviour for adult females.

Bathing: Wild flying-fox ‘bathe’ during rain and it is an important part of grooming. Flying-fox may also participate in urine baths where they urinate on themselves to help spread lubricating oils on to the wings, and as a mechanism to cool down in hot weather by fanning their wet fur.

Ejecting spat: The process of spitting out a dense pellet of indigestible fibrous tissue from fruit that has been chewed, to extract the liquid that is swallowed.

Hanging alert: Animals is hanging stationary, but is awake and alert.

Moderately colonial: Animals are often found roosting alone or in small groups.

Play chase: Seen among immature animals. Two or more animals fly from one location to another and rapidly leave again.

Play wrestle: Usually seen among immature animals. Involves close ventral contact between individuals while giving inhibited bites to neck area of the other animal. More than 2 animals may be involved. Thumb hook pokes and punches have also been observed in juvenile flying-fox, and may be important play behaviours used in aggressive adult interactions.

Roosting: Animal is asleep, with wings wrapped around the body, suspended by one or both feet from a perch.

Scent marking: Rubbing neck and chin glands against perches or walls of cage. Seen most frequently by adult males marking their territories.

Static flying: Flapping wings as if to fly but retaining hold of the perch with the feet. Seen mainly among infants who cannot fly yet.

Walking on the ground: Animals will descend to the ground to search for fallen food. Limb sequence is typical for quadrupedal gait and claw of the thumb is used to pull the animal along.

Wing fan: A gentle fanning with a half-folded wing. Seen during hanging alert. May be thermoregulatory.

Wing shake: Aggressive action. One or both wings held with arms outstretched from the body. Wings shaken with thumb pointing at addressee. Claw can be used in physical encounters and is capable of inflicting injury.

Wing flick: Short flick of the distal half of wings achieved by moving digits. Seen sometimes during courtship by males.

5.1 Optimal Social Grouping

Although the Christmas Island flying-fox has been described as moderately colonial, the majority of flying-fox species are social and we therefore recommend that no flying-fox be maintained singularly. The only exception to this recommendation is if an animal requires special medical care or quarantine and housing in this situation is temporary.

Squabbles among flying-fox are common in establishing social hierarchies but aggression is generally ritualised and rarely leads to injury or death (Jackson, 2003). Fighting may result in minor injuries, or bullying individuals lower in the pecking order away from feed and water stations. The ideal number of individuals, and the sex ratio of animals within the group, will depend on the size of the enclosure, and desired management outcomes. If densities are too high, or low, stress may result in detrimental physiological and/or behavioural changes (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995).

Male to female ratios of 1:3, 2:6, or 2:8 are recommended (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995; Jackson, 2003). If husbandry objectives are to increase the size of the population in an organised breeding program, housing groups with one male and 3 – 4 females is recommended.

As captive male flying-fox have been observed herding their harems into corners of the enclosure, do not have more breeding groups in one enclosure than there are distinct territories (or corners) available to each male.

Tidemann (1985) reported that the population of Christmas Island flying-fox was heavily skewed towards a higher proportion of mature females and theorised that their breeding structure is most likely polygamous, with females reaching maturity more quickly, and living longer, than males. More recent research suggests that there may have been a population structure shift with more males being captured than mature females (Hall, et al., 2014), however more research is needed into this area.

Excess males resulting from breeding, may need to be kept in bachelor groups. Too many males in a mixed sex, breeding population may result in decreased reproduction, and juvenile males pulling neonate pups off mothers (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995).

Bachelor groups do however have problems associated with them, such as increased incidence of aggression, and some reports that sub-ordinate males may become irreversibly reproductively

senescent (Lengel & Oberlin, 2016). Proximity to breeding groups may also contribute to increased stress and aggression, so bachelor groups should be housed away from females if possible.

In the wild, Christmas Island flying-fox are estimated to have a generation length of 10 years (with a range of 6 – 12 years (James, et al., 2007).

5.2 Age of Dispersal, Removal of Young

Young flying-fox are usually easily integrated into the colony.

However, if breeding is the goal of captivity, juvenile flying-fox, especially males, should be separated from their mothers after they are weaned but before they reach sexual maturity. For many *Pteropus* species this is between 6 months and 2 years of age. Be sure that juveniles are eating solid foods before removing them from the dam as flying is not always indicative of independence.

5.3 Introductions & Removals

Introducing new animals and removal of animals from a captive population is generally not problematic, however, introductions should take place early in the day to allow keepers time to observe the colony. As new animals may initially spend time away from the group before ultimately being accepted, and some bickering to determine social hierarchy may occur, presentation of food or novel enclosure furniture may distract the animals from the new cagemate and help facilitate introductions. Extra food stations should be included with the introduction of new animals, as permanent residents may act aggressively defending their territory feed station. Weekly weights should be obtained for new individuals for a minimum of two weeks until weight stabilizes (Lengel & Oberlin, 2016).

Females tend to accommodate new members more easily than males (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995). If prolonged aggression towards new individuals occurs, one of these individuals should be removed from the group to prevent stress and major physical injury.

While quarantine guidelines should be upheld, it is important not to overextend quarantine of individual animals as their social behaviour may be adversely affected. If possible, quarantine flying-fox in groups rather than singly.

If an animal needs to be treated for medical purposes it is best, where possible, to provide veterinary care while leaving the animal in its enclosure. If it is necessary to remove the animal for treatment, effort should be made to minimise the time spent separated from the group, and reintroduction should take place as for any new animal.

Removing flying-fox from colonies causes minimal stress. If dominant animals are removed from the colony, social hierarchy will be re-established and reproduction may be affected, but this is usually only for a short time.

If maintaining the colony for genetic management, the breeding male should be removed immediately after the first young are born, or earlier if pregnancy is known. However, care should be taken not to stress the entire colony while attempting to capture males (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995).

New males or females should be introduced two to three months after the colony's females give birth so that juveniles are large enough to fend off aggressive behaviour.

5.4 Behavioural Ontogeny

Flying-fox behaviour is formed largely through observational learning (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995).

Young flying-fox are generally poor flyers and will often crash into things as they learn how to fly. Static flying is common among infant and juvenile flying-fox as they exercise their wings and prepare for flight. Pups are very playful and their need for environmental enrichment is great. They will interact with other pups, adults, and exhibit furniture as they learn.

At weaning, a pup will first learn to eat solid food by licking its mother's lips as she feeds before sampling solid foods on its own. Juveniles up to one year of age will return to their mothers if they are frightened.

Once reaching sexual maturity, adult female flying-fox spend most of their year invested in reproducing, first with gestation, and then with lactation and weaning.

5.5 Parental Care

Parenting is done by the female. Allo-parenting is rare. In the wild it is quite common to find maternity colonies, and captive colonies can replicate this behaviour by removing adult males just prior, or immediately after birth.

Mothers carry and hold their infants in a variety of positions including front and back. They will also crèche their infants for various lengths of time especially during weaning and while foraging. Time crèched is species specific.

Should an infant fall or get knocked down, mothers will usually pick their infants off the floor. However, in overcrowded situations maternal neglect is not uncommon. Infanticide can occur in flying-fox colonies, and while the causes are unknown, it does not appear to be directly related to overcrowding or having males present in the colony while pups are quite young (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995).

Mother-infant fights are common, especially during weaning, with the pup frequently getting attacked by the mother presumably to promote independence.

5.6 Behavioural Indicators of Social Stress or Social Changes

Behavioural indicators of stress are usually passive and may include increased alertness, huddling as a group with knees bent and wings held at the sides, and increased lip licking and tongue rolling (Griffith, 2004). Aggressive indicators of stress may include excessive chasing, wing tears, vocalisations, alertness accompanied by wing flicks and thumb punches, moving away from the threat, and urination and defecation. Cowering, biting, shivering, jerking, twitching, lethargy, exclusion from roosting with the group, and a lack of appetite are also reported behavioural indicators of stress. Individuals that consistently eat last, or wait for other members of the group to finish eating may be experiencing chronic aggression and associated stress.

Chronic stress may cause anorexia and weight loss.

In the wild, pregnant females generally establish female camps prior to parturition. There have been reports from mixed sex captive colonies, of stillborn and aborted foetuses, and neonatal deaths due to trauma, from males displaying aggressive behaviour towards female cagemates (A Lorenzo, Pers. Com.). High incidence of infant mortality may also be an indicator for overcrowding and social stress (Lengel & Oberlin, 2016).

Individual *P. poliocephalus* have been reported to form social bonds with other members of a colony, preferring to spend time with certain individuals, while actively avoiding others (Griffith, 2004).

'Play' has been observed in hand-reared juvenile flying-fox (Griffith, 2004) and it is thought to be important in learning social behaviour. Hand-reared juveniles will form strong bonds with their human parent if they are not socialised with other flying-fox. Abnormal behaviours and stress may result in failure to integrate a non-socialised flying-fox into a shared captive environment.

5.7 Behavioural Signs of Illness

Individual animal behaviour should be monitored daily to pick up subtle changes that may indicate health issues, or changes to social hierarchy that may require direct management. Often, clinical signs of poor health can be subtle, so knowing your animals is vital to maintaining population health.

Daily health checks do not mean daily hands on interaction with animals. Observation should be adequate to determine if an animal requires further monitoring, or hands on intervention. While carrying out daily routines, take note of group interactions, individual posturing and activity, basic body condition, condition of fur, vocalisations and sounds, and signs of general ill health including changes in faecal consistency, urine colour, general smells, and signs of blood and unusual discharge.

Behavioural signs of illness may include, but are not limited to: a decreased appetite, weight loss, unusual posture, lethargy, reluctance to move, solitary roosting, and abnormal flight.

Anecdotal reports of hypoglycaemia with animals found collapsed on the ground have been reported in a captive Australian population. This event followed cold nights and episodes where access to food was limited due to dominant animals crowding feed stations. This was resolved by placing additional food stations around the enclosure (P Eden, Pers. Com.).

All behavioural signs of illness should be recorded on the record sheets so that personnel working on different days are able to refer to these notes and determine if changes are new or ongoing, if medicines have been prescribed/administered, or if there are individual animals that require extra attention throughout the day.

5.8 Enrichment

Environmental and behavioural enrichment is important for flying-fox mental health, and keepers and managers should spend time developing new ways to stimulate their colonies. Methods of environmental enrichment that are known to be successful include:

- Naturalistic foods: native flowers, fruits and browse
- Varying food presentation: fruit kebabs (Image credit: Stanioch, 2016), whole fruits, wire cages containing whole soft fruits like banana or halved stone fruit (Image credit: Griffith, 2004), whole

skinned mangos suspended from ropes or flexible poles, and plastic buckets suspended from the roof

- Novel foods that are rarely available: Wombaroo Leadbeater's or Wombaroo Nectavore mix in a drip feeder, and hard to source native foliage or novel fruits
- Supplying olfactory stimulation: fresh herbs, perfumes, oils, non-toxic plants and flowers, honey drops, peanut butter smears
- Adding props to enclosures such as ropes, branches, mirrors, non-abrasive materials, and keeping in mind individual territories and flight paths.
- Providing adequate flight space
- Supplying fresh leafy browse

Note: Food based enrichment needs to be measured and incorporated into the colonies daily food intake. Treating food enrichment as extra food may result in unhealthy weight gain, and contribute to food wastage.



6.0 Reproduction

Pteropodids reproduce sexually, producing live young (pups) that females nurse from one or two mammae located either side of their thorax.

Christmas Island flying-fox, like other flying-fox, have low reproductive rates, giving birth to a single pup each year. For the Christmas Island flying-fox the natality rate is less than 1 pup per mature female per year (James, et al., 2007). Much of the information on flying-fox reproduction is anecdotal.

6.1 Age-specific Fecundity

Sexual maturity and age at first breeding is dependent on species. Generally, smaller species are able to reproduce much sooner than larger species (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995). Smaller species have been recorded as reproductive at 4 – 8 months for females and 1 – 2 years for males, while females of larger species typically reach breeding age between 1 – 2 years with some males being 18 months to 2 years.

Female *P. poliocephalus* reach sexual maturity at 18 months of age, but tend not to breed until the second breeding season after their birth, at around 2 years, where they exhibit a birth rate of around 20% (McIlwee & Martin, 2002). Males reach breeding age at approximately 2.5 years (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995; Griffith, 2004). Female *P. alecto* breed in their third year, but may breed earlier, while males become mature at 16 – 18 months of age (Stanioch, 2016). Both female and male *P. rodricensis* reach reproductive maturity at 12 months to 2 years of age (Mickleburgh, et al., 1992).

Female Christmas Island flying-fox have been reported to fall pregnant as early as 6 months of age (Tidemann, 1985), however it is not known if this pregnancy resulted in successful parturition. There are also suggestions that individuals are not considered adult until approximately 2 - 3 years of age (Hall et al., 2011; J Welbergen, Pers. Com.). Males are suspected to have a lower life expectancy than females (James, et al., 2007).

