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Assisted colonization of the Western Swamp Turtle *Pseudemydura umbrina* into novel wetlands: macro-invertebrate communities, biomass and turtle diet

verfasst von / submitted by

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2. Zusammenfassung

Der natürliche Lebensraum von *Pseudemydura umbrina* (Siebenrock, 1901), ein saisonales Feuchtgebiet nördlich von Perth (Australien), ist durch zahlreiche Gründe, wie Habitatfragmentierung oder Klimaerwärmung, stark gefährdet. Der jährliche Niederschlag hat sich die letzten fünf Jahrzehnte, stark verringert. Dies hat zu einer Verkürzung der Aktivitätsperiode der Schildkröten geführt. Aufgrund dessen wurde ein 12-monatiger Versuch zur Ansiedlung dieser stark gefährdeten Schildkrötenart, außerhalb, und weiter südlich ihres natürlichen Lebensraumes, durchgeführt. Es wurden Biomasse, Biodiversität und Mageninhalte der Schildkröten aller Untersuchungsflächen ermittelt, um die beiden südlichen Habitatflächen auf einen optimalen Lebensraum für *P. umbrina* zu testen. Biomasse und Biodiversität in den Versuchsflächen und in der Referenzfläche unterscheiden sich nicht wesentlich. Dennoch werden die südlichen Flächen als geeignete Lebensräume für *Pseudemydura umbrina* in den nächsten 50-70 Jahren eingestuft. Die Methode, durch Menschenhand durchgeführte Verlagerung eines Lebensraumes einer Spezies auf einem optimalen Lebensraum zu testen, wird in Zukunft immer wichtiger werden, um Arten vor dem Aussterben zu bewahren.

3. Abstract

The natural habitat of the Western Swamp Turtle (*Pseudemydura umbrina* Siebenrock, 1901), ephemeral, winter wet swamps north of Perth, Australia, is in danger due to climate change and habitat fragmentation. Rainfall in this area has declined over the last five decades. This trend is predicted to continue, increasingly shortening the annual activity period for the turtles and potentially shifting their optimal climate zone a few hundred kilometres to the south. Therefore, a project testing assisted colonization of this rare reptile was initiated to evaluate the food availability, biomass and biodiversity of three test sites. Both biomass and biodiversity did not differ significantly between the southern sites, with cooler climatic conditions, and the references site north of Perth. Prey items were investigated by single flushing of the stomach of each turtle. Results indicate that the southern areas may be suitable future habitats for *P. umbrina* in 50-70 years as each site provided the invertebrates and vertebrates that the turtle will eat. Assisted colonization into wetlands, in which *Pseudemydura umbrina* have not been known to occur naturally, may become a reasonable conservation tool to ensure the long term survival of the species.

Keywords: Stomach flushing, Aquatic invertebrates, Chelidae

4. Introduction

Nineteen percent of the world´s reptiles are endangered with the risk of extinction. Of these, fifty percent are turtles and tortoises [1]. One of these species is the Western Swamp Turtle (*Pseudemydura umbrina* Siebenrock, 1901), which is listed as critically endangered [2] and is vulnerable to climate change because of its unusual biology [3].

Pseudemydura umbrina is an omnivorous and short-necked freshwater turtle. It is the smallest of all Australian Chelidae and has a very low rate of reproduction (three to five eggs per year, females reach maturity with 8 - 15 years) [4, 5]. *Pseudemydura umbrina* is endemic to the south-western corner of Western Australia [2, 6, 7] and is the only representative of the subfamily Pseudemydurinae [7, 8]. The species inhabits shallow, ephemeral swamps over clay or sand over clay soils [6, 9], in a Mediterranean climate with hot, dry summers and cold, wet winters [3, 9, 10]. High invertebrate species richness and biomass as well as a diverse flora are typical features of these wetlands [3]. The Western Swamp Turtle has a unique biology, when the swamps start to dry out in late spring, the turtles go underground and aestivate during summer for 4-5 months [11]. During aestivation the turtles are inactive which brings behavioural and physiological benefits to avoid desiccation and heat stress during summer [12]. When the temperatures drop and rainfall increases, usually in April/May, *P. umbrina* becomes active again [11]. *Pseudemydura umbrina* is active and feeds at a water temperature between 14 and 28°C [6, 9, 13]. Therefore, the species has a limited period of feeding, which concerns the cooler months of June to November [9].

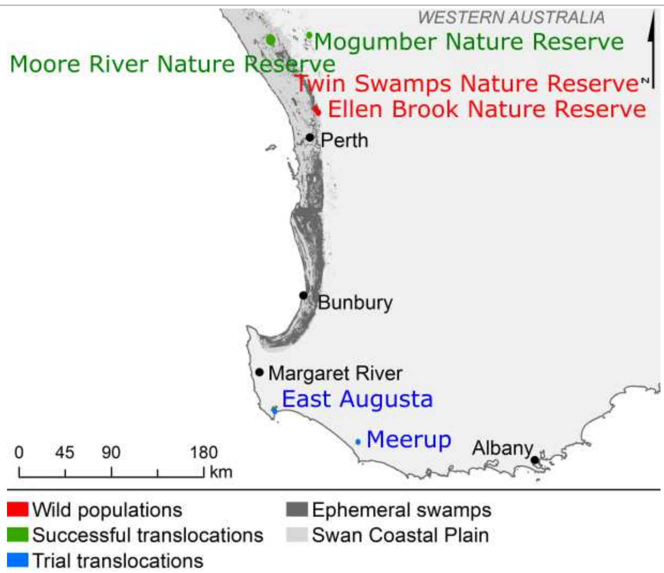

Figure 1: Map of wild populations in Ellen Brook Nature Reserve and Twin Swamps Nature Reserve, already successful translocations sites Mogumber Nature Reserve and Moore River Nature Reserve and trial translocations sites in Meerup and East Augusta (adapted from Kuchling *et al.* 2018)

The only wild populations occur in the Swan Coastal Plain north of Perth (Figure 1) [2, 6, 7] where two habitat areas, Ellen Brook Nature Reserve (31°45’S) and Twin Swamps Nature Reserve

(31°43’S), are protected since 1962 [3, 7, 14]. Translocations of captive-bred *P. umbrina* in order to establish new populations happened, in Twin Swamp Nature Reserve since 1994, in the Mogumber Nature Reserve since 2000 and in the Moore River Nature Reserve (31°11’S) since 2007 [11, 15, 16, 17].

