

Notes on the biology, captive management and conservation status of the Lord Howe Island Stick Insect (*Dryococelus australis*) (Phasmatodea)

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Received: 31 January 2008 / Accepted: 12 March 2008 / Published online: 8 April 2008
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Abstract The Lord Howe Island Stick Insect (*Dryococelus australis*; Phasmatodea: Phasmatidae: Eurycanthinae) is a large, flightless stick insect once thought to be extinct but rediscovered on an island (Balls Pyramid) near Lord Howe Island in 2001. A captive population at Melbourne Zoo is now in its fourth generation and aspects of the biology of the species are discussed. Observations focussed on the eggs as indicators of the health of the population and inbreeding depression, but included data on the juveniles where possible. Behavioural observations reveal that this species is very different from other Australian stick insects, but similar in many ways to overseas members of the Eurycanthinae. Veterinary interventions and post mortems have provided substantial information about the captive population and its environmental stresses, and have wider implications for captive invertebrate populations, particularly those involved in conservation programs. Evidence of inbreeding and the conservation significance of this species is discussed in context with other programs and their implications.

Keywords Inbreeding · Insect husbandry · Insect pathology · Ex situ conservation · Captive management

Introduction

The Lord Howe Island Stick Insect (LHISI) was once abundant on Lord Howe Island, 700 km off the coast of New South Wales, Australia. It apparently became extinct on Lord Howe Island within a few years of the accidental introduction of rats in 1918, following the grounding of the

supply ship *Mokambo* (Gurney 1947). In 2001, a small population of the stick insects was rediscovered surviving on a rocky outcrop, called Balls Pyramid, 25 km off Lord Howe Island (Priddel et al. 2003).

The LHISI was categorised at the time as endangered under the New South Wales Threatened Species Conservation Act 1995 and presumed extinct in the IUCN Red Data List (IUCN 1983). A Draft Recovery Plan was developed by the New South Wales Department of Environment and Climate Change (NSWDECC) (Priddel et al. 2002), and in 2003 two adult pairs were removed from Balls Pyramid for captive breeding. One pair was delivered to Insektus, a private breeder in Sydney, the other pair to Melbourne Zoo. At that point almost nothing was known of their biology and ecology, other than observations made by Lea (1916). The remaining wild population is now thought to be less than 40 individuals, living on a few bushes on the side of a cliff on Balls Pyramid (Priddel et al. 2003).

Description

The LHISI is a large, flightless phasmatid, with males reaching 120 mm (more commonly 106 mm) at adulthood and females 150 mm (more commonly 120 mm) (Zompro 2001). Both sexes are uniformly black at maturity, often with a reddish-brown tinge. The body is generally smooth and shiny, and the intersegmental membranes between joints are pale grey (Fig. 1). Adult males are distinguished by two conspicuous spines on the enlarged hind femur. Females have a long, pointed sheath (the operculum or subgenital plate) underneath the last segment, with a wider and more terminally tapering abdomen.

Upon hatching, LHISI are pale to mid-green, and become darker green as they moult. Juveniles are pale

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Fig. 1 Adult female LHISI on its only recorded natural food plant, Lord Howe Island Melaleuca (*Melaleuca howeana*)

brown, becoming darker brown with age, then very dark brown to black in the final instars (Fig. 2).

Habitat

The only known population of LHISI is amongst a group of melaleucas (*Melaleuca howeana*) on the north-west face of Balls Pyramid. The melaleucas cover an area of about 30 by 10 m, and are the only vegetation on the pyramid other than groundcovers. Smaller islands around Lord Howe Island have been extensively searched for LHISI, without success.

Lord Howe Island is covered with a range of subtropical habitats, from cloud forest at the tops of the mountains to coastal vegetation on the dunes. The temperature on Lord Howe Island ranges from 15 to 25°C throughout the year, and the humidity is generally high year round. There is a great range of plant species throughout the habitats in which LHISI were once known to occur, but on much of the island the largest tree is the Lord Howe Island Fig (*Ficus macrophylla columnaris*). In the forested areas, the canopy is high and there may be little lower level vegetation or ground cover, and the soil is deep and often sandy.

Balls Pyramid is much more exposed than Lord Howe Island, with a greater range of temperatures. The humidity



Fig. 2 Second instar LHISI. This stage is dark green-brown, becoming brown in later instars and then black

is also high due to its exposure to the sea, but the rock itself is very dry and there are no sources of fresh water. Consequently there is very little vegetation and almost no soil. The melaleucas on which the LHISI survive are very old and stunted, and growing very densely close to the rock. Due to the large numbers of sea birds which nest on the bushes, the foliage is covered with guano, and many of the plants are also being threatened with smothering from Morning Glory (*Ipomoea indica*). There are quantities of melaleuca leaf litter at the bases of the bushes, in some places quite deep, but this is very friable and dry.

Prior to its extinction on Lord Howe Island, the only information on LHISI was recorded by Lea (1916), but this did not include biological or dietary data. On Balls Pyramid, LHISI emerge at night to feed on the outer foliage of melaleucas and are presumed to shelter during the day at the bases of the shrubs.

Methodology

The original, wild caught pair (P1) was kept free ranging in a glasshouse at Melbourne Zoo and observations made of their behaviour nightly for the first month. This included time spent feeding, mating, exploring, egg-laying or remaining inactive. The egg-laying medium was sifted daily and eggs measured by length, width and weight. Subsequently, four egg-laying media (sand, vermiculite, peat and a 50:50 sand-peat mixture) were trialled in a glasshouse housing 130 adults. Over four generations, 3,093 eggs were set up for incubation and, of these, 2,627 were weighed, measured and their development monitored. The depth of egg deposition of the first 10 batches was measured by uncovering eggs with a fine camel hair brush.

Eggs were kept in labeled groups in sand for up to 5 months until they could be measured and weighed. They

were then incubated in four different media (sand, soil, peat and vermiculite) under three moisture regimes. These included wet (media sprayed daily), moist (sprayed once a week) and dry (unsprayed). Eggs were placed 2.5 cm below the top of the media with the operculum facing upwards. Newly hatched nymphs were measured from the front of the head to the tip of the abdomen with electronic calipers on the day of emergence and a subjective assessment made of their condition (poor, good, excellent).

Nymphs were initially set up on Lord Howe Island Melaleuca, but other plant species were later included as additional choice or non-choice trials. Nymphs were kept singly or in groups of up to 40 in plastic pet paks or wooden, aluminium or plastic enclosures with mesh screening. Plants and insects were sprayed daily and a Petri dish of free water was available at all times to older juveniles and adults. Adults were kept in similar but larger enclosures to the nymphs, usually in groups of 10 females to two males. The enclosure included a wooden box (variable in size) as a daytime retreat, egg-laying media and a piece of wood or bark soaked during the day in water to increase humidity within the enclosure. Additionally, up to 200 adults were kept free-ranging in a glasshouse 5 m × 3 m × 3 m (L × W × H). Observations were variously made of feeding, moulting, intraspecific interactions and other behaviours.

