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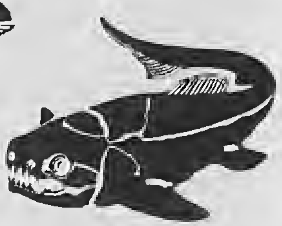

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Cover design: The four subjects symbolize the diversity of sciences embraced by the Royal Society of Western Australia. Mangles' kangaroo paw (*Anigozanthos manglesii*) and the numbat (*Myrmecobius fasciatus*) are the floral and faunal emblems of Western Australia, and stromatolites are of particular significance in Western Australian geology (artwork: Dr Jan Taylor). The Gogo Fish (*Mcnamaraspis kaprios*) is the fossil emblem of Western Australia (artwork: Danielle West after an original by John Long).

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Aquatic fauna and water chemistry of the mound springs and wetlands of Mandora Marsh, north-western Australia

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Abstract

The Mandora Marsh system, adjacent to Eighty-mile Beach, in the northwest of Western Australia supports many mound springs as well as several other permanent and seasonal wetlands. Although listed under the Ramsar Convention, little was known of the non-waterbird ecological values of the system. A survey of the water chemistry and aquatic fauna of the wetlands and springs was conducted in October 1999, which identified a new species of fish and bathynellid syncarid, occurrence of stygofauna, as well as a relatively diverse aquatic invertebrate fauna. Overall, the aquatic fauna is of considerable conservation value. The survey also identified pressures from cattle, feral camels and possible future developments adjacent to the Marsh, which could threaten the future ecological health of these mound springs if not managed appropriately.

Keywords: aquatic fauna, mound springs, wetlands, endemism, Mandora Marsh

Introduction

Mound springs and spring-swamps are known from several parts of the world but are most significant when located in deserts. Notable arid-zone systems are the artesian Nubian Aquifer in north-eastern Africa, the carbonate aquifers of eastern Nevada and the Great Artesian Basin (GAB) in north-eastern Australia (Ponder 1986, 2003; Nobel *et al.* 1998). The GAB underlies approximately one-fifth of Australia and is the best studied within that continent. However it is not the only spring fed system within Australia. There are many other smaller systems known to support permanent springs, including the Canning Artesian Basin and the North Kimberley Mounds in the north of Western Australia, and the Gnangara Groundwater Mound, a superficial aquifer overlying deeper confined aquifers in the south-west (Jasinska *et al.* 1996; Knott & Jasinska 1998; Kern *et al.* 2004). Permanent springs are particularly important because they provide long term, stable habitat in which aquatic fauna may survive periods of aridity. As a result, the aquatic fauna of such springs is often characterised by relictual species, with these populations representing remnants of a more widespread distribution in earlier geological times (Leys *et al.* 2003; Murphy *et al.* 2009). The springs may also contain locally endemic species (Ponder 1986). For instance, springs in Mexico and western parts of the United States are notable for their endemic aquatic invertebrate fauna (Ponder 1986; Erman 1998). Similarly, species have been found that are apparently restricted to springs, as reported for those on the edge of the GAB in South Australia (Mitchell 1985; Ponder 2003) and Queensland (Ponder & Clarke 1990), as well as those on the Gnangara Groundwater Mound in south-west Western Australia (Jasinska *et al.* 1996; Jasinska & Knott 2000) and in the Pilbara region of north-west Western Australia (Pinder *et al.* 2010).

Springs can occur in many physical settings, including that of mound springs that appear as low (1–3 m high) mounds from which water seeps. The mounds are formed over thousands of years by the deposition of fine clay brought to the surface by the water and by the long term accumulation of organic material derived from vegetation dependent on the water source. The types, modes of formation, physical, chemical and geological characteristics of artesian springs have been described by Ponder (1986). While many detailed studies have been conducted in South Australia (see Ponder 2002), there have been few comprehensive assessments of spring systems in other states, including Western Australia. The Mandora Marsh area, in the arid north-west of Western Australia, is characterised by numerous wetlands, the majority of which are well vegetated mound springs fed by artesian water. Little is known of the aquatic

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invertebrate fauna of the wetlands and mound springs of Mandora Marsh, but as noted by Kay *et al.* (1999), permanent springs would be expected to have high conservation importance because they support richer faunas than ephemeral water-bodies. Therefore, as part of a larger survey to document terrestrial fauna and flora, as well as wetland-dependent flora of the Marsh (see Graham 1999), the aquatic invertebrate fauna and water chemistry of a representative suite of springs and wetlands were sampled in October 1999. The aims of the aquatic sampling were to determine water quality, species composition, conservation significance and effects of current land use.

Study Area

The Mandora Marsh system (105 000 ha) lies within the La Grange South groundwater subarea of the Canning Basin, on the northern edge of the Great Sandy Desert. It is 140 km south-south-west of Broome, approximately 40 km inland from Eighty-mile Beach, and lies at the transition between the Pilbara and Kimberley regions (Fig. 1). It is part of a larger palaeo-drainage system that extends from the Northern Territory, through Lake Gregory in the eastern Kimberley, to Eighty-mile Beach on the west coast (Wyrwoll *et al.* 1986). Mandora Marsh is located on what is considered to be the palaeo-estuary for this system.

The climate of the region is semi-arid monsoonal with hot, wet summers (mean daily max./min. temperatures approximately 35/25°C) and warm, dry winters (mean daily max./min. temperatures approximately 28/12°C). Mean annual rainfall is 390 mm, however, as reported by Halse *et al.* (2005), annual rainfall is highly variable, ranging from ~ 30 mm in dry years to > 1500 mm in wet years, with rainfall mostly derived from cyclonic events which occur with a high frequency. Since 1910, 22 cyclones have crossed the coast in the vicinity of Eighty-mile Beach, and at least one cyclone occurred each year between 1994 and 1999; Cyclone Annette (1994), Gertie and Chloe (1995), Kirsty (1996), Rachel (1997), Thelma and Les (1998), and Gwenda (1999). When total annual rainfall exceeds 800 mm, widespread flooding usually results. These wet years appear to lead to substantial recharge of shallow groundwater aquifers around Mandora Marsh so that moderate rainfall in years following a major flood will also result in flooding (Halse *et al.* 2005).

The marsh is listed in the Directory of Important Wetlands of Australia (Environment Australia 2001), and also forms part of the Eighty-mile Beach Wetland of International Importance listed under the Ramsar Convention (Jones 1993). Ramsar nomination was based principally on the number of migratory birds utilising Eighty-mile Beach, but also for the landform values of Mandora Marsh (Jones 1993). It has subsequently been shown that the marsh periodically also supports very high waterbird values (Halse *et al.* 2005). The marsh consists of Mandora Lake (which is a large saline pan), samphire flats, an endoreic salt water creek (Salt Creek), salt and freshwater mound springs, and the underlying aquifer. Mandora Lake (known as Walyarta in the traditional Karajarri language) forms a broad, open basin up to 10 km wide that holds shallow water (< 0.5 m) following substantial rainfall in the catchment.