In the long term, climate change, in combination with the very fragmented habitat, is potentially the biggest threat to *P. umbrina* [5, 18, 19]. The annual mean temperature in the southwest of Western Australia has risen over the last five decades from 23.1°C in 1966 to 25.8°C in 2012, rainfall has decreased from 575.2 mm to 479.8 mm and swamp hydroperiods have shortened [10, 20]. This leads to a shortened duration of periods in which the swamps retain sufficient water, resulting in less feeding time for adults and insufficient hatchling growth: they have to reach a body mass of approximately 18 g during their first wet season, to survive their first summer [3, Gerald Kuchling unpublished]. Healthy turtle development is necessary to allow for the sufficient energy needed to reproduce, maintain basic bodily functions and to build up enough fat reserves for the forthcoming summer of aestivation [3, 9]. The turtles feed only under water which means that the swamps must carry water to support live food including small crustaceans, insect larvae and tadpoles [7, 17]. Food availability is therefore an important factor for the survival and reproduction of *P. umbrina* [21]. Climate models indicate that future change will continue to shift the climatic zone in which *P. umbrina* can live. Such zones are located a few hundred kilometres south [10, 18].

The Western Swamp Turtle has a very small geographical range making it nearly impossible to change this without any human support [22]. *Pseudemydura umbrina* is a strong candidate for assisted colonization [10]. In the mid-1980s *P. umbrina* was the world´s most endangered chelonian species [7], since then, translocation projects caused an increase of the number of animals in the wild [2, 14]. This assisted translocation is a great option to relocate species that are threatened by disadvantageous changes in the local climate [10]. This study is part of the first twelve-month trial release of juvenile turtles to test two potential future habitats, East Augusta (34°17’S) and Meerup (34°39'S), with cooler climatic conditions outside their known or suspected distribution as a response to climate change to decrease the chance of extinction of the species in the wild [2, 11, 17, 18]. These conservation actions are needed due to habitat fragmentation, agricultural use, a small geographic range of the turtle and climate change [2, 7]. As a reference site we used a wetland in the Moore River Nature Reserve. The reason for choosing Moore River Nature Reserve as a reference site was the successful introduction of 135 juvenile *P. umbrina* which suggested that the swamp life of Moore River Nature Reserve is suitable to support the Western Swamp Turtle [15]. The capacity of Twin Swamps Nature Reserve had reached the maximum of turtles, no translocations are taking place in the Ellen Brook Nature Reserve due to genetic management and to temporally secure individuals, and swamps in the Mogumber Nature Reserve were too dry during the time of collecting [2, Gerald Kuchling unpublished]. Previous evaluations of the suitability of translocation sites up to 300 km south of Perth in the Augusta/Walpole region were already theoretically tested to find suitable long-term habitats for *P. umbrina* [22]. We wanted to augment this abstract modelling of an optimal living area in the southwest of Western Australia by studying the supply of food in practice, by collecting aquatic invertebrates, tadpoles and fish to demonstrate the biomass and biodiversity of all three areas. It is nearly impossible to monitor turtles feeding under entirely natural conditions. For this reason we used the standard method of "stomach flushing" and subsequent analysis of the stomach contents [23, 24]. With this method, we had attempted to provide insights into prey choices of *P. umbrina.* Knowledge of diet and food resources was needed to assess the two wetlands in East Augusta and Meerup for assisted colonization. We tested the hypothesis that wetlands in the Moore River Nature Reserve, Meerup and East Augusta do not have big differences in biodiversity or biomass values. That is, do all three wetlands have enough variety of food and are therefore equally suitable living areas for *Pseudemydura umbrina*?

5. Material and Methods

5.1. Study animals and release sites

Test wetlands, where in total 35 captive-bred juveniles from Perth Zoo with a body mass of over 100 g were released, were the Moore River Nature Reserve at the Swan Coastal Plain, Meerup at the D´Entecasteaux National Park and East Augusta near the Scott River National Park, where they have not been known to occur naturally (twelve turtles in Meerup and East Augusta, eleven turtles in the Moore River Nature Reserve – one was not released there because of a missing eye). Turtles were marked by filing notches into their marginal scutes as well as with Trovan ® microchips. They were also fitted with a VHF radio-transmitter and a button temperature logger [2].

Turtles were released in the south-eastern part of the Moore River Nature Reserve which contains a system of seasonal clay swamps [15]. It is a widespread swamp area with thick bush vegetation. The two southern swamps, East Augusta and Meerup, are seasonal swamps with sand over clay base. They are characterized through a low canopy of bush vegetation (Figure 2) [2, 18].

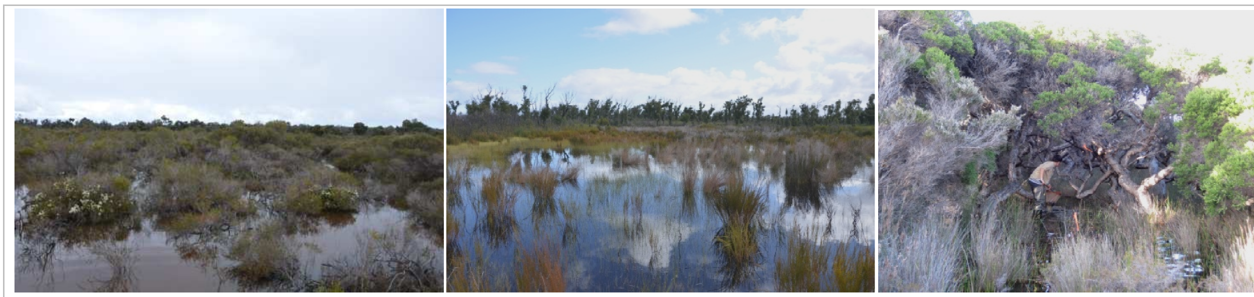

Figure 2: Swamps in Moore River Nature Reserve, Meerup and East Augusta in early spring (left to right).

5.2. Sample collection

Fifty-four macro-invertebrate samples (which included tadpoles and fish) in total were taken with a 50 cm x 50 cm x 50 cm triangular net during spring when all three wetlands carried water. The fieldwork was organised twice per wetland: in September and October in the Moore River Nature Reserve and in October and November in East Augusta and Meerup. Sampling time was adjusted according to weather conditions, water levels and the seasonal drying of swamps. Henceforth, the first collection of samples will be referred to as early spring, and the second collection of samples as late spring.

In each area, we took three biomass, three plankton and three benthic samples on each of the two sampling occasions (early and late spring). Depending on the type of sample different mesh width were used: plankton 53 µm mesh net, biomass 90 µm mesh net and benthic 250 µm mesh net. The net was swept one meter through the water 10 times per biomass sample and 15 times per plankton and benthic sample. The net was drawn along the wetland sediments, including under bushes and in open water. This action stirred up the mud and through that opaque water the net was swung to collect the raising invertebrates as well as tadpoles and fish. For plankton samples, we swung the net only from the surface top to the sediment without stirring up the mud [25]. Once caught, invertebrates and vertebrates were transferred to plastic bottles containing 100% ethanol for preservation. Preserved invertebrates were then identified in the laboratory.

