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ORIGINAL ARTICLE

A Preliminary Investigation of Insect Succession Patterns on Decomposing Carrion on Rottnest Island (WA)

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ABSTRACT

Introduction: Pivotal component of medico-legal investigations is the determination of time since death, or (minimum) postmortem interval, $_{(min)}$PMI. Forensic entomology can provide reliable estimates of $_{(min)}$PMI using carrion insect species. Carrion insect arrival and departure occurs in predictable and sequential waves, however, successions may vary depending on geographical location and environmental conditions. Hence, it is crucial to refer to insect succession data of the same region where the death scene occurs. A current gap in forensic entomology research is on carrion-breeding species and succession on decomposition remains/carrions on small islands. Rottnest Island is located off the south-west coast of Perth. The aim of this study was to establish baseline data of carrion insect fauna present on this island, and to provide entomological evidence that may aid in any future investigations on the island. **Methods:** A list of the insect taxa associated with two decomposing swine hindquarters during the summer season was compiled over a 15-day study period and compared with published mainland data obtained during the same period of the year. **Results:** Ten insect taxa representing four families were collected and identified in association with the decomposing carrion. The first wave of colonising Diptera species included Calliphoridae species and Sarcophagidae species. The second wave of Diptera comprised Muscidae species, in addition to beetles. Furthermore, ants and spiders were noted throughout the trial. **Conclusions:** Comparing the results on Rottnest Island with currently available data from mainland WA, showed that the composition of insect species and the patterns of succession were similar.

KEYWORDS: Forensic entomology; Postmortem Interval; small island; globalization

INTRODUCTION

Forensic entomology involves the use of arthropods associated with decomposing remains and carrion, as evidence in criminal investigations (1, 2). Knowledge of the community composition and succession patterns of forensically significant insects assists in the reconstruction of criminal events by providing estimates of the elapsed time since death. Based on the circumstances of the case under investigation, such time can be referred to as the minimum postmortem interval, $_{min}$PMI, or actual postmortem interval, PMI (3, 4).

Where there are no physical, chemical or ecological impediments, colonisation by insects on a fresh carcass can occur within minutes (3). Blow fly species (Order Diptera) are among the first to ovi/larva-posit larvae and directly feed on decomposing tissue (5). Other necrophagous fly species (e.g. Order Sarcophagidae, Muscidae, Piophilidae) and beetles (Order Coleoptera) begin their colonisation subsequently as decomposition progresses (6). The community assemblages and sequence of insects colonising decomposing carrion can be unique to a given geographical location and the prevailing environmental conditions (7). Once these have been established for a given geographic location, insect evidence recovered from forensic investigations can be compared to data compiled in that region to help provide an accurate estimation of the $_{(min)}$PMI (4, 7).

Several publications have highlighted the impact of climatic variation and environmental characteristics on the composition of insect taxa and succession patterns (5, 8, 9). Factors such as climate, vegetation, soil type and urban development can influence the preference of insect species to certain habitats (10, 11). Succession studies performed worldwide, focusing on necrophagous Diptera at contrasting geographical locations within a given region, have revealed variability in the abundance and composition of insect fauna communities at contrasting habitat types (5, 8, 9). However, these and other fly trapping studies are rarely performed on islands, and currently only large islands such as Hawaii and Marshall Islands (USA), Sicily (Italy) and Tasmania (Australia) have been documented (8, 9, 12-14). Research carried out at three locations of different altitudes in Oahu (Hawaii), displayed variations in insect succession patterns (8).