Many flying-fox experience a post-partum oestrus (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995).

6.2 Breeding Seasons

Changes in day length appear to indicate the commencement of the breeding cycle in the majority of *Pteropus* species (Griffith, 2004; Olsson & Woods, 2008).

Pteropodids may be monoestrous with one oestrus cycle per year (e.g. *P. giganteus*, *P. poliocephalus*), or polyoestrus with multiple cycles per year (*Rousettus aegyptiacus*, *Cynopterus brachiotis*). In general, larger species seem to be monoestrous while smaller species generally polyestrous (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995).

In an outdoor captive environment, where animals are exposed to natural light cycles, breeding seasons seem to match those of wild populations. In the wild and in captivity, *P. poliocephalus*, *P. alecto*, and *P. conspicillatus* breed between February and April (Griffith, 2004; Olsson & Woods, 2008; Stanioch, 2016). *P. scapulatus* breed from November to December (Olsson & Woods, 2008). In

captivity, *P. rodricensis* show no seasonal breeding patterns, while in the wild pups are born August – February after a 150 day gestation period (Lengel & Oberlin, 2016).

Christmas Island flying-fox are reported to breed between July and September, however this has not been substantiated by any research and there appears to be considerable spread, and variation in birthing periods between years (James, et al., 2007).

There has been a single reported case of possible sperm storage in *P. giganteus* in a captive environment (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995), and reports of delayed pregnancy to control the timing of births in other species (Olsson & Woods, 2008).

Homosexual mating has been reported in *P. poliocephalus* (Griffith, 2004).

6.3 Mating System, Courtship, Copulation and Parturition

Little is known about the mating behaviour of Christmas Island flying-fox. It has been suggested that only a handful of mature males mate with the majority of females in camps (Tidemann, 1985), however as we have discussed elsewhere, social structure in relation to sex ratios within the population may have been misreported or have changed since these studies, and mating systems may have changed as a result (see section 5.1). More research is needed in this field.

Wild flying-fox are seasonal breeders, with timing tending to be species specific, but generally coinciding with food abundance. Seasonality may decrease in captivity (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995).

During the breeding season, male scent marking and general odour, vocalisations, and aggression towards other males increases (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995; Griffith, 2004). Increases in weight, testicular size, and interactions with females during mating season have been described in male *P. poliocephalus* (Griffith, 2004). Many *Pteropus* species form harems, and males will follow receptive females, vocalising at them and hooking them with their thumbs (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995; Griffith, 2004).

The colony hierarchy does not always hold for breeding, so dominant males do not necessarily sire all the offspring in that harem or group. Females may also breed with more than one male so sires can be difficult to ascertain without genetic testing for paternity if groups contain more than one male (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995).

Courtship often involves male initiated allogrooming, especially around the genital area, followed by much vocalising, and then mating. Females may choose whom they mate with, and reject the male by showing aggression or by actively moving away (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995; Griffith, 2004).

In other *Pteropus* species, individuals will copulate repeatedly, and with multiple mates during the breeding season. Males will attempt to copulate year round, though females are less inclined and generally display aggressive or avoidance behaviours.

As part of the mating ritual, the interested male may flick his wings towards the female and vocalise loudly, before grooming the female and orientating himself to her dorsal surface. The male will clasp the female with his wings and thumbs, and grasp the nape of her neck with his teeth. Intromission is short and is accompanied by a quick ejaculation. Copulation is generally ventral/dorsal, but on occasion may be ventral/ventral. Copulation is often very loud and boisterous and flying-fox will mate several times in one day.

There is little behaviour change in the weeks and days prior to parturition.

6.4 Gestation

As a general rule, flying-fox carry only 1 young per gestation period. Twins are possible, but rare, and survival of both pups seems unlikely without intervention (Griffith, 2004; Stanioch, 2016).

Gestation in the Christmas Island flying-fox has been estimated at around 5 months, with females giving birth in the rainy season, generally between December and February, but with occasional births occurring sporadically throughout the year (Tidemann, 1985; James, et al., 2007).

6.5 Parturition

A typical birth and placental delivery may last a few minutes, to several hours and generally occurs during the day (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995; Griffith, 2004).

Two to three weeks prior to parturition, the foetus shifts from a sprawled lateral position to a more centralised, ventral position directly above the vaginal area which may cause a slight swelling. It is common, in the hours prior to parturition, for the female to separate herself from the rest of the group and fan herself with her wings. As delivery starts, the female will hang from her thumbs, and strain hard as if attempting to void. The pup will emerge head first, with the wings folded around the head. The female will then adopt a horizontal position to lick and groom the genital area and pup. Eventually, the female will use her wings like a net to protect the pup from falling as the pup uses its feet and thumbs to crawl to the nipple located on the side of the upper thorax. The dam, or occasionally cagemates, will eat the placenta.

Caesarean section has been successful in flying-fox though care is needed to ensure wound healing is successful, and hand-rearing of the pup is often required.

6.6 Infant development

Physical development of infant flying-fox can be negatively affected by poor nutrition, illness, or injury (Griffith, 2004). Typically, pteropodids are born fully furred with the exception of the ventral surface, with well developed claws for attachment to their mothers, and recurvant milk teeth that are replaced by adult teeth as the pup is weaned (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995).

Once pups become too large to carry, females will crèche pups together while they forage. Pteropodid offspring are generally weaned by 5-6 months, at approximately 60 – 70% of adult weight (Olsson & Woods, 2008).

Very little is known about Christmas Island flying-fox birth or neonate development. Females are reported to become volant at an earlier age than males (James, et al., 2007).

6.7 Neonatal/Infant Mortality

A survey of North American Zoos showed a 33% mortality rate in captive frigivorous bats before 2 months of age (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995) including stillbirth. The reported mortality rate for wild *P. alecto* and *P. poliocephalus* in juveniles under 2 years is around 43-80% and 30% respectively (McIlwee & Martin, 2002). The annual mortality rate for wild *P. conspicillatus* is approximately 35% however this is for the entire population, not just juvenile animals (Fox, 2006).

In captive colonies, major causes of neonatal death include: environmental stress, cagemate inflicted trauma, hazards within enclosures (e.g. drowning in pools), and medical causes (e.g. infections and caesarean section related deaths). Inexperienced mothers result in more recorded neonatal deaths than experienced females. Cannibalism has been recorded when overcrowding is an issue, or when a pup is stillborn (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995).

In the wild, pregnant females establish female camps prior to parturition. There have been reports from mixed sex captive colonies of stillbirth, aborted foetuses, and neonatal deaths due to trauma from females receiving aggressive behaviour from male cagemates (A Lorenzo, Pers. Com.).

Northern Queensland care facilities have reported episodes of neonatal congenital palatine defects ranging from pinpoint holes, to complete rostrocaudal separation (Olsson & Woods, 2008). Poor fur growth and bone deformities, particularly of the feet, were also recorded during these events. Euthanasia was elected in most circumstances, though some animals with small palatine defects were successfully treated surgically (Olsson & Woods, 2008). The cause of these defects is unknown.

Also reported in neonatal flying-fox is imperforate anus, where the natural opening of the anus is absent or inadequate. This can be indicated by an absence of faeces production, bulging perineum, and abdominal distension (Olsson & Woods, 2008). While surgical correction is an option, euthanasia should be considered if immediate veterinary intervention is not possible.

6.8 Contraception

Methods for controlling reproduction in a captive environment include: separating sexes, surgical sterilisation, and contraceptive implants.

Maintaining single sex colonies is recommended if breeding is not desired. However, maintaining bachelor groups may not be a viable long-term strategy of population control as subordinate animals may become irreversibly reproductively suppressed (Lengel & Oberlin, 2016). More research is needed in this area.

“Little or no information is available on reversible chemical contraception methods in bats” (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995). However, melengestrol acetate (MGA) implants have been used successfully in *P. rodricensis* to control reproduction with little reported effect on social behaviour (Jackson, 2003; Asa, 2009; Lengel & Oberlin, 2016). Reported side effects of implants have included 22% rejection/loss of implant, weight gain, and retarded hair growth at implant site (Jackson, 2003).

6.9 Hand-rearing

Hand-rearing may be desirable if a pup is found to be abandoned, or if a mother is sick and/or unable to care for her pup. Pups found with serious injuries or deformities should be euthanased as treatment options are limited. Pups with signs of infectious disease, such as discharges, diarrhoea, sneezing, unexplained neurological dysfunction, or difficulty breathing, may not be suitable for hand-rearing due to the concern of potential zoonotic disease. Fostering abandoned pups to other lactating females with recently weaned pups has rarely been successful (Lengel & Oberlin, 2016). Luckily, successful hand-rearing of flying-fox is well documented.

Hand-rearing of pups should be limited to personnel with current rabies vaccinations.

Strict hygiene principles are crucial when hand-rearing young flying-fox pups. Biting, crying, restlessness, and poor feeding are all indicators of stress in the hand-reared flying-fox, and care should be taken to provide a calm quiet environment for housing the pup while it develops (Griffith, 2004). Olfactory cues are an important social mechanism for flying-fox and rearing multiple pups at the same time may cause stress for the individual. Hands should be washed between handling individual pups, and some carers recommend changing clothes between animals (Griffith, 2004).

Neonatal pups should be wrapped in a small towel or soft cloth to keep them warm (28°C) as they are unable to regulate body temperatures effectively.

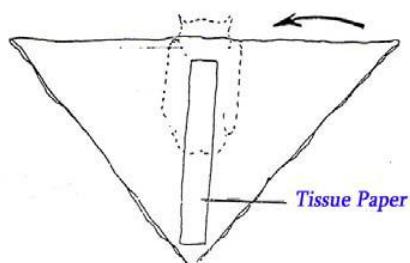


Figure 1

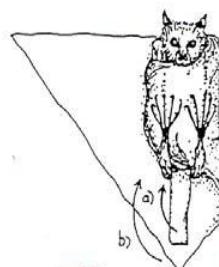


Figure 2

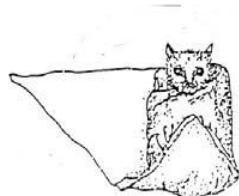


Figure 3



Figure 4

Figure 1: Wrapping neonate pups for hand rearing (Griffith, 2004).

A folded tissue can be used as a nappy for small flying-fox, and skin and wings should be kept clean of urine and faeces to prevent fungal and bacterial infections. Young hand-reared pups will require toileting before every feed until they learn to do this on their own. Gentle manual stimulation of the anogenital area with a moist cotton ball will induce urination and defecation. Overstimulation can cause irritation and cotton wool should be used rather than tissue as tissue can become quite abrasive.

The pup should be toileted prior to, or part way through feeding. Invert the pup so that its head is raised, and rub a warm damp cotton ball gently over the genital area, wiping away urine and faeces produced. It is also important to clean around the pup's mouth after feeding to remove any residual milk from the fur as poor hygiene can result in fungal infections. Pups should also be given daily sponge baths with a damp cotton ball or cloth to reduce odour, prevent infections, and maintain suppleness of wing membranes. It is important to ensure that animals are completely dry before wrapping them back in their towels. Drying an animal by exposing them to natural sunlight is ideal.

The 'Mumma Method' is also adopted by a lot of carers hand raising flying-fox whereby the pup is placed on a rolled up hand towel wrapped in absorbent material. The pup is placed on the towel on its belly with its wings wrapped around the towel. It will often grab the towel with one or all feet. A piece of soft flannelette (approx. 40 cm²) is then folded in half and wrapped around the flying-fox and towel so that the head is covered but the feet are left exposed. The 'Mumma' can be placed in a basket supported at 40° head down and the whole system can be covered with a breathable blanket (Jackson, 2003; Griffith, 2004). Urine and faeces soak into the 'Mumma' keeping the pup relatively clean and dry and the pup can move itself up and down the 'Mumma' to thermoregulate.