5.3. Analysis

In the laboratory the biomass specimens were separated using three sieves (90 µm, 250 µm and 2 mm). Plankton were separated with 53 µm, 90 µm and 250 µm sieves and benthic samples with 2 mm, 500 µm and 250 µm sieves.

For taxonomic separation, portions of the samples were placed into a large petri dish and viewed under a microscope. The aim was to pick representative specimens of all species. For biomass samples we removed all invertebrates, tadpole and fish. The animals were separated into sixteen groups: Annelida, Nematoda, Turbellaria, Isopoda, Amphipoda, Ostracoda, tadpoles, fish, Acarina, Gastropoda, Diptera, Odonata, Hemiptera, Ephemeroptera, Trichoptera and Coleoptera.

For the biodiversity (including plankton and benthic) samples the number of animals was estimated and Isopoda, Amphipoda, Ostracoda, Diptera, Acarina, Annelida, Gastropoda, Odonata, Hemiptera, Ephemeroptera as well as Coleoptera were identified at the family level.

For biomass, animals were dried in the oven for 48 hours at a temperature of 60°C. The samples were allowed to cool down and were weighed to determine their dry mass using Chyo JL-180 balance which had an accuracy of 0.0001 g.

5.4. Stomach Flushing

To investigate prey items of the Western Swamp Turtle, stomach flushing was performed once on each turtle either concurrently with or some days after the sampling of invertebrates and vertebrates. The Animal Welfare approval allowed stomach flushing only once per turtle. The stomach flushing procedures were carried out by Gerald Kuchling.

For stomach flushing, turtles were caught through radio tracking. All turtles were flushed as soon as they were captured [26] and were released again within one hour. A modified pressure spray bottle was filled up with swamp water. The head of the turtle was manually restrained and the neck slowly extended to minimize the risk of injury [Gerald Kuchling unpublised]. With a blunt probe, the mouth was opened, and the hose was inserted carefully. It was important to place the tube in the stomach (not beyond the pylorus which could be realized through a resistance feeling). The technique aims to displace stomach contents out through the esophagus [23]. During flushing, a continuous flow of water was required to mobilize items stuck in the esophagus. Flushing was ended when mucus-coated mass appeared [Gerald Kuchling unpublished, 23]. We counted the number of items in the stomach to each taxonomic group.

6. Results

6.1. Biomass

In early spring the highest biomass value of vertebrates and invertebrates was the cumulative biomass of the Meerup site (Figure 3). The Moore River Nature Reserve exhibited the lowest biomass.

During the second collection period in late spring, the Moore River and Meerup testing areas showed similar biomass results. During late spring the cumulative biomass value of East Augusta was greater than twice that of the Meerup and Moore River testing areas (Figure 3). Notable was a large difference in the total biomass at East Augusta between early and late spring. There was no significant difference between the biomass results of all three areas during early and late spring (Kruskal Wallis test: chi-squared = 6.635, df = 5, p-value = 0.2492).

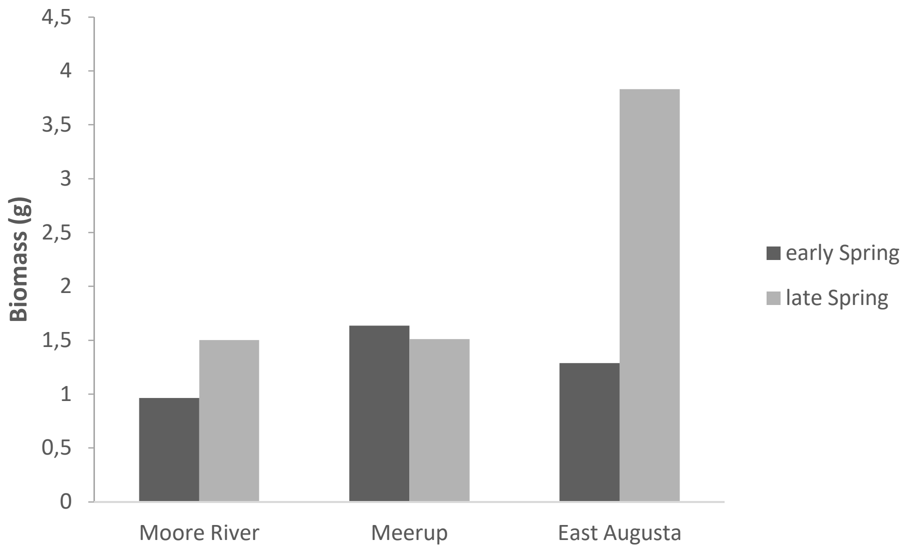

Figure 3: Biomass during collections in early (first collection) and late spring (second collection) in the Moore River Nature Reserve north of Perth, Meerup, at the D´Entecasteaux National Park and East Augusta, near the Scott River National Park.

In early spring the total biomass of Moore River comprised 0.5880 g vertebrates (tadpoles), 0.2602 g crustacea and 0.0959 g insects (Figure 4). The largest portion of the invertebrate biomass was the crustacean group Isopoda. Conchostraca and Culicidae were also a noticeable part of the biomass (Table 1). Tubellaria, Oligochaeta, Nematoda, Gastropoda and Acarina comprised a very small portion of the total biomass.

In late spring the Moore River Nature Reserve showed an overwhelming majority of insects, followed by vertebrates and crustacea (Figure 4). During late spring in the Moore River Nature Reserve, the largest biomass value was Insecta, belonging mostly to the family Notonectidae (Hemiptera) (Table 1). The vertebrates comprised the second largest part of the total biomass. This was more than the total biomass value of early spring in the Moore River Nature Reserve. There was an increasing number of Corixidae, dragonflies, damselflies and Isopoda. In the Moore River Nature Reserve, the collecting dates showed a clear increase in the insect biomass value from early to late spring. A striking decrease in the total biomass value was obvious in the three categories of vertebrates, crustacea and Acarina (Table 1, Figure 4).