Early colonising Calliphoridae species *Eucalliphora lilaea* (Walker), *Calliphora vomitoria* (Linnaeus) and *Dyscritomyia* species were observed to only occur at the high and mid-elevation sites, but not the low-elevation site, appearing at the first day of decomposition at mid-elevation site and third day at the high elevation site (8). Variations in insect assemblages observed in this study were driven by the different characteristics of the three contrasting locations on the Oahu island, where the regions varied in sun light exposure, tree density and vegetation species (8). Another study conducted during the winter season on the largest Mediterranean island, Sicily, monitored insect colonisation activity on decomposing animal carrion in a cave environment (12). Observations of delayed colonisation and development rates of blow fly species, as well as low survival rates were recorded (12). The observations were reported as a result of the absence of sunlight and very low temperatures (−8 to 10°C) in comparison to the corresponding terrestrial site (1 to 7°C) (12). Additionally, this study reported the presence of *C. vicina* Robineau-Desvoidy at both the cave and terrestrial site, however, *C. vomitoria* was only observed in the cave environment (12).

To date, the studies conducted in Australia are largely focused on mainland geographical locations, underrepresenting small island fauna in the forensic entomology literature (5, 15-19). Only two publications in Australia are based on island insect succession patterns in association with decomposing carrion, and both of these studies were performed in Tasmania (9, 13). The first publication reported blow fly succession in north-east Hobart, southern Tasmania, on possum carrion during the autumn/winter period, where the study identified eight species of blow flies associated with decomposing possum carrion (13). A more recent study was conducted at two locations in the northern Tasmania (Devonport, a rural environment and Launceston, an urban environment) over the summer months (9). The study identified variations in community composition of forensically significant insect species at the two contrasting locations with a total of ten insect species identified at the rural location in comparison to eleven species at the urban location (9).

Currently, no forensic entomology research has been published on any small islands. Western Australia (WA) has a chain of nine coastal islands in proximity to mainland Perth, and Rottnest Island is the largest and situated the furthest in distance from the mainland (18 km) (20). Rising sea levels separated Rottnest Island from the mainland ~7,000 years ago (20). The Island consists of varying habitat types, including salt lakes, coastal lakes, salt swamps, woodland, heathland and urbanised areas (20). The island attracts thousands of tourists every year and is widely known for its native quokkas (*Setonix brachyurus*, Lesson) (20). Rottnest Island is a reserve, where all plants and animals are protected by law (20). Several ecological studies have been performed on the island, but none to specifically address forensic entomology questions. In contrast, since 1930 several succession studies focusing on the necrophagous entomofauna associated with decomposing carrion have been conducted on mainland WA (5, 17, 19, 21-28). Over the past few years, investigations on suspicious deaths of humans and animals have taken place on the island. In 2013, dismembered human remains relating to a murder case were discovered at a south-east location on the island (29). Additionally, cases of animal cruelty involving the killing of quokkas by burning, inflicting deliberate physical harm and poisoning, have also been reported (30). Entomological evidence was overlooked in all of these cases.

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The present study aimed to monitor the succession patterns of insect species on Rottnest Island and compare insect assemblages to historical decomposition studies and trapping data obtained on mainland WA during the same season. The present study, although preliminary, focuses on remediating the gap in the forensic entomology literature based on small islands, by constructing reference data on forensically relevant insect fauna associated with the decomposition of carrion. Although preludial at this stage, it is envisaged that results of this research will be considered in any future investigations of suspicious deaths and criminal activity involving wildlife on Rottnest Island.

Figure 1 A: Rottnest Island and Forbes Hill location (source: Google maps https://www.google.com/maps/); B: Satellite image of Forbes Hill (source: Google maps https://www.google.com/maps/); C: General view of study site.

MATERIALS AND METHODS

The research trial was conducted over a period of 15 days on Rottnest Island (WA), during the southern hemisphere summer season 2020 (6^{th} to 20^{th} January). The size of the island is approximately 19 sq km. The study site was Forbes Hill, a woodland habitat situated approximately 3 km towards the centre of the island, and at a 12-meter elevation level (32° 0' 15" S, 115° 30' 55" E) (Figure 1). The study field is dominated by pines (*Callitris* sp.), tea trees (*Melaleuca* sp. and *Acacia* sp.) and low shrubland (*Acanthocarpus preissii*) (31).