Supplementary feeding trials included the young seeds and ‘cabbage palm heartwood’ of the Kentia Palm (*Howea forsteriana*), as well as Orthopteran mix. The latter is used as supplementary feed for a range of insects in captivity, particularly Orthopterans and stick insects (see Rentz 1996 for recipe).

Direct observations on LHSI feeding and indirect observations on feeding marks were used to determine the effectiveness of the Kentia Palm products. Three small Petri dishes of Orthopteran mix were weighed and changed daily and placed in three enclosures with 32 adult LHSI for 1 month, plus a control dish outside the enclosures but within the same glasshouse.

Individual nymphs and adults were tagged using queen bee markers for behavioural observations. These markers are small coloured plastic discs which are fixed to the backs of individuals using a non-toxic glue (Fig. 3). Nymphs as young as 3 months old were tagged, but tags were lost at each moult and insects required retagging. Tagged individuals were kept in groups, and every day the group checked to ensure that any individual that moulted overnight was retagged. The identity of many individuals was lost, however, as multiple insects moulted overnight and could not be individually distinguished.

The bee markers were fixed to the dorsal surface of the thorax, slightly to one side for nymphs so it did not interfere when the thorax split down the middle during



Fig. 3 A pair of adult LHSIs in the daytime retreat. The male (lower) is facing the opposite way to the female (top), with two of his legs over her body. Note the yellow bee markers on the back of each thorax

moulting. LHSI have a tendency to choose to spend the day in damp, mouldy conditions and the tags quickly became covered in frass and dirt which was removed by gentle scrubbing. The bee markers lasted several months on the back of adults.

LHSI deaths were recorded and selected specimens were post mortemed by Melbourne Zoo veterinarians.

Biology and life history

Eggs

The gestation period, the ability of females to store sperm and the influence on the eggs of multiple matings with different males is not known. Males of this species do not transfer an obvious spermatophore as do other stick insect species.

Females begin laying eggs about a fortnight after reaching adulthood, whether a male is present or not. Whether this species is able to reproduce parthenogenetically is not yet known. A number of adult females have begun producing eggs before being given access to a male, but none of these eggs has hatched. The number of these eggs produced (42) is not sufficient to adequately determine viability, as any group of 42 eggs may or may not produce any offspring, particularly at the beginning of a female’s egg-laying period. Female *Extatosoma tiaratum* will mate if given access to males but produce eggs parthenogenetically if no male is available within 33 days of maturity (Carlberg 1981, 1982). Whether a similar process occurs with LHSI is not known and requires further research.

The first several batches of eggs produced by each female tend to be smaller than those produced later on, and

the very first eggs may be slightly misshapen, particularly the micropylar plate. The eggs produced at the end of a female's life may also be smaller than those produced previously, and females will continue producing eggs until death, although the rate of egg production declines dramatically towards the end.

The eggs (Fig. 4) are relatively large, whitish to pale cream and covered with a fine, raised, net-like structure (Hennemann and Conle 2006). On contact with moisture, eggs become very dark in colour, ranging from dark grey-brown to black, and if sitting on a moist surface the egg may be black on the lower side and white on the dry side. The dark colour fades as the egg dries out, suggesting the egg wall is very porous and/or absorbent. Unviable eggs also become very dark as they age, and this has led to erroneous descriptions based on old museum specimens.

The average length of an egg is 6.36 mm (range 3.6–7.1 mm, $n = 2627$) and the width 3.95 mm (range 2.1–4.45 mm, $n = 2627$). Most specimens are about 6.2 mm long and 3.6 mm wide. The average weight of an egg is 0.058 g (range 0.005–0.08 g, $n = 2627$). At the top is a flat operculum through which the nymph emerges, and on the side towards the opposite end is a tear-shaped micropylar plate, about 2.4 mm long. One specimen, which subsequently hatched successfully, possessed one micropylar plate on each side of the egg, a state that has not been previously recorded for Phasmatodea (Clark Sellick 1998).

Females generally produce eggs in batches of 9–10, about a week to 10 days apart. Smaller batches are commonly produced, and single eggs may be deposited in the intervening periods. The sex ratio of successfully hatched young appears to be about 50:50 overall. There is some evidence that the early eggs from any individual female will produce female offspring, and later eggs tend to be male, although this requires further confirmation. This



Fig. 4 LHSI eggs. Most are laid subterraneously but some are deposited on the soil surface

situation leads to dramatic changes over time in the sex ratio of the entire population, with the adult population at Melbourne Zoo varying between 10% female in one period and 98% female in another. Eggs are deposited in the egg laying medium at an average depth of 2.5 cm (not including those deposited on the surface of the medium) (range 0–3.8 cm, $n = 48$).

The average number of eggs produced per female was 149.1 (range 52–257, $n = 28$ females studied). The number of eggs produced by P1, the wild-caught female, was 257 and was the highest number produced by any female so far. The average number of eggs produced per female from subsequent generations was 161.58, the difference possibly due to inbreeding issues (see below).

The average incubation time for LHSI in captivity was 209.1 days (range 175–241 days, $n = 624$). There was no significant difference between the incubation media trialled, and the moisture regimes ($P < 0.05$). Vermiculite was the most successful incubation media and has been used continuously since the completion of the trial.

Just under half (47%) of the eggs so far analysed have hatched. The hatch rate depends strongly on an egg's parentage (and generation number—see below). The percentage hatch rate of eggs produced by individual females over all generations varied from 5% to more than 70%. For all 22 females from F2, for example, the percentage varied from 22.2 to 73.6%.

There was little difference in volume, weight or morphology between eggs that hatched and those that did not, and there was no significant difference between the two groups ($P < 0.05$). However, more of the smaller and lighter eggs did not hatch than hatched. For eggs that hatched, only 32.92% were 0.05 g or less ($n = 404$ eggs). For eggs that did not hatch, 50.67% were 0.05 g or less ($n = 1,115$ eggs) (1,519 eggs total).

Conversely, more of the larger and heavier eggs hatched than did not hatch. For eggs that emerged, 67.08% were 0.06 g or greater ($n = 404$ eggs). For eggs that did not emerge, only 49.33% were 0.06 g or greater ($n = 1115$ eggs) (1,519 eggs total) (Fig. 5).

Similar results were obtained when eggs were analysed by weight (Fig. 6).

Eggs which did not hatch within the above incubation period can be classified into four states:

- (1) those that remain intact and appear to be still viable;
- (2) those with an intact operculum but with a thick yellow exudate around the operculum to which the incubation media adheres;
- (3) those that have lost the operculum and contain, within the body of the egg, a thick yellow fluid; and
- (4) those that have lost the operculum and are completely empty.