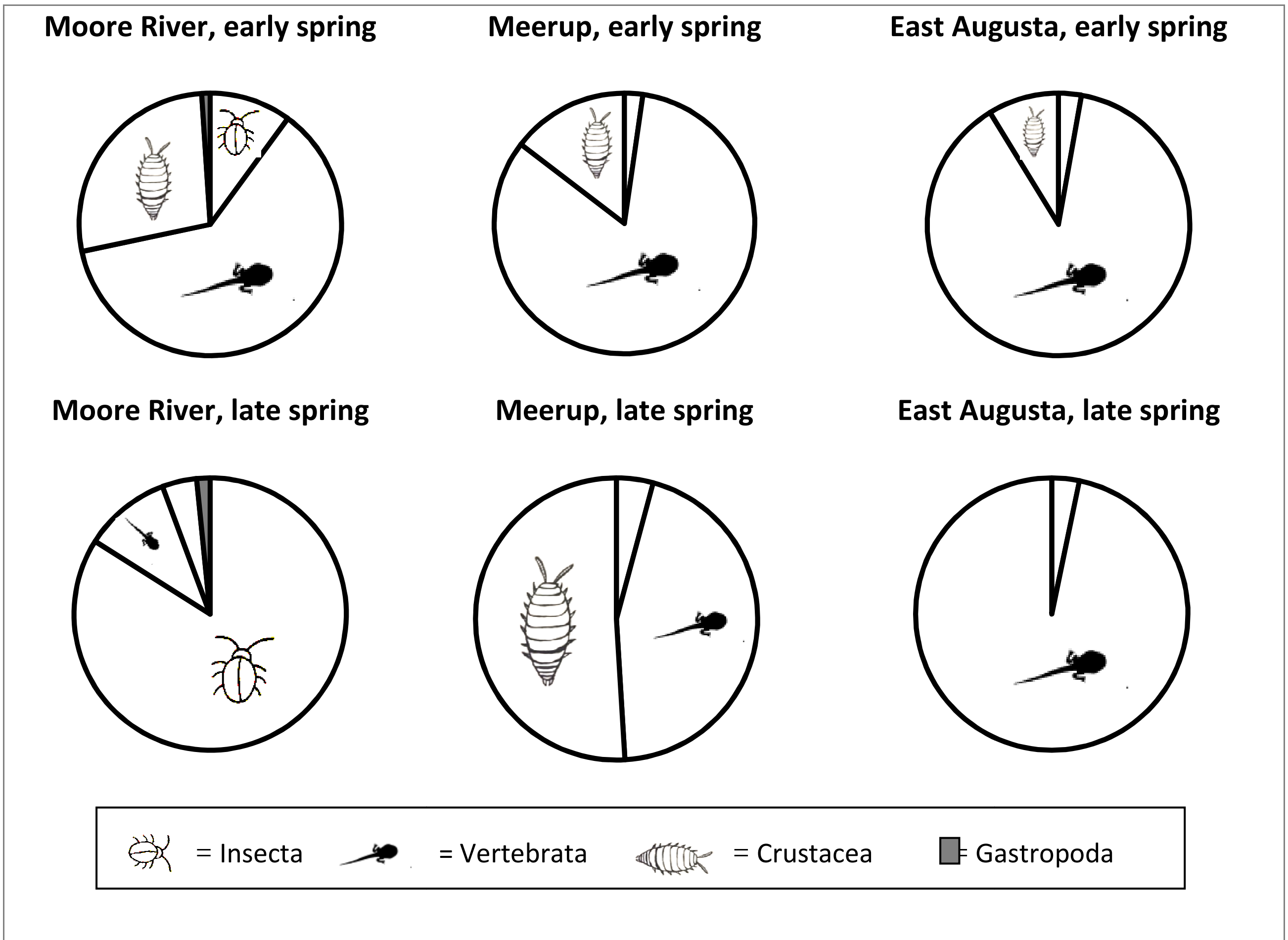

Figure 4: Percentage of Insecta, Vertebrata, Crustacea and Gastropoda in the biomass values (g) of Moore River Nature Reserve, Meerup and East Augusta in early and late spring.

The total biomass of Meerup in early spring comprised 1.3559 g vertebrates and 0.2753 g invertebrates, mainly composed of crustacea and insects (Figure 4). Of the vertebrate component, tadpoles comprised the majority and fish the minority. The remaining 17% of total biomass comprising invertebrates was largely consisted of Decapoda (Table 1). Insects comprised a notably small portion of the total biomass.

In late spring the biomass value of crustacea and insects increased (Figure 4). The invertebrate component of the total biomass results of Meerup in the late spring was characterized by a large quantity of Decapoda. The vertebrates, including tadpoles and fish, decreased during the second collection. Of the vertebrate component of the total biomass, a large quantity of tadpoles and small quantity of fish were observed (Table 1).

Table 1 Biomass value (g) of Moore River Nature Reserve, Meerup and East Augusta in early and late spring.

Order	Group	Moore River, early spring	Moore River, late spring	Meerup, early spring	Meerup, late spring	East Augusta, early spring	East Augusta, late spring
Insecta	Damselflies	0.0080	0.0315	0.0073		0.0113	0.0039
	Dragonflies	0.0001	0.1409				
	Coleoptera larvae	0.0055	0.0027	0.0107	0.0109	0.0001	
	Coleoptera adults		0.0015	0.0012	0.0045	0.0121	0.0103
	Ephemeroptera	0.0005	0.0001				
	Hemiptera-Notonectidae	0.0179	0.9673				0.0895
	Hemiptera-Corixidae	0.0011	0.0929	0.0013	0.0159	0.0024	0.0077
	Hemiptera-others	0.0001	0.0009				
	Trichoptera		0.0006	0.0007	0.0217	0.0003	0.0074
	Culicidae	0.0332					
	Chironomidae	0.0049	0.0054	0.0140	0.0101	0.0073	0.0051
	Ceratopogonidae	0.0003	0.0006			0.0006	0.0002
	Diptera-others	0.0245	0.0004	0.0001	0.0001		0.0004
Crustacea	Amphipoda			0.0013	0.0013	0.0061	0.0001
	Conchostraca	0.0478	0.0101				
	Decapoda			0.2372	0.7676	0.1062	
	Isopoda	0.2124	0.0502				
Gastropoda	Molluscs	0.0099	0.0238				
Arachnida	Hydracarina	0.0007	0.0003	0.0001	0.0015	0.0001	0.0004
Turbellaria	Flatworms	0.0070	0.0146		0.0001	0.0004	
Oligochaeta	Annelida	0.0022	0.0032	0.0004	0.0003	0.0018	0.0014
Nematoda	Nematoda	0.0004	0.0012	0.0016	0.0007	0.0004	0.0003
Vertebrata	Tadpoles	0.5880	0.1534	1.2670	0.5776	1.1358	3.7017
	Fish			0.0889	0.0991	0.0016	0.0011
Total Biomass		**0.9643**	**1.5017**	**1.6354**	**1.5121**	**1.2887**	**3.8299**

In early spring in East Augusta, the biomass was composed of 1.1374 g vertebrates and 0.1484 g invertebrates. The invertebrate portion of the total biomass comprised mainly crustacea and insects (Figure 4).