As many other countries, field-based research in Western Australia concerning the decomposition of human remains is legally prohibited; therefore, bait, sticky-based fly traps and animal models are the only option available for decomposition studies (9). Typically, full or partial swine carcasses (*Sus scrofa domesticus* Erxleben) are the preferred animal model, as reasonably inexpensive and readily available in large numbers (32).

Due to the specific permissions and restrictions put in place by the Rottnest Island authorities to perform decomposition studies on the island, the overall cost and the constrictive means of transportation from mainland to the island (commercial ferry boat), two swine hindquarters (referred as SH1 and SH2 in this study) were used instead of whole carcasses. The SHs, weighing 5±0.43 kg, were purchased fresh from an abattoir, placed in a sealed container and transported under refrigeration to Rottnest Island on the same day. On arrival at the island port, the sealed container was removed from the refrigeration, so that the SHs were at ambient temperature before being exposed. Using this protocol, no insect colonization occurred prior to the commencement of the experiment.

At the study site, the SHs were placed on the ground between 1230 and 1300hours on the first day of the experiment, approximately 15 meters apart. Each SH was tethered between two tree branches, using nylon ropes of 1cm thickness, in order to prevent movement by scavenging animals and birds. Cages were not required to protect the SHs, as no large scavengers are present on the island. Daily temperature and relative humidity records were obtained for the duration of the

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experimental period from weather loggers (Easy Log®) at the site. Loggers were launched at 1200hours on the first day of the experiment, recording at five-minute logging intervals. Wind data were obtained through the Bureau of Meteorology of the Australian Government, with data sourced from Rottnest Island, station 009193.

The collection, preservation and analyses of the insect samples was conducted in accordance with the current standards and guidelines accepted by the forensic science community (1). A representative number of samples were collected on a daily bases for the 15-day duration of the experiment, between 0900 and 1100 hours. Samples were collected from the different parts of the colonized carrion, as well as sieved from the soil associated with the SHs. Each sample was placed in separate aerated vials, stored in containers and refrigerated until the arrival to the laboratory on the mainland, within 8 hours from collection (1). At the laboratory, each sample was divided into 2 subsamples, one sacrificed for morphological identification (1) and one reared to complete the life cycle to adult stage for identification purposes (33). In regards to the latter, fly immatures were placed on beef liver substrate in plastic boxes (22×40×20 cm) containing sand 5 cm deep, sealed with mesh covers and elastic bands and maintained in the laboratory at a temperature of 25±1.3˚C (33). Samples of live adult flies were sacrificed by placing them in the freezer (-20˚C) for 48 hours (1). Sample vials and containers were labelled according to sampling date and time, hindquarter number (SH1 or SH2) and site of collection (soil sample or carcass surface sample) (1). Specimens were observed via stereomicroscope (Leica® MZ8) and pictures were taken using Dino Light microscope (Dino Lite-Edge 3.0/5mp AM73915MZT). Insect samples were taxonomically identified to family, genus and species by using available literature and consulting expert entomologists. Relative species abundance was considered by simply identifying and counting individuals of every species collected. As a consequence of this being a small-scale experiment involving only 2 SHs, the insect collected were only identified and recorded, but the obtained data were not statistically analysed.

RESULTS

a) Environmental data

Overall, sunny and dry weather conditions were observed for the entire experimental period. Average daily temperature throughout the 15-day experimental period was 23.0 ± 1.1°C, with a maximum temperature on day 6 of the experiment (34.8°C) and minimum temperature on day 5 (16.7°C). The average relative humidity recorded was 63.7 ± 8.7%. The maximum humidity was 83.5% (day 12) and the minimum humidity was 50.1% (day 6). Rottnest Island also experiences high wind velocities with the daily average lowest speed recorded during the experiment was 48 km/h (day 2, 6, 11), while the highest was 81 km/h (day 4).

b) Decomposition process, insect community composition and succession

During the 15-day experiment the SHs passed from fresh stage of decomposition (days 1-3) to active decay (day 4-7) and advanced decay (day 8-13), and started the post-dacay/dry stage on day 14 (34). The lack of the abdominal part in the carrion used for the experiment prevented the production of putrefactive gas by the gut anaerobic bacteria, therefore the bloat stage of decomposition didn't occur. However, the high humidity recorded throughout the experimental period allowed both the SHs to remain hydrated and attractive for the insects as oviposition site (35).