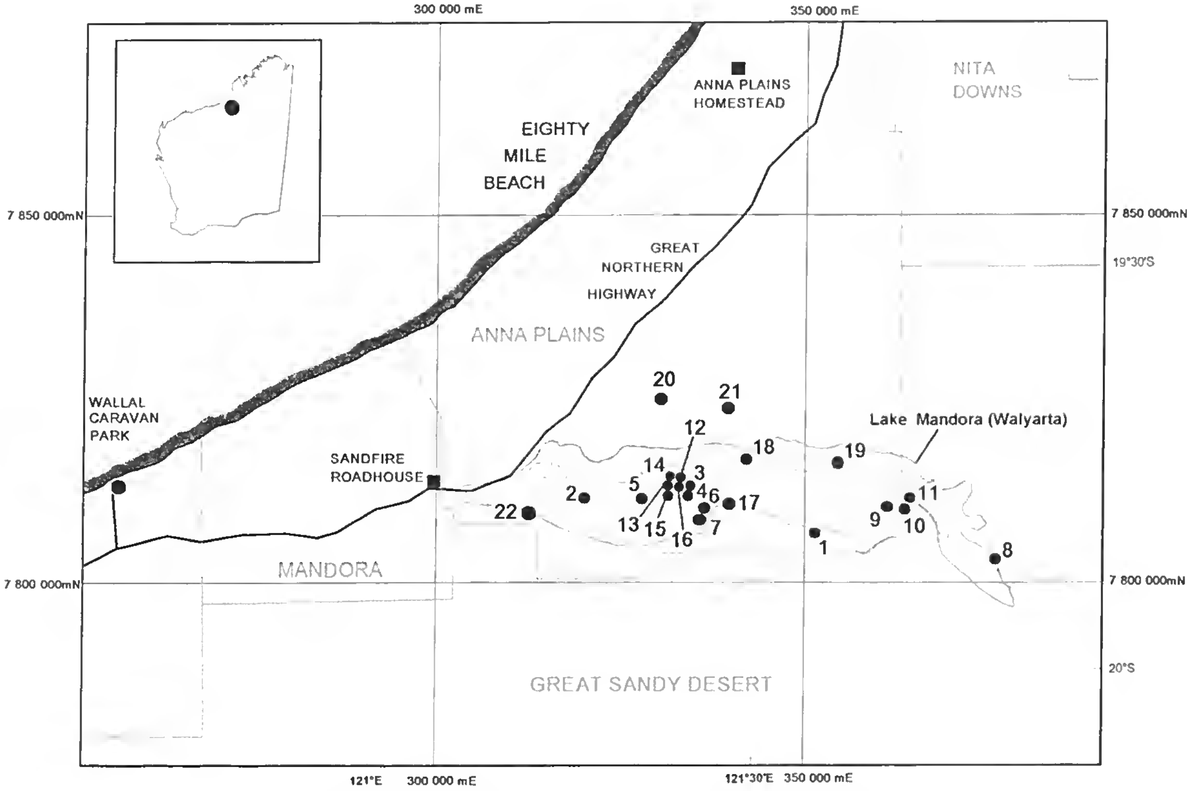

Figure 1. Location of sampling sites (numbered) within Mandora Marsh. Refer to Table 1 for key to site numbers.

Episodic inundation to a depth of ca. 1 m occurs when cyclones pass close to the lake. The area around the lake supports samphire vegetation (*Halosarcia indica* [Willd.] Paul G.Wilson, *H. halocnemoides* [Nees] Paul G. Wilson and *Frankenia* sp.) and *Sporobolus virginicus* (L.) Kunth grassland. Salt Creek, a small permanent saline creek seeps into the eastern end of Mandora Lake and features the most inland occurrences of mangroves (white mangrove, *Avicennia marina* [Forssk.] Vierh.) in Western Australia. The creek is 10–20 m wide, 1–2 m deep, 5 km long and is supplied by groundwater discharge and saline seeps from adjacent claypans.

Scattered along Mandora Marsh is a series of permanent springs that form mound springs and small swamps. Eil Eil Spring, also referred to as Mandora Soak (Jones 1993) and Mandora Swamp (Wyrwoll *et al.* 1986), is the most spectacular of these spring-swamps. It takes the form of a 'raised bog' rising 2 m above the surrounding country and supports a 20 m high forest of silver cadjeput, *Melaleuca argentea* W. Fitzg. over a tussock grassland of *Fimbristylis ferruginea* Vahl (Wyrwoll *et al.* 1986). A 3.3 m thick accumulation of organic sediment overlies a sand substrate, and carbon dating suggests it began accumulating 7000 yrs B.P. (Wyrwoll *et al.* 1986). Other plants occurring in the spring-swamps include dragon flower trees, *Sesbania formosa* (F.Muell.) N.T. Burb., the rush *Schoenoplectus litoralis* (Schrad.) Palla, the bulrush *Typha domingensis* Pers. and the mangrove fern *Acrostichum speciosum* Willd. Cyclonic winds are known to physically damage the vegetation in the spring-swamps. For example, Cyclone Annette crossed the coast at Mandora Station in December 1994, with wind speeds of 217 km hr^{-1} (Australian Bureau of Meteorology) and de-crowned *Sesbania* trees in Saunders Spring (Storey pers. obs.). Surface water tends to be limited in the springs, typically consisting of small areas of shallow water (5–10 cm) amongst the vegetation and organic debris on top of the mound spring, and a shallow (~ 20 cm deep) moat around the perimeter of the mound.

Methods

A total of 22 sites (Table 1) were sampled between the 18th and 21st of October, 1999. Sites were selected with the emphasis on mound springs, but to help interpret the significance of the mound-spring data, sampling was also undertaken at Mandora Lake, saline springs feeding Salt Creek, a seasonal claypan (Coolabah Claypan), and the underlying aquifer accessed by collection at outflows from windmills on bores/wells (Friday and Lyngett Well). At each site, a basic suite of water chemistry variables was measured *in situ* using a Yeo-Kal Model 611 multiprobe water quality analyser. Variables included temperature, pH, dissolved oxygen (DO % saturation), total dissolved solids (TDS g L^{-1}) and electrical conductivity (mS cm^{-1}) (Table 1). Water samples were also collected from 12 of the sites for more comprehensive laboratory analysis of water chemistry, *i.e.* total soluble nitrogen (TN), nitrate (N-NO$_3$), ammonia (N-NH$_3$), total soluble phosphorous (TP), soluble reactive phosphorus (PSR), ionic composition, total soluble iron (Fe), silica (SiO$_2$), colour (TCU), alkalinity, hardness and turbidity (NTU). Samples for nutrient analysis were pre-filtered in the field through

Table 1

Sites sampled in Mandora Marsh, grouped by wetland type, with GPS-determined latitudes and longitudes, indicating if sampled for a basic or comprehensive suite of water chemistry variables, and if aquatic macro- and/or microinvertebrates were sampled.