In late spring, East Augusta biomass had a total majority of vertebrates and minority of invertebrates, including mostly insects. During late spring, a threefold increase of the total biomass value of tadpoles was observed (Table 1). Within the invertebrate group the Hemiptera family, Notonectidae had a notably large biomass value. Insects and Acarina quantities present within the total biomass value for East Augusta decreased from early to late spring. All invertebrate groups experienced an increase in biomass between first and second survey, with the exception of Oligochaeta, Turbellaria and Crustacea. The biomass of Crustacea has declined by three quarters between early and late spring. The vertebrates' biomass value increased in East Augusta during the second collection, in comparison with the Moore River Nature Reserve and the test swamp in

Meerup in which the biomass of vertebrates experienced a drop during the second survey. Additionally, the biomass value for insects in Moore River was extremely high in comparison with the two southern areas.

6.2. Biodiversity

The northern study area, Moore River Nature Reserve, had the highest biodiversity of all three areas. In Moore River, we also found the highest number of families that were unique among the three test swamps (Table 2). The number of individuals of the family Chironomidae (Diptera), was extraordinarily high in Moore River, Meerup and East Augusta.

Regarding families, the highest number was observed in Moore River Nature Reserve where the late spring samples (with 28 different families) had more families than the early spring samples with 27 different families. This also occurred in Meerup where the earlier collection showed 21 different families and the later collections 22 different families. East Augusta had the fewest families, and was the only area that had more families in the first sampling period with 20 different families than in the second with 19 different families. The data showed a strikingly high number of insect families in each of the three wetlands. Gastropoda were only present in Moore River and East Augusta. Polychatea were found only in the southern swamps of East Augusta.

Table 2 Families present (x) in Moore River Nature Reserve, Meerup at the D´Entecasteaux National Park and East Augusta, near the Scott River National Park.

Group	Family	Moore River	Meerup	East Augusta
Polychaeta	Aeolosomatidae			x
Arachnida	Arrenuridae	x		
	Aturidae		x	x
	Hydryphantidae		x	
	Limnesiidae	x	x	
	Paristiformes	x	x	x
	Pezidae			x
	Pionidae	x		
	Unionicolidae		x	
Crustacea	Amphisopodidae	x		
	Ceinidae		x	x
	Limnadiidae	x		
	Lynceidae	x		
	Parastacidae		x	x
	Perthidae		x	x
Gastropoda	Ancylidae			x
	Glacidorbidae	x		
	Planorbidae	x		
Insecta Coleoptera	Dytiscidae	x	x	x
	Haliplidae	x		
	Hydraenidae	x		

	Hydrophilidae	x	x	x
	Scirtidae	x		x
Insecta Diptera	Ceratopogonidae	x	x	x
	Chironomidae	x	x	x
	Culicidae	x		
	Muscidae	x		x
	Tipulidae	x	x	x
Insecta Ephemeroptera	Baetidae	x		
Insecta Hemiptera	Corixidae	x	x	x
	Hebridae	x	x	
	Hydrometridae		x	
	Mesoveliidae	x		
	Notonectidae	x		x
	Veliidae	x	x	
Insecta Odonata	Aeshnidae	x		x
	Argiolestidae		x	x
	Corduliidae	x		
	Lestidae	x	x	x
Insecta Trichoptera	Ecnomidae		x	
	Leptoceridae	x	x	x
Nematoda	Nematoda	x	x	x
Oligochaeta	Enchytraeidae	x	x	x
	Naididae	x	x	x
	Opisthopora	x		x
	Phreodrilidae			x
	Pristinidae	x	x	x
Turbellaria	Turbellaria	x	x	
Vertebrata	Tadpole	x	x	x
	Fish		x	x

6.3. Stomach Contents

Ten turtles were stomach flushed in the Moore River Nature Reserve, five of them in September 2016, two in October 2016 and three in November 2016 (Table 4). Crustacea was the main prey item, the second most abundant was Coleoptera (Figure 6). Ostracoda were the most common food item at Moore River Nature Reserve (Figure 5). The second most prey item invertebrate group was Isopoda (Amphisopodidae). Another prey item invertebrate group was Cladocera. The turtles in Moore River Nature Reserve had twelve different groups of animals in their stomachs (additional to the already mentioned ones are: Coleoptera – Dytiscidae, Diptera – Culicidae, Copepoda, Hemiptera – Corixidae and Veliidae, Odonata – Corduliidae, Conchostraca – Limnadiidae, Turbellaria). The stomach flush results of turtles in all three areas showed that turtles consumed the most (number of animals and families) in the Moore River Nature Reserve.

In Meerup, stomach flushing was performed with six turtles, three in October 2016 and three in November 2016 (Table 4). Only four groups of animals were found in the stomachs of turtles in this swamp. Insecta (Trichoptera - Leptoceridae) were consumed in the greatest quantities, followed by the micro-invertebrate group Collembola (Insecta). Arachnida (Frontipodopsidae) and Coleoptera (Hydraenidae) were consumed additionally (Figure 6).

We stomach flushed eleven turtles in East Augusta, two in October 2016 and nine in November 2016 (Table 3). East Augusta had the second highest quantity of eaten individuals. The main prey items were Insecta, followed by Crustacea and Vertebrata. Of this testing area, seven groups of invertebrates and tadpoles were found in the stomachs of the turtles. Tadpoles were the outright majority, followed by Coleoptera (Dytiscidae), and Trichoptera (Leptoceridae) and Amphipoda (Amphisopodidae) (Figure 6). Additional consumed animals were: Odonata (Lestidae) and Crustacea (Perthidae).

Table 3 Stomach Flushing details of all flushed turtles in the three test sites Moore River Nature Reserve (MRNR), Meerup and East Augusta (EA).

Date	Time	Test site	Number of eaten animals	Turtle ID
18.09.2016	2.30pm	MRNR	11	1134
21.09.2016	5.25pm	MRNR	0	1099
21.09.2016	4.00pm	MRNR	64	1191
21.09.2016	4.30pm	MRNR	37	1170
21.09.2016	5.00pm	MRNR	13	1127
21.10.2016	2.30pm	MRNR	7	1115
21.10.2016	3.45pm	MRNR	5	1138
07.11.2016	3.00pm	MRNR	2	1029
07.11.2016	3.15pm	MRNR	30	1028
07.11.2016	4.00pm	MRNR	40	963
22.10.2016	3.15pm	Meerup	0	1193
22.10.2016	3.15pm	Meerup	0	1132
22.10.2016	3.00pm	Meerup	0	1141
22.11.2016	3.15pm	Meerup	10	1193
22.11.2016	3.45pm	Meerup	17	1135
22.11.2016	1.15pm	Meerup	0	1185
23.10.2016	1.00pm	EA	10	1128
23.10.2016	3.00pm	EA	6	1052
23.11.2016	12.45pm	EA	21	1123
23.11.2016	3.00pm	EA	8	1137
23.11.2016	3.30pm	EA	3	1184
23.11.2016	2.45pm	EA	5	1106
23.11.2016	2.30pm	EA	4	1089
23.11.2016	12.30pm	EA	4	1088
23.11.2016	12.30pm	EA	4	1190
23.11.2016	11.45pm	EA	10	1143
23.11.2016	12.30pm	EA	2	1139