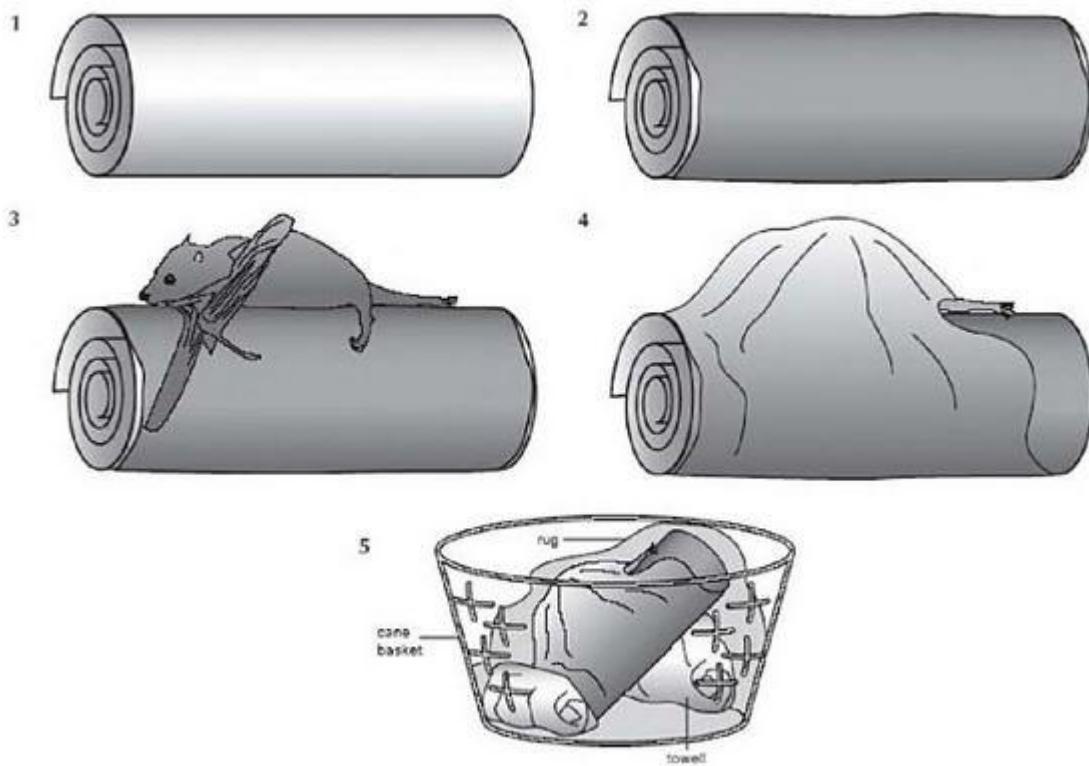


Figure 2: The 'Mumma Method' or wrapping neonatal flying-fox pups (Jackson, 2003).

Swaddled pups should be fed with the head slightly lower than the feet, and with the animal placed on its side to prevent aspiration (Olsson & Woods, 2008). Never feed a pup that is cold. Warm pups slowly if they are found in this state using a hot water bottle wrapped in a towel or similar. Neonatal

animals should be fed with a warmed (body temperature) milk replacer every 2 hours from 6am until midnight with feedings decreasing gradually over time, to be replaced with fruit juice, and then diced fruit until weaned (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995). Always use cooled, pre-boiled water when making up formula, and discard unused milk (do not reheat it for the next feed). Microwaving small quantities of liquid to body temperature can be very difficult and often results in the milk being too hot. We recommend placing the bottle of formula in a mug containing hot water to heat the milk slowly, checking the temperature on the inner wrist as you would do for a human baby.

There are a number of commercially available flying-fox milk replacers, including, Wombaroo flying-fox milk replacer (<http://www.wombaroo.com.au/native-wildlife/Flying-Fox>). Make sure to follow the guidelines for the specific replacer being used as each brand has different recipe, volume, and storage recommendations.

Flying-fox teats are commercially available, but will require a hole to be created at the tip by piercing the tip with a hot needle. The hole should be large enough for milk to drip out slowly when inverted, but not flow freely. If the pup is very small, a smaller teat (possum sized) may be more appropriate. Fitting the nipple to a syringe can provide more control over milk flow for smaller animals, and some experimentation may be required to find an appropriate sized nipple. A blind teat (one without a hole) may also be used as a comforting dummy in very young, stressed, animals that would otherwise be permanently attached to its mother's nipple (Griffith, 2004). Do not use teats with holes in them as a dummy as the pup may swallow excess air and become bloated.

In other pteropodid species, at 1 week of age, feeds should be given every 4 hours, and by 6 weeks feeds should happen every 6 hours. Recurved baby teeth are replaced by adult teeth at around 6 weeks of age (Griffith, 2004). General uneasiness, changes in faecal colour and consistency, reluctance to feed, and chewing on the teat are all behaviours associated with teething. Weaning can begin once the adult teeth have begun to grow in, at approximately 7 weeks.

By 7 weeks, the pup will also begin displaying static flying behaviours and may be placed on a clothes airer fitted with hanging fabrics in a loose hammock to practice flapping its wings. As it is able to gain horizontal lift, the pup may be encouraged to fly short distances and should be given something soft to land on. Usually this is the shirt front of its carer. The airer should be placed in a safe environment where crashing and uncoordinated flying is unlikely to result in injury.

Pups should be exposed to sunlight for a minimum of 10-15 min every day to promote skin and bone health (Olsson & Woods, 2008). From 4-6 weeks of age pups will begin to exercise their wings by static flying, before beginning to fly short distances at 8-12 weeks. Once flying-fox begin flying confidently, they can be moved to a larger enclosure.

Pups may have difficulty feeding for a number of reasons. These include; incorrect formula type, dehydration, colic, being overfed, and systemic disease (Olsson & Woods, 2008). Monitoring faecal output may be useful in monitoring a pup's tolerance to the milk replacer being used.

A guide to pup faeces includes (Griffith, 2004):

- Normal = golden yellow, thin porridge consistency
- Smelly or mucousy = incompatible with milk, stress
- White lumps = malabsorption/undigested milk, reduce volume
- Black specs = the pup has started to groom itself
- Diarrhoea = incompatible with milk formula, poor husbandry, poor hygiene, sterilise all feeding equipment
- Constipation = offer warm water instead of milk, bathe abdomen in warm running water, seek veterinary advice if faeces not produced in more than 12 hours

Ensure all feeding equipment is sterilised using suitable sterilisation agents such as Milton (Griffith, 2004) after each use.

7.0 Nutrition

In most cases, it is impossible to replicate a wild diet in the captive setting, but nutritional needs can be met with carefully chosen, commercially available or selected natural foods and dietary supplements. Considerations will need to be given to locally available food sources, seasonality of available food items, and commercial nutrient supplements. As little is known about the wild diet of the Christmas Island flying-fox, extrapolations from other captive pteropodids have been made where data is deficient.

Nutrient targets will need to be maintained to deliver a suitable captive diet. Monitoring body weight is essential for assessing adequate nutrition and hydration. Weight loss, as well as significant weight gain, can indicate numerous health issues in individual flying-fox.

7.1 Foraging Ecology

The majority of foraging ecology data for *Pteropus* species has been described using wild feeding behaviours. Some studies involve collecting the food items to examine nutrients contained within them, while others analyse excreta, however these methods are limited.

Christmas Island flying-fox, like many other flying-fox species, are frugivores that feed on a variety of plants species, and their parts; fruits, flowers, nectar and leaves. They are also forest pollinators and examination of faeces shows that they ingest pollen as part of their normal diet. The extent to which each forage item contributes to overall nutrition is unknown. More work is required to determine nutrient requirements for this species.

Flying-fox extract the majority of their nutritional needs from the liquid portion of the foods they eat and they swallow very little of the solid portion of these foods. Fruit and leaves are chewed to extract the juice, while the fibrous portion is squeezed into a firm pellet and it spat out or ejected. This pellet is commonly referred to as 'spat'.

Foraging changes seasonally with the availability of food sources, and the wet season delivers the greatest abundance of fruit (James, et al., 2007; Cochrane, 2011).

Known food plants of Christmas Island flying-fox are listed in Appendix F.

Unlike many *Pteropus* species, the Christmas Island flying-fox are not only active at night, but also actively forage during the day.

7.2 Nutritional Requirements

Flying-fox, like other mammals, require specific nutrients to maintain a healthy metabolism. Low levels of important nutrients can have detrimental effects on the population, especially with regards to fecundity and survivorship (Nelson, et al., 2000). Target nutrient requirements will be estimated by utilising data from other *Pteropus* species until those for Christmas Island flying-fox can be determined.

A study of wild Samoan flying-fox (*Pteropus samoensis*) found that several important nutrients, including fat, nitrogen, sodium, iron and calcium, were found at higher levels in native food sources than in agricultural fruits (Nelson, et al., 2000). What agricultural fruits lack in the naturally high levels of nutrients found in native fruits however, can be overcome by their relative abundance.

The American Zoo and Aquarium Association, Chiropteron Advisory Group, compiled an estimate of target nutrient requirements for captive frugivorous Chiroptera using National Research Council guidelines for a number of domestic and laboratory animals, and successful pteropodid captive diets (American Zoo and Aquarium Association Chiropteron Advisory Group, 1995). These estimates are shown below:

Preliminary Target Nutrient Levels (dry matter basis)	
Nutrient	Daily Diet Composition
Crude Protein (%)	2.0 - 15.0
Fat (%)	5.0 – 9.0
Vitamin A (IU/g)	4.0 – 14.0
Vitamin D2 & D3 (IU/g)	0.2 – 2.0
Vitamin E (mg/kg)	11.0 – 56.0
Calcium (%)	0.5 – 1.0
Phosphorous (%)	0.4 – 0.9

Most fruits are low in calcium, with the exception of figs, and calcium supplements may be beneficial as low calcium can affect reproductive success (Nelson, et al., 2000; Jackson, 2003; Olsson & Woods, 2008). Insufficient dietary sodium may also affect reproduction and survivorship of young (Nelson, et al., 2000). Fruit purees can be offered in an emergency but the high indigestible fibre content can have implications on health such as malnutrition, gastrointestinal disease, and rectal prolapse (Olsson & Woods, 2008).

Commercial supplements designed specifically for flying-fox, or other species with a comparable diet, are available and often utilised in a captive diet. Popular supplements include Wombaroo High Protein Supplement, Complan, and VetaFarm Blossom Nectar.

Preventing obesity in captive *P. alecto* at Territory Wildlife Park was achieved by replacing some fruit with lettuce (20% by weight) to control calorie intake, and increasing natural browse to promote foraging behaviour and increase activity (Stanioch, 2016).

Salt or mineral licks are also popular supplements, especially in hot weather.

7.3 Working Diet Composition

An adult flying-fox may consume a total of 10-15% body weight per day (dry matter basis), or between 25 – 120 % of body weight on an as fed basis (American Zoo and Aquarium Association Chiropteron Advisory Group, 1995; Griffith, 2004; Stanioch, 2016). Nutrient intake may increase for lactating females. In the wild, pteropodid pups will suckle for at least 6 months so adding high protein supplements to the captive diet during this time is very important.

As the Christmas Island flying-fox is also an active day feeder, it is recommended that some food items, presented as enrichment, are offered during the day, while the bulk of the diet is offered in the late afternoon to mimic wild foraging behaviour. By offering food later in the day, it is also less likely to spoil.

Water receptacles should be cleaned routinely, usually during morning husbandry, to remove dropped food, spat, faeces and urine from water bowls, and again at the end of the day to ensure fresh water is available overnight.

Sufficient feed stations should be provided so that subordinate individuals have access to food and not be bullied from feed stations by dominant individuals. Philadelphia Zoo recommends one feed station per flying-fox, or two per flying-fox in all male groups (Lengel & Oberlin, 2016). Placing feed stations at least 1 – 1.5 m apart should reduce food related aggression (Stanioch, 2016). The number of food stations will increase with an increasing population.

Captive diets for Australian flying-fox typically consist of:

- Fruit (including skin and seeds): 350 g chopped per animal, per day: apples, grapes, pears, pawpaw, figs (fresh or dried), mangoes, raisins, sultanas, melons, kiwi fruit, stone fruit, etc. At least 3 varieties of fruit should be offered daily, with 2/3 of the diet made up of hard fruits (apples, pears), and 1/3 soft fruits (melons, grapes, mangos) (Griffith, 2004; Olsson & Woods, 2008; Stanioch, 2016). Hard fruits are important for dental health.
- High protein supplements (daily, as per manufacturer instructions)
- Fresh native browse (i.e. flowers and leaves, as available)

Banana should only be offered sparingly (Griffith, 2004), or avoided altogether (Stanioch, 2016), due to its high fibre content.

Fruit bowls should be offered in a sheltered location so that supplements are not washed off by rain.

Diets in some institutions may also be supplemented with:

- Vegetables: carrots, green beans
- Starchy vegetables: sweet potatoes, corn, etc.
- Citrus (in small amounts): mandarins, oranges
- Leafy greens: lettuce, spinach, kale, Bok Choy
- Fresh herbs: parsley, thyme, mint (fresh herbs are also a useful form of enrichment if placed in bunches around the enclosure)

Food is presented in shallow trays, spill proof dishes, as whole fruits (where appropriate), and as part of behavioural enrichment strategies. To prevent food wastage, fruit should be chopped into 1.5 cm bite sized pieces.