Site no.	Type	Site name	Latitude S	Longitude E	Basic water chemistry	Detailed water chemistry	Macro-inverte-brates	Micro-inverte-brates
1	Claypan	Coolabah Claypan	19° 48' 50"	121° 30' 38"	✓	✓	✓	✓
2	Main lake	Mandora Lake (Walyarta)	–	–	✓	–	–	–
3	Mound spring	Fern Spring	19° 46' 01"	121° 23' 29"	✓	✓	✓	✓*
4	Mound spring	Melaleuca Spring	19° 46' 15"	121° 23' 15"	✓	✓	✓	✓
5	Mound spring	Saunders Spring	19° 47' 05"	121° 20' 15"	✓	✓	✓	✓*
6	Mound spring	Little Eil Eil Spring	19° 47' 40"	121° 26' 33"	✓	✓	✓	✓*
7	Mound spring	Eil Eil Spring	19° 47' 53"	121° 26' 49"	✓	✓	✓	✓*
8	Mound spring	Linear Spring	19° 49' 07"	121° 37' 53"	✓	✓	✓	–
9	Mound spring	Top Spring	19° 48' 48"	121° 36' 52"	✓	✓	✓	–
10	Mound spring	Sump 300m from Top Spring	–	–	✓	–	–	–
11	Mound spring	Sporobolus Spring	19° 48' 18"	121° 37' 30"	✓	–	–	–
12	Mound spring	Stockyard Main Spring	19° 45' 34"	121° 23' 06"	✓	–	–	–
13	Mound spring	Small spring with mangrove at stockyard	19° 45' 34"	121° 23' 06"	✓	–	–	–
14	Mound spring	Mangrove/*Typha* spring 100m W of stockyard	19° 45' 34"	121° 23' 06"	✓	–	–	–
15	Mound spring	Small spring 200m ESE of stockyard	19° 45' 34"	121° 23' 06"	✓	–	–	–
16	Mound spring	Spring with *Typha* and moat 150m W of stockyard	19° 45' 34"	121° 23' 06"	✓	–	–	–
17	Riverbed spring	Bretts Spring	19° 47' 23"	121° 27' 06"	✓	✓	✓	✓
18	Salt creek	Salt Creek Claypan Spring	19° 44' 03"	121° 28' 41"	✓	✓	✓	✓
19	Salt creek	Salt Creek Stromatolite Pool	19° 44' 36"	121° 32' 29"	✓	–	–	–
20	Windmill	Friday Well (bore)	19° 42' 44"	121° 20' 24"	✓	✓	✓	✓
21	Windmill	Lyngett Well (bore)	19° 42' 27"	121° 27' 42"	✓	✓	✓	✓
22	Windmill	Coolgardie Well	19° 47' 15"	121° 13' 25"	✓	–	–	–

* Denotes microfauna was also extracted from a shallow core sunk into the peat.

a 0.45 µm Millipore™ filter. For interpretations of biological significance, concentrations of major cations and anions were converted to milliequivalents (meq L^{-1}) prior to statistical analysis. The concentration of bicarbonate (and carbonate) ions was estimated as the difference between the equivalent sum of the cations and that of chloride and sulphate ions.

Aquatic macroinvertebrates and microinvertebrates were each sampled at 12 of the 22 sites, with both components sampled at 10 sites (Table 1). Macroinvertebrates were sampled using a standard 250 µm mesh net to kick/sweep over a discontinuous 50 m distance, with the aim of maximising species collected. Microinvertebrates (Protozoa, Rotifera, Copepoda, Ostracoda & Branchiopoda) were sampled using a 53 mm mesh plankton net passed through the water column without disturbing benthic sediments. Sampling the mound springs was difficult due to the limited area and shallow nature of surface water, with sampling conducted in shallow pools on the top of the mounds and in the surrounding moat. To collect invertebrates from the groundwater aquifer, plankton nets were suspended under the outflow pipes of windmills. Nets were left in place for at least 24 hrs. Hyporheic fauna was sampled at four of the mound springs. This was achieved by extracting a one metre deep core of the consolidated peat using a 40 mm diameter PVC pipe corer. The resultant hole was allowed to fill with porewater and was then pumped through a 110 mm net using a bilge pump, and the material retained for sorting. All samples were fixed in 5% formalin.

Fish were collected opportunistically throughout the study area by a combination of sweep (FBA pond net with 1 mm mesh aperture) and cast netting (213 cm diameter net, with 2 cm stretched mesh). Specimens that could not be identified in the field were fixed in 5% formalin and forwarded to the Western Australian Museum (Dr Gerry Allen) and/or the Museum of the Northern Territory (Dr Helen Larson) for identification.

Analysis

Relationships between sites in terms of physico-chemical characteristics and fauna assemblages were analysed using multivariate procedures in the PRIMER (v6) software package (Clarke & Gorley 2006). Sites were assigned to categories according to salinity (as TDS; < 1 g L^{-1}, 1 – 4.5 g L^{-1}, > 4.5 g L^{-1}). Mutually correlated physico-chemical variables were identified using Spearman rank correlation (SPSS software version 17.0) and strongly correlated ($\rho \geq 0.95$) variables were excluded from further analyses: electrical conductivity (µS cm^{-1}; correlated with salinity g L^{-1}), hardness (mg L^{-1}; correlated with Ca^{+}) and Mg^{2+} (correlated with salinity, hardness and ion equivalents Ca, K and SO_4). Physico-chemical data were transformed where appropriate and normalised as recommended for multivariate analysis on mix-type variables (Clarke & Warwick 2001). Physico-chemical data and invertebrate presence/absence data were examined using both hierarchical agglomerative clustering (UPGMA) and non-metric multidimensional scaling (MDS) ordination (Clarke & Gorley 2006). Cluster and ordination based on Euclidean distance was used in preference to Principle Components Analysis (PCA) for physico-chemical data so that the hypotheses of differences in habitat types could be tested using the ANOSIM ($p < 0.05$) procedure in PRIMER. Cluster and ordination based on Bray-Curtis similarity matrices (Bray & Curtis 1957) was used to analyse species data, with infrequently occurring species (species occurring in < 5% of samples) omitted to avoid 'low-occurrence' taxa having a disproportionate effect on the results (Gaugh 1982; Belbin 1995). The SIMPROF (similarity profile) routine within PRIMER was used to test the significance ($p < 0.05$) of CLUSTER site groupings while ANOSIM was used to test the significance ($p < 0.05$) of the separation of salinity groupings in MDS ordination space. Similarity percentage analysis (SIMPER) was used to determine those species contributing most to the similarity/dissimilarity between sites. Similarity matrices from physico-chemical and species data were correlated using RELATE analysis. The relationship between abiotic and biotic data was further assessed using the BIOENV routine within PRIMER to calculate the smallest subset of physico-chemical variables that explained the greatest percentage of variation in the taxa ordination pattern, as measured by Spearman rank correlation (ρ) (Clarke & Warwick 1998). Unless indicated, default values or procedures otherwise recommended by Clarke and Gorley (2006) were employed for PRIMER routines.