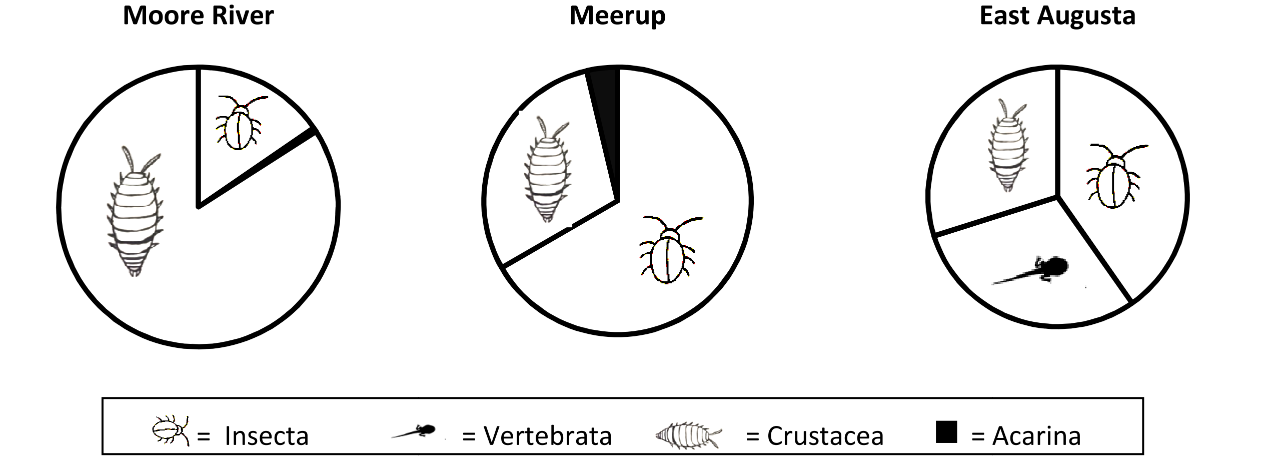

Figure 5: Prey item components in Moore River, Meerup and East Augusta.

Some turtles ate many invertebrates and tadpoles (maximum = 62 eaten animals per turtle) and some ate very little or none (minimum = 0 eaten animals per turtle) (Table 3 and 4). The main prey items of all tested turtles were Ostracoda, Isopoda, Cladocera and Coleoptera.

Table 4 Total number of stomach-flushed specimens (N_{Total}), number and percentage (in parenthesis) of full stomachs (N_{Full}), total number of prey items (n), mean number and standard deviation (S.D.) of prey items/stomach of adults of P. umbrina in Moore River, Meerup and East Augusta.

	Moore River	Meerup	East Augusta	Total
N_{Total}	10	6	11	27
N_{Full}	9 (90%)	2 (33.33%)	11 (100%)	22 (81.48%)
n	207	27	77	311
Mean ± S.D.	21± 20.98	4.5 ± 7.31	7 ± 5.37	11.52 ± 14.83

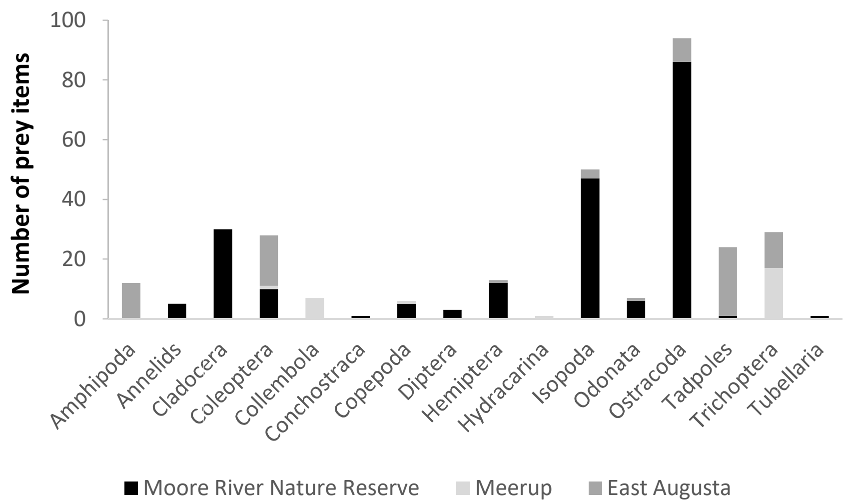

Figure 6: Number of prey items in each test site: Moore River Nature Reserve north of Perth, Meerup at the D´Entecasteaux NP and East Augusta, near the Scott River NP.

In observing the presence of various invertebrate groups and tadpoles in the stomach flush samples of *P. umbrina* at the three release sites, it was evident that turtles in the Moore River Nature Reserve consumed the largest variety of families (Figure 6). The diet of turtles in East Augusta largely consisted of tadpoles, but also included five other invertebrate families. Juveniles of *P. umbrina* had Amphisopodidae (Isopoda, Crustacea) in their stomachs, but no single Isopoda was found in any biomass or biodiversity sample in East Augusta. Meerup turtles had consumed a limited variety of families.

Coleoptera was the only group of invertebrates/vertebrates which were found in stomach contents of turtles in all of the three test swamps (Table 5). It was also the group which was eaten of the most of the flushed turtles, 10 of 27 reptiles had beetles as part of their diet. Nine turtles of all flushed ones had tadpoles in their stomach contents but just one of these turtles was in Moore River Nature Reserve. Eight of them were found in East August in late spring, that area which had the largest biomass value of tadpoles (3.7017 g in late spring, Table 1).

Table 5 Frequency of occurrence (%) of other prey taxa encountered in stomach contents of juveniles (juv.) of P. umbrina in Moore River, Meerup and East Augusta (both sampling periods combined).