Food can be used as a form of enrichment by offering whole fruits or large pieces of fruit, food puzzles, threading cubes of fruit and vegetables onto metal kebabs or wire (use a cork on the end to prevent injury), and by presenting food at a variety of heights around the enclosure.

Favourite food items can be restricted and used for training and conditioning purposes.

7.4 Feeding sick animals

Sometimes, sick or injured animals may require assistance to maintain weight and promote the healing process. It is vital to monitor weight in sick animals.

Some animals may take additional supplements and treatments directly from a feeding syringe by licking it from the tip, however, severely debilitated animals may require tube feeding to maintain weight during treatment and rehabilitation. Care should be taken to avoid being bitten or scratched

and firmly wrapping the flying-fox in a soft cloth or towel is a useful way to restrain an animal for such procedures (See section 4.6).

For short periods of time, injectable fluids containing 5% dextrose and vitamin supplements can be provided subcutaneously however this is not suitable for long term treatment. Tube feeding high-energy supplements (e.g. Poly-Aid Plus, 1 g/10g body weight q 12 hr), or a puree of low-fibre fruits with supplements, may be necessary for debilitated animals. Probiotics added to the diet of debilitated flying-fox, and animals treated with antibiotics, may be useful in restoring normal gut flora (Olsson & Woods, 2008). Unsweetened acidophilus yogurts can be used for this purpose (Griffith, 2004).

Tube feeding (gavage) is performed by force feeding a liquid or pureed diet by passing a 14-18G stainless steel bird crop feeding needle, size 3-4 nasogastric human infant feeding tube, or 14-18G IV cannula, down the throat to the stomach (Olsson & Woods, 2008). A gag may be required to keep the animal's mouth open while passing the tube and preventing the animal from biting (potentially severing and swallowing) the tube. This procedure should be done with the flying-fox in a head up position. Approximately 2-3 mL/100 g body weight of the food or supplement should be administered every 3-4 hours (Olsson & Woods, 2008). This may be replaced by voluntary feeds as the flying-fox recovers, possibly from syringe at first, followed by solid foods/normal diet.

8.0 Health

As described above, all flying-fox should be individually identifiable, and each animal should have a written husbandry/medical record. All observational notes about individual animals should be written onto their own medical file including changes to diet, behaviour, physical condition, and weight, etc.

Veterinary advice should always be sought before treating flying-fox. Non-irritating disinfectants should be used. Pteropodids are well known for self-trauma so wounds, bandages, sutures, and anything else that may cause irritation, should be monitored closely, and pain relief administered if required. Elizabethan collars may be necessary to prevent self-mutilating behaviour.

Good hygiene is essential for the prevention of disease and disease transmission. *Pteropus* species are vectors for several potentially fatal zoonotic diseases and should be managed with this in mind, even if the individuals health status has been confirmed.

In a population study carried out by Hall et al. (2010) there was little evidence that any of the 28 Christmas Island flying-fox examined were suffering from infectious disease (Hall et al., 2014).

8.1 Inoculations

There are no recommended vaccinations for flying-fox. It is essential however, that all personnel working directly with flying-fox have a protective rabies titre and always use appropriate personal protective equipment (See section 4.1).

Flying-fox should not be vaccinated against rabies because: there is no vaccine available for use in flying-fox, the vaccine may interfere with tests for rabies detection, and a vaccinated animal may still shed the virus (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995).

8.2 Physical Examinations

Provided the animal is adequately restrained, physical examinations on a conscious animal should be relatively straightforward, however, chemical restraint is recommended for aggressive flying-fox, or animals undergoing prolonged examination.

A visual examination of an unrestrained flying-fox is usually the first step to assessing the physical health of any animal. If the animal appears in general good health, it may be wrapped in a towel, or restrained by someone wearing thick welding gloves, as previously described (See section 4.6).

General physical examination should assess:

- General condition, as indicated by gently massaging the chest muscles and the area over the scapula spine to feel if these muscles are adequate or if the animal appears thin, or overweight.
- Fingers, toes, and wings can be assessed by gently unfolding and extending them. Lumps, damaged toes, missing claws, unusual grip, breaks, dislocations, abrasions, and cuts should be noted. Dry, papery wings can indicate dehydration. Slimy coating, unusual odour, or spots on wings may indicate fungal infection.
- Assess coat condition for alopecia, ectoparasites, and underlying trauma.
- If oral cavity can be assessed, check the condition of the teeth, colour of the mucus membranes, and tongue. The mouth can be coaxed open by gently applying pressure to the

corners of the jaw while tilting the head back (gripping from behind to allow clear vision into the mouth). Lips may be lifted with a cotton bud while the head is secure to further assess teeth and gums if required. Be sure to have the head secure before placing hands anywhere near the flying-fox's mouth to avoid being bitten.

- Assess the eyes and ears. Eyes should be clear, bright and alert, with no discharge
- Assess genitals, and nipples, for abnormalities.
- Check the anus for signs of diarrhoea, unusual discharges, trauma, etc.
- With a stethoscope, assess lung sounds and observe respiratory rate. Assess heart rate. Normal heart rate in the Christmas Island flying-fox has not been established, but is reported as 100 – 400 beats per minute for active megachiropterans (Jackson, 2003), and 130 bpm at 30°C in *P. poliocephalus* (Griffith, 2004).
- Temperature can be taken rectally. While normal resting body temperature for Christmas Island flying-fox has not been established, other adult pteropodids maintain a body temperature between 35 – 40°C (Jackson, 2003; Griffith, 2004; Olsson & Woods, 2008).
- Record weight by placing an adult flying-fox in a soft cloth bag, in a plastic tub placed on scales, or by dangling the cloth bag from a hanging scale. Subtract the weight of the bag.

Some species may also respond well to positive reinforcement and can be trained to perform specific natural behaviours like spreading their wings, which may be useful in conducting routine physical exams and decrease the need for physical restraint (Lengel & Oberlin, 2016).

8.3 Neonatal Examinations

All births, complications of birth, and neonatal deaths should be recorded on the record sheet of the dam. Dam and Sire identification should also be recorded if known.

Neonates should be observed without disturbing the female to ensure normal behaviour is exhibited (pup is suckling and tightly held to dam). If close assessment of the neonate is necessary, it is recommended that the mother be anaesthetised, rather than physically restrained, to decrease the threat of injury to both the female and the pup.

8.4 Life Span

More data is needed to effectively estimate the life span of Christmas Island flying-fox.

Various captive flying-fox have been reported to have a life span of 15 – 20 years, while *P. rodricensis* has been reported to have a captive life span of 28 years, and a single captive male *P. giganteus* has been reported as living more than 40 years. Captive animals generally live longer than their wild counterparts, but this is species specific and may not always be the case. Wild *P. alecto* are reported to live up to 15 years but may live more than 20 years in captivity (Stanioch, 2016). *P. poliocephalus* have been reported to live around 10-15 years in the wild but more than 20 years in captivity (Griffith, 2004).

Tooth wear is a generally good indication of flying-fox age, with young animals having clean pointed teeth, and older animals having much more rounded canine teeth and increasing molar wear (Jackson, 2003, p. 302). Very old animals may have teeth worn all the way to the gum line. This type of assessment is quite subjective however, and is best if done by an experienced person with a good knowledge of the species in question.

8.5 Parasites

Some effort should be given to preserving the unique parasites that naturally infect the flying-fox host to maintain natural immune responses if animals are to ever be returned to the wild. All wild animals are host to a variety of external and internal parasites that live on them without detriment. Often, it is only when an animal is in a significant state of physiological stress that parasites can become a problem and should be actively treated.

Observing increased skin irritation, decreased weight, or changes in faecal consistency are all indicators that parasitic burden may require treatment. As many parasites can exist and reproduce in the environment, treatment protocols should include changing and disinfection of exhibit furniture and substrate as appropriate.

Ectoparasites

Ectoparasites can be collected directly off the individual and placed into 70% ethanol for identification. In any event where wing webs or skin develop lumps or nodules, parasitic infection should be considered and veterinary opinion sought. Lesions may require a skin scrape, sticky tape prep, or biopsy to confirm parasitic causality, identification, and development of a treatment plan. Mites, lice, fleas, ticks, and flies have all been recorded as ectoparasites of flying-fox (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995).

The bat mite, *Meristaspis calcaratus* (Arthropoda: Parasitiformes: Spinturnicidae) has been identified previously on the Christmas Island flying-fox. Prevalence in healthy animals was recorded at about 10% (Hall, et al., 2014). This family of mites is considered common in flying-fox and has been recorded from several species of *Pteropus* from PNG, Malaysia, Madagascar, Vanuatu, and Guam (Baker & Delfinado, 1964).

The pathology of *Meristaspis* mites is poorly understood. They may act as vectors for blood parasites but these have not been recorded in the Christmas Island flying-fox. *Meristaspis* mites may be readily transferred between individuals within a colony and there are also reports of transfer to human skin.

Nycteribiid and streblid flies (wingless, spider like) have also been reported on numerous bat species (Olsson & Woods, 2008). Tidemann (1985) reported finding a Hippoboscid fly *Olfersia aenescens* on a single Christmas Island flying-fox, but considered this incidental as this species normally parasitises sea birds.

Ivermectin (200-400 g/kg PO, IM, SC) every 10 to 14 days for three treatments can be used to treat heavy ectoparasite burdens (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995). Care should be taken to ensure correct dosage, and veterinary advice should be sought prior to engaging in any treatment plans. Incorrect dosage can result in toxicity, which may be indicated by lethargy, decreased appetite, or paralysis 24 to 48 hours post treatment.

Blood Parasites

No blood parasites have been observed in blood smears from Christmas Island flying-fox and no blood parasites could be identified from PCR from a very low number of wild caught individuals (Hall, et al., 2014).

Trypanosoma spp. have been identified in several Australian flying-fox species but have not been associated with clinical disease (Ladds, 2009, p. 219). The significance of identifying trypanosomes in blood smears is unknown.

Endoparasites

Faecal analysis is the tool generally used for assessing internal parasite burden and identification. See Appendix G for faecal analysis procedures. Faecal examination for internal parasites should be carried out every 6 months for each individual in the colony. While analysis can be carried out on faeces collected from the colony as a collective whole, this will not allow for identifying individuals that may be suffering with a higher than acceptable parasite burden.

Coccidia and an unidentified ascarid have been identified in faeces from the Christmas Island flying-fox in the past (Hall, et al., 2014). Both are considered incidental, with little health risks for a healthy individual.

Coccidiosis

Coccidia (Apicomplexa; Conoidasida; Coccidiina) are single celled intracellular parasites that inhabit the gastrointestinal tract of their definitive host, and pass their oocysts in faeces. These have been found to infect 25% of Christmas Island flying-fox (Hall, et al., 2014).

Intestinal coccidiosis in herbivorous mammals is considered common, but there are very few reports in *Pteropus* spp. There are only 15 species of described coccidians in bats worldwide; all are of the genus *Eimeria* (Duszynski, 1997). There is a single report of *Sarcocystis* sp. (suspect) in the grey-headed flying-fox, published reports from four non-Australian flying-fox species (unknown genus), and renal coccidiosis has been reported as an incidental finding in big brown bats (*Eptesicus fuscus*) (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995; Wunschmann, et al., 2010; Hall, et al., 2014).

Coccidians are highly species specific and as such, are not considered to carry a zoonotic risk.

Ascariasis

There has only been a single report of an ascarid-like ovum infecting a single, clinically healthy Christmas Island flying-fox (Hall, et al., 2014). The ascarid, *Toxocara pteropodis*, has been reported from all Australian and south-east Asian pteropodids (Olsson & Woods, 2008). The parasite is usually reported from suckling pups, and adult worms may be present in the faeces of weaning juveniles. Infection is generally considered of little consequence to the population, but there have been reports of airway obstruction, biliary tract obstruction, intestinal volvulus, poor weight gain, and morbidity (Prociv, 1990; Heard, et al., 1995; Olsson & Woods, 2008).

More research is required to determine the implications of each of these gastrointestinal parasites in Christmas Island flying-fox and where available, faeces should be collected and examined regularly for the presence of gastrointestinal parasites and associated ova.

8.6 Major Disease Problems and Their Treatments

Dermatological diseases

- Band constrictions - Care should be taken when applying identification bands to ensure they do not cause immediate injury, and periodic checks should be carried out to ensure that

bands remain functional. If swelling or ulceration around the band site occurs, remove the bands immediately and treat wounds topically as required (dependant on severity). Other thumb injuries including torn nails, simple fractures, and abrasions should also be treated in accordance with severity.