A total of ten insect taxa were collected from the decomposing SHs during the experimental period. Table 1 reports the species of insect collected on SH1 and SH2 during the 15-days experiment, with days grouped together when the insect assemblage was similar. Nine taxa were Diptera, which were observed to colonise the SHs in primary and secondary waves, while only one species of Coleoptera was observed from day 5. Overall, the insect community present and the succession of their arrival was similar between both localities. However, a difference was observed with the presence of *Musca vetustissima* Walker (Diptera: Muscidae) on SH1 (Table 1).

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Table 1 Diptera and Coleoptera colonisation of the two experimental swine hindquarters (SH1 and SH2) on Rottnest Island during the 15-days summer experiment (6-20th January). Experimental days were grouped together when the insect assemblage was found to be similar. Insects life stages are identified as A (Adult), E (Egg), L (Larvae), PF (Post-feeding) and FPu (Full Puparia). X = Specimen collection. Experimental days were grouped based on observed changes in insect activity.

	Order	Family	Species	Experimental Day																							
				Day 1-2			Day 3-4			Day 5-6				Day 7-8				Day 9-10					Day 11-15				
				A	E	L	A	E	L	A	E	L	PF	A	E	L	PF	A	E	L	PF	Fpu	A	E	L	PF	Fpu
SH1	Diptera	Calliphoridae	*Lucilia sericata*	X	X	X	X	X	X	X		X				X	X			X	X	X			X	X	X
			Calliphora dubia	X		X			X			X				X	X			X	X					X	X
			Chrysomya rufifacies	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Chrysomya varipes	X			X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Chrysomya megacephala	X	X		X	X	X	X		X		X		X	X										
		Sacrophagidae	sp.	X		X	X		X			X				X	X				X	X				X	X
		Muscidae	*Musca domestica*				X			X				X				X					X				
			Musca vetutissima				X			X				X				X					X				
			Australophyra rostrata							X				X				X					X				
	Coleoptera	Dermestidae	sp.							X				X				X					X				
SH2	Diptera	Calliphoridae	*Lucilia sericata*	X	X	X	X	X	X	X	X	X				X	X			X	X	X				X	X
			Calliphora dubia	X		X			X			X	X			X	X				X	X					X
			Chrysomya rufifacies	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Chrysomya varipes	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Chrysomya megacephala	X		X	X	X	X	X		X		X		X	X					X					
		Sacrophagidae	sp.	X			X		X	X		X				X	X			X	X	X					X
		Muscidae	*Musca domestica*							X				X				X					X				
			Australophyra rostrata							X				X				X					X				
	Coleoptera	Dermestidae	sp.							X				X				X					X				

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Chrysomya rufifacies (Maquart) (Diptera: Calliphoridae) and *Ch. varipes* Robineau-Desvoidy were the dominant colonisers throughout the entire trial. Adults of *Ch. rufifacies* were observed to oviposit on both SHs from day 2 (Table 1). *Ch. varipes* adults were also present amongst the initial colonisers. Larval samples of these species were collected all throughout the trial from both SHs. Similarly, *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) were common at the beginning of the trial. This species was observed arriving minutes after the placement of the SHs at the study site. Larval mass formation of *L. sericata* was observed throughout the early days of the trial and lasted until day 8 (Table 1).

Other Calliphorid species such as *Calliphora dubia* (Maquart) adults were active at the beginning of the trial, where initial colonisation of first instars were observed from day 1 (Table 1). *Calliphora dubia* were not observed in as large numbers as *L. sericata*, *Ch. rufifacies* and *Ch. varipes*. The post-feeding stage of *C. dubia* on the SH2 was recorded to start earlier in comparison to SH1 (Table 1).

