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Captive management of the Frégate Island giant tenebrionid beetle *Polposipus herculeanus*

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Abstract: The Frégate Island giant tenebriond beetle *Polposipus herculeanus* is a Critically Endangered species restricted to Frégate Island, Seychelles. The ex-situ conservation programme at the Zoological Society of London and the European Endangered Species Programme are described. Captive propagation started in 1996 and has been highly successful with the programme holding 980 adult beetles by the end of 2003. Reproductive data is described and the finding of pathological infections of the fungus *Metarhizium anisopliae* var. *anisopliae* is discussed.

Key words: Coleoptera, conservation breeding, Seychelles, Tenebrionidae

The Frégate beetle (*Polposipus herculeanus* Solier, 1848) (Fig. 1) is one of the world's most remarkable, and endangered, tenebrionid beetles. Currently restricted to Frégate Island, Seychelles, it has a highly restricted distribution and is listed as Critically Endangered (A2e) in the IUCN Red List 2004 (IUCN 2004). In 1995 the accidentally introduced brown rat (*Rattus norvegicus*) became established on Fregate Island, initiating concerns about the potential predation threat to the native wildlife. In 1996, with the support of the Government of Seychelles and Frégate Island Private, the Zoological Society of London's (ZSL) Invertebrate Conservation Unit (ICU) was approached by the Nature Protection Trust of Seychelles (NPTS) and BirdLife International and asked to establish an ex situ captive group of Frégate beetles. As a result 47 beetles

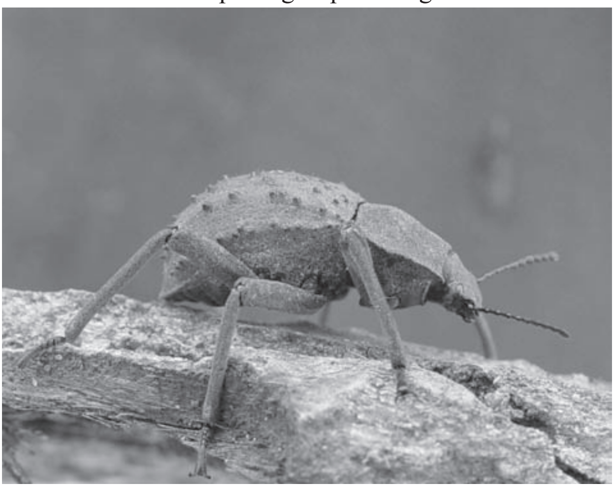


Fig. 1 Adult Frégate beetles (photo: A. Ferguson)

were collected and transferred to ZSL in 1996 (Pearce-Kelly 1997), and a further 20 beetles were collected in 1999 (Ferguson 2000). The remit of the ex-situ conservation programme was to establish a proven husbandry protocol for maintaining and breeding Frégate beetles in captivity, to conduct research into their life history and to investigate disease profiles of the species.

The ex-situ programme has proved successful; its husbandry remit has been realised and it continues to provide much needed lifecycle and health information. Additional breeding groups have been established at a limited number of institutions. In 2002 European Association of Zoos and Aquaria (EAZA) committee officially elevated this initiative to European Endangered Species Programme (EEP) status. The management of the ex-situ populations is described here, summarised from the "Management Guidelines for the Welfare of Zoo Animals – Frégate Island Beetle" (Ferguson & Paul Pearce-Kelly 2004).

Distribution and status

These beetles are endemic to Frégate Island in the Seychelles archipelago. Situated at 55.93°E and 04.58°S, Frégate Island is the most easterly and isolated of the granitic Seychelles group. There is some debate as to the historic range of the Frégate beetle. It was first described by Solier in 1848, from a specimen supposedly collected from Bengal on the Voyage of Duvaucel (Solier 1848; Marshall 1982). This record was refuted by Gebien (1922) and the beetle has not been found on the Indian mainland since. Collins (1983) puts forward a theory that the beetle could have colonised from India, as stowaways in bundles of sandragon *Pterocarpus indicus* wood. However, it seems much more credible that the specimen from 'Bengal' was mislabelled (M.V.L. Barclay, pers. comm.).

In 1869 Lt.-Col Pike collected two specimens, apparently from his visits to Round Island, off Mauritius. Both of these (one consisting of only a head and thorax), are in the collection of the Natural History Museum, London (M.V.L. Barclay, pers. comm.). The beetle has not been found in this location since and the source of these specimens has also been doubted (Marshall 1982). The collection of B.G. Nevinson (1852-1909) in the Natural History Museum includes six Fregate beetle specimens labelled 'Seychelles Is.' without further data. The first known authentic collection of the beetle from Frégate Island was in 1905, and again in 1908, by the Percy Sladen Trust Expeditions. All subsequent specimens are known to have been collected from Frégate Island and today this is the only location where the beetle exists (Lionnet 1971). Although the validity of the source location of the two earlier specimens has been questioned and despite lack of solid evidence, some authors do argue for the species once having a wider distribution, encompassing other islands in the Seychelles and possibly Round Island as well (Gerlach *et al.* 1997). The Frégate beetle inhabits forest stands and it is possible that extensive clearance of native trees for agriculture could have caused the beetle's extinction from other islands in the Seychelles (Cooke 1997). This theory could be investigated further by researching the ground litter in likely historic range, beetles of this kind are robust and it is possible that pieces of the exoskeleton might be easily found if ground litter were sifted for extraction of beetle parts from the substrate (M.V.L. Barclay, pers. comm.).