- Wing tears may occur from time to time, and will generally heal on their own without treatment. Large wing tears or damage to the leading or supporting wing edges may require sutures to heal effectively, but prognosis should be guarded (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995; Griffith, 2004; Olsson & Woods, 2008). Bandaging wing webs may obstruct blood flow causing subsequent necrosis, fungal infections, or self-trauma, and should be avoided.
- Alopecia (hair loss) – may be a normal physiological event associated with moulting, but is more likely to indicate ectoparasitism or malnutrition.
- Allergies to certain plants or insect bites may result in conjunctival or facial swelling, scratching or ulceration, or respiratory distress, and should be treated with antihistamines or corticosteroids.
- Trauma – overcrowding, fighting, poor hygiene, burns, and poor enclosure and furniture design may result in bites, alopecia, wing wounds and scarring and treatment should be applied topically as appropriate but all efforts should be put into preventing these kinds of interactions.
- ‘Slimy wing’ – a fungal infection of the wing membrane which causes a malodorous slime to build up on the wing membranes but may spread to other areas including the mouth and fur. Infection is most common in young, hand-reared animals. Budding yeasts of *Candida albicans* can typically be seen on an impression smear of the affected skin. Infection can be avoided by maintaining good hygiene, improving airflow and providing access to direct sunlight. Washing the affected areas with warm running water and drying the flying-fox thoroughly, before applying a 1-3% chlorhexidine solution can be used as treatment if infection occurs.
- Other fungal infections may occur and topical antifungal creams may be applied. Ringworm can be a zoonotic disease, but handlers should already be wearing appropriate personal protective equipment, including gloves and gauntlets.
- Skin nodules associated with nematode infection of unfurred skin have been described in the Southern Bentwing Bat (*Miniopterus schreibersii bassanii*) (McLelland, et al., 2013). Anecdotal reports of skin nodules associated with mite infestations in several Australian flying-fox species have also been received by the Australian Registry of Wildlife Health and investigations are pending. Topical treatment of severely affected animals has not been successful.
- Infected scent glands may respond to antibiotics, but in severe cases, surgical removal may be required (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995).

Gastrointestinal diseases

- Diarrhoea, straining to defecate, bloating, inappetence, anorexia, vomiting, rectal prolapsed and abdominal pain are all symptoms of gastrointestinal tract dysfunction.
- Oral lesions may occur in hand-reared young if milk is too hot.
- Oral candidiasis (thrush) may occur in hand-reared pups with clinical symptoms including white oral plaques, reluctance to feed, rust-coloured salivary stains, halitosis (bad breath),

mouth ulcers, and microscopic evidence of yeast. "Treatment includes nystatin, fluconazole or itraconazole" (Olsson & Woods, 2008). This infection can continue down into the pharynx and oesophagus and may not be limited to the mouth.

- Bloat - Young hand-reared animals may experience abdominal discomfort if they swallow a lot of air while feeding, eat too much, or are fed an inappropriate milk supplement (Olsson & Barnard, 2009). The onset of bloat can be rapid, and life threatening but the risk can be minimised by appropriate teat size selection, correct posture while feeding, and controlling milk flow.
- Tooth wear and gingivitis may affect weight and body condition and should be considered if an animal is reluctant to feed or shows consistent weight loss. Treatment options will depend on the severity of dental disease.
- Coccidiosis and Ascariasis – see above. Where available, faeces should be collected and examined for gastrointestinal parasites and associated ova. Other, undescribed gastrointestinal parasites may be present, yet unidentified, in the wild Christmas Island flying-fox population.
- Vomiting is uncommon and usually occurs in the terminal patient (Olsson & Woods, 2008). Ingestion of noxious substances may also induce vomiting.
- Bacterial overgrowth from feeding an inappropriate diet may cause bloating and abdominal discomfort. Blood or excessive mucus in faeces may also be an indicator. Treatment is based on culture to identify bacterial agent and sensitivity testing to determine effective treatment options.
- Probiotics to restore healthy gut flora is recommended for any animal treated with antimicrobial therapy (antibiotics). Unsweetened acidophilus yoghurt may be used (Griffith, 2004).
- Straining to defecate, and/or rectal prolapse, may be seen secondary to constipation and this is often seen in animals fed a blended diet where they are unable to eliminate fibre as spat (Olsson & Woods, 2008). Partial rectal prolapses are often self-correcting once diet is altered, however severe prolapses may require intervention in the form of manual or surgical correction. Sugar and electrolyte powder can be applied to the prolapse to reduce swelling before using a well-lubricated cotton bud to gently reinsert the prolapsed tissue (Olsson & Woods, 2008).

Cardiovascular disease

- Hypovitaminosis E causing dilated cardiomyopathy has been reported in captive pteropodids (Heard, et al., 1996; Olsson & Woods, 2008).
- A decrease in oxygen can result in changes in mucus membrane colour.
- Cardiovascular disease, hypoproteinaemia, and protein losing enteropathy can result in oedema (swelling) to the head and neck (Olsson & Woods, 2008).

Respiratory disease

- Reluctance to fly, exercise intolerance, weight loss and failure to thrive are all symptoms of possible respiratory dysfunction. Sneezing and an associated clear nasal discharge are described as common and should be of no concern (Olsson & Woods, 2008).
- Aspiration pneumonia caused when liquids are accidentally inhaled into the lungs may occur in pups fed with their head higher than their feet. Non-steroidal anti-inflammatory drugs are

recommended, however antibiotics to treat secondary bacterial infections may be required (Olsson & Woods, 2008).

- *Histoplasma capsulatum* is a common fungus in soil and can cause benign respiratory disease in flying-fox. Histoplasmosis is a zoonotic disease that can be transferred to people by inhalation of infected guano dust. Flying-fox are not the infective agent here, but the fungus does have a propensity to grow in flying-fox and bird faeces. The risk of infection is low and can be minimised further by good hygiene and husbandry protocols.

Urogenital diseases

- Poor hygiene during hand-rearing may cause urinary tract infections.
- Penile bleeding is uncommon, while vaginal bleeding occurs at times of oestrus and parturition. Bleeding from the genitals should be investigated. Urinary tract infection, trauma, neoplasia, obstruction, and infection may cause urinary or genital bleeding (Olsson & Woods, 2008).
- Extreme heat events, exposure to toxins, and highly stressful natural events such as cyclones may induce abortion.

Neurological diseases

- Trauma, parasitism, congenital defects, ear infection, viral disease, bacterial infections, and toxicoses may cause nervous system dysfunction illustrated by head tilt, abnormal posture, paralysis, seizures, abnormal vocalisations, increased aggression, and blindness.
- Australian Bat Lyssavirus (ABLV) is fatal to flying-fox, closely related to the rabies virus, and is a zoonotic disease transmissible to humans via contamination of skin or mucous membranes (mouth, eyes and open wounds) with saliva through bites or scratches. All personnel handling or interacting with flying-fox must have a protective rabies titre and wear appropriate personal protective equipment. There are no vaccinations against ABLV for flying-fox. ABLV should be ruled out for any animal presenting with neurological disorders. ABLV causes fatal encephalitis in both flying-fox and people. Flying-fox as young as 8 weeks can be infected. Symptoms can include unusual vocalisation, difficulty swallowing, salivation, increased and non-targeted aggression, severe depression, blindness, convulsions, inability to fly, hind limb paresis, abortion, muscle weakness, generalised trembling, and death (Ladds, 2009, p. 17).

There is an Australian Veterinary Emergency Plan (AUSVETPLAN) disease strategy for ABLV in captive flying-fox colonies and this should be referred to in order to obtain the most recent guides for management of this disease (<https://www.animalhealthaustralia.com.au/our-publications/ausvetplan-manuals-and-documents/>).

If exposure through a flying-fox bite or scratch occurs, wounds should immediately be scrubbed with betadine and clean running water for a minimum of five minutes, before bandaging the wound with betadine. Exposure of mucous membranes to bodily fluids should be treated by rinsing the affected area for 5 minutes. Medical treatment should be sought as soon as possible in both instances. A post-exposure rabies vaccination will be required. Povidone-iodine has been recommended by the Australian Veterinary Association as the preferred disinfection agent for flying-fox bites/scratches due to its virucidal activity (Bodley & Eden, 2014).

Viral material has not been recovered from faeces, therefore faeces does not pose a risk of exposure (Bodley & Eden, 2014).

- Lead or zinc poisoning can occur if flying-fox ingest heavy metals from poorly chosen enclosure materials, inhalation from industrial and vehicle emissions, or from insecticides applied to fruit (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995; Jackson, 2003; Olsson & Woods, 2008). Clinical symptoms are similar to those for ABLV and may include inability to fly, anorexia, emaciation, diarrhoea, excess salivation, weakness, ataxia, incoordination, muscle tremors, aggression or receiving aggression from cagemates, anaemia, and death (Olsson & Woods, 2008; Ladds, 2009, p. 395). Blood lead levels greater than 8-10 ppm are considered diagnostic (Ladds, 2009, p. 395) though these results have not been validated for the Christmas Island flying-fox.
- *Angiostrongylus cantonensis*, a parasite of rodents, can infect other vertebrates including flying-fox, causing neurological disease similar to that exhibited by animals infected with ABLV. Non-descript symptoms including weakness, debility, anorexia, inability to fly or hang, progressive paralysis, respiratory distress, and abortion of stillborn pups have all been attributed to *A. cantonensis* infections (Ladds, 2009, p. 295). Limb paresis, poor reflexes and coordination, poor bladder control (retention and incontinence), depression, ocular changes (nystagmus, unequal pupils, and poor light response), head tilt, muscle tremors and frothing at the mouth are all common neurological indicators of infection (Ladds, 2009, p. 295). Infection is acquired by unintentional ingestion of infected slugs and snails, and arguably their slime. There is no clinical test for *Angiostrongylus* infection and diagnosis is made by histopathology of the brain and spinal column. There is no treatment. Pest proofing enclosures against rats and snails, and thorough washing of food items is recommended.
- *Toxoplasma gondii* has been diagnosed in captive juvenile *P. conspicillatus* and *P. scapulatus* displaying respiratory and neurological disease (Sangster, et al., 2012). Toxoplasmosis is a protozoal disease spread by cats, which can also infect humans. Suitable pest control to eliminate cat faeces from entering exhibits, good hygiene, and the adoption of mandatory personal protective equipment when conducting routine husbandry will prevent infection.

Musculoskeletal disease

- Joint swelling can result from trauma induced by inappropriate enclosure materials, infection, foreign body penetration, poor nutrition, toxicoses, or age-related degenerative joint disease. Treatment will depend on identifying the cause. Surgery may be required if antimicrobial or anti-inflammatory therapy is ineffective.
- Fractures may occur during capture and restraint. In young flying-fox, rough handling may result in injury to growth plates and long bones causing growth abnormalities as the animal ages (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995). All fractures need to be evaluated by a veterinarian to determine appropriate treatment options. Exposed bone may require multiple surgeries for amputation and/or treatment (American Zoo and Aquarium Association Chiropteran Advisory Group, 1995; Olsson & Woods, 2008; P Eden, pers. com.). Forearm fractures are usually repairable, with the exception of fractures close to the wrist (Griffith, 2004). Bandaging may obstruct blood flow causing subsequent necrosis or self-trauma, and should be avoided.
- Degenerative joint disease, sometimes seen in aged individuals, can be treated with non-steroidal anti-inflammatory drugs for pain relief (Olsson & Woods, 2008).

Nutritional diseases

- Metabolic bone disease – may be seen in animals with nutritional deficits, and is especially prominent in hand-reared animals with unsuitable nutritional supplementation. Inadequate dietary calcium and/or vitamin D can result in soft bones, pathological fractures, swollen joints, and bone deformities. Exposure to natural sunlight, appropriate diet, suitable dietary supplements and milk formulas are necessary to avoid metabolic bone disease. Care should be taken however to avoid over-supplementation, which may also lead to clinical bone deformities, kidney damage or seizures (Olsson & Woods, 2008).
- Vitamin B12 deficiency occurs when diets consist only of washed and peeled fruits without adding mineral supplements, or when antibiotic therapy is prolonged destroying normal gastrointestinal flora (Olsson & Woods, 2008). Abnormal flight and ataxia are indicative of vitamin B12 deficiency.
- Protein deficiency may be caused by stress, malnutrition, chronic diarrhoea, infection, haemorrhage, trauma, and critical illness as a result of anaemia, muscle atrophy, oedema, depressed growth, and an impaired immune response (Olsson & Woods, 2008). Adequate diet and dietary supplements are important to avoid protein deficiency.
- Hypoglycaemia and collapse has been reported in a captive flying-fox colony exposed to cold temperatures and limited access to food when dominant animals prevented subordinates from accessing food stations (P Eden, Pers. Com.). This problem was remedied by the addition of extra food stations around the exhibit.
- *P. rodricensis* appear to be sensitive to low level exposure to dietary fluoride that may result in bone lesions indicative of toxicity (Lengel & Oberlin, 2016).
- Haemochromatosis, an iron storage disease that can cause mortality in flying-fox, has been reported from captive diets supplemented with improper levels of iron and ascorbic acid (Crawshaw, et al., 1995).