Fig. 5 Percentage eggs which hatched or did not hatch in relation to egg volume

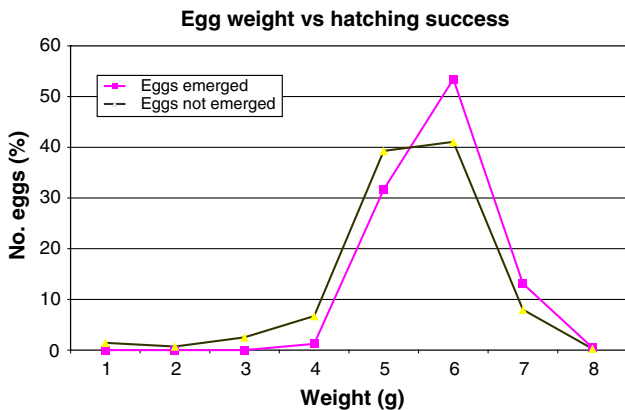
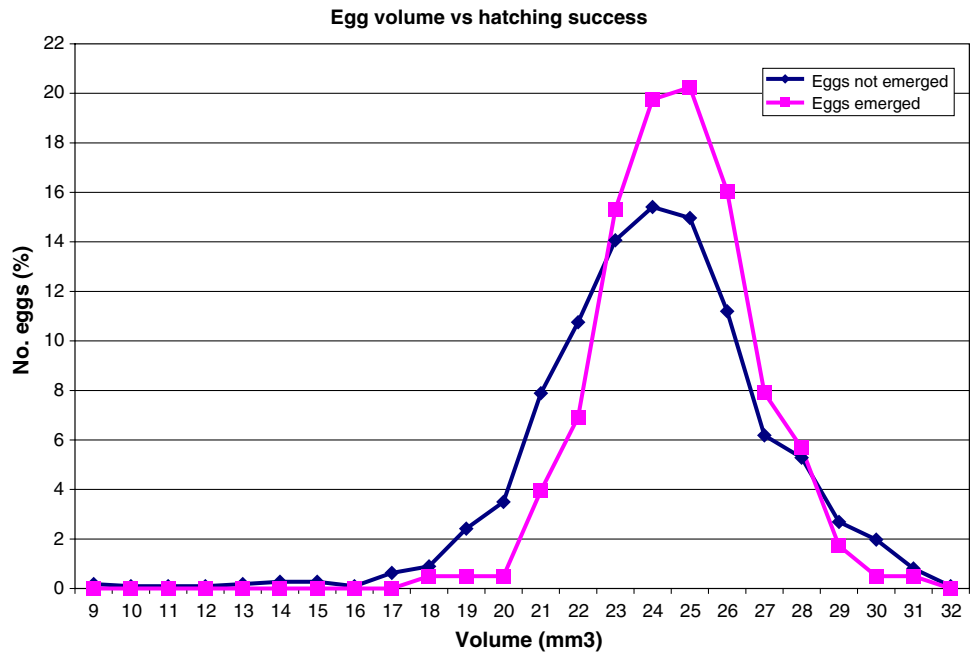


Fig. 6 Percentage eggs which hatched or did not hatch in relation to egg weight

LHISI’s closest relative, *Eurycantha calcarata* from Papua New Guinea, is known to deposit its eggs subterraneously and in captivity the most successful egg-laying media is peat. For LHISI the best egg-laying media (in order of success) are peat (26.2%), sand (23.5%), peat-sand mixture (14.2%) and vermiculite (3%) ($n = 1,250$). However, the largest proportion of eggs (28.1%) were produced within the daytime retreats, deposited amongst frass. It is not known whether these eggs were produced during the day, or in the evening before the females left the retreat to feed etc. In addition, 5% of eggs were collected from the floor of the enclosure, presumably produced during nightly activities.

Juveniles

The average body length of newly hatched LHISI was 19.73 mm (range 13.61–23.57, $n = 1,235$). Hatchlings

usually emerge from the egg underground, and burrow to the surface to climb the nearest large object. Most individuals appear to hatch during the night, but will also hatch throughout the day, including the afternoon.

There are five instars between egg and adult, but the length of the each instar can vary significantly between individuals. Moulting takes place at night and is generally completed by 5 am. The process takes approximately 25 min, with the juvenile hanging from a branch of the food plant or from the top of the enclosure. Occasionally an individual may become caught in the skin and is unable to successfully complete ecdysis, which results in the death of the individual. More commonly, the forelegs of an individual may become caught in the old skin and either be lost or slightly deformed. This may be rectified during subsequent moults.

Nymphs may moult within a fortnight of emerging from the egg, but there is overlap between different stages of different individuals throughout the rest of the instars. The intermoult period in the later instars may be as little as 10 days. LHISI reach maturity at between 201 and 224 days, averaging 210 days ($n = 32$). Adults may live for up to 18 months after maturing.

Behaviour

LHISI appear to be a particularly gregarious species. Lea (1916) reported that 68 nymphs were collected from a single tree hollow on Lord Howe Island, and there are also reports that large numbers sheltered within the roof spaces of houses on the island. When given the option of several

daytime nesting boxes in captivity, groups of insects tend to crowd into a single nesting box rather than spread out into smaller groups. Both adults and nymphs shelter together during the day in groups of mixed age.

Adult males are endowed with large spines on the hind femora, the exact purpose for which is unclear. They sometimes use these to squeeze a finger when handled by humans, and they may be used against other males (Bedford 1975, Minott 2006). In captivity, males are not kept with other males in the presence of females, following the death of a female which may have been caused by one of several males sharing her enclosure. Adult female Eastern Goliath Stick Insects (*Eurycnema goliath*) have twice been observed to fatally injure other adult females by (inadvertently) squeezing the victim's body in the crook of the well-spined hind legs (unpubl. obs.). Male LHISI have been kept together in groups without females and without incident, and single males have been kept successfully with groups of up to 10 adult females.

Feeding behaviour

LHISI feeding on *Melaleuca howeana* will consume leaves of all ages, from those at the base of the plant to the tips of the stems. LHISI will methodically consume every leaf on a branchlet, moving from the tip to the base, so extended bare patches are left after feeding. The leaves are consumed right down to the petiole and the insect will often continue on to chew the bark, leaving small raised scars in the stem. Small branchlets may be chewed through as the insect pushes the stem right into the base of the mandibles. Feeding appears to be in sessions of about 1–1.5 h (although they may extend up to 260 min), followed by extended periods of inactivity.

LHISI will also chew non-plant material, such as plastic. Despite vigorous and audible chewing, the material is usually left unmarked, so the significance of this behaviour is unknown. They will chew bark of other trees and plants such as tree ferns, but again it appears that little or no material is actually consumed.