The natural vegetation of Frégate Island has been extensively altered by human activity and little is known of the island's original vegetation structure and habitat dynamics. Continuous human settlement is thought to have commenced from the early 19th century with resultant clearance of the native *Pisonia grandis* woodland to make way for plantations in the late 1800's, largely of coconuts *Cocos nucifera* (Robertson & Todd 1983). Introduced species, especially the cinnamon (*Cinamomum verum*) and cashew (*Anacardium occidentale*), have now replaced much of the natural woodland (McCulloch 1996). Approximately 75-80% of the island is now covered with this mixed species forest (Fig. 2) and when the monsoon changes this often produces an autumnal effect on the trees, with leaves being lost creating a thick leaf litter layer which the beetles are often found in (B. Sachse, pers. comm.).

Wild ecology and behaviour

The Frégate beetle is arboreal and nocturnal. Lloyd (1971) carried out a survey in 1971 and reported finding the beetle only 'on trees or decaying logs'. Lionnet (1971) reports that although beetles had been found on the ground 'under fallen palm leaves', he collected them from the 'cracks and holes' of trees. Captive studies confirm the beetles are nocturnal, spending the daylight hours hidden in crevices in trees, coming down to the ground to feed during the night (Slater 2001). As well as clustering in crevices and under bark during the day beetles also hide in tree nodes and on the underside of horizontal branches where they meet the trunk (B. Sachse pers. comm.). Predator avoidance might have been the stimulation for the evolution of this behaviour. Predators of the adults include Wright's skink (*Mabuya wrightii*) (Mellor 2002). The larvae are taken by magpie robins and skinks (*M. wrightii* and *M. seychellensis*) (J. Millett pers. comm.). Adult beetles produce a chemical from defensive glands at the posterior of the beetle (Marshall 1982), this chemical has a musky smell and stains the skin purple/brown but does not cause any irritation (pers. obs.).

Fig. 2 Frégate beetle breeding room at ZSL, showing large and small tubs (photo: A. Ferguson)

Although sightings have occurred of beetles on a huge variety of tree species, a preference for several different tree species is shown (Lloyd 1971). As well as being found on native species like badamier (*Terminalia catappa*) the adult beetles appear to have adapted successfully to many introduced species and are found associated with sandragon, cashew, *Alstonia macrophylla* and mango (Lloyd 1971; Cooke 1997). They occur in a variety of woodland types (sandragon, mixed and coastal), but are infrequently found in scrub and coconut plantation, and are absent from grassland, agricultural areas and settlements (Gerlach 1999). Beetles are most abundant in mixed woodland dominated by mature cashew, which has heavily fissured bark (N. McCulloch, in litt. 1993). It appears that niche preference could be based predominantly on tree trunk and branch structure; species with flaky bark, fissures, cracks and crevices being favoured as they provide refuges in which to hide during the day. Mellor (2002) found the beetles had a clumped distribution, and were present more often on trees with a larger diameter (i.e. more mature trees); in mixed woodland the beetles prefer *Alstonia macrophyla*, this appears to contradict other studies as this tree species has a smooth, pale bark offering little shelter or camouflage. Other species the beetles seem to associate with are *Dracaena reflexa*, *Calophyllum inophyllum*, *Paraserianthes falcataria*, cinnamon and mango (B. Sachse, pers. comm.). Species the beetles appear to avoid are coconut, *Ficus benghalensis* and *Artocarpus altilis*.

The beetles occur from ground level to 15m above ground (C. Mellor pers. comm.). Cooke (1997) recorded beetles most frequently at 1.5–2m height. They are often seen aggregated in groups of up to 21 individuals when roosting during the day (Lloyd 1971; Murray & Nicoll 1998). Whether this is due to the availability of refuges or whether the beetles obtain some benefit from this gregarious behaviour is unknown. Being fairly large and flightless it seems reasonable to assume they have low powers of dispersal; a 10 week mark-recapture study (May-July) found that 6.5m was the mean distance beetles moved from the marking site (Mellor 2002). The furthest a beetle travelled was 19m and half the animals were found still on their original tree.

In captivity the beetles sometimes display an unusual behaviour when being sprayed with a fine mist; standing tall, they lower their head, raise their abdomen and then 'flick' their body quickly from one side to the other (pers. obs.). What this behaviour serves to achieve is unknown, possibly it helps deflect water off them in the case of heavy rain or might assist with clasping onto the tree. It would be interesting to discover if this behaviour occurs in the wild.

Management in captivity

Frégate beetles are kept successfully in large colonies in captivity but at denser levels the potential risk of stress and disease affecting the population increases. ZSL uses large plastic tubs measuring 90cm length, 70cm width, 60cm height, with a large branch sticking up vertically, for populations up to 100 beetles (usually less) (Fig. 3). Large, round plastic plant containers (80cm diameter) have also been used as well as a smaller oblong glass tank. Small numbers (approximately 12) survive well and have bred when kept in smaller containers (50x20cm, and 15cm substrate depth). Frégate beetles have fused elytra and cannot fly so there is no requirement for the enclosure to

have a lid. However, they are adept climbers. A smooth surfaced vertical edge to the enclosure of at least 16cm height from the substrate is sufficient to keep the beetles inside.
At ZSL, a 'coir' substrate (based on coconut fibre) is used, mixed with layers of leaf litter and chunks of wood. Most ZSL containers have a 50cm substrate depth: the substrate needs to be at least 30cm deep to allow the larvae optimum room for tunnelling and pupating, although breeding has successfully occurred in only 12cm. The bottom of the container has holes to facilitate drainage, covered by a thin layer of pea shingle (c3-4cm) the soil substrate placed on top of this. Larval development is fastest, with the highest weight gain and the most number of instars (measured by length) in 17 weeks in a substrate consisting of 40% soil, 40% rotten wood and 20% decomposing leaves (Veen & Berdouni 2003).