Other diseases

- Neoplasia: Craniofacial tumours (soft tissue sarcomas, carcinomas) and osteosarcomas have been reported in aged pteropodids, but neoplasias in Australian flying-fox appear to be rare (Olsson & Woods, 2008). There has been one reported case of a microchip associated leiomyosarcoma in a 7 year old captive Egyptian fruit bat (*Rousettus aegyptiacus*) (Siegal-Willott, et al., 2007).
- Hendra virus (HeV) (Paramyxoviridae), a notifiable disease in Australia, is carried by all mainland Australian flying-fox species at a low prevalence, though they show no clinical signs of infection. Though this virus is zoonotic, transmission to humans is made through exposure to oral and nasal secretions of infected horses. Direct flying-fox to human infection has not been documented. Abortions in flying-fox may be associated with HeV infection, but most flying-fox show no clinical signs of HeV infection (Olsson & Woods, 2008; Ladds, 2009, pp. 17-18). Although a small number of Christmas Island flying-fox have been tested for HeV, and found to be negative, the sample size for these tests was small and further investigation is warranted (K. Rose pers. com.).
- Menangle virus (Paramyxoviridae) is a notifiable disease in Australia. Again, though carried by pteropodids, and associated with flu-like illness in humans, a pig vector is needed to complete the infection cycle. The clinical presentation in flying-fox is unknown, but presumed asymptomatic (Olsson & Woods, 2008; Ladds, 2009, p. 18).

- *Pasteurella multocida* bacterial infections are common in rescued flying-fox that have sustained cat bite wounds. Cellulitis, abscesses, osteomyelitis, septicaemia and bacteraemia are possible sequellae, but these can be treated with wound care and antibiotics.
- *Leptospira* spp. have been identified in numerous Australian and Asian flying-fox, but details about clinical symptoms and pathological changes are lacking (Olsson & Woods, 2008; Ladds, 2009, p. 83). Leptospiral DNA has been found in pteropodid kidney and urine specimens (Ladds, 2009, p. 83). Appropriate hygiene and safety protocols should be maintained to avoid this zoonotic infection.
- Phosphate/Cadmium Toxicity: The health repercussions for Christmas Island flying-fox from historic and ongoing phosphate mining on Christmas Island are unknown. Dust fallout from the mining industry has been implicated in the abandonment of seabird nesting colonies on the island (James, et al., 2007), and there is some concern about ingestion of heavy metals on dust smothered food resources. More research is needed.
- Other toxicities: Insecticides and pesticides used around enclosures to control weeds and those that are sprayed on fruit, rodenticides used in pest control, and parasiticides such as Ivermectin, Frontline and Revolution, are the most common chemicals of concern to flying-fox (Shepherd, 2009). Toxicity can be avoided by restricting use of these chemicals around flying-fox enclosures, maintaining strict husbandry protocols, and following the treatment guidelines for any drugs used for the treatment of flying-fox.

8.7 Physiological Reference Values

Not all diseases have obvious physical symptoms. It is important therefore to establish an understanding of baseline health parameters for any animals coming into the captive environment in order to interpret health data obtained from sick or injured individuals. Hall et al. (2014) established baseline health data from a small subset of wild Christmas Island flying-fox including morphological, haematological, and biochemical reference ranges. Data did not include adult females. This data is being updated with ongoing studies of wild Christmas Island flying-fox and will be expanded in the future.

Life stages of wild flying-fox, where specific age is unknown, can be broken into four distinct age classes (Jackson, 2003):

Neonate: From birth until rear-facing milk teeth begin to be replaced with adult teeth. Milk dependant.

Juvenile: Not fully weaned but somewhat independent, may still have some milk teeth present but the majority have been replaced by clean pinpoint sharp teeth, capable of flight, usually less than 40 days old, less than 80% of expected adult body weight, no visible nipple (females), finger joints are unfused with large epiphyseal cartilage bands.

Subadult: May be 80 – 100 % of expected adult body weight, teeth are sharp (may be slightly stained), nipples are undeveloped (females), finger joints are not fully fused.

Adult: Fully grown and sexually mature, teeth may appear stained and worn, nipples are large and visible (females), finger joints are fused and no epiphyseal cartilage bands are visible.

While severe tooth wear is usually indicative of an aged animal, tooth wear can also be exacerbated by poor nutrition and trauma. Conversely, heavily worn teeth may affect an older animal's ability to chew and maintain healthy nutritional levels.

Identification of adult females can further be classified as nulliparous, lactating, post lactating and regressed, based on the morphology of the nipple.

Nulliparous females have not bred yet, and as such have small, undeveloped nipples. Lactating females have large nipples, often with calluses and visible milk glands. Post-lactating females typically have pendulous nipples surrounded by a circular patch of wrinkled skin where milk glands have regressed, while regressed females generally have smaller, flattened nipples with smooth, darkly pigmented skin.

Morphological Reference values

(Hall, et al., 2014)

Sex	Age	N	Weight	Forearm	Ear length	Foot length	3 met length	5 met length	Skull max length
Male	Adult	12	395.6 ±52.2	130.4 ±2.7	28.7 ±2.5	36.9 ±2.2	81.3 ±3.5	86.9 ±2.6	55.6 ±1.5
Male	Juvenile	6	243.3 ±35.4	117.5 ±4.3	28.4 ±0.6	35.2 ±2.0	71.9 ±3.8	77.5 ±4.4	51.8 ±1.5
Female	Sub-adult	5	315.8 ±65.3	125.4 ±6.1	28.6 ±2.6	35.6 ±1.7	78.4 ±4.1	83.6 ±5.6	53.8 ±2.4
Female	Juvenile	5	200 ±29.9	112.7 ±4.4	27.8 ±1.3	33.6 ±4.3	70.5 ±2.7	74.3 ±3.5	50.2 ±2.9

Standard morphologic measurements for each animal includes weight, forearm length, ear length from notch to tip, hind foot length from heel to toe tip excluding claws, length of metacarpal in third digit of wing, length of metacarpal in fifth digit of wing and maximum skull length (Churchill, 2008, pp. 70-78, 255).

The Christmas Island flying-fox is small compared to other members of the Pteropidae family and results from a 2010 survey (Hall, et al., 2011) found that individuals were smaller in almost every respect to those reported by Churchill (2008). It is suspected that there may be true morphologic population changes and further study is warranted.

Hematologic and biochemical reference values

(Hall, et al., 2014)

Parameter	Mean (Range)
Weight (g)	217 – 546
Alkaline phosphatase (ALP) (U/L)	1478.56 (248.3 – 2708.82)
Alanine transaminase (ALT) (U/L)	9.94 (2.74 – 17.14)
Aspartate aminotransferase (AST) (U/L)	34.06 (17.88 – 50.24)
Albumin (g/L)	40.19 (36.63 - 43.75)
Amylase (U/L)	1701.92 (1219.36 – 2184.48)
Total Bilirubin (umol/L)	2.89 (1.77 – 4.01)
Creatinine kinase (CK) (U/L)	393.89 (0 – 836.53)
Calcium (mmol/L)	2.08 (1.98 - 2.18)
Cholesterol (mmol/L)	0.43 (0.15 - 0.71)
Creatinine (umol/L)	46.11 (33.35 – 58.87)
Gamma-glutamyl transferase (GGT) (U/L)	7.31 (4.77 – 9.85)
Globulins (g/L)	25.29 (21.91 - 28.67)
Glucose (mmol/L)	3.36 (2.55 – 4.98)
Lactate dehydrogenase (LDH-P) (U/L)	219.09 (70.33 – 367.85)
Lipase (U/L)	13.47 (0 – 43.87)
Magnesium (mmol/L)	0.69 (0.61 - 0.77)
Phosphate (mmol/L)	2.43 (1.39 - 3.47)
Total Protein (g/L)	65.21 (58.93 – 71.49)
Triglycerides (mmol/L)	0.51 (0.13 - 0.89)
Urea (mmol/L)	0.98 (0 – 2.58)
Uric Acid (mmol/L)	0.01 (0 - 0.03)
White Blood Cells (10^9 /L)	8.39 (2.6 – 13.2)
Haematocrit (%)	40.7 (36 – 44)
Neutrophils (10^9 /L)	3.74 (1.69 – 5.71)
Lymphocytes (10^9 /L)	4.58 (0.91 - 7.66)
Monocytes (10^9 /L)	0.08 (0.00 - 0.18)
Eosinophils (10^9 /L)	0.10 (0.00 - 0.26)
Basophils (10^9 /L)	0.00

All of the hematologic values of the Christmas Island flying-fox fall within the normal range for all other comparable *Pteropus* species (Hall, et al., 2014).

Growing, juvenile flying-fox tend to have higher concentrations of circulating ALP and phosphate (Heard & Whittier, 1997; Hall, et al., 2014). Christmas Island flying-fox tend to have higher ALP, amylase, and lipase plasma concentrations (and to some extent phosphate) compared to other *Pteropus* species (Hall, et al., 2014).

8.8 Zoonotic Diseases

In recent years, flying-fox as a vector for zoonotic diseases has formed a large component of virus research globally. While we have come to understand a lot about the viruses carried by flying-fox, which may cause disease and death in humans, there are many unknown elements to each of these diseases, and many emerging diseases we are only beginning to understand.

- Personal Protective Equipment should always be used and maintained when handling flying-fox, including wearing appropriate gloves, even while handling anaesthetised or otherwise restrained flying-fox.
- Establish a protocol to record all flying-fox bites and scratched from flying-fox claws, even if the skin is not broken. Contact sites should be washed vigorously for a minimum of 5 minutes.
- Minimise the personnel working with flying-fox as well as the need to capture and handle animals.
- Maintain appropriate quarantine procedures when introducing new flying-fox to the colony

Zoonotic diseases of note include:

1. Australian Bat Lyssavirus (ABLV) - as described above (Ladds, 2009, p. 17).
2. Hendra virus (HeV) (Paramyxoviridae), as described above.
3. Menangle virus (Paramyxoviridae), as described above.
4. Histoplasmosis: as described above.
5. *Leptospira* spp. as described above.
6. Ringworm (a fungal infection), as described above.

In a study carried out in 2010, 10.7% of Christmas Island flying-fox urine samples (3 of 28) tested positive for previously unknown paramyxovirus using a Respirovirus-Morbillivirus-Henipavirus nested PCR (Vidgen, et al., 2015). The Christmas Island flying-fox population has not been tested for Australian Bat Lyssavirus (ABLV). The potential for viruses as a zoonotic disease in the Christmas Island flying-fox population is unknown. While other defined zoonotic diseases of flying-fox were not identified during 2010 and 2015 field studies, testing has not yet been extensive enough to rule these diseases out entirely (K. Rose pers. com.).

8.9 Recommended Routine Medical Procedures

Ideally, each flying-fox should be thoroughly examined at least once per year. This would include a physical exam, identification checks, and blood collection for haematology, biochemical analysis, and serology/viral studies. Faecal analysis for monitoring parasite surveillance should also be carried out routinely, a minimum of once per year.

8.10 Immobilization and Anaesthesia

The need to anaesthetise an animal should weigh up the risks from anaesthesia, and the risk of human and flying-fox injury (Bodley & Eden, 2014). Minor procedures may be performed under manual restraint, while more complex and lengthy procedures should be carried out with the animal under anaesthesia.

The preferred method of anaesthesia is isoflurane in oxygen and this has been applied to Christmas Island flying-fox without complication (Hall et al. 2014):

- Mask down animal at 2-5% isoflurane in 1L/minute oxygen until the animal is relaxed, and then decrease to 2-3% isoflurane.
- Maintain oxygen flow at 1 L per minute.

If gas anaesthetic is unavailable, intramuscular injectable agents may be used, but veterinary advice should be sought before administration to ensure correct dosages.

8.11 Euthanasia

Euthanasia, and the decision to euthanase an animal, should be carried out by a veterinarian. When a veterinarian is not available, the most current AVMA Guidelines for the Euthanasia of Animals (<https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>) should be followed.

8.12 Necropsy Protocol

A necropsy (post mortem examination, autopsy) is very important in the event that any flying-fox dies, even if cause of death is known (e.g. trauma, euthanasia). By examining the carcase of every available flying-fox, we will be able to learn things about the health and diseases of this species that are otherwise unattainable in live specimens. We will also be able to further evaluate base line health data for the species, which is important for any incidence where health wavers from the normal. We will also be able to identify and describe any unusual physiological anatomy for this species.