Mating behaviour

Mating takes place usually with the female horizontal on the ground and the male above her, but it will sometimes occur with both hanging vertically on a plant or at an angle of 45° (Fig. 7). If mating vertically, the male may lose his grip on the female and hang downwards by the abdomen until he is able to gain a footing and return to the upright position, still attached to the female (Honan 2007b).

Mating episodes take between 14 and 25 min, and there may be up to three episodes per night, usually with one to two nights in between episodes. The female may continue



Fig. 7 A pair of LHISI mating on the side of a plant pot (photo turned sideways for clarity). The male (top), identifiable by his thickened hind femora, is curling his abdomen over and then underneath that of the female (bottom)

to feed during mating, but generally both sexes remain completely immobile, with not even the antennae moving. The male may remain on the back of the female for some time following cessation of mating.

Egg-laying behaviour

When about to lay an egg, a female moves her body backwards slightly and immediately probes with the tip of her abdomen into the soil. She arches her body as she pushes the tip down into the soil and after a period of usually only a couple of seconds, begins to grind her entire abdomen back and forth sideways. This continues for a variable period until she moves her body forward slightly and removes her abdomen from the soil, leaving behind an egg.

She will then pat down the soil with her abdomen. Every time the abdomen touches the soil it moves to one side slightly to smooth the soil, and the end also curls under the abdomen towards the front slightly, further smoothing the soil. This is repeated a variable number of times, sometimes with pauses in between. She then remains immobile for a couple of minutes, during which time another egg will appear at the tip of her abdomen. The process is then repeated.

Pair bonding

'Pair bonding' is unusual in insects and not clearly defined, but there are reports that adult males and females of *Eurycantha horrida* form bonds if kept together for a period.

The behaviour of individual pairs of LHISI that have been kept together for a long period suggests a bond between some pairs. When a male and female are kept

together in the same enclosure, the pair characteristically spends the day in the retreat with the male alongside the female and two or three of his legs over the top of her body (Fig. 3). Nine pairs kept together as individual pairs at Melbourne Zoo for several months were observed daily over a month to investigate the relationship between male and female, determined by the location of each in relation to the daytime nesting box. There were four possible combinations:

- male inside nesting box and female outside;
- female inside nesting box and male outside;
- both sexes within nesting box; and
- both sexes outside nesting box.

Behaviour differed markedly between pairs, but remarkably consistent within each pair over time. In one pair, both sexes were found together in the nesting box every day of observation, with the male's body lined up beside that of the female and with three of his legs stretched over her; in other pairs both were outside the nesting box on most occasions, or the male outside the box and the female inside. Of the total 270 observations, never once was the male found inside the nesting box and the female outside.

Diet

The diet of the stick insects on Lord Howe Island is not known, as no records were kept before they became extinct there. The only related published information is that juvenile LHISI were found in large numbers during the day in hollows of tree trunks, presumably of the dominant Lord Howe Island Figs (*Ficus macrophylla columnaris*) (Lea 1916). On Balls Pyramid, they are known to feed on Lord Howe Island Melaleuca (*Melaleuca howeana*), but they may have other additional plant sources there.

In captivity, LHISI have largely been fed on Lord Howe Island Melaleuca (*Melaleuca howeana*). They have also been successfully reared on Tree Lucerne (*Chamaecytisus prolifer*), Blackberry (Bramble) (*Rubus fruticosus*) and Moreton Bay Fig (*Ficus macrophylla*) (Table 1). They show signs of being adaptable to a range of food plants. All stages have done particularly well on Tree Lucerne, with several generations now having been reared on it.

As they may have had other food sources on Lord Howe Island or even Balls Pyramid that are not yet known, a supplementary diet of Orthopteran mix was offered to LHISI in captivity at Melbourne Zoo. Of the 90 replicates, there was no evidence of feeding by any stick insects.

In his 1852–1855 journal of the voyage of the HMS Herald which berthed at Lord Howe Island in 1853, John MacGillivray noted “colonies of a singular cricket-looking wingless insect between 4 and 5 inches in length which the

people on the island have designated the ‘land lobster’” and which “feeds on rotten wood” (Etheridge 1889). Local sources suggested this was the ‘heartwood’ of the Kentia Palm. Consequently, pieces of Kentia Palm trunk and heartwood were offered to LHISI in captivity. Up to 10 pieces of wood, either dry or soaked in tap water, were placed in enclosures for 2 years, but there was no direct or indirect evidence of feeding. Following observations of the P1 female chewing fibres of Tree Fern trunks (*Cyathea australis* and *Dicksonia antarctica*), 5 cm long pieces of tree fern trunk were offered for 2 years, also with no evidence of feeding.

Discussions with several locals on Lord Howe Island revealed that their parents had observed LHISI feeding on young seeds of the Kentia Palm. Seeds of all ages (including unripe specimens) were subsequently collected and 50 were offered on dishes to free ranging LHISI in captivity. After 2 months there was again no evidence of feeding.

Clinical cases

Due to the conservation value of LHISI specimens held in captivity and the difficulty in obtaining further specimens, veterinary interventions have been undertaken on a number of ailing and dead specimens to determine the cause of morbidity or death.

The first case occurred within 2 weeks of the P1 female being collected from the wild. The female ceased feeding and started to become inactive about a week after being in captivity, following her first episode of egg laying. Over several days her activity, particularly feeding activity, was notably reduced and for 5 days she ceased feeding altogether (Fig. 8). During this period she was x-rayed to determine if she was egg bound due to a possibly inappropriate egg-laying substrate, and six eggs were clearly seen inside her abdomen (Fig. 9). These eggs were subsequently deposited by the female and later developed very thin, brittle shells, and eventually disintegrated entirely, presumably due to the effects of the x-ray, but all further eggs appeared undamaged. Other analogous stick insects (*Eurycnema goliath* and *Extatosoma tiaratum*) x-rayed at the same time showed dozens of eggs in the abdomen, so the LHISI female was apparently not egg bound. Her foregut was also seen in the x-ray to be full of air, suggesting aerophagy, a sign of distress in vertebrates, particularly birds (H. McCracken Dr, Senior Veterinary Surgeon, Melbourne Zoo, personal communication).