Frégate beetles are arboreal and are provided with 1.5–2m upright tree trunks/branches with nooks and crannies, fissures or flaky bark. Additional pieces of wood are buried completely, half buried and placed on top of the substrate, as food for larvae. Leaf litter added to the substrate provides food for adults and larvae and refuge areas for adults.

Mites can build up in the substrate and in high numbers this can adversely affect the viability of the larvae and pupae; this is thought to be the cause of one captive population dying out (R. Ratajszczak, pers. comm.). Regular replacement of the substrate prevents mites from building up in large numbers, but this must be offset against the damage done to larvae and pupae whilst sorting them out from the soil. To some extent, mite level can be controlled by not allowing the substrate to become too wet. Daily inspection, prompt removal of dead beetles and regular replacement of the substrate, are important health management measures.

Fig. 3 Beetles clustering (photo: A. Ferguson)

The environmental parameters the beetles are exposed to are within the range experienced under field conditions; however seasonality effects have yet to be studied in the field and therefore have not been incorporated into management regimes. The ZSL environmentally controlled room has air temperature ranging between 25°C (night) to 28°C (day). Tub soil temperature is 22-24 °C (measured at 12cm below surface level). Relative humidity is generally 65-75 %, but can rise to approximately 95% after the tubs are sprayed. One group of beetles at ZSL is on public show in an exhibit tank, this has a similar temperature range as above but is not as humid (45-55% relative humidity).

The photoperiod is set to a 12 hour cycle, the reverse of natural daylight. From 8am-8pm red fluorescent lights are on; most insects appear insensitive to the deeper shades of red light, so it is effectively dark for the beetles (Wigglesworth 1972). From 8pm–8am, white TLD 36W/35 fluorescent lights illuminate the room, giving lux readings ranging from 1500 (top of branch) to 150 (bottom of branch, in shade). These fluorescents are in the process of being replaced with natural spectrum lights emitting 2% UVB and 10% UVA. Ultraviolet radiation has been shown to adversely affect the culturability and germination of fungal conidia of *Metarhizium anisopliae* var. *anisopliae* (Braga *et al.* 2001), a fungus that can kill the beetles (see below).

Newly emerged beetles are transferred into the appropriate generation enclosure. No aggression has been seen when these are transferred (pers. obs.). Experience at ZSL indicates that group structure can be mixed and changed with no problems. Always be aware of the risk of transferring disease between different groups, if any show signs of disease that enclosure should be quarantined.

ZSL has an 'on show' enclosure comprising a mixed species exhibit, housing beetles in with Seychelles millipedes (*Sechelleptus seychellarum*) and enid snails (*Pachnodus fregatensis*). This system has worked very well for the beetles and millipedes, however it is drier and less humid (45-55% humidity) than in the breeding rooms, so has not proved to be as successful for the snails. The beetles breed successfully under these conditions, however millipede density is very low (4 individuals), at higher levels the burrowing millipedes would undoubtedly disturb pupating beetle larvae.

The ZSL programme animals are kept in a dedicated room as single generation, single species groups in separate tubs (enclosures). In this way the beetles can be monitored easily and appropriate conditions for the beetles care are not compromised in any way by other species presence in the enclosure. Working practice includes certain barrier and hygiene precautions being taken e.g. wearing dedicated laboratory coats, washing hands and using gloves. In the same room are housed other Seychelles invertebrates; Seychelles millipedes (*Sechelleptus seychellarum*), enid snails (*Pachnodus fregatensis*) and Seychelles scorpion (*Chiromachus ochropus*). These require the same environmental conditions but are kept in separate enclosures.

Diet and feeding

Very little has been published on the wild diet of these beetles. Adults eat fruit and fungi (Millet 1999) and leaves (Mellor pers. comm.). Observations of the larvae (and their presence as indicated by their distinctive large bore holes) show they will feed on most types of rotten, decaying wood, including; sandragon, cashew (Lloyd 1971), *Pisonia grandis*, *Terminalia catappa*, *Albizia lebbek* (GERLACH 1996) and also coconut logs (N. MCCULLOCH, in litt. 1993). The current ecologist on Frégate has not observed larvae in the coconut stumps although larvae of the rhino beetle use this resource (B. SACHSE, pers. comm.). Larvae have even been found in the thatch of the house roofs when the thatch was removed and replaced in 2003 (B. Sachse, pers. comm.).

In captivity, a variety of fruit and vegetables (produce) is offered including; apple, carrot, potato, sweet potato, sweetcorn, mushroom, cucumber, banana and lettuce. Two or three of these items are offered daily, on a rotational basis. Adults have been seen eating the bark of branches and leaf litter in their enclosure (pers. obs.). A variety of types of broadleaf tree species leaf litter is used such as London plane (*Platanus x acerifolia*), horse chestnut (*Aesculus hippocastanum*) and birch (*Betula* sp.). The litter is frozen for 24 hours or immersed in water for a few days in order to minimise the introduction of pests. Soaking the litter may increase palatability (I. ROMA, pers. comm.). They are also reported eating moss, lichen and bark chippings (K. VELTMAN, pers. comm.). A nine week study comparing one group of beetles fed on the latter diet with another group fed the same diet but additionally offered fruit and vegetables found no significant weight difference between the two groups (VEEN & BERDOUNI 2003). Captive beetles in Mauritius are reported to have eaten fungi, and the larvae 'any rotten wood' (GERLACH 1996).