Results

Physico-chemistry

Wetlands varied in several physico-chemical parameters. Salinities ranged from fresh (< 1 g L^{-1}) at Coolabah Claypan, Bretts Spring and Top Spring to saline (27.2 g L^{-1}) at Mandora Lake, Salt Creek Claypan Spring (38.5 g L^{-1}) and Salt Creek Stromatolite Pool (> 60 g L^{-1}) (Table 2). The mound springs ranged from fresh to brackish (ca. 0.5–5.8 g L^{-1}), with the brackish sites tending to be lower in the landscape and closer to the lake. Analyses of ionic composition indicated Na and Cl to be the dominant ions in most waters (Table 2), accounting for 60–87 % of the total cation and anion equivalence (Table 3). The exceptions were the freshwater Linear Spring, Top Spring and Coolabah Claypan, where HCO_3^- was the dominant cation. At Coolabah Claypan, HCO_3^- constituted 72 % of the anion equivalence. The dominance or sub-dominance of HCO_3^- and the sub-dominance of Ca^{2+} suggested ionic composition of waters at most sites to be influenced by limestone aquifers rather than accumulation and concentration of marine derived salts alone. Mg^{2+} and SO_4^{2-} were subdominant in waters with TDS ≥ ~ 5 ‰ and SO_4^{2-} was also relatively high at Bretts Spring (Tables 2 and 3). The Ca^{2+}:$HCO3^-$ ratio was typically < 1, but higher ratios were recorded for Fern Spring (1.3) and Salt Creek Claypan Spring (4.3) (Table 3). The Na^+:Cl^- ratios typically ranged from 1.0 to 1.5, but with a ratio of 2.1 at Coolabah Claypan.

In most instances waters were alkaline (pH 7.21–9.61). The open, shallow and unvegetated sites (*i.e.* Salt Creek Claypan Spring, Mandora Lake, Saunders Spring and Melaleuca Spring) had high daytime water temperatures ranging from 33.7 °C to 36.3 °C. When combined with high nutrient levels, as at Saunders Spring (5.2 mg L^{-1} TN, 0.71 mg L^{-1} TP) and Melaleuca Spring (17 mg L^{-1} TN, 2.0 mg L^{-1} TP), this often resulted in extremely high

Table 2

Physico-chemical analyses for each of the 22 sites sampled in Mandora Marsh.

Site no.	Site name	Temp °C	pH [H^+]	DO %	EC mS cm^{-1}	TDS g L^{-1}	Na mg L^{-1}	Ca mg L^{-1}	K mg L^{-1}	Mg mg L^{-1}	Cl mg L^{-1}	HCO_3^{A} mg L^{-1}	SO_4 mg L^{-1}
17	Bretts Spring	30.3	9.17	136.0	1335	0.74	294	28	9	12	390	132	107
1	Coolabah Claypan	27.1	8.58	101.2	303	0.14	70	29	12	8	52	240	3
7	Eil Eil Spring	29.1	7.60	49.4	2541	1.4	539	72	20	37	660	488	191
3	Fern Spring	26.0	7.46	85.7	10500	5.78	1910	201	82	123	2900	461	766
20	Friday Well (bore)	33.6	7.05	70.5	2480	1.46	384	105	82	61	600	521	172
8	Linear Spring	20.6	8.41	99.9	1105	0.62	187	54	20	31	210	408	61
6	Little Eil Eil Spring	25.3	7.46	31.3	2112	1.22	446	67	17	29	620	350	112
21	Lyngett Well (bore)	28.0	7.21	47.6	2366	1.30	463	74	61	40	570	507	206
4	Melaleuca Spring	36.3	9.61	>200.0	7342	4.71	1750	96	63	59	2500	344	538
18	Salt Creek Claypan Spring	34.5	8.44	>200.0	57400	38.50	14700	286	1740	608	21000	200	7320
5	Saunders Spring	35.8	9.21	>200.0	2200	1.27	353	48	26	24	460	237	169
9	Top Spring	19.1	8.32	106.0	975	0.54	178	35	12	17	180	320	42
22	Coolgardie Well	39.3	7.76	52.9	907	0.49	–	–	–	–	–	–	–
2	Mandora Lake (Walyarta)	33.7	8.84	108.4	42200	27.20	–	–	–	–	–	–	–
19	Salt Creek Stromatolite Pool	35.6	7.81	>200.0	>80000	>60.00	–	–	–	–	–	–	–
11	Sporobolus Spring	19.4	8.66	92.8	2099	1.25	–	–	–	–	–	–	–
12	Stockyard Main Spring	24.6	8.85	134.9	6825	4.39	–	–	–	–	–	–	–
13	Small mangrove spring	21.9	7.89	30.8	6000	3.83	–	–	–	–	–	–	–
14	Spring100m W of stockyard	26.8	7.97	55.0	12300	7.02	–	–	–	–	–	–	–
15	Spring 200m ESE of stockyard	25.4	6.81	18.0	6333	4.04	–	–	–	–	–	–	–
16	Spring 150m W of stockyard	22.7	8.15	64.4	7711	5.02	–	–	–	–	–	–	–
10	Sump 300m from Top Spring	18.1	8.14	69.7	1257	0.72	–	–	–	–	–	–	–

> Denotes *in situ* value outside the measurable range for hand–held field meters;

^{A} Estimated from the difference between the equivalent sum of the cations and that of $Cl^- + SO4^{2-}$.