After 5 days she became completely immobile and did not react to touch or light, and a solution of melaleuca leaves (mashed with a mortar and pestle), glucose and calcium in distilled water was administered to her with an

Table 1 Plant species offered to LHSIs in captivity

Scientific name	Common name	Comments
<i>Melaleuca howeana</i>	Lord Howe Island Melaleuca	Reared several complete generations through egg to adult and egg again. Can be fed long term on this species alone
<i>Chamaecytisus prolifer</i>	Tree Lucerne	
<i>Rubus fruticosus</i>	Blackberry	Reared one generation through egg to adult and egg again
<i>Ficus macrophylla</i>	Moreton Bay Fig	
<i>Rubus</i> species	Native Blackberry	Hatchlings did not feed at all (in non-choice tests)
<i>Coprosma repens</i>	Mirror Bush	
<i>Nerium oleander</i>	Oleander	
<i>Lantana montevidensis</i>	Lantana	
<i>Tagetes lemonii</i>	Mexican Marigold	
<i>Abelia grandiflora</i>	Glossy Abelia	
<i>Pipturis argentius</i>	No common name	
<i>Ligustrum vulgare</i>	Privet	
<i>Gardenia angusta</i>	Gardenia	Hatchlings fed initially but did not survive past second instar (in non-choice tests)
<i>Pollia crispata</i>	Rainforest Spinach	
<i>Asystasia bella</i>	River Bell	
<i>Clutia pulchilla</i>	No common name	
<i>Alnus jorullensis</i>	Evergreen Alder	Hatchlings fed well but did not survive to adulthood (in non-choice tests)
<i>Acacia iteaphylla</i>	Flinders Ranges Wattle	Hatchlings fed well and are still surviving provided very soft tips are used (in non-choice tests)
<i>Photinia robusta</i>	Red Tip	
<i>Rubus laudatus</i>	North American Blackberry	
<i>Sida rhombifolia</i>	Paddy's Lucerne	Adults did not feed at all (in the presence of other plant species)
<i>Prostanthera lasianthos</i>	Mint Bush	
<i>Prostanthera rotundifolia</i>	Australian Mint Bush	
<i>Westringia fruticosa</i> (wynyabbie gem)	Native Rosemary	
<i>Mellicope elleryana</i>	Pink Euodia	
<i>Kunzea erycoides</i>	Burgan	
<i>Rubus parvifolius</i>	Native Raspberry	
<i>Alpinia caerulea</i>	Native Ginger	
<i>Alocasia</i> species	Cunjevoi	
<i>Howea forsteriana</i>	Kentia Palm	Adults fed to some degree (in the presence of other plant species)
<i>Hibiscus tiliaceus</i>	Sandalwood	
<i>Correa laurenciana</i>	Mountain Correa	
<i>Allocasuarina</i> species	Casuarina	
<i>Bougainvillea</i> species	Bougainvillea cultivar	
<i>Ficus longifolia</i> (sabre)	Long Leafed Fig	
<i>Ficus benjamina</i>	Small Leafed Fig	

Table 1 continued

Scientific name	Common name	Comments
<i>Callistemon viminalis</i>	Callistemon Hanna Ray	Adults fed extremely well (in the presence of other plant species)
<i>Leptospermum lanigerum</i>	Woody Tea Tree	
<i>Rosa</i> species	Domestic Rose	
<i>Ficus benjamina</i>	Weeping Fig	
<i>Schefflera actinophylla</i>	Umbrella Tree	
<i>Alphitonia excelsa</i>	Red Ash	
<i>Cullen adscendens</i>	Mountain Psoralea	
<i>Omalanthus populifolius</i>	Queensland Poplar	Hatchlings did not feed at all (in non-choice tests) and adults did not feed at all (in the presence of other plant species)
<i>Citrus limon</i>	Lemon Tree	Hatchlings fed well and are still surviving provided very soft tips are used (in non-choice tests) and adults fed extremely well (in the presence of other plant species)

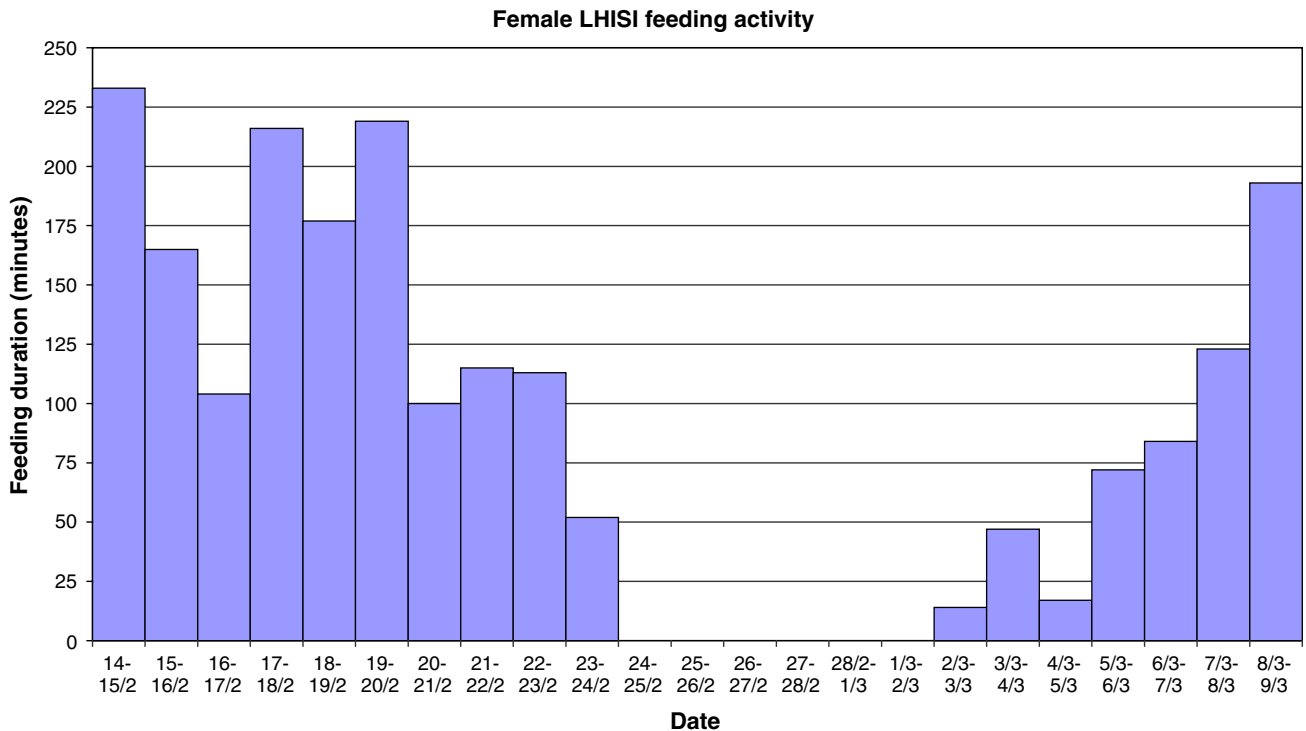


Fig. 8 Time spent feeding by the P1 female collected from Balls Pyramid for the first month in captivity. Note the cessation of feeding for 5 days before treatment

eyedropper on her mouthparts. Within a few hours, she became active again and resumed normal activity, subsequently living for another year. The cause of her morbidity and the reasons for the success of the treatment are still unknown.