The larvae require dead wood to feed on, appearing to prefer the more decomposed wood (pers. obs.). The larvae will burrow right inside food items and decaying wood, and pupae have been found in chambers made in decomposing wood (pers. obs.). In an experiment to test whether the larvae expressed any kind of preference between wood types, HARDING (2002) found there was no significant difference between larval association with horse chestnut, sycamore (*Acer pseudoplatanus*) and palm wood (*Trachycarpus fortunei*). He found that larvae reared on sycamore alone had better survivability, larger weight gain and increase of body length over 79 days than larvae reared on the other two types. The reasons for this can not be determined as there are undoubtedly a number of interacting factors affecting digestibility such as water content, stage of decay and type of mycological breakdown.

On two occasions a beetle has been observed cannibalising a large pupa (pers. obs.). In one case the pupa had been seen on the substrate surface earlier that day, indicating all was not well with it (they rarely stay on the surface); then later on a beetle was seen eating this from the anterior whilst it was still alive. This might indicate these beetles eat animal protein in the wild (by scavenging) and raises the suggestion that a protein rich food should be provided in the diet. At Riga Zoo the beetles are also offered granulated cat food. Both adults and larvae have been observed eating this (I. ROMA, pers. comm.), suggesting that the larvae might also scavenge for protein. It would be valuable to carry out further investigations in this area.

These animals are extremely sensitive to pesticide chemicals. As a precaution against potentially harmful chemicals all produce is peeled, washed and the top 2cm of carrots are discarded (as this area is most likely to be contaminated). Water is provided in shallow, small containers (4cm diameter), containing soaked cotton wool to prevent drowning. The cotton wool is changed once weekly or more often if necessary. The beetles are watered and the enclosure sprayed daily with a fine mist (reverse osmosis, filtered rain or filtered tap water) providing droplets of water the beetles can drink from in the branches.

Captive behaviour

Frégate beetles will live in a large group with little aggression (pers. obs.). During the day (light hours) they cluster tightly together in crevices on branches or under bits of wood on the substrate, they prefer to climb upwards in daylight hours if provided with the opportunity. It is possible that clustering density is related to the availability of crevices.

Lee (2003) observed a beetle 'butt' and push another out of the way with lifting head movements, whether this related to food or territory is not known. On occasions beetles have been seen interfering with a mating pair (Slater 2001). They will cluster round a food source (pers. obs.), and the food needs to be in large chunks and sufficiently dispersed throughout the enclosure to ensure that many individuals have access to it. At higher densities these interactions are likely to become more frequent.

Reproduction

Little has been recorded of the reproduction of this species in the wild. Frégate beetles were first hatched and reared in captivity by Mr R. Pope, at the Natural History Museum (NHM), London in 1977. 12 live adult specimens were sent to the NHM and ova were present on pieces of sandragon bark which were included in the consignment. The beetles and eggs were kept in a propagator at 29.5°C and 80% relative humidity. Several larvae were reared and preserved at different instar stages, and one adult emerged (Marshall 1982). Carl Jones in Mauritius has also bred captive Frégate beetles (Gerlach 1996). Beetles have bred regularly since 1998 at ZSL and all members of the EEP breed this species successfully.

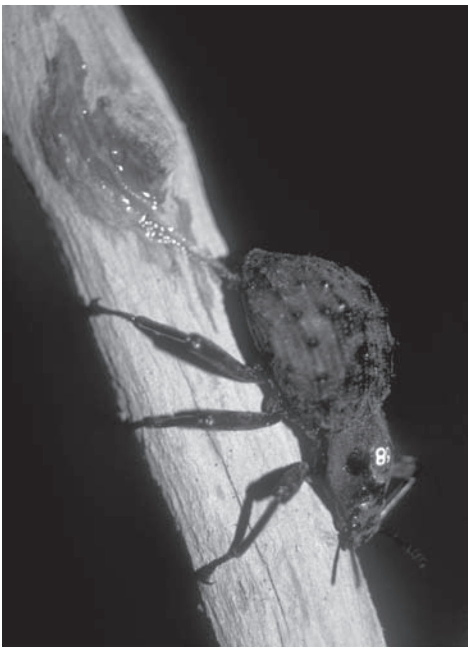
The beetles show no obvious sexual dimorphism. Morphometric measurements collected at ZSL of known sex beetles has been statistically analysed and shows no significant difference in size between the sexes, so this can not be used as a diagnostic tool for sexing (Slater 2001; Lee 2003).

Very little courtship has been observed; mating occurs more frequently at night (dark hours) (Slater 2000), mostly in the early hours of darkness (Harding 2002), as would be expected given that this is the main activity period for this species. Males initiate 72% of matings, but females have some control over mating as 80% of failures were due to female rejection (the other 20% being due to disturbance by another individual) (Slater 2000). Mating has been observed on the substrate surface but 60-80% of observed matings occur in the tree branches (Harding 2002; Lee 2003). Copulation has been observed lasting 21 minutes (pers. obs.). A possible form of mate guarding has been observed, where the male remains standing over the female for approximately 20 minutes after mating (Lee, 2003).