Table 2 continued

Site no.	Site name	Alkalinity mg L^{-1}	Hardness mg L^{-1}	Turbidity NTU	Colour TCU	Fe mg L^{-1}	SiO_2 mg L^{-1}	TN mg L^{-1}	N-NO_3 mg L^{-1}	N-NH_3 mg L^{-1}	TP mg L^{-1}	PSR mg L^{-1}
17	Bretts Spring	100	120	4.1	21	<0.05	19	0.4	0.07	0.04	0.05	0.04
1	Coolabah Claypan	170	100	5.2	27	0.06	8	2.2	<0.02	0.02	0.10	<0.01
7	Eil Eil Spring	335	330	12.0	170	<0.05	95	2.7	<0.02	0.14	0.15	0.06
3	Fern Spring	243	1000	2.5	160	0.13	40	1.4	0.03	0.02	0.21	0.21
20	Friday Well (bore)	333	520	<1.0	8	<0.05	66	13.0	9.70	0.02	0.02	0.02
8	Linear Spring	308	260	48.0	95	<0.05	100	5.2	0.15	<0.02	0.67	0.03
6	Little Eil Eil Spring	225	290	2.2	110	<0.05	54	2.0	0.04	0.31	0.49	0.27
21	Lyngett Well (bore)	355	350	<1.0	6	0.30	75	16.0	16.00	0.03	0.04	0.04
4	Melaleuca Spring	268	480	46.0	270	0.08	36	17.0	0.06	0.14	2.00	0.04
18	Salt Creek Claypan Spring	240	3200	6.4	7	<0.50	29	2.8	1.20	0.29	0.06	0.03
5	Saunders Spring	198	220	32.0	66	<0.05	3	5.2	0.11	0.02	0.71	0.01
9	Top Spring	243	160	16.0	120	<0.05	77	5.4	0.05	0.02	0.58	0.04
22	Coolgardie well	–	–	–	–	–	–	–	–	–	–	–
2	Mandora Lake (Walyarta)	–	–	–	–	–	–	–	–	–	–	–
19	Salt Creek Stromatolite Pool	–	–	–	–	–	–	–	–	–	–	–
11	Sporobolus Spring	–	–	–	–	–	–	–	–	–	–	–
12	Stockyard Spring	–	–	–	–	–	–	–	–	–	–	–
13	Small mangrove spring	–	–	–	–	–	–	–	–	–	–	–
14	Spring 100m W of stockyard	–	–	–	–	–	–	–	–	–	–	–
15	Spring 200m ESE of stockyard	–	–	–	–	–	–	–	–	–	–	–
16	Spring 150m W of stockyard	–	–	–	–	–	–	–	–	–	–	–
10	Sump 300m from Top Spring	–	–	–	–	–	–	–	–	–	–	–

< Denotes value less than analytical detection limit.

daytime dissolved oxygen levels (> 200% saturation), indicative of eutrophication. The presence of dung and pugging from cattle and wild camels on the mounds, and in the surrounding moats, suggested stock and feral animals were most likely the cause of high nutrient levels. Nutrient enrichment was most obvious at Friday and Lyngett wells, both of which had excessively high nitrate (> 9.7 mg L^{-1}) and total nitrogen (> 13 mg L^{-1}) concentrations, suggesting groundwater contamination in the vicinity of the bores. Turbidity ranged widely with highest levels (> 30 NTU) encountered at Linear, Melaleuca and Saunders springs. Silica levels were mostly within the range 29–100 mg L^{-1}, with relatively low levels recorded from Saunders Spring (3 mg L^{-1}) and Coolabah Claypan (8 mg L^{-1}).

Cluster analysis on physico-chemical data did not

Table 3

Ionic composition (% meq L^{-1}) and equivalent ratios.

Site no.	Site name	%Ca	%K	%Mg	%Na	%Cl	$\%SO_4$	$\%HCO_3$	Ca:Na	Ca:Mg	$Ca:HCO_3$	Na:Cl	Na:K	$HCO_3:Cl$	$Cl:SO_4$
17	Bretts Spring	9.1	1.5	6.4	83.0	71.4	14.5	14.1	0.1	1.4	0.6	1.2	55.5	0.2	4.9
1	Coolabah Claypan	26.5	5.6	12.1	55.8	26.9	1.1	72.0	0.5	2.2	0.4	2.1	9.9	2.7	23.5
7	Eil Eil Spring	11.7	1.7	9.9	76.6	60.9	13.0	26.1	0.2	1.2	0.4	1.3	45.8	0.4	4.7
3	Fern Spring	9.5	2.0	9.6	78.9	77.7	15.1	7.2	0.1	1.0	1.3	1.0	39.6	0.1	5.1
20	Friday Well	18.0	7.2	17.3	57.5	58.2	12.3	29.4	0.3	1.0	0.6	1.0	8.0	0.5	4.7
8	Linear Spring	19.4	3.7	18.4	58.6	42.6	9.1	48.2	0.3	1.1	0.4	1.4	15.9	1.1	4.7
6	Little Eil Eil Spring	13.1	1.7	9.3	75.9	68.4	9.1	22.5	0.2	1.4	0.6	1.1	44.6	0.3	7.5
21	Lyngett Well	12.9	5.4	11.5	70.2	56.1	15.0	29.0	0.2	1.1	0.4	1.3	12.9	0.5	3.7
4	Melaleuca Spring	5.5	1.8	5.6	87.1	80.7	12.8	6.5	0.1	1.0	0.8	1.1	47.2	0.1	6.3
18	Salt Creek Claypan Spring	1.9	6.0	6.7	85.5	79.2	20.4	0.4	0.02	0.3	4.3	1.1	14.4	0.01	3.9
5	Saunders Spring	11.8	3.3	9.7	75.3	63.6	17.3	19.1	0.2	1.2	0.6	1.2	23.1	0.3	3.7
9	Top Spring	15.6	2.7	12.5	69.2	45.4	7.8	46.8	0.2	1.2	0.3	1.5	25.2	1.0	5.8

reveal strong grouping of sites according to salinity categories (Fig. 2A). Four significant ($p < 0.05$) cluster groupings were identified: group 1 comprised the most saline site, Salt Creek Claypan Spring; group 2 comprised the mound springs at Melaleuca, Saunders and Bretts springs; group 3 comprised Coolabah Claypan, and group 4 comprised Friday and Lyngett wells together with all other mound springs for which there was detailed water chemistry (Fig. 2A). MDS ordination revealed a similar pattern, again with the distinct separation of Salt Creek Claypan Spring (Fig. 2B). ANOSIM detected no significant differences between salinity groups in ordination space (Global $R = 0.194$, $p = 0.094$).