The main aim for conducting a necropsy is to determine the cause of death. It also gives us the opportunity to retain tissues for investigation by various researchers and bank tissues for genetic and disease research that has not yet been explored/is not yet available.

Necropsy examinations are best conducted on freshly dead specimens. Refrigeration for a day is acceptable if absolutely necessary. Freezing of the whole carcass severely limits the interpretation of tissues and should be avoided. Carcasses should never be placed into a refrigerator or freezer containing food.

Accurate necropsy records are essential for assessing population health and mortalities should be reviewed on a yearly basis. All necropsy data should be submitted to the Australian Registry of Wildlife Health for long-term management of population health data.

Only personnel with protective rabies titres should conduct necropsy assessment of flying-fox. Adequate personal protective equipment should always be used.

The Necropsy Protocol can be seen in Appendix H.

8.13 Quarantine

Quarantine procedures and protocols need to be written and implemented before any animals are brought into captivity. As these animals are destined for on-island management, and no other mammals are being cared for in this facility, quarantine procedures should be fairly straightforward. Sick or new animals entering the established colony however, will pose a quarantine risk and these scenarios should be considered and a management plan put in place, before the need arises.

Quarantine is defined as a period of physical isolation imposed for the purpose of detecting disease. Quarantine is implemented in conjunction with physical examinations and laboratory testing to monitor the health of the animals with the objective of preventing or controlling introduction or spread of infectious diseases.

There are three distinct reasons for implementing quarantine on the captive population. These include:

1. Quarantine of the founder population will identify baseline health data for the individual, and captive population, and allow for veterinary intervention where necessary. We are unsure of the current health status of the wild population and a mandatory closed quarantine of at least 21 days will ensure that all founding animals are healthy and suitable for ongoing management in a captive setting. Quarantine should commence from the date that the last individual enters the population.

2. Any animals entering the captive population to replete the population if there has been mortality, to introduce additional founders, or to house injured wild flying-fox will be required to undergo mandatory quarantine to ensure the integrity and health of the entire captive population. Quarantine conditions should closely mimic those of the rest of the captive colony. All new animals should also undergo the minimum 21 day quarantine period and be subject to the same physical exam outlined for founder animals. During this time, quarantine animals must not be allowed contact with the rest of the population, including mesh to mesh contact. Equipment used for animals in quarantine must not be used in the general population, and vice versa. Preferably staff caring for quarantine animals will be different from those caring for the remaining captive population. If the same people are looking after both populations, barrier keeping methods should be employed to prevent the potential transfer of pathogens². Testing and monitoring during post-arrival quarantine will allow clinical, subclinical and incubating diseases to be detected and eliminated prior to the animal entering the captive population. Animals can only be reintroduced to the population once health has been determined by a veterinarian.

3. Maintenance of strict quarantine for the duration of the program will aid in the reintroduction of individuals to the wild should this be required. Quarantine will ensure that no new disease processes have been introduced to the captive colony that may jeopardise the health of the wild population. If management of the population is moved to mainland facilities, quarantine will be required to prevent the introduction of exotic diseases into Australia. By maintaining strict and documented quarantine practices, the time and effort required for transfer or release of animals from on-island facilities will be minimised significantly.

All animals entering quarantine will undergo physical examination under anaesthesia, will be given a unique identification, undertake blood, swab and faecal collection for diagnostics, have faeces collected for parasite screening and identification, and be weighed.

While physical examinations, and some basic laboratory testing can be conducted on Christmas Island, many of the tests for infectious diseases of concern are only available in certified laboratories on mainland Australia. Acquiring permits for sample export, and the shipping of biological samples in a timely fashion, presents another level of difficulty. Samples may need to be stored on site until permits and shipping can be arranged. Animal samples cannot be kept in the same facilities (refrigerators, freezers) as food.

In Australia testing for Australian Bat Lyssavirus (ABLV) is only conducted by Australian Animal Health Laboratories, CSIRO Division of Animal Health, Geelong. Samples required for definitive

² Barrier keeping is an approach to animal care that eliminates the potential for the spread of pathogens between animals by direct or indirect transmission. It encompasses: the effective isolation of animals (and their excretory/secretory products) of undefined health status from at-risk animals; and eliminating the potential for the transmission of pathogens by being transported on inanimate objects (e.g. boots, clothes, gloves, equipment, vehicles, etc.).

diagnoses include fresh brain for direct antigen detection, virus isolation by tissue culture, and PCR, and fixed brain for immunocytochemical examination. Other samples that may be submitted include salivary gland and cerebrospinal fluid. Non-haemolysed serum from a live flying-fox may be subject to the serological rapid fluorescent focus inhibition test (RFFIT) or Enzyme Linked Immunosorbent Assay (ELISA), and a Real-Time PCR has been developed for testing on saliva samples, however interpreting results from these tests are difficult (Australian Government Department of Health 2004, Animal Health Australia 2009). Testing can take between 2 and 5 days once it reaches the laboratory, and samples from Christmas Island will be subject to import permit restrictions and shipping delays.

No tools or cleaning equipment should be used for multiple enclosures and individual sets of equipment should be supplied for each. Gloves and catching equipment should always be changed or disinfected between uses.

Due to the social nature of flying-fox, it is recommended, if possible, that no individuals should be kept singularly for a prolonged period of time. Minor treatments may be administered while individuals remain part of the group, and ‘buddies’ may accompany animals to holding if regular medical treatments are needed that cannot be administered in the normal group setting.

All personnel must have a sound understanding of the principles of quarantine and must practice due diligence in their daily tasks, following quarantine principles such as strict hygiene, appropriate use of dedicated personal protective equipment (PPE) and equipment, and be mindful at all times that their actions do not breach quarantine practice.

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Healesville Sanctuary, Australia (8.13.0 Grey-headed flying-fox *Pteropus poliocephalus*)

Philadelphia Zoo, USA (15.15.5 Rodrigues flying-fox *Pteropus rodricensis*)

Parc Zoologique et Forestier, New Caledonia (5.1.0 Ornate flying-fox, *Pteropus ornatus*; 1.3.0 Insular flying-fox, *Pteropus tonganus*; 1.1.0 New Caledonia flying-fox, *Pteropus vetulus*)

Territory Wildlife Park, Australia (3.7.0 Central (Black) flying-fox, *Pteropus alecto gouldi*)

Drusillas Park, UK (16.0.0 Rodrigues flying-fox, *Pteropus rodricensis*)

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Chester Zoo, UK (75.92.0 Rodrigues flying-fox, *Pteropus rodricensis*)

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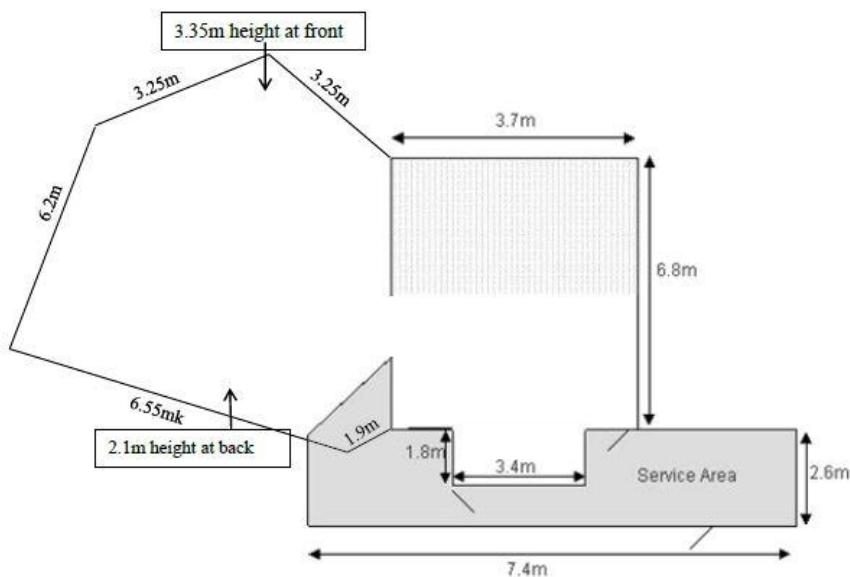
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APPENDIX A - Enclosure designs at other institutions

Auckland Zoo, New Zealand

4.8.0 (non-breeding population) Little red flying-fox, *Pteropus scapulatus*

MAF Biosecurity New Zealand Standard 154.03.04: containment facilities for zoo animals, 29 January 2007, clause 15.



Two interconnected mesh aviaries. Established vegetation provides vertical, horizontal, and linear climbing opportunities, environmental enrichment, and visual barriers. Ground space available under vegetation to allow terrestrial locomotion. Behavioural and environmental enrichment is provided daily.

Aviary 1: 4.35 m wide x 8.15 m long x 3.4 m high (at the highest point).

- Fully enclosed 1 x 1 cm square mesh enclosure with 4.95 x 1.7 m clear corrugated plastic (along roof) and 4.35 x 0.57 m clear Perspex (near roof) to provide elemental shelter. Heaters provided.
- Two sections of shade cloth secured to mesh on roof to provide shade and security.
- Remainder of roof open to elements.
- Connecting service area with mesh slide door. Mesh door to public area (padlocked).

Aviary 2: Added to provide opportunities for flight. Fully meshed 6.2 m wide x 6.55 m long x 3.5 – 2.1 m high.

Territory Wildlife Park, Australia
Black flying-fox, *Pteropus alecto* (Stanioch, 2016)

Large natural display Aviary: Unsuccessful large naturally furnished public display aviary:



- 2.8.0 (non-breeding population)
- 10 m wide x 6 m long x 4 m high
- Steel and wire mesh aviary. Natural rock creates permanently flowing creek with a small rock pool at the base.
- Females in oestrus have been impregnated by wild males through fine mesh.
- Despite natural perches, flying-fox roost entirely on the wire roof itself. Exhibit furniture only used during territorial disputes and when food enrichment placed on it.
- External walk in feeding station attached to the back of the aviary by an externally operated slide to limit contact between keepers and flying-fox in contact with wild population. Lessons learnt: Feed station only opened during feeding as animals began to spend all of their time in this space, even after food was consumed.

• Lessons learnt: Despite access to harvested browse, flying-fox quickly stripped large quantities of planted vegetation. Several major re-plantings proved expensive and futile.

Holding enclosures: Long term off display holding:



- Complex of 12 enclosures constructed of steel and 0.8 x 0.8 mm zinc coated parrot wire.
- Central walkway with power, lights, fans, hot water and infra-red video equipment. Entry via a main door. Central sink and food preparation area. Little temperature required but overhead irrigation system cools building and surrounding area on extremely hot days.
- Peak tin roof covers entire walkway and 1/3 of each enclosure.

- Groups of >5, enclosure measures 4.5 x 7.6 x 3m. Groups <5, enclosure measures 2.4 x 6.1 x 3 m.
- Floor – 1/3 concrete and 2/3 mesh covered in substrate.
- Enclosure furniture is not utilised and flying-fox tend to hang directly from the roof.
- Each exhibit contains automatic watering troughs and taps for cleaning.

Public Nocturnal Enclosure:

- 8.5 x 5 x 4 m of three solid walls of block and fibre-cement sheeting (1 with double door access system), and a forth wall of glass viewing windows.
- Bank of lights above viewing window on a 12/12 reverse lighting system.
- Air circulation via vented fans.
- Several large trees with branches provide roosting sites.
- Substrate of thick mulch.
- Other exhibit furniture includes hollow logs, stumps, termite mounds, grass tussocks, low light plants.
- Exhibit has been shared successfully with other bird and small mammal species.

Casela World of Adventures, Mauritius

0.025 Greater Mascarene flying-fox, *Pteropus niger*

- Mesh and concrete with mesh ceiling, 9.15 m long x 3.05 m wide x 3 m high
- Trees inside enclosure

South Australia Government Guidelines

(Government of South Australia, Department of Environment, Water and Natural Resources, 2011)

Two species of Flying-fox are recorded in South Australia: the Little Red (*P. scapulatus*) and the Grey-headed Flying-fox (*P. poliocephalus*).

South Australian Minimum Flying Fox Rehabilitation Enclosure Sizes:

Enclosure Type	Enclosure Width (m)	Enclosure Length (m)	Number of Individuals	Enclosure Height (m)
Intensive Care	0.5	0.5	1	0.5
Intermediate Care	1	1	1	0.7
Crèche	2	5	5 – 20	2
Pre-release	4	10	30	2.5

South Australian Minimum Enclosure Sizes for Permanently Captive Flying Foxes (up to 6 adults):

Enclosure Width (m)	Enclosure Length (m)	Additional Area (m) per Flying Fox > 6	Enclosure Height (m)
4	4	1.5 x 1.5	2.5

APPENDIX B – Example Individual Record Sheet (Taronga Zoo)

< MASTER PROBLEM LIST - TARONGA ZOO >

Taxon Name: _____ ARKS No.: _____

Common Name: _____ Birth Date: _____

< CURRENT STATUS >

Age: _____

Sex: _____

House Name: _____

ID: _____

Arrival Date: _____ Enclosure: _____

Origin: _____

< PROBLEMS LIST >

APPENDIX C - Example Daily Enclosure Report (Taronga Zoo)

TARONGA ZOO DAILY REPORT

Please use ARKS Specimen ID

DATE ..