On one occasion after a mating pair was disturbed, the female was seen to have a white, thread like structure protruding from her rear (pers. obs.). Beetles have also been observed producing a long white strand in a blob of clear liquid from their posterior (Fig. 4), then immediately turning to eat this discharge, and others also joining in to eat (pers. obs.). Samples taken and examined microscopically contained a tubular body or plug, with approximate dimensions 0.2x5mm (observed by phase contrast magnification x10 and x40). Within and around the plug were numerous live, very motile spermatozoa, with very long flagella and small heads (Fig. 5) (MacGregor, pers. comm.), confirming that these exudates are sperm plugs or spermatophores. How long after mating the sperm plug is expelled is unknown.

Mating frequency appears not to be particularly high given the density of beetles in enclosures at ZSL. In 24 hours total time spent observing beetles (made during their active period over 6 weeks, 300 beetles in 4 tubs) only 17 matings were recorded (T. Lee, pers. comm.). Under controlled captive conditions there appears to be no seasonality of breeding (pers. obs.) and it is unknown whether this species shows breeding seasonality in the wild. Monitoring mating partners over a number of years has shown that males and females mate with different partners (Ferguson 2002), suggesting a polygamous mating system. No post-natal parental care has been observed.

The eggs (Fig. 6) are oblong, approximately 1.5-2mm long and a pale yellow in colour. They are most often laid on or near wood just under the substrate surface. Occasionally eggs have been seen on the wood branching above the substrate and also on the substrate surface, not near any wood. The interval between egg laying and hatching has been observed to be less than 2 weeks at an average temperature of 24° Celsius (pers. obs.) and five days at 26-27°C. The high temperatures produced white, motionless larvae which turned yellow by the fourth day and on the fifth day bored holes into the soft rotten wood provided (I. Roma, pers. comm.).



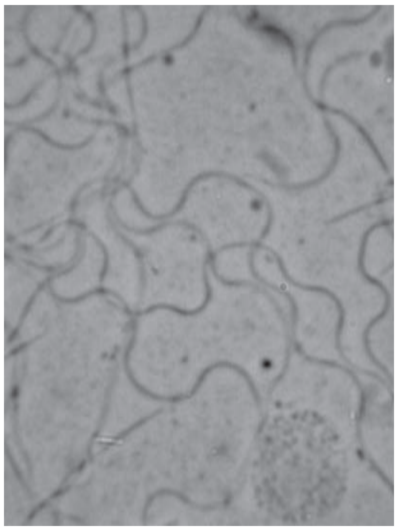


Fig. 4 Female passing sperm plug (photo: A. Ferguson)

Fig. 5 Spermatozoa x400 (photo: A. Ferguson)

The larvae spend all their time beneath the substrate surface. They tunnel in the soil eating fruit, vegetables and rotten wood, as well as the substrate medium (Ferguson 2002). There are seven larval instars (Fig. 7) (Marshall 1982), the last reaching 5cm in length (Ferguson 2002). At this stage the larva excavates a chamber in the substrate approximately 2x4x3cm high in which it remains motionless for an average of 9 days (n=34) before pupating (Fig. 8). After 17 days (n=35) it metamorphoses into a beetle, pale brown and lying on its back. It gradually darkens off, turns the right way up and then and tunnels its way to the surface to emerge an average of 8 days later (n=42) as a fully formed adult. The whole development process from the larva making a chamber to the adult beetle emerging takes an average of 34 days (Ferguson, in prep.).

If pupae are disturbed i.e. the chamber collapses or they are removed from the chamber, they are unlikely to continue development successfully (Ferguson 2002). An obvious sign a new beetle has emerged is the evidence of a tunnel. Occasionally, a new emerged beetle will retreat to its tunnel during the day (light hours) within the first 24 hours after emergence. When they leave the tunnel, they are often seen to climb as high as possible on the branches provided. Adults have also been observed emerging from rotting wood (pers. obs.; I. Roma, pers. comm.), so can make chambers in decomposing wood as well as soil substrate.

Wild collected beetles have produced next generation adults 10 months after their arrival and other generations have emerged in 9 months (Ferguson pers.obs). Larvae (1cm long) were seen in a tub seven and a half weeks after the first two beetles of a new generation emerged and were placed in a breeding tub, thus the adults are fertile and able to breed by 4-6 weeks after emergence with a generation time of 7-10 months (larvae take 6–8.5 months to emerge as adults).

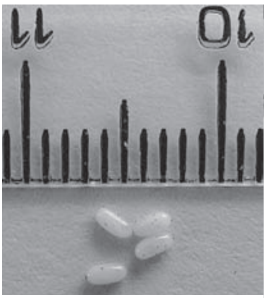


Fig. 6 Frégate beetle eggs (photo: A. Ferguson)

Fig. 7 Frégate beetle larvae (photo: R. Williams)

Fig. 8 Frégate beetle pupa (photo: A. Ferguson)

Longevity

Longevity in the wild is unknown. Two founder individuals, collected as adults of unknown age for the ex situ breeding programme, survived over seven years in captivity, although the average age appears to be approximately half this (pers. obs.).