Prey taxa	Moore River 10 (juv.)	Meerup 6 (juv.)	East Augusta 11 (juv.)	Total 27 (juv.)
Amphipoda			27.27	11.11
Annelida	20			7.40
Arachnida		16.67		3.70
Cladocera	40			14.81
Coleoptera	50	16.67	36.36	37.03
Collembola		16.67		3.70
Conchostraca	10			3.70
Copepoda	50	16.67		22.22
Diptera	20			7.40
Hemiptera	30		9.09	14.81
Isopoda	40		18.18	22.22
Odonata	10		9.09	7.40
Ostracoda	80		9.09	33.33
Tadpole	10		72.72	33.33
Trichoptera		33.33	27.27	18.52
Turbellaria	10			3.70

The main time in which we found the most of stomach contents in the turtles in the Moore River Nature Reserve was in early spring (September) (Table 3). Larger quantities of stomach contents were found during late spring (November) compared with mid spring (October). In East Augusta both months had similar data regarding feeding activity. In Meerup, the only stomach contents were in November during the second collection.

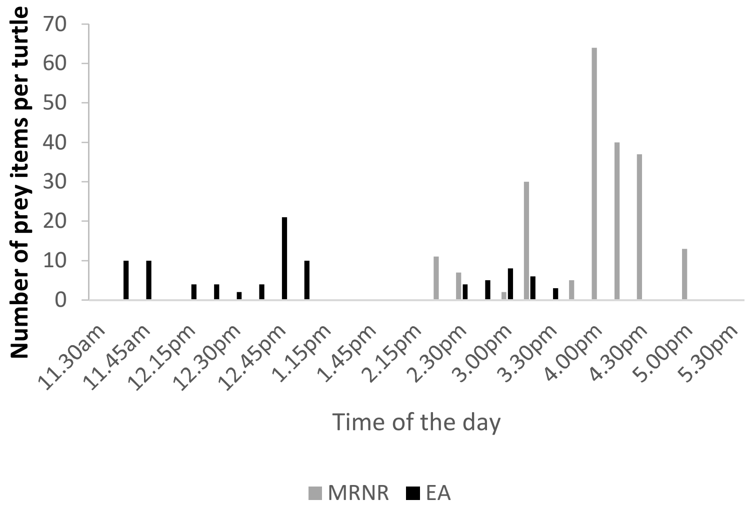

Figure 7: Number of prey items per turtle in the Moore River Nature Reserve (MRNR) and in East Augusta (EA).

The timeframe in which the Western Swamp Turtle ate the most in our study occurred between 2pm and 4.30pm (Figure 7). The peak of feeding was at 4.00pm. In East Augusta, stomach flushing was performed around midday and between 2.30pm and 3.30pm, whereby more stomach contents were found in the afternoon. The data of Meerup were not shown on Figure 7 due the small sample number. There was no significant correlation between the number of animals eaten and the time at which the stomach flush was performed (Kruskal Wallis test: chi-squared = 1.2222, df = 2, p-value = 0.5427).

7. Discussion

We maintain the hypothesis that there are no differences between Moore River Nature Reserve, Meerup and East Augusta in either biodiversity or biomass values.

7.1. Biomass

However, there are small differences regarding the total biomass of all three wetlands.

Firstly, the Moore River Nature Reserve had the lowest biomass of all three wetlands. This is because Moore River biomass value is mostly invertebrates (mostly insects) rather than vertebrates, in opposition to Meerup and East Augusta. Invertebrates will often have less mass than vertebrates (tadpoles and fish). Thus, the average invertebrate biomass was less in the two southern areas of Meerup and East Augusta than in Moore River.

Secondly, the rising temperatures and shrinking pools and the consequently concentrated aquatic invertebrates and vertebrates in Moore River and in East Augusta between early and late spring could be a reason for the increased biomass during late spring in these locations [Gerald Kuchling unpublished]. Moore River increased by 4.2°C and East Augusta by 3.3°C [20]. Additionally, aquatic invertebrates developed after the winter season. For this reason, invertebrates were not just larger but also more animals in later spring than in early spring leading to a higher biomass in late spring [Adrian Pinder unpublished]. Consequently, in East Augusta, with an annual rainfall of 856.0 mm in 2016 [20], the aquatic invertebrates and vertebrates were constrained in small water holes leading to higher biomass values. Therefore, Meerup had a higher water level in late spring compared to East Augusta. The data of the Bureau of Meteorology have shown that Meerup had slightly warmer climatic conditions than East Augusta [20]. The same situation was evident with the average daily maximum shallow water temperature [Alexandra Bouma unpublished]. Both areas had cooler median temperatures than the Moore River Nature Reserve during the collection times [20].

Thirdly, the vertebrate mass also increased in East Augusta in late spring, whereas in Moore River Nature Reserve and Meerup they did not. This may have been caused by the development cycle of different frog species, such that by late spring the tadpoles may already have developed into frogs [Gerald Kuchling unpublished].

Lastly, in Meerup, the temperature rose 4.9°C between early and late spring [20] which is the largest difference in temperature in all three test sites, even though the biomass in Meerup decreased in late spring. This may be due to an extended hydroperiod as a result of the high annual rainfall of 1443.5 mm in 2016 (median rainfall between 1970 and 2016 was 1193.7 mm) [20], meaning that the water level was higher in late spring. Therefore, the aquatic animals were more widely scattered.

7.2. Biodiversity

The Moore River Nature Reserve has higher biodiversity than Meerup and East Augusta. This might be attributable to warmer shallow water conditions [Alexandra Bouma unpublished] and the wet winter and spring in 2016 on the Swan Coastal Plain. The average annual rainfall for 2016 at the Moore River Nature Reserve was very high with 743.4 mm compared to the median rainfall in the years from 1970 till 2016 which was 631.4 mm. The high rainfall in Moore River in 2016 was unusual because it was also the highest annual rainfall since 1999 [20].

Fish were found in Meerup and East Augusta but none was found in Moore River. At both southern swamps, we identified the Salamanderfish (*Lepidogalaxias salamandroides)* and the Black-Stripe Minnow *(Galaxiella nigrostriata)* [Gerald Kuchling unpublished]. Decapoda such as crayfish, some of which can be predators of *P. umbrina* [27], were also found in Meerup and East Augusta. This is a potential risk for those populations and should be considered in further translocation activities [27].

7.3. Stomach contents

We also maintain the hypothesis that all three wetlands are having enough variety of food for *Pseudemydura umbrina*.

Differences in the number of flushed turtles in all three test swamps are due to the loss of radio-transmitters. Based on the information of stomach contents samples we did get, turtles ate the most (μ = 21 animals consumed per individual) in the Moore River Nature Reserve (Figure 6) which is closer to the natural habitat of *P. umbrina* [15]. Next came East Augusta and then Meerup.

In the Moore River Nature Reserve, juveniles of *P. umbrina* caught prey items mainly in early spring (September). This is possibly due to the warmer weather conditions in early spring which means they were more active. In Meerup and East Augusta, turtles ate more food during late spring than early spring. This is most likely also because the weather was warmer in these locations in late spring. Again, this leads to increase feeding activity. Some turtles have not had any food in their stomachs; this may be due to the cold weather conditions and time of day when sampling took place which might have discouraged feeding activity [Gerald Kuchling unpublished]. However, almost twice as many turtles were tested in Moore River (10 turtles) and East Augusta (11 turtles), then in Meerup (6 turtles). This could account the smaller number of families/prey items detected at Meerup.