Chrysomya megacephala (Fabricius) flies were present in small numbers on day 2, ovipositing eggs on both SHs (Table 1). At the same time, flesh flies (Diptera: Sarcophagidae) visited both the SHs in large numbers. Adult sarcophagid species were observed to start colonisation and larviposit on day 2 on SH1, and on day 3 on SH2 (Table 1).

Adult muscid species, *M. domestica* L., *M. vetustissima* and *Australophyra rostrata* (Robineau-Desvoidy) were observed in small numbers during the later period of the trial. *M. domestica* were present on SH1 from day 4 but arrived at SH2 on day 5 (Table 1). Meanwhile, *M. vetustissima* were observed on SH1 from the fourth day of the trial (Table 1). *A. rostrata* were also late to appear and were only seen from day 5 on both SHs (Table 1). All three muscid species were low in abundance, and no apparent oviposition of eggs or larvae were observed or collected throughout the trial.

Dermestids (Coleoptera: Dermestidae), commonly known as 'larder beetles' were observed on day 5, mostly present on the surface below the SHs (Table 1). The colonisation of adult dermestid species at both SHs lasted till the end of the study trial, day 15 (Table 1), when the carrion was almost dry.

Ants (Hymenoptera: Formicidae) were observed to infest the SHs to feed throughout the entire study period, and at times were observed carrying fly eggs and larvae. Additionally, spiders were observed in close proximity to the SHs feeding on adult blow fly species.

Voucher collections from this study are held at Murdoch University (Perth, Western Australia).

Table 2 A summary of the studies conducted in mainland Western on insect assemblages found on decomposing carrion and fly traps

Author (Year)	Animal model	Season of the experiment	Diptera	Coleoptera	Other insect species	Notes
Newman *et al.* (1930) [30]	Fly Traps	All seasons	*Chrysomyia rufifacies* *Lucilia sericata* *Calliphora australis* *Microcalliphora varipes*			Observational study on blow flies associated with sheep strike using fly traps
Bornemissza (1956) [24]	Guinea pig	All seasons	Blowfly Piophilidae	Dermestidae *Ptomaphila* sp Histeridae Staphylinidae	Tineid Tyroglyphid Mites Gamasid mites Hymenoptera	A study on soil modifications by carrion frequenting fauna

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Murray (1978) [29]	Sheep	All seasons	*Lucilia cuprina* *Lucilia sericata* *Calliphora nociva* *Calliphora albifrontalis* *Chrysomya rufifacies* *Peronia rostrata* Sarcophagidae			A study on blow flies initiating sheep strike
Monzu (1980) [28]	Sheep	All seasons	*Lucilia cuprina* *Calliphora nociva* *Calliphora albifrontalis*			Primary species of sheep strike blow flies
Dadour and Cook (1992) [26]	Fly Traps	Not provided	*Lucilia cuprina* *Lucilia sericata* *Calliphora nociva* *Calliphora albifrontalis* *Calliphora varifrons* *Chrysomya rufifacies* *Chrysomya megacephala* *Chrysomya varipes* *Musca domestica* *Musca vetustissima* Sarcophagidae Anthomyiidae Tephritidae Drosophilidae Heleomyzidae Lonchaeidae Platystomatidae Sepsidae	Nitidulidae Curculionidae Dermestidae Chrysomelidae Lygaeidae Formicidae Cydnidae Staphylinidae Scarabaeidae Tenebrionidae Histeridae	Psychodidae Pyralidae Noctuidae Sphecidae Braconidae Chalcididae	The effectiveness of commercial fly traps
Cook *et al.* (1995) [25]	Goat & Sheep	All seasons	*Lucilia cuprina* *Chrysomya rufifacies* *Chrysomya varipes* *Calliphora dubia* *Musca vetustissima* *Hydrotaea rostrata* Sarcophagidae			A study on blowfly species associated with sheep strike
Voss *et al.* (2008) [18]	Pig	Summer and Autumn	*Lucilia sericata* *Calliphora albifrontalis* *Calliphora dubia* *Chrysomya rufifacies* *Chrysomya varipes* *Chrysomya megacephala* *Hydrotaea rostrata*	*Dermestes ater* *Dermestes maculatus* *Saprinus* sp. *Creophilus erythrocephalus* *Onthophagus taurus* *Necrobius rufipes* *Omorgus tatei*	Chalcididae Iridomyrmex	1) Insect succession on decomposing carrion inside a vehicle; 2) The effects of carbon monoxide poisoning on insect succession