Health and disease

Post-mortem examination of a number of beetles at ZSL has shown an association with a fungal infection *Metarhizium anisopliae* var. *anisopliae* (Fig. 9). This is a known entomopathogen capable of causing a fatal systemic mycosis (A. Cunningham, pers. comm.). Identifying the source of this infection is complicated. *Metarhizium anisopliae* is a common mycopathogen of soil insects in temperate regions (Charnley 1997) and is probably ubiquitous throughout the tropics as well (J. Nicklin, pers. comm.). The source of infection could originate from the coir substrate and leaf litter used in the beetles' enclosures, or, the original wild collected adults may have been exposed to this fungus in situ before being transferred to ZSL. So far this fungus has not presented on post mortem examination of any other invertebrate species at ZSL, further suggesting a pre-existing association.

The situation is further complicated by the use of this fungus as a biocontrol for a number of invertebrate species (the method of infection is discussed by Clarkson & Charnley 1996). It has been found in *Oryctes* sp. beetles (Scarabaeidae) in Kenya and was also introduced to Mauritius in the 1930s as part of investigations into control methods for the sugar cane white grub *Clemora phyllophaga* (Scarabaeidae) (Greathead 1971). It would be valuable to investigate whether in the past this biocontrol has been used on Frégate, or other islands in Seychelles. In the 1928 Seychelles Department of Agriculture's 'Annual Reports on Agriculture' it notes "Fungus parasites are helping in insect control [referring to scale insects]. A programme is being drawn up for the introduction of other fungus parasites and predators" in the 1933 edition it says "Another parasitic fungus was obtained from India" (J. Gerlach, pers. comm.). There is a possibility it has been used on rhino beetles (*Oryctes monoceros*) in coconut plantations on the Seychelles (Gerlach pers. comm.), although there is no documented record of its use in Lionnet's (1959) review of the biological control of agricultural pests in the Seychelles.

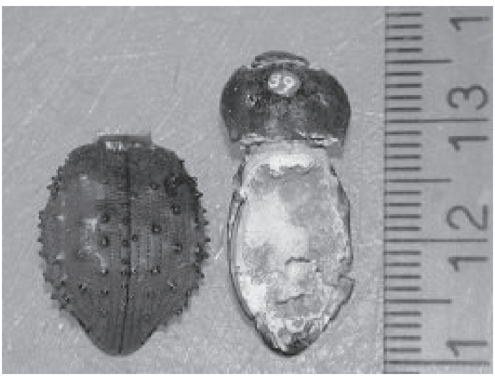


Fig. 9 *Metarhizium anisopliae* on necropsy examination (photo: A. Pocknell)

There are thought to be different subspecies (transgenic strains) of *Metarhizium anisopliae* var. *anisopliae*, with differing host specificities (J. Nicklin, pers. comm.). Identification of the strain found at ZSL and compared to that found (if any) in the fresh substrate and on Frégate Island would help elucidate the picture. Further collaborative investigations are planned between ZSL and Birkbeck College, London University.

It has not been established whether this fungus occurs in wild beetles. One in situ wild Frégate beetle has been observed with a suspected fungus growing around the joints implying there might be a presence in the wild population (R. Lucking, pers. comm.). The beetle was not collected, so the fungus identification remains unknown. It would be valuable to carry out disease profiling on the wild beetle population and would be an essential action should translocation be considered as a conservation option.

Fortunately, this infection appears not to be particularly virulent in healthy adult Frégate beetles. The mortality rate in the population as a whole is relatively low and not all deaths are attributed to this organism. Of 112 post-mortems in 2002, 30% were positive for the fungus, suggesting a low pathogenicity of this particular fungus isolate in the Frégate beetle (Elliot 2003) and it is possible that the individual beetle only succumbs to this fungal infection if it is compromised or stressed in some other way (J. Nicklin pers. comm.). Death rates and causes are constantly monitored at ZSL. Prompt removal of dead individuals, preferably before generation and dispersal of conidia, and regular replacement of the substrate should help minimise exposure to fungi. Larvae have also died infected with *Metarhizium anisopliae* var. *anisopliae*. Having intimate contact with the substrate, it is to be expected that the larvae would be affected, and this might provide an explanation for the relatively few adults that emerge from the hundreds of larvae seen in the tubs (pers. obs.), although there could be other causal factors.

Other post mortem findings include prolapse and possible egg binding, *Geotrichium candidum* in larva (this is thought to be a secondary agent infecting larva after trauma and not the primary pathogen), possible gout, rhabditoid nematodes (from a non-pathogenic genus) in an adult beetle abdomen, *Aspergillus niger* and *Candida pelliculosa*.

Generation size

It appears that some of the populations at ZSL show a slight decrease in average size through the generations. However, there is great variation in the size of the wild collected adults so investigations are currently being carried out to indicate whether this is a significant difference in size or not.

Marshall (1982) reports that the single F1 generation bred at the NHM was noticeably reduced in size compared to the wild collected parents. She speculated on a number of factors which might have caused this: insufficient food, a growth inhibiting effect from salicylic acid in the willow bark provided for larval food and presence of symbiotic eugregarine protozoa in the guts of the larvae although these protozoa might assist with the digestion of wood (A. Pocknell, pers. comm.). At ZSL individual adult beetles have had their faeces screened and the results were negative for gregarines. It would be valuable to investigate whether the wild population carries this protozoan.

Other possibilities that may cause size changes are high levels of lectins (sugar binding proteins that have an inhibitory effect on growth; Gurjar *et al.* 2000), the increased level of fruit and vegetables offered in captivity might cause the shortening of development time and protein level in the diet might be too low when compared to a wild diet which may include carrion. It has also been postulated that keeping larvae at high density may adversely affect their viability and might cause smaller adult size (I. Roma, pers. comm.). This theory requires further investigation.