Aquatic invertebrates

Excluding the two bores, 134 taxa were recorded from 10 sites (Appendix). Approximately half are known to be described species and at least some of the larvae not formally identified are likely the same species as represented by adults. Many of the microinvertebrate specimens however, likely represent undescribed species. The aquatic invertebrate fauna of the Mandora wetlands was dominated by Insecta, which comprised ~53 % of the total fauna. Coleopterans were the richest of the insect groups (23.7 %), followed by dipterans (13.7 %) and hemipterans (8.6%). Microinvertebrates comprised 23 % of all taxa collected, predominantly rotifers (11.5 %) and rhizopods (10.1 %). Microcrustacea were also relatively species rich; ostracods (5 %), copepods (5 %) and cladocerans (3.6 %). Only one species of macro-crustacea was collected, a bathynellid syncarid, subsequently described as a new genus and species of stygofauna, *Kimberleybathynella mandorana* (Cho *et al.* 2005). Other rare components included a new species of assimineid snail, *Assiminea* sp. nov., present at Fern and Linear springs. Ephemeropterans, odonates and trichopterans were also under represented and mostly restricted to Bretts Spring, Coolabah Claypan and Linear Spring.

The greatest number of taxa (48) was collected from the freshwater Coolabah Claypan and the fewest (15) from the saline Salt Creek Claypan Spring. An average of 30 taxa was collected for sites at which both macro- and microinvertebrates were sampled. Fifty-eight percent of taxa were recorded from single sites only. Both bores were depauperate with Lyngett Bore containing a juvenile unionicolid water mite, and two ostracods; *Cypretta baylyi* McKenzie and *Limnocythere ?dorsosicula* (juvenile specimen). Friday Well contained juvenile cyclopoid copepods and the new bathynellid syncarid. The fauna of the cores was also depauperate in comparison with surface waters at the same sites (Appendix), containing less than 29 % of the taxa richness of the corresponding wetland. A total of 10 taxa were collected from the cores, three of which were not recorded in the surface water samples; *Arcella* sp. B, *Difflugia* cf *ventricosa* and an indeterminate foraminifera.

Five species appeared restricted to the saline Salt Creek Claypan Spring, including the rhizopod *Arcella* cf *discoides*, the copepod *Ameira* sp., the ostracod *Diacypris spinosa* De Deckker, the chironomid *Tanytarsus barbitarsus* Freeman and an unidentified empidid. There were 40 species that were only collected from freshwater (< 1 g L^{-1}) sites, including seven of the eight rotifers, two rhizopods, four of the six branchiopods, two ostracod species, five chironomid species, three odonates and all four trichopteran species.

Classification of invertebrate data (Fig. 3) produced three significant ($p < 0.05$) site groupings: group 1 comprised the saline (38.5 g L^{-1}) Salt Creek Claypan Spring, group 2 comprised the saline mound springs (Melaleuca 4.7 g L^{-1}, Fern ~ 5.8 g L^{-1}) together with the freshwater Saunders Spring (~ 1.3 g L^{-1}), and group 3 comprised the remaining freshwater mound springs (Eil Eil 1.4 g L^{-1}, Little Eil Eil 1.2 g L^{-1}, Bretts ~ 0.7 g L^{-1}) and Coolabah Claypan (~ 0.1 g L^{-1}). Analysis of similarity profiles (SIMPROF) revealed significant differences between salinity groups 2 and 3. However, the average Bray-Curtis pairwise similarity was generally low; ~ 36 % within the group 3 freshwater mound springs and ~ 51 % within the group 2 springs. Low similarity levels were not unexpected given the large number of singletons collected. Between-group similarity levels were ~ 20–33%. Salinity groupings were not significantly different in ordination space (ANOSIM, $R = 0.374$, $p = 0.10$), however similarity matrices for physico-chemical and invertebrate data were correlated (RELATE, $\rho = 0.581$, $p = 0.002$). Water quality variables that best explained the variance in invertebrate community composition among sites were dissolved oxygen concentration (% saturation), salinity

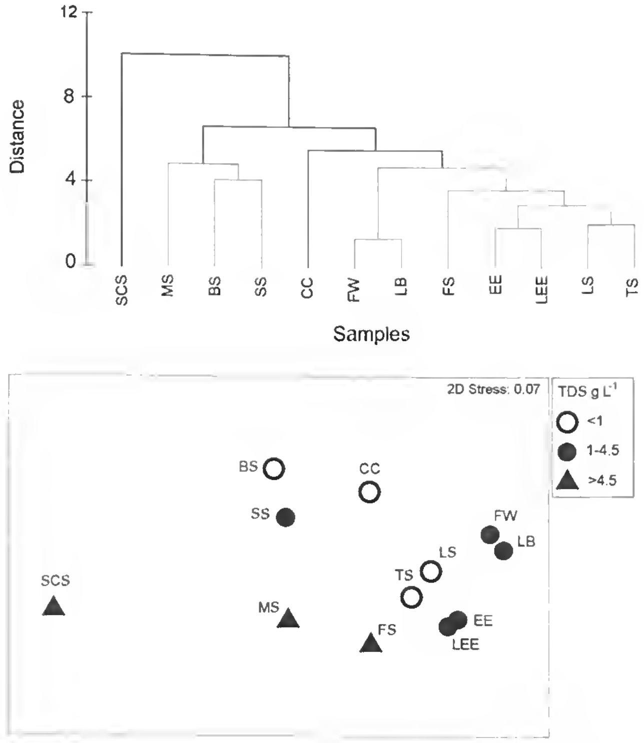

Figure 2. UPGMA classification dendogram (A) and MDS ordination plot (B) on physico-chemical data for all sites for which there were comprehensive water chemistry data (n = 12). Sites or groups of sites joined by a red line on the cluster dendogram are not significantly different (SIMPROF, p >0.05). Ordination is in two dimensions indicating salinity (TDS g L^{-1}) groupings. Site codes: BS = Bretts Spring; CC = Coolabah Claypan; EE = Eil Eil Spring; FS = Fern Spring; FW = Friday Well; LEE = Little Eil Eil Spring; LS = Linear Spring; MS = Melaleuca Spring; SCS = Salt Creek Claypan Spring; SS = Saunders Spring; TS = Top Spring.

(log$_{10}$ transformed TDS) and %HCO$_3^-$ (BIOENV, ρ = 0.771). Of these variables, salinity appeared the most influential on the number of species found at each site, with 30 % of species apparently restricted to freshwater springs.