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			Musca domestica *Musca vetustissima* *Stomoxys calcitrans* Sarcophagidae Piophilidae	*Helea castor*		
Voss *et al.* (2009) [5]	Guinea pig	All seasons	*Lucilia sericata* *Calliphora albifrontalis* *Calliphora dubia* *Chrysomya rufifacies* *Chrysomya varipes* *Chrysomya megacephala* *Hydrotaea rostrata* *Musca domestica* *Musca vetustissima* Sarcophagidae Piophilidae	*Dermestes ater* *Dermestes maculatus* *Saprinus* sp. *Creophilus erythrocephalus* *Aleochara* sp. *Necrobia rufipes* *Ptomaphila lacrymosa* *Omorgus tatei*	*Nasonia vitripennis* *Tachinaephagus zealandicus* *Aphaereta* sp. *Spilomicrus* sp. *Iridomyrmex* sp.	Seasonal insect succession study on decomposing carrion
Voss *et al.* (2011) [31]	Swine	Autumn	*Lucilia sericata* *Calliphora albifrontalis* *Calliphora dubia* *Chrysomya rufifacies* *Chrysomya varipes* *Chrysomya megacephala* *Australophyra rostrata* *Musca domestica* *Musca vetustissima* Sarcophagidae *Piophila casei*	*Dermestes* sp. *Saprinus* sp. *Creophilus erythrocephalus* *Helea castor* *Necrobius rufipes* *Necrobius ruficollis* *Ptomaphila lacrymosa* *Omorus tatei*		Insect succession on clothed and unclothed carcasses
McIntosh *et al.* (2017) [27]	Swine	Autumn	*Lucilia sericata* *Calliphora dubia* *Chrysomya rufifacies* *Chrysomya varipes* *Chrysomya megacephala* *Australophyra rostrata* Sarcophagidae Piophilidae	*Dermestes ater* *Dermestes maculatus* *Saprinus* sp. *Necrobia rufipes* *Necrobia ruficollis* Staphylinidae		Insect succession on burnt and unburnt decomposing carcasses
Magni *et al.* (2019) [16]	Swine	Autumn	*Lucilia sericata* *Calliphora albifrontalis* *Calliphora dubia* *Chrysomya rufifacies* *Muscina stabulans* *Fannia canicularis* *Megaselia scalaris*	*Carcinops pumilio* *Creophilus erythrocephalus* Carabidae *Diamesus* sp.	Vespidae Formicidae	Effect of suitcase concealment on insect colonisation

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Table 3 Succession patterns of colonising insects on decomposing animal carrion in Western Australia at similar habitat types: Rottnest Island (Woodland); South-West Perth (Coastal bushland) [1]; South Perth (Bushland); and North Perth (Agricultural) [2]. Insects colonizing the swine carcasses on mainland [1, 2] or hindquarters on Rottenest Island were grouped as primary colonisers (Prim), secondary colonisers (Sec) and tertiary colonisers (Ter).