Population magagement

The total population in Europe in captivity at 31st December 2003 was 980 individual adults in five institutions (Table 1). All beetles originate from those bred at ZSL, where beetles have now been bred to the fifth generation. The Frégate beetle breeding project was initiated in 1996 and became an official European Endangered Species Programme (EEP) in October 2002. The EEP coordinator is currently Amanda Ferguson at ZSL.

Beetles wild collected in 1996 are kept separate from those collected in 1999 and different generations are kept separately. Daily checks are made for new emerged beetles which are removed from the enclosure, marked with an identification disc (Fig. 12) and a series of standardised physical measurements taken before being transferred to a different tub holding beetles of the same generation. A coding system is employed, consisting of: generation/year founders were collected e.g. F2/96 is the second generation from the 1996 collected beetles. It is essential that accurate records are kept of each adult's emergence and death. Individually labelling beetles allows data to be collected on age at death, which is useful when looking at disease epidemiology. Institutions participating in the EEP are asked to carry out post mortem examinations on deceased specimens so the disease status of the populations can be monitored.

The Frozen Ark project, initiated in July 2004, is aimed at preserving DNA and tissue samples of endangered species to ensure basal genetic data is not lost. Stored under optimum conditions this genetic material provides a resource for research and conservation as well as informing best population management practice for still living but threatened species. The EEP programme has provided 30 post mortem larval specimens, representing the generations closest to the original wild founder groups (15 larvae of F2/96 and 15 of F1/99). For more information see http://www.nhm.ac.uk/news/items/frozen_ark270704.html.

Conservation Status

Being endemic and having such a restricted distribution the Frégate beetle is considered especially vulnerable to extinction from events such as the introduction of disease or alien species and natural disasters. It is listed in the IUCN Red List and the Seychelles Red Data Book as 'Critically Endangered A2e' (NPTS 1997; IUCN 2004) Critically Endangered (A2e). In the early 1970's and 1980's the beetle was reported as 'fairly common' or 'abundant' (Lionnet 1971; Lloyd 1971; Collins 1983). A basic sample survey of 50 trees in each of six areas in August 1993 determined that the beetles were 'not common in the main plantation areas below the 10m contour', despite mature trees being present (McCulloch 1993).

Although previously brown rats had occurred sporadically on Frégate, it was their colonisation in 1995 that caused the conservation community great concern. In 1996 Birdlife International staff (Lucking & Lucking followed by Murray & Nicoll) initiated monthly sample surveys of the beetle numbers which continued regularly until 2001, then on an ad hoc basis in 2002 (J. Millet, pers. comm.) using a similar protocol to that used in 1993 (Strachan & Dunn 1997). Lower altitude sites consistently held the lowest number of beetles and an apparent seasonal variation in beetle abundance was noted, with numbers being highest between November-May, the wetter season, possibly due to a change in behaviour increasing their detectability (Lucking & Lucking 1997). Increases in August 1998 may have been partly due to the beetles clustering under an increased number of nest boxes for the magpie robins (Murray & Nicholl 1998). Subsequent monthly transects showed a significant decline in beetle abundance between March 1996 and December 1999 attributed to predation by rats (Millet 1999; Parr 1999). In 2000, Frégate Island was effectively de-ratted by poison baiting (Shah 2001). Measures are in place to prevent the accidental re-invasion of rats, which remains an ever-present possibility.

Gerlach (1999) calculated a total population estimate of 74,521–104,940 based on data collected in 1997 by Strachan & Dunn (1997) (61,490 in mixed woodland, 11,583–38,610 in *Pterocarpus* woodland, 1,448 – 4,840 in coastal woodland and some scarce sightings in other habitats). He notes that this estimate is 'vulnerable to errors in density estimate, area calculations and the biased distribution of survey sites'. The Birdlife monthly transects in 2001 (post rat eradication) showed an increase in beetle abundance (J. Millet, pers. comm.). Two recent studies have attempted to estimate total population size. A combination of the McCulloch/Birdlife methods in 1999 and 2002 (before and after rat eradication) estimates the population at 57,060 (±9,038) in 1999 and 50,390 (±3,288) in 2002 (difference not significant) (Gerlach 2005). Transects in sandragon and mixed woodland in May-July 2002 estimate a population size of 22,750 with the total population being not much higher than this value as the beetle 'hardly occurs' in habitats outside the sample types (C. Mellor, pers. comm.).

The above surveys have been carried out during the day, which might bias the results towards recording daytime visibility levels rather than numbers present. The variation in methodology, effects of observer bias and variations in beetle seasonal activity pattern mean it is difficult to compare the results of these surveys over time. It would be valuable to develop a more robust, standardised methodology for assessing population size. Surveying at night might yield some valuable results when the beetles are more active and dispersed.

In 1998 Angasana wilt (a fungal disease caused by *Fusarium oxysporum*) was discovered attacking sandragon trees on Mahé (Boa 2002). The subsequent spread to Frégate resulted in a die off of most of the Island's sandragon trees by 2004 (M. MacQuitty and B. Sachse, pers. comm.). This is likely to have a profound effect on vegetation dynamics as sandragon is a major woodland component and is one of the principal Fregate beetle associated tree species. Many dead beetles can be found in these dead stands and they appear to be dispersing out to less favoured habitat (B.

SACHSE, pers. comm.).