A number of other specimens, both those that have died of apparently old age and those that have died unexpectedly, have been subsequently post mortemed. Post mortem

examinations included gross necropsy, histological examination of tissues by veterinary pathologists and scanning electron microscopy. The gross necropsy was particularly useful, despite the fact that the Melbourne Zoo veterinary practitioners were not at the time well acquainted with the internal anatomy of invertebrates. Normal anatomic structures are easily identifiable and gross changes in the gastrointestinal tract, body condition and exoskeleton of

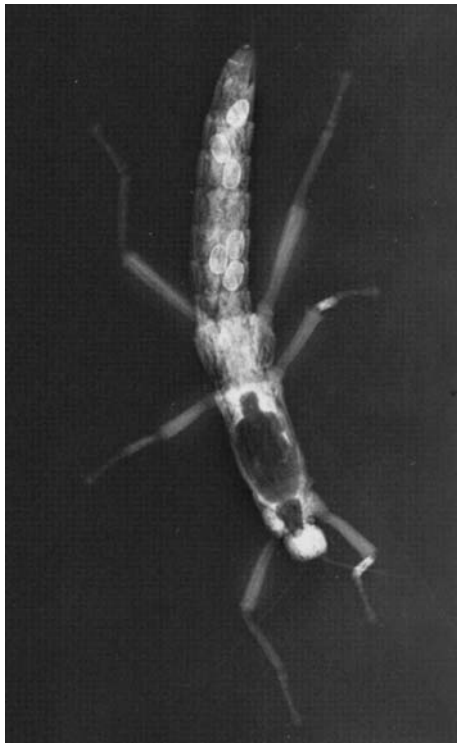


Fig. 9 X-ray of the P1 female collected from Balls Pyramid. Note the black area within the thorax (a symptom of aerophagy) and the six white eggs in the abdomen

healthy specimens which have died of old age are readily comparable to subadult or adult specimens which have died unexpectedly.

For example, the location and quality of food in the gut gives an indication of when the insect last fed. Watery and/or foul-smelling gastrointestinal contents are suggestive of possible infection or other pathology. Dull, slightly discoloured body fluids and intracoelomic fat indicate an aged specimen, whereas a smaller than usual quantity of fat indicates illness. The complete absence of intracoelomic fat in the abdomen indicates an extended period of illness preceding death. The size and condition of the ovaries or testes also gives an idea of the overall health of adults.

These factors help determine whether the cause of death is acute or chronic. A chronic illness may indicate immunosuppression as a result of exposure to environmental stress, toxins or disease. Environmental stress was indicated in one adult male that died prematurely. Upon dissection, his foregut was full of newly chewed leaves, his hindgut full of well-processed leaf material, his testes well-developed and plenty of fat throughout the body, suggesting a healthy condition and that he was feeding well right up to the point of death. However, his internal organs appeared very dry, with almost no free fluid in the body cavity, suggesting general desiccation. The enclosure in which he was being kept was moved off the floor to an area

in the glasshouse where humidity was higher, the mesh of the enclosure was changed for a smaller mesh size, and humidity was increased in the glasshouse throughout the night. There have been no subsequent deaths attributable to desiccation.

On another occasion, an adult female was discovered near death and attempts were made to revive her using the treatment administered to the P1 female during her period of morbidity, without success. The Melbourne Zoo veterinarians also administered a modified form of Ringer's solution (Schultz and Schultz 1998), also without success. An x-ray revealed nine well-developed eggs in her abdomen and signs of aerophagy. Upon dissection, the foregut was found to be stretched like a balloon, and the foregut was almost empty, suggesting she had not eaten for some time. There was a reasonable spread of fat throughout her body, but not as much as seen in previously dissected specimens. On the inside of the gut, at the junction of the fore- and hindgut was a small area of green pigment, which appeared to be part of, or embedded in, the gut wall. This was analysed by pathologists without result. The pigment may have come from a pelletised fertilizer used on the potted melaleuca plants, as the colour was identical to that of Greenjacket Osmocote, perhaps consumed inadvertently by the female. Greenjacket Osmocote has been removed from the potting mix and there have been no subsequent cases attributable to this.

Multiple deaths occurred during two incidents in small LHISI enclosures without obvious cause and without stick insects in nearby enclosures being affected. The food plants were tested for insecticide and herbicide but none was found. There were less than usual fat bodies spread throughout the abdomen, air in the foregut without any leaf material and only brown fluid with air bubbles in the hindgut. The brown fluid was also found on the floor of the enclosure. Under histological examination, a number of LHISI specimens had multifocal cuticular lesions (including pallor and erosion) apparently associated with a fungus or unusually large bacterium (spirochaete), but scanning electron microscopy and culture failed to identify them further. The overgrowth of fungi (or bacteria) in dead specimens in part occurred after death, but the location and extent of the growth within the body cavity but outside the gastrointestinal tract suggested that it occurred because the animals were immunocompromised before death.

Several individual deaths also subsequently appeared to be caused by a fractured cuticle (supported by a strong epithelial and hypercellular reaction at the site(s) observable only with high powered microscopy) and ulceration in the midgut, both associated with bacterial growth. These fractures were found all over the body, including on whole mounts of the head. The cuticular fractures had a yellow, serous fluid with a superficial cap of protein and bacterial colonies, again with cuticular widening and pallor. Due to

the internal scarring, this was interpreted as an old fracture that had allowed access of fungi or bacteria (possibly *Klebsiella pneumonia*) into the coelomic cavity, resulting in subacute ulceration around the gastrointestinal tract leading to terminal sepsis. Coupled with evidence of recent feeding, this suggested mechanical damage and/or acute stresses caused by other insects (possibly due to overcrowding), transport or handling.

Although only a small proportion of deceased LHISI have been analysed by post mortem, five separate pathology reports from specimens collected at Melbourne Zoo and Healesville Sanctuary have reported or inferred that the direct or indirect cause of death was cuticular fractures which allowed bacterial infection and, subsequently, terminal sepsis. This is a condition not reported previously in the limited but growing literature on invertebrate medicine (Lewbart 2006). Given the gregarious nature of LHISI and their habit of clinging to each other tightly with their exceptionally strong tarsi within their daytime retreats, this is an area that requires further investigation.

One of the most important features of veterinary interventions of endangered invertebrates is that practitioners and pathologists have access to, and become familiar with, healthy specimens. This enables identification of normal versus abnormal tissues and recognition of pathological changes, and builds up a bank of detailed digital images from necropsies that can be used for referral.

Genetic management

The original population of LHISI on Lord Howe Island, anecdotally comprising many thousands of individuals, was significantly larger than the surviving population on Balls Pyramid, which is estimated to be less than 40 individuals. The captive population is founded on two pairs, one taken to Melbourne Zoo and the other to Insekus in Sydney.