Sequence of insect arrival on the decomposing remains			**Rottnest Island (Woodland)**			**South-West Perth (Coastal bushland) [16]**			**South Perth (Bushland) [4]**			**North Perth (Agricultural) [4]**		
Primary/ Secondary/ Tertiary														
Order	**Family**	**Species**	**Prim**	**Sec**	**Ter**	**Prim**	**Sec**	**Ter**	**Prim**	**Sec**	**Ter**	**Prim**	**Sec**	**Ter**
Diptera	Calliphoridae	*Lucilia sericata*	X			X			X			X		
		Calliphora dubia	X			X			X			X		
		Chrysomya rufifacies	X				X		X			X		
		Chrysomya varipes	X				X		X			X		
		Chrysomya megacephala	X			X				X		X		
	Sarcophagidae	sp.	X			X			X			X		
	Muscidae	*Musca domestica*		X				X		X		X		
		Musca vetutissima		X				X		X			X	
		Australophyra rostrata		X			X				X		X	
Coleoptera	**Dermestidae**	sp.		X			X			X			X	
	Histeridae	sp.	N/A	N/A	N/A		X			X			X	
	Staphylinidae	sp.	N/A	N/A	N/A		X				X			X

DISCUSSION

Comparing the results of the current research with previous WA mainland studies (Table 2), research by Voss *et al*. (2008) and Voss *et al.* (2009) (5, 19) were considered the best fit based on season of the study, habitat type and the use of swine as the animal model. Both Voss *et al* studies were conducted over a longer period of time (5, 19). Accordingly with Table 3, the preliminary outcomes of the Rottnest research show strong similarities between assemblages of significant Dipteran species reported in the WA mainland studies (5, 19).

The overall community composition and order of colonisation of both Diptera and Coleoptera species was observed to be consistent across both SHs. Blow flies such as *L. sericata*, *C. dubia*, *Ch. rufifacies*, *Ch. varipes*, *Ch. megacephala* and flesh flies were the initial colonisers in association with the SHs. Meanwhile, secondary colonisers included the muscid flies *M. domestica*, *M. vetustissima*, *A. rostrata* and the larder beetle species.

However, some differences between mainland and Rottnest Island were noted. While *Chrysomya* species have been observed as primary colonisers on Rottnest Island, they were secondary colonisers in the previous WA succession studies (Table 3) (5, 19). To note, in other parts of Australia (e.g. Queensland) when temperatures are high as experienced in this trial, *Ch. rufifacies* is a primary strike fly (36). The same is typically observed in the Old World for *Ch. albiceps* (37).

The high temperatures and relative humidity recorded throughout the duration of the current summer study may be a potential factor contributing to the early arrival time of this species (15). Additionally, several Coleoptera species reported to occur during the summer months on the mainland (e.g. *Ptomaphila lacrymosa* (Schreibers), *Omorgus tatei* (Blackburn) and *Helea castor* (Pascoe) (5) were not recovered during the

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Rottnest study (Table 3). Also, clown beetles (Histeridae) and rove beetles (Staphylinidae) were reported only in mainland studies (Table 3). The former appeared during the bloat stage of decomposition, while the latter appeared during the wet stage (5, 19). Unfortunately, a hindquarter does not pass through the bloat and putrefaction stage. As a consequence, a longer duration of study and the use of whole animal carcasses passing through all the stages of decomposition would be an advantage, as it allows researchers to observe changes in insect community structure throughout the different stages of decomposition (38).

The similarities observed between data compiled on Rottnest Island and mainland WA are potentially the result of the globalisation phenomenon suggested by Turchetto and Vanin (2004) (39). It is indicated that geographic barriers between regions are overcome via the migration of humans and the exchange of raw and food material between regions (39). This has resulted in a shift in the worldwide distribution for some forensically significant Diptera (39, 40). Consequently, community assemblages, patterns and developmental rates of faunal species in some regions may not align with faunal succession data originally compiled in the 19th and 20th centuries (39, 40). An example of globalisation has been reported for *Synthesiomyia nudiseta* (van der Wulp). This muscid species is distributed across the United States and East-Asia, and in more recent years was recovered from forensic cases in Spain (40). In 2015, *S. nudiseta* was recovered from five cases that occurred in north-west Italy (40). A phylogenetic sequencing analysis indicate the sequences of *S. nudiseta* recovered from cases in Italy are directly related to sequences of this species in China (40). The study indicates the strong potential of the introduction of this species into this region through sea cargo at one of Italy's ports (40). Another example is provided by the worldwide spread of *Hermetia illucens* (L.) (Diptera: Stratiomyidae) (41), originally native of the Americas, now present in both tropical and temperate regions (42, 43). The spread of this species is due to its lack of hardiness to the cold, but also its presence on an enormous variety of organic materials and waste (41).