Fish

Two native fish species were recorded from the Salt Creek system, spangled perch *Leiopotherapon unicolor* (Günther) and a new species of goby *Acentrogobius* sp. nov. Spangled perch were abundant in the Salt Creek channel and in Mandora Lake. Large numbers of dead fish were also observed in dry channel pools to the east. The goby was taken from amongst mangrove pneumatophores in small pools in a channel running from a saline spring into Salt Creek. The habitat was difficult to sample and only a limited number of juveniles and adults were caught [1]. No fish were observed in any of the springs, bores or Coolabah Claypan.

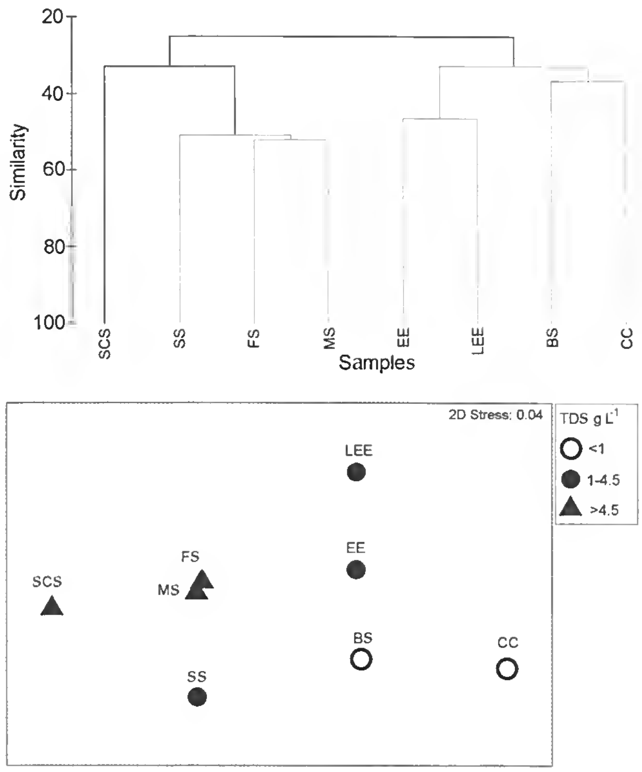

Figure 3. UPGMA classification (A) and MDS ordination plot (B) on invertebrate species presence/absence data for all sites at which both macro- and microinvertebrates were sampled (n = 8). Sites or groups of sites joined by a red line on the cluster dendogram are not significantly different (SIMPROF, p >0.05). Ordinations in two dimensions indicating salinity (TDS g L^{-1}) groupings. Site codes: BS = Bretts Spring; CC = Coolabah Claypan; EE = Eil Eil Spring; FS = Fern Spring; LEE = Little Eil Eil Spring; LS = Linear Spring; MS = Melaleuca Spring; SCS = Salt Creek Claypan Spring; SS = Saunders Spring; TS = Top Spring.

Discussion

Fauna composition

In terms of total number of invertebrate taxa (138), Mandora Marsh may be considered relatively rich when compared with other Australian spring systems. Similarly, the proportion of Insecta (~ 50%) is comparatively high for Australian inland waterways, although microcrustacea may be under-represented because sampling for microinvertebrates was restricted due to the shallow, organic-rich water. The Department of Environment and Conservation subsequently surveyed the invertebrate fauna of Saunders Spring in May 2008 (Daniel *et al.* 2009), as part of resource condition monitoring of the Eighty-mile Beach Wetland of International Importance (Ramsar site). Forty-five macroinvertebrate taxa were recorded from three sampling locations within the Spring. The macrofauna was dominated by cosmopolitan dipteran, coleopteran and hemipteran species. Microfauna were not sampled.

Mitchell (1985) sampled 13 mound springs in northern South Australia and recorded 59 taxa, comprising 27 Crustacea and 32 Insecta, however, not all groups were taken to species level, and microfauna other than

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[1] On 11th August 2001, Sally Black, Tim Willing and David Dureau (Department of Environment and Conservation) collected additional specimens of *Acentrogobius* sp. nov. from the Salt Creek palaeochannel, 15 km east of the current study site (location 19°44'33" S, 121°37'20" E).

microcrustacea were not sampled. Therefore, the fauna likely supports additional taxa, but even so, it appears less rich than the Mandora wetlands. Halse *et al.* (2000) sampled three peaty spring sites in the Carnarvon Basin, with taxa richness ranging from 15, in a very small site with virtually no free water, to 38. These sites had a high proportion of Insecta (*i.e.* up to 90%), reflecting the difficulty in sampling the shallow, peaty habitat. Halse *et al.* (2002) collected 159 species during a one-off sampling of five springs in the Pilbara region of north-west Western Australia, with an average richness of 60 species per spring and an average proportion of Insecta of 61%. The springs, however, were not mound springs but were on river channels, subject to frequent wet season flushing, resulting in a coarser substrate and deeper surface flow. The sites were also widely distributed across the Pilbara region (*i.e.* separated by 100s of kilometres), and across catchments which would increase the suite of species that could be present.

Pinder *et al.* (2010) similarly surveyed a comprehensive diversity of wetland habitats across the Pilbara, including 18 flowing springs/spring fed creeks. Between 2003 and 2006, a total 189 samples were collected from one-off sampling of 100 sites in spring and autumn. Average richness per sample was 94, with microinvertebrate richness ranging from 3 to 57 and macroinvertebrate richness ranging from 4 to 170. Insects comprised 42% of all taxa collected. By comparison, average richness from the 36 samples collected at springs/spring-fed creeks was 94, with microinvertebrate richness ranging from 5 to 45 and macroinvertebrate richness ranging from 45 to 144. Insecta were again dominant, comprising 61% of the total fauna of the springs/spring-fed creeks. Pinder *et al.* (2010) found numerous endemics (*e.g.* amphipods, isopods, riffle beetles) and rare non-endemics (*e.g.* assimineid snails, leptophlebiid mayflies, species of water mite) that appeared restricted to a sub-set of the springs/ spring-fed creeks. One of these groups, the assimineid snails, was also present in Mandora Marsh, represented by a new species, *Assiminea* sp. nov.

Further south, Pinder *et al.* (2006) surveyed seven tumulus mound springs in the northern Wheatbelt Region of Western Australia, recording 88 taxa in March and 80 taxa in August 2001, giving a total of 103 taxa. The fauna of the springs comprised common and widely distributed insects, some regionally rare and a suite of groundwater-dependent species including a bathynellid syncarid, an amphipod, and several species of ostracod and copepod. The fauna was dominated by Insecta (58 %), with the conservation value lying with the restricted groundwater-dependent fauna, as well as some species that were considered uncommon in the region, being more characteristic of the wetter south-west (Pinder *et al.* 2006).