Population fragmentation

There is some evidence that fragmented insect populations are able to adapt to reduced variation in the long term and that even rapid fragmentation can evoke evolutionary change in less than 100 years (Hanski and Poyry 2007). LHISI have been present on Balls Pyramid for at least 80 years, since the founder population became extinct on Lord Howe Island, probably in the early 1920s. Given that LHISI are flightless, that Balls Pyramid has never been connected by land to Lord Howe Island, and that the two are separated by 23 km of open ocean, how the population came to establish there is not known. However, there are three known possibilities:

- (1) floating across on vegetation. The sea is generally treacherous between Lord Howe Island and Balls Pyramid, and there are no sloping shores on Balls Pyramid for vegetation to wash up, only sheer cliff faces. This may have occurred any time over the last several thousand years;
- (2) carried across by seabirds. Thousands of seabirds nest on Balls Pyramid but, as there is no vegetation there, nesting material must be carried across from Lord Howe Island. In the 1960s, a dead LHISI was found incorporated into a seabird nest (McAlpine 1967; Smithers 1966). If a moribund or dead female was taken across by a bird, viable eggs in her abdomen in theory may be able to hatch. At Melbourne Zoo, 40 eggs in total have been collected from the abdomens of dead females but none of these have hatched. Once again, this method of establishment on Balls Pyramid may have occurred any time over the last several thousand years.
- (3) carried across by fisherman. Lord Howe Island was officially discovered in 1788 but not settled until about 1834 (Etheridge 1889). Since then, fishermen have been travelling from Lord Howe Island to the rich fishing grounds around Balls Pyramid (Nicholls 1952) and, anecdotally, used LHISI for bait before they became extinct. Transfer by fishermen may therefore have occurred between 80 and 170 years ago.

Under the latter scenario, the LHISI population on Balls Pyramid would be relatively young and, because of the lack of host plants there, must have remained small during its entire history. Whether this population is like some studied, that have adapted readily to rapid fragmentation or like others, that have shown no evolution or microevolution whatsoever (Hanski and Poyry 2007), requires further study.

LHISI were collected for museums in large numbers before becoming extinct on Lord Howe Island. One notable difference between the museum specimens and the current captive population is the size of the hind femora of the adult males. In the museum specimens, the femora are often at least as wide as the abdomen and the femoral spines are stout and elongated, whereas the captive males have femora half the width of the abdomen or smaller, with significantly smaller and narrower spines.

There are two possible explanations for this situation:

- (1) as LHISI were very common on Lord Howe Island, collectors were able to freely choose the largest, most impressive specimens for their collections, strongly biasing the sample;
- (2) wide femora and long spines in adult males are traits that positively select for their survival and

reproduction, useful either in defending themselves against predators or fighting off other males.

If the first explanation is true, enlarged femora may be a rare trait which nevertheless should, over time, appear in captive populations. If the second explanation is true, enlarged femora are no longer useful in a captive, predator-free environment, but may appear more frequently in the population if those that bear them are afforded access to more females.

Interestingly, the P1 male collected from the wild on Balls Pyramid had large femora and femoral spines (once again attributed to collector bias) but almost all of its descendants have notably smaller femora.

Inbreeding depression

Various authorities on conservation genetics state that a viable population must contain between 50 and 1,000 individuals to prevent inbreeding (Thompson et al. 2007; Nunney and Campbell 1993) and up to 5,000 individuals to preserve genetic variation and avoid the expression of deleterious genes (Lande 1995). Inbreeding depression operates on small populations in two ways: by lowering overall fitness through increased homozygosity and by reducing adaptive variation and therefore the animals' ability to deal with environmental stresses, exposure to toxins and disease (Thompson et al. 2007). LHISI in captivity have demonstrated both increased homozygosity and lowered fitness, and are exposed to environmental stresses and other factors, such as occasional overcrowding.

Attempts have been made previously to measure inbreeding in insect populations in captivity. Saccheri et al. (1996) measured fecundity, egg weight, hatching rate, adult

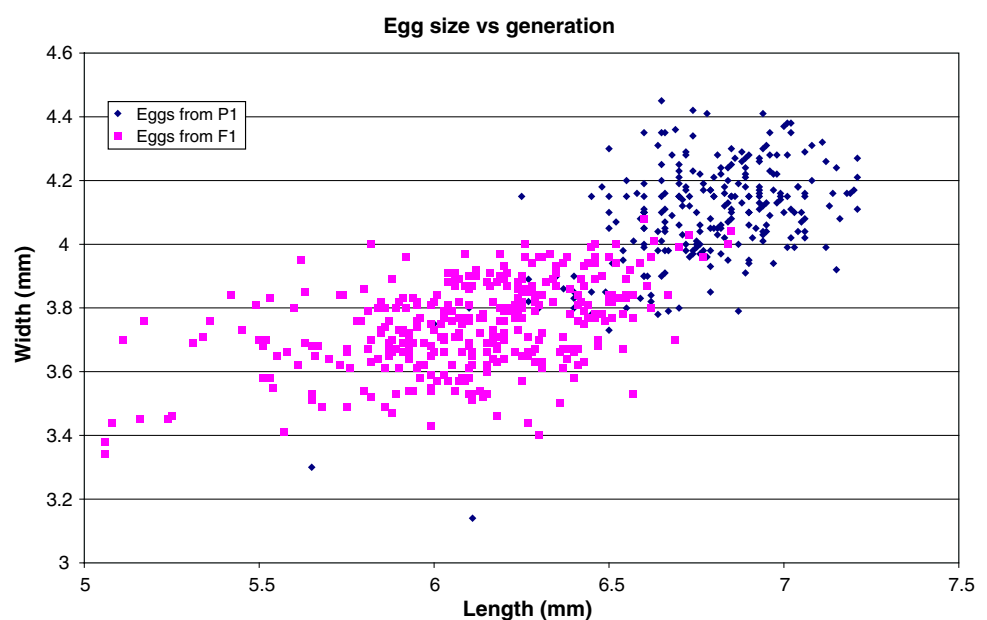
emergence rate, adult size and frequency of morphological deformities in the Bush Brown butterfly (*Bicyclus anynana*) in the laboratory, and found evidence of inbreeding at surprisingly high levels in just a few generations. There has long been evidence of inbreeding in vertebrates in captivity, but it has been assumed by many that insect populations contain sufficient inherent variation and other traits that help them avoid inbreeding depression, although there have been few practical studies (Thompson et al. 2007).

For LHISI in captivity, fecundity, egg size and weight, hatching rate and nymphal size were measured over four generations (P1 + F1 – F3). Morphological deformities and nymphal survival rate were also noted but not rigorously measured. The size of the eggs produced by F1 females was dramatically smaller than those produced by the P1 female (Fig. 10). This was true even when adjusted for hatched versus unhatched eggs.