While Rottnest Island and mainland WA have maintained a similar climate, given the close proximity of the two, as well as the frequency of tourist and product transfer from mainland to the island and vice versa within a single day, insect species are highly likely to be transferred between the two regions (20). Approximately 300,000 recreational boats and ferries are recorded to visit Rottnest Island per year (20), facilitating the transfer of insect species via food materials delivered to the island. As well, the fact that Rottnest Island and the mainland split approximately 7000 years ago suggests that the insect species on Rottnest Island are merely a subset of the mainland species (20). Population genetic analyses comparing insects collected on Rottnest Island and mainland would be able to clarify this point.

The history of cases regarding dead humans and animals found on Rottnest Island sheds light on the significance of generating baseline data of forensically significant insects active on the island (29, 44). As an example, Rottnest Island is famous for its native quokka and cruelty inflicted on these marsupials is not uncommon (30, 44). In 2015 six quokkas were found dead near a camping site, five of which were stuffed into a tree (44). The WA Department of Parks and Wildlife initiated an investigation into the killing of the quokkas, however, there are no reports regarding the progression of the investigation (44). Additionally, a case involving the discovery of human remains have also been reported on Rottenest Island (29). In 2013, a severed head of a Caucasian man was discovered at a location south-east of the island (29). As to whether investigations of animal or human death on Rottnest Island in the past have utilised entomological evidence is a moot point because up until this study there is no baseline data in the literature. This could be further compounded by Frye or Daubert's rule of evidence, which without scientific literature to support forensic entomology evidence recovered from Rottnest Island, such evidence may be deemed inadmissible in a court of law (45, 46).

Being a preliminary study, this research suffered a number of limitations. Firstly, the use of only two SHs rather than repetitions of whole animal carcasses due to permission, cost and transportation issues. Ideally whole animal carcasses are preferred for the study of insect succession studies, as this would allow researchers to monitor changes taking place caused by insect activity, as well as changes in

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community structure throughout the stages of decomposition (fresh stage, bloat stage, active and advanced stage, dry stage) (34, 35, 38). Secondly, this should be accompanied by a longer study on the island running it concomitantly with a mainland study. These studies would provide more reliable outcomes regarding annual and seasonal patterns of colonising insect species and improve the applicability of baseline data in conducting $_{(min)}$PMI estimates. Furthermore, repetitions using whole carcasses would provide data to validate the present observations and produce statistically more robust $_{(min)}$PMI estimations for future reference (47, 48).

CONCLUSION

The results of the insect fauna collected in this preliminary study only highlights and supports the global discourse that there is a need for more complete decomposition trials in more localities. As noted, most research studies in WA and in the rest of the world are not based on islands, so this study aimed to provide a preliminary insight into insect succession patterns of forensically significant insects on Rottnest Island. We speculate at this stage, baseline succession data generated on adjoining mainland regions in corresponding seasons in WA could be applied to conduct future $_{(min)}$PMI estimates on the island.

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Conflict of Interest

Authors declare none.

Authors Contibutions

I.R.D and R.C.O. conceived the project and obtained the permissions, A.E. carried out the experiment under the supervision of I.R.D, R.C.O. and P.A.M..

A.E. performed all the laboratory analyses under the supervision of P.A.M..

A.E. wrote the manuscript in consultation with P.A.M and all authors discussed the results and contributed to the final manuscript.

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