Turtles require a certain water temperature to feed [19]. The main activity of *P. umbrina* occurs at the water temperatures between 26-28°C and turtles are inactive if it is cooler than 14°C and leave the water when it is warmer than 30°C [11, 13]. Feeding is therefore restricted to spring and early summer. When the water temperatures are too warm during the active period of the turtle, they select cooler microclimates in the shade (e.g. under bushes) or dig themselves into the mug [Gerald Kuchling unpublished].

During the wet season the Western Swamp Turtle spends most of its time in water, leaving only to bask, to move between swamps, to mate, or to find ideal aestivation or nesting sites [28]. Hatchlings fed at all times of the day during the wet season, but mostly between 2pm and 4pm, and they responded to warmer water temperatures by increasing their feeding activity [19]. This is why we took stomach flush samples mainly around midday, to test the main feeding time of *P. umbrina*. Since our results indicate that most food was consumed between 3pm and 4.30pm, future diet studies should concentrate to collect samples at this time of the day (Figure 7).

According to Gilbert, turtles do not exhibit significant prey selection [21]. Our results confirm this. The turtles ate a wide variety of live food (Figure 6). The food source Coleoptera was available in all of the three test swamps and was found in the stomachs of turtles from all three locations (Table 5). In the Moore River Nature Reserve, mostly Crustacea were consumed (Ostracoda and

Isopoda), in East Augusta primarily tadpoles were consumed, and in Meerup the major food consumed were insects (Trichoptera). No evidence was found regarding the selection of invertebrate families (Table 5). Thus, we maintain the hypothesis that all three wetlands have enough variety of food for *Pseudemydura umbrina*.There was a low quantity of Conchostraca consumed at the Moore River Nature Reserve compared to the studies of Gilbert in other nature reserves like Mogumber Nature Reserve, where turtles ate mostly this group [21].

We found Isopoda in the samples of two different turtles in East Augusta, but no single animal of this group has been collected during sampling in this wetland. Isopoda may be rare or hiding in structures (e.g. bushes) accessible to turtles but not to sampling nets.

Even though the Salamanderfish (*Lepidogalaxias salamandroides)* and the Black-Stripe Minnow *(Galaxiella nigrostriata)* were present in Meerup and East Augusta they appeared to be absent from the diet of *P. umbrina.* Therefore, the Western Swamp Turtle does not seem to count as a predator of the endangered *Lepidogalaxias salamandroides* [Gerald Kuchling, unpublished].

The availability of food is associated with the duration of the hydroperiod, which limits the time for feeding and the likelihood of surviving the aestivation period [19]. The daily energy requirements for a Western Swamp Turtle with a weight of 500 g (fasted and at rest) would be 3.8 kJ at a temperature of 20°C and 20 kJ at 30°C because of the increased cost of walking and swimming [28]. Turtles in other nature reserves (Ellen Brook Nature Reserve) feed primarily on invertebrates in the water column [21]. The feeding cost decreases towards the end of the wet season when the swamps shrink to small waterholes and turtles expend little effort catching prey [28]. Conversely, the costs of feeding increase at the beginning of the wet season if rainfall is irregular and turtles have to move from one marshland to another in order to obtain sufficient food but digestion is then constrained by low temperatures. This is potentially why the energy needs of *P. umbrina* will increase with climate change [28].

Climate change is predicted to decrease the annual rainfall and therefore the lifecycles of aquatic prey, restricting food availability [19]. This means that finding food requires more energy [21, 28]. With rising temperatures, the necessary energy requirements might not be enough, which could lead to a decreased survival rate during the aestivation period [19]. By 2030, the length of the hydroperiod may become much shorter in the current habitat of *P. umbrina* than in the southern wetlands [29]. This means that the hatchlings have less time to reach the necessary 18 g body mass to survive the forthcoming summer [10, 19]. The southern translocation wetlands are cooler and experience increased water levels earlier in the year compared with the northern sites [29]. Subsequently, this means that hatching will likely occur earlier in the year [10].

7.4. Assisted Colonization

Climate change may allow the southern sites to become more advantageous habitats for the turtle by 2070 [10]. Meerup in the D´Entecasteaux National Park is not only the coldest site, but presenting also the least convenient of all tested areas for the embryonic development of turtles [10]. This will, however, change in future and it is indeed a suitable wetland for assisted colonization regarding vegetation composition, current land use, site size and food availability [22].

The region of East Augusta is the most suitable habitat in terms of activity potential by 2030 [29]. There was also a high overall nest suitability predicted for East Augusta under 2070 climate [10]. The release site in East Augusta is very small and accessible, which is ideal for monitoring during the trial release but not for long term [2]. We found out that East Augusta had the least biodiversity but had some of the greatest biomass. Local traffic and adjacent private properties are a major threat regarding the establishment of a new population in this region [Gerald Kuchling unpublished]. Turtles may cover several kilometres to find a mating partner, a new habitat and an ideal place for aestivation [11], as such these conditions may prove problematic. To find an ideal site for *Pseudemydura umbrina*, an interaction of all necessary factors should be met. However, East Augusta was only a test site probably there will be an establishment of a population of *P. umbrina* at the adjacent Scott River National Park [Gerald Kuchling unpublished].

The comparative area, the Moore River Nature Reserve, which is on the Swan Coastal Plain North of Perth and already a translocation site, helped us to demonstrate the possibility of shifting the habitat of *P. umbrina* further south beyond the historical range [2, 10]. Theoretically, the southern coast of Western Australia would be the best for assisted colonization and would be the most promising solution to prevent the extinction of the species [22, 29]. Other studies have stated that food availability is the most important factor when considering future translocation sites [22]. Potential food availability determines turtle’s abundance [30]. Assisted colonization becomes more and more essential to *P. umbrina*, where investigations have shown that the most convenient wetlands will soon be several hundred kilometres south of the historical range of the species [22]. The first step is done, to test the practicability of assisted colonization in the field to cooler and moister areas [2]. In respond specifically to climate change impacts, this was the first known vertebrate species which was translocated through assisted colonization [5]. The present study demonstrated that food availability for *P. umbrina* will be sufficient at the southern sites. We have shown that Meerup and East Augusta can be suitable translocation sites which will offer good conditions for assisted colonization in 50-70 years, inside the potential life span of juveniles of *Pseudemydura umbrina* [Gerald Kuchling unpublished].

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9. Table of figures

10. List of tables