DIVISION

APPENDIX D – Example Hand Raising Record Sheet (Taronga Zoo)

HAND-RAISING DAILY REPORT

Spec. _____ Arrival No. _____ Enc Date _____

ID_____ Sex_____ HR by_____

APPENDIX E - First Aid Response for Exposure to ABLV

In the event of a flying-fox bite, scratch, needle stick injury, or contamination of an open wound

- Wash the exposed area with a Betadine scrub for a minimum of 5 minutes.
- After 5 minutes, apply Betadine ointment to the wound, and then apply dressing to protect the injury.
- Go to the hospital's emergency department. It is better if someone can accompany you.

In the event of contamination of a mucous membrane (eye, nose, mouth)

- Rinse the exposed area liberally with running water or eye wash solution for 5 minutes.
- Go to the hospital's emergency department. It is better if someone can accompany you.

Advice surrounding appropriate first aid response for exposure to ABLV may change. Please refer to the most up to date medical advice by consulting state or commonwealth government health department guidelines.

<http://www.health.gov.au/internet/main/publishing.nsf/Content/ohp-rabies-consumer-info.htm>

APPENDIX F – Wild Diet of the Christmas Island Flying-fox

Known food plants of the Christmas Island flying-fox include (James, et al., 2007) (Cochrane, 2011)

Taxonomic name	Common Name	Nutritional Source	Source
<i>Anacardium occidentale</i> #	Cashew	Fruit	Cultivated (no longer available)
<i>Annona muricata</i> #	Soursop	Fruit	Cultivated
<i>Annona reticulata</i> #	Custard apple	Fruit	Cultivated
<i>Arenga listeri</i> ^	Argena palm	Flowers	
<i>Atrocarpus heterophyllus</i>	Jackfruit	Fruit	Cultivated
<i>Barringtonia asiatica</i>	Box mangrove	Flowers	Native
<i>Barringtonia racemosa</i> ^#	Powder-puff Tree	Flowers	Native
<i>Carica papaya</i> *	Papaya	Fruit	
<i>Celtis timorensis</i> ^#	Stinkwood	Fruit	Native
<i>Cocos nucifera</i> ^	Coconut Palm	Flowers	Native
<i>Citrus maxima</i>	Pomelo	Fruit	Native & Cultivated
<i>Citrus sinensis</i>	Orange	Fruit	Cultivates (unconfirmed)
<i>Dendrocnide spp.</i>	Stinging Tree	Flowers	
<i>Dispyros digyna</i>	Black Sapote	Fruit	Cultivated
<i>Dysoxylum gaudichaudianum</i> ^	Ivory Mahogany	Flowers	Native
<i>Erythrina variegata</i>		Flowers	(Unconfirmed)
<i>Ficus macrocarpa</i> #	Strangler Fig	Fruit & Leaves	Native
<i>Guettarda speciosa</i>		Fruit & Flower	(Unconfirmed)
<i>Hernandia ovigera</i> ^	Buah Keras Laut		Native
<i>Hoya</i>		Flowers	(Unconfirmed)
<i>Inocarpus fagifer</i> ^	Tahitian (Indian) Chestnut	Fruit	Native
<i>Macaranga tanarius</i> #	Nasturtium Tree	Flowers	Native
<i>Maclura cochinchinensis</i>	Cockspur Thorn	Fruit	Native
<i>Mangifera indica</i>	Mango	Fruit	Cultivated
<i>Magnifera odorata</i>	Mango	Fruit	Native & Cultivated
<i>Manihot glaziovii</i>	Ceara rubber tree	Flowers	Native
<i>Manilkara zapota</i>	Sapodilla	Flowers	Cultivated
<i>Melia azedarach</i>	White Cedar	Flowers	
<i>Morinda citrifolia</i>	Cheese fruit	Flowers	
<i>Muntingia calabura</i> *	Jamaican Cherry	Fruit	
<i>Musa spp.</i>	Banana	Fruit & Flower	Cultivated (unconfirmed)
<i>Pandanus</i>		Fruit	(Unconfirmed)
<i>Persea Americana</i>	Avocado	Fruit	Cultivated
<i>Physalis sp.</i>		Flowers	
<i>Pipturus argenteus</i>		Flowers	
<i>Planchonella nitida</i> ^		Fruit & Flowers	Native
<i>Psidium guajava</i>	Guava	Fruit	
<i>Schefflera actinophylia</i>	Umbrella Tree	Fruit & Flowers	Cultivated (unconfirmed)
<i>Spondias cythere</i>	Hog Plum	Fruit	Native & Cultivated (unconfirmed)
<i>Syzygium nervosum</i> *^#	Daly River Saltinash	Fruit & Flowers	Native
<i>Syngium aqueum</i>	Water Apple (Wax Jambu)		Cultivated (unconfirmed)
<i>Terminalia catappa</i> ^#	Indian Almond	Fruit & Flowers	Native
<i>Tristiropsis acutangula</i> ^	Fern Leaved Tamarind	Fruit & Flowers	Native
Unknown spp.	Tree	Leaves	Native

*Sources reported to be favoured by Christmas Island flying-fox

^*Chiropterophilous* (exclusively reliant on Christmas Island flying-fox for pollination). Five of these are considered wholly reliant on Christmas Island flying-fox for conservation within their Australian range.

Known roosting trees (also *Bruguiera gymnorhiza*, *Guettarda speciose*, *Gyrocarpus americanus*, *Hibiscus tiliaceous*, *Kleinhowia hospita*, *Ochrosia ackeringae*, *Pisonia grandis*, vine tangles, and cultivated fruit trees).

APPENDIX G - Faecal sample examination method

Wet preparation - for protozoa (trophozoites and cysts), helminths, etc.

- Place plastic tube on Ovitector© base and place faecal sample in cylinder
- Place enough formal saline in the cylinder to cause faeces to disperse when agitated with an applicator stick
- Using the applicator stick place a few drops of the faeces suspension onto a slide, place a cover slip on the slide and examine the whole slide for larvae, ova, flagellates, etc.

Flotation method for helminth ova.

- Half fill same cylinder with saturated sodium nitrate
- Mix well with applicator stick
- Push the plastic Ovitector© sieve down the cylinder as far as it will go
- Fill the cylinder with sodium nitrate solution
- Place a glass slide over the cylinder
- Allow 15 minutes for ova to float to the top of the cylinder, then remove slide, flip over and place cover slip on top
- Examine the whole slide for ova (can be done at 10x magnification)

Saturated sodium nitrate

- Place 500 grams sodium nitrate in a large jug (at least 1.5 litres)
- Slowly add 750 mls of hot tap water, mixing well to dissolve
- There should be some slurry of sodium nitrate solution remaining
- Store in a dark bottle

Formalin fixed faecal examination method (Adams et al. 2008)

- Using an applicator stick place faeces into a 10 ml centrifuge tube up to the 1.5 - 2 ml mark
- Fill centrifuge tube up to the 10 ml mark with distilled water
- Emulsify faeces with an applicator stick and centrifuge at 2000 rpm for 2 minutes
- Pour off supernatant
- Refill tube to the 10 ml mark with Sugar Sodium Chloride Solution and remix. Centrifuge at 1000 rpm for 2 minutes (Do NOT Use Brake)
- Heat a wire loop and allow to cool, then transfer 2-3 loops from the surface of the centrifuge tube to a slide and add cover slip (22 x 22). Reheat the wire loop to sterilize.
- Scan slide on 100x, also scan a small area at 400x

Sugar Sodium Chloride Solution

- Weigh out 500 mls of white domestic sugar into a beaker/flask
- Place onto a magnetic stirrer and then add the saturated salt solution until you have a total volume of 1000 mls
- Drop in a magnetic flea and mix until all contents are dissolved
- Decant solution into a storage bottle

Saturated Salt Solution

Hot tap water is added to 350 g of salt to make up one litre (SG 1.20)

APPENDIX H - Necropsy Protocols

Morphological data collection

- Record species
- Record individual identification
- Record weight
- Record date of death, and date of necropsy examination
- Record circumstances of death (found dead, euthanasia, recent medical history)

External Examination

- Note general body condition particularly signs of sub-optimal nutrition - muscle wasting
- Check skin for lesions including ulcers, lumps, redness, exudates, discoloration or parasites.
Note location of lesions.
- Check eyes
- Check orifices and note any lesions including mucosal colour (e.g. pale), tooth wear and colour, genital lesions
- Take swabs from any external lesions
- Describe and photograph any lesions or parasites
- Collect a sample of haired skin into a cryovial containing ethanol (for genetic analysis)
- Collect any ectoparasites in 70% ethanol

Internal Examination

- Wet the skin over the chest and belly and push the hair into a part down the centre. It may be easier to use a spray bottle filled with 70% ethanol for this task.
- Cut through the skin along the midline of the ventral body with a scalpel blade or small scissors from mandible to pelvis. Do not enter the body cavity at this stage. Try to keep fur out of the incision as much as possible to prevent contamination of the site. Reflect the skin to expose the muscles of the abdomen, chest and neck.
- Stop to assess hydration, fat deposits and muscle mass.
- Use clean instruments to open the body cavity to expose the internal organs.
- Try not to touch the internal organs with your instruments as you use scissors to make a T shaped incision along the end of the rib cage and then along the centre-line from the end of the rib cage to the pelvis to open the abdomen.
- Use a pair of heavy scissors (chicken shears or bandage scissors) to cut through the breast muscle and ribs of the thorax and expose the heart and lungs.
- Again, use a clean and disinfected set of instruments to prevent contamination.
- If the carcass is fresh, collect blood by cutting either the greater vessels or the apex of the heart and collecting blood with a capillary tube. Make a blood film, label it appropriately, and retain the remaining sample in 90% ethanol or on FTA™ paper for bacterial culture or for PCR for blood parasites.
- Without touching anything, note the position, size and colour of the internal organs. Take photographs and describe any irregularities. It is important to resist handling or crushing the tissues as this may cause contamination or damage the tissues being taken for histopathological examination.

- Using the clean scissors and forceps collect samples of liver, kidney and half of any lesion into individual cryovials and freeze.
- Remove the internal organs by cutting through the oesophagus, trachea and greater vessels and gently peeling out the organs (leaving the rectum attached). Inspect the internal organs. If possible, open the lungs to check the internal surfaces for thickening, exudates, or parasites. Then open the length of the intestinal tract looking for any irregularities in the mucosal surface, parasites or lumps. Collect parasites into diluted formalin (3% formalin). Collect half of any lesion into a cryovial and freeze.
- Gently handle the tissues to collect a 5mm portion of each into 10% neutral buffered formalin. Try not to crush the tissues with your forceps or cut off the portion of crushed tissue as you drop the remainder into formalin.
- Ensure that you sections going into formalin are not thicker than 1cm (as formalin only penetrates 5mm in any direction) and that your bottle contains 10x the volume of formalin compared with tissue.
- Remove the head from the spine by cutting through the ventral side at the spot where the first vertebrae meets the base of the skull. Skin the head and try to remove any remnant bits of fur. Obtain a clean pair of fine tipped scissors or shears. Insert the tip of the scissors gently into the back of the skull into the hole exposed when removed from the first vertebrae (foramen magnum). Use the scissors to gently cut through the bone of the skull essentially creating a round cap around the edge of the brain. Remove the skull cap to expose the brain, and then use clean instruments to remove 1 cerebral hemisphere into a sterile cryovial. Remove the rest of the brain and place it into the formalin. Formalin can be decanted after 2- 3 days to allow the samples to be shipped for histological examination.
- Instruments can be cleaned with a detergent, rinsing and then placing them in a disinfectant for 15-20 minutes, rinsing, and drying them thoroughly.

In neonatal animals, preserve the umbilicus and placenta if available. A quick test to determine if the neonate was stillborn is to drop a sample of the lung into 10% neutral buffered formalin. If the lung floats, the animal was breathing at the time of birth (not stillborn). Necropsy examinations of neonate animals follows the same procedure as for larger specimens. If a full necropsy is not possible on aborted or stillborn pups, a small piece of skin (perhaps an ear) is removed and placed in ethanol for preservation of genetic material, the abdominal skin may be opened, a portion of lung, liver and kidney collected with clean instruments into individual cryovials, and the entire foetus may then be placed whole in formalin.