The adult fecundity, egg weight, hatching rate and nymphal body length also decreased from the P1 to F1. There was also evidence of morphological abnormalities, particularly to the abdomens of adult males, and dramatically lower survival rate of newly hatched nymphs.

In June 2004, four adult males from F1 were sent to Insektus in Sydney and four adult males from Insektus were incorporated into the Melbourne Zoo population. After the new males were introduced, no further abnormalities were observed, the size of the eggs and nymphs increased, the hatching rate increased from less than 20% to about 80%, and the survival rate of nymphs and adults rose to almost 100%. Consequently the population at Melbourne Zoo, which had been around 20 surviving adult individuals at any time during the first 3 years of captive

Fig. 10 Comparison of egg sizes produced by P1 and F1 females



management, rose to 600 within 12 months of the males' genes taking effect (Honan 2007a).

Following this apparent inbreeding depression, F2 individuals at Melbourne Zoo were kept separated into 18 genetic lines which have been maintained in the intervening period. Adult females are kept in groups of 10, to which two adult males from other genetic lines are added and rotated at irregular intervals. Given that the detailed reproductive biology and behaviour of this species is not known, the effect of this rotation and potential alleviation of inbreeding is also unknown. Despite these attempts at genetic management, there is currently evidence of further inbreeding and, as the captive population at Insektus in Sydney no longer exists, in the long term the only viable solution may be the introduction of new genetic material from the remaining wild population.

Conservation significance

The LHISI provides a significant example of the benefits, as well as the tribulations of captive breeding in invertebrate conservation. On the negative side, conservation workers are often forced to deal with species about which there is no background biological information, and in addition there is often no biological information from related or analogous species as there might be for vertebrates. If a threatened invertebrate species is to be taken into captivity, there is also usually no husbandry information, which can make rearing and breeding more difficult.

There is also very little funding available for zoo-based invertebrate conservation projects, particularly in comparison to programs focussing on threatened vertebrate species. Apart from labour by zookeepers, the funds required by the LHISI project were minimal and most of the funds provided were as charitable donations. Three small glasshouses currently house more than 10 times the known wild population of LHISI, with room to grow the collection. This is the case for most invertebrate conservation programs (Pearce-Kelly et al. 2007), for which the requirements for space, finances and other resources are low, but where skills and labour are at a shortage, particularly due to the labour-intensive nature of invertebrate husbandry.

Consequently, although there are sufficient resources at Melbourne Zoo to maintain a population of LHISI in captivity, these are not adequate to effectively study the species' biology and genetics. This is the case for many invertebrate conservation breeding projects and has consequences for the long term management of each species, leading to suboptimal husbandry and genetic management. Post mortems of unexpectedly deceased specimens, for

example, are worth little unless the results can be analysed and used to improve husbandry techniques. Although results are currently gained from post mortems and pathology analyses, the significance of most of them are still completely unknown. On the positive side, some invertebrate conservation programs genuinely do require almost no funding and, due to the ability of many invertebrate species to recover from threatening processes and population fragmentation, they can be returned to the wild as soon as the threats have been removed.

In some cases, a conservation program based on a single species of invertebrate can act as a taxonomic surrogate for a range of both vertebrate and invertebrate species within the same habitat, as demonstrated by LHISI. Up to 15 species or subspecies of vertebrates (and untold numbers of invertebrates) have become extinct on Lord Howe Island since rats were accidentally introduced in 1918. Some of these vertebrates still survive on tiny islets around Lord Howe Island or on other islands in the Pacific and are themselves endangered to varying degrees.

Planning for a rodent eradication project is currently underway on Lord Howe Island, and in this case invertebrates (LHISI) rather than the vertebrates are the impetus for the project. Rodent baiting trials were successfully conducted on islands analogous to Lord Howe Island in early 2007, and trials using non-toxic, dye-laced baits were undertaken on Lord Howe Island in August 2007. The full baiting program is scheduled for 2010, after which LHISI eggs, juveniles and adults will be returned to the island, followed by other vertebrate species in the longer term.

Another positive element to the LHISI project is its high profile, similar in Australia to that of the Eltham Copper Butterfly (*Paralucia pyrodiscus lucida*) and Richmond Birdwing (*Ornithoptera richmondia*), and similar in many ways to the profile generated by vertebrate conservation programs. This is in part due to the unusual appearance and biology of the stick insects, their apparent extinction for several decades and the story surrounding their rediscovery and collection from the wild. Due to media coverage in Australia and particularly overseas, the LHISI project has high recognition amongst the general public and this has contributed in some degree to raising the profile of invertebrate conservation in Australia.

One of the most useful roles of zoos in invertebrate conservation is to raise the profile of a range of insects and other species, highlight their positive contribution to humans and the ecology, and destigmatise them as a group (Lewis et al. 2007). Zoos are also able to undertake formal and informal education programs and involve the public directly in community action, which can be one of the most effective means of preserving species and habitats (Sands and New 2002). In addition, zoos have some role to play in direct conservation of invertebrates,

particularly single species conservation (Honan 2007a; Pearce-Kelly et al. 2007), despite assertions by some authorities that captive breeding has little or no role in effective conservation programs (Collins 1990). This is evidenced by the fact that some invertebrate species would no longer exist in the wild or would not survive long term in their natural habitat but for ongoing conservation programs (New 1995).

With the rodent eradication program in process, the LHSI recovery program utilises both in situ and ex situ conservation measures, the captive management component being particularly important due to the perilous state of the wild population. Although invertebrate conservation programs are now tending away from the single species approach to a more holistic habitat approach (Yen and Butcher 1997), there is merit in attacking the problem at both levels (Clarke 2001). Apart from current issues surrounding inbreeding depression, the LHSI project has served as insurance for the wild population, with currently more than 400 adults and juveniles spread across seven captive populations in Australia and overseas, and more than 13,000 eggs under incubation. With the rodents removed from Lord Howe Island, a viable population should be returned to their original habitat within the next few years to colonise the island in a threat-free environment.

Acknowledgements The LHI Stick Insect captive breeding program is currently being undertaken by Rohan Cleave, Norman Dowsett, Robert Anderson, Zoe Marston and other invertebrate keepers at Melbourne Zoo. Special thanks to Rohan Cleave for his tireless management and analysis of thousands of LHSI eggs. Thanks to Dr David Priddel (Senior Researcher, New South Wales Department of Environment and Climate Change) for his oversight and foresight of the project, Dianne Gordon (Team Leader, Discovery and Learning, Healesville Sanctuary) for discussions and educational advice and Dr Tim New for reviewing the manuscript. Post mortems and pathological analyses were undertaken by Dr Kate Bodley, Dr Helen McCracken, Dr Michael Lynch, Dr Paul Ramos (Veterinarians, Melbourne Zoo) and Dr Peter Holtz (Veterinarian, Healesville Sanctuary).

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