One potential conservation measure is the translocation of Frégate beetles to other islands in their considered former range such as Cousin, Cousine or Aride in Seychelles, and Round Island, off Mauritius (COOKE 1997, LUCKING & LUCKING 1997). This course of action could only occur if the target islands have suitable habitat remaining, or restored habitat that satisfies the beetles' ecological requirements, as well as being free of alien predators. Disease profiling of the beetles would need to be fully investigated before any translocations were carried out. Any reintroduction, release or translocation programme would have to comply with the IUCN guidelines, which have been developed 'to help ensure that re-introductions achieve their intended conservation benefit, and do not cause adverse side effects of greater impact' (see http://iucn.org/themes/ssc/pubs/policy/reinte.htm).

The beetle remains vulnerable due to its restricted range, so still warrants conservation focus. Health related investigations are essential when considering the possibility that the translocation of Frégate beetles to former historic range islands might become a future conservation option.

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Appendix I. Other Fregate Island invertebrates maintained at ZSL

Husbandry & captive management at ZSL - environmental parameters

- Room temperature range 25-28°C. Substrate temperature range 22-24°C.
- Room humidity 65% (65-75%).
- Spraying and watering daily is carried out using reverse osmosis water which has been left standing in the room for 24hrs to warm up and thus avoid 'cold shock'.
- Photoperiod set to a 12 hour cycle on reverse lighting. From 08.00–20.00hrs red fluorescent lights are on; from 20.00-08.00hrs white TLD 36/W/35 fluorescent lights provide illumination, giving lux readings of 1500–150 in different parts of the room. These are in the process of being replaced with natural spectrum lights emitting 2%UVB and 10% UVA.

Seychelles millipedes (*Sechelleptus seychellarum*(DESJARDINS, 1834))

Husbandry: The millipedes are kept in large tubs or smaller glass tanks with a thick layer of substrate (coir and leaf litter in layers). They spend most of the light hours underground. They are active in the dark and will occasionally climb branching if provided. A variety of food is offered and placed on the soil surface. Potato, sweet potato, apple, carrot, cucumber, mushroom, sweetcorn, lettuce, and banana. Where possible the food is peeled in case of pesticide contamination. All the food is washed in reverse osmosis water. Cuttlebone is also given in small chunks and it is also ground up and mixed into the substrate to provide extra calcium.

Observations: Average width and length of adult males is 13.4mm and 176mm; and females 14.5 and 189 mm (n=10 in each case). Average weights; males 31.5g (n=9) females 34.1g (n=10) – different individuals from the previous. The original adults received from the wild all died within 2 years, so longevity is assumed to be 2-3 years. They moult underground. They can be handled but about 50% of the time will ooze a

chemical which stains hands yellow. Young of 2cm were seen in the soil 11-12 months after the adults arrival.
Other holders: Bristol, Shaldon, Dudley, Drusillas, Rotterdam, Artis, Poznan & Riga.

Enid snails (*Pachnodus fregatensis* Van Mol & Coppos, 1980)
Husbandry The snails are kept in plant propagators 22x33x17cm high. A 5cm layer of coir is provided with moss covering ~½ of the area. The snails are fed 'partula diet no 13' spread on a perspex slide placed on the substrate as well as washed lettuce and a piece of peeled fruit/veg (potato, sweet potato, apple, carrot, sweetcorn & lettuce). Food is changed and the propagator lids cleaned every other day. Cuttlebone pieces are provided. The propagator vents are kept open and fine plastic mesh siliconed over the holes to prevent escapes. Deaths have occurred due to room overheating (max 38°C).
Observations: Eggs are laid in batches of approximately 50 under moss.
Other holders: Martin Mere & Riga.

Seychelles scorpion (*Chiromachus orchropus* Koch, 1838)
Husbandry: The scorpions are kept singly; unless in a breeding pair. Adult pairs are kept in glass tanks 30x60x30cm with a 5cm layer of coir substrate. Stones, wood and cork bark are provided for refuge. A shallow water container is provided. Sub-adults are kept singly in plastic containers (16x28x10cm) with a shallow (2cm) layer of soil, with bark, plastic tubing or ½ flowerpots for refuge and water containers (as above). They are fed weekly with crickets usually, but also waxmoth larvae, mealworms or locusts. Smaller ones are fed more frequently. Containers are watered and sprayed whenever necessary and the substrate kept with a gradient of wetness. The scorpions are not disturbed at all whilst moulting and for a few days afterwards.
Observations: Breed fairly easily. After introducing male and female mating occurs soon (hours or days later). Young have been produced 10 months after being introduced. Between 60-100 young are born, white, and crowd on the female's back. They stay on the females back for at least 4-6 weeks; however some stay on for a few weeks after this (some have also been seen on the male!). The first moult has been seen 12 days after birth. To get maximum numbers reared they need to be separated from each other and the parents, or cannibalisation occurs (albeit at a low rate). Slight sexual dimorphism visible. Number of pectin teeth varies between 8-10 and can be different on the left and right side of the same individual. A number of adults and young are being sent to Museum Nationale d'Histoire Naturelle, Paris scorpion expert, Wilson Lourenço to conduct further lifecycle and development research.
Other holders: Bristol, Poznan & MNHN Paris.

Table 1. Frégate beetle EEP summary

	London	Poznan	Bristol	Artis	Riga	TOTAL
1996	43	0	0	0	0	43
1997	34	0	0	0	0	34
1998	56	0	0	0	0	56
1999	117	10	12	0	0	139
2000	285	?	13	20	0	318
2001	361	?	26	20	0	407
2002	380	0	124	14	15	533
2003	619	30	130	18	183	980