Tumulus mound springs on the Gnangara Groundwater Mound, north of Perth have been sampled more frequently, with taxa richness ranging from 40 species from King's and Muchea springs (Jasinska & Knott 2000), 43 from King's, Muchea and Egerton springs (Pinder, unpub. dat.), and 72 from repeated seasonal sampling of Muchea and Egerton springs (Jasinska & Knott 1994).

When compared with other arid-zone non-spring wetlands, the Mandora system is again comparable in terms of richness. Halse *et al.* (1998) undertook invertebrate sampling of Lake Gregory – a large (380 km^2), mostly fresh, semi-permanent lake on the edge of the Great Sandy Desert, with similar methods to those used in this study and considered the 174 taxa (42% Insecta) recorded over three sampling occasions to represent a highly diverse fauna for inland waters. Sampling, however, spanned a range of salinities as the lake evapoconcentrated, which would encourage different species to inhabit the lake. Storey & Creagh (unpubl. data) collected 124 taxa (37 % Insecta) from freshwater pools during one-off sampling of the highly ephemeral Jones Creek in the northern Goldfields of Western Australia. Timms *et al.* (2007) recorded more than 100 invertebrate taxa over four sampling occasions from saline and fresh wetlands (> 750 km^2) in the episodically filled Lake Carey complex in the eastern Goldfields, though their study did not target rotifers or micro-crustaceans. Amongst other substantially larger, more extensively surveyed arid systems, riverine waterholes of the Lake Eyre basin reportedly support 136 macroinvertebrate taxa (Costelloe *et al.* 2004) and at least 400 microinvertebrate taxa (Shiel *et al.* 2006), and arid-zone wetlands on the Paroo River floodplain in south-west Queensland support at least 200 invertebrate taxa (Timms & Boulton 2001; Hancock & Timms 2002).

The considerable richness of Mandora Marsh was considered due to the different types of permanently inundated wetlands within the one system. This concurs with the suggestion by Halse *et al.* (1998) that increased richness will occur in inland systems when there is regular, prolonged inundation coupled with waters of varying solute composition. Faunal composition of the Mandora wetlands was closely linked to salinity, though even within freshwater sites, spatial variation in community assemblage was high. However, it is likely the current survey underestimates species diversity and number of restricted species, as not all sites were sampled for microinvertebrates, but also the shallow, peaty seeps on the mound springs were difficult to sample, especially for microinvertebrate fauna.

Fauna endemism

The macroinvertebrate fauna of the Mandora Marsh was dominated by highly vagile predators capable of traversing the region and easily able to move between wetlands within the marsh. Dispersion within the marsh of less mobile elements of the aquatic fauna would be facilitated by widespread flooding that regularly occurs following cyclonic activity. Taxa that appeared endemic to the springs (*e.g.* the snail *Assiminea* sp. nov.) are likely to have habitat requirements that restrict their occurrence. Ponder (unpubl. data) undertook a survey of 30 springs in the northwest of Western Australia in September 1987, sampling Salt Creek, Eil Eil Spring and three unnamed springs at Mandora. The most significant fauna recorded was *Assiminea* sp., which appeared to be restricted to the springs of Mandora Marsh. Insecta were not identified below ordinal level, however all orders recorded by Ponder were present in this survey.

Though the cores contained taxa not recorded from adjacent surface waters, the rate of occurrence of these taxa was low and comparable to elements of the microfauna taken from each wetland. These taxa were therefore considered to be rare, rather than restricted to the pore water of the peat mounds. The presence of groundwater species such as the bathynellid syncarid *Kimberleybathynella mandorana*, copepods *Mesocyclops brooksi* and *Metacyclops mortoni* Pesce *et al.* and the ostracods *Vestalenula marmonieri* Rosetti & Martens and ?*Candona* sp., does suggest the potential for more stygal material from this aquifer. The Pilbara region has previously been found to be rich in stygal species (Halse *et al.* 2002; Eberhard *et al.* 2005; Cooper *et al.* 2007).

Rhizopoda

Rhizopods are ubiquitous in damp situations, from soil and moss to fully aquatic environs. Taxonomy has historically been based on test morphology. Because testates were long-thought to be cosmopolitan, names applied have been 'cosmopolitan' on all continents, even when the bearers are only superficially (or not at all!) similar. Australasian Rhizopoda are poorly known, particularly those of Western Australia. The only detailed work in any Australian region remains that of Playfair (1917) in NSW. The group needs critical revision by modern standards using biometry, scanning electron microscopy and genetic analysis. Evidence from widespread collections across southern Australia (Meisterfeld & Tan 1998; Shiel & Tan unpubl. data), shows that the Australian Rhizopoda have a cosmopolitan component, a Gondwanic component and an endemic component, but, given the poor taxonomic resolution of the rhizopods in Australasia, the extent of each component remains unknown. Only 14 taxa in five families were encountered in the Mandora Marsh samples, most from the genera *Arcella*, *Centropyxis* and *Difflugia*, with *Arcella* sp. A and *Centropyxis aculeata* (Ehrenberg) the most common (present at four sites). Richness was similar to that reported by Pinder *et al.* (2010) for other springs and spring-fed creeks within the Pilbara region. However, richness and density per site were very low relative to collections from eastern Australia (Meisterfeld & Tan 1998), although collecting methods may be partly responsible for this. Most Mandora Marsh rhizopods either could not be identified from current literature, and may well be undescribed, or resembled to some degree known species, but in-depth population analysis is needed to confirm the identification. Only two species were unequivocally the nominate (cosmopolitan) species.

Rotifera

Rotifers are among the smallest metazoans and are largely associated with freshwater, although a few species are adapted to athallasic saline or oceanic waters. They were long thought to be cosmopolitan, however recent detailed studies on all continents have demonstrated regionalism in rotifer faunae. Australia has approximately 15% endemicity in the known Rotifera (~ 700 spp.) (Shiel & Koste 1986). The few reports of rotifers from Western Australia suggest that it has a higher degree of endemicity than found in eastern Australia, with some Tasmanian affinities in southwest Western Australia and Indo-Malaysian affinities in the northwest of the state (*e.g.* Halse *et al.* 1998; Shiel & Williams 1990; Storey *et al.* 1993). At least 16 rotifer taxa in seven families were recognised in the Mandora Marsh samples. More are likely in view of the difficulty of identifying bdelloid rotifers from preserved material. Nevertheless, the number of 'hidden' species is likely to be few and the total estimated (~ 20) is low relative to other Western Australian sites. For example, Pinder *et al.* (2010) recorded 105 taxa from springs/spring-fed creeks across the Pilbara, Halse *et al.* (1998) recorded ~ 40 species at Lake Gregory, and Shiel *et al.* (2006) recorded 20 to 30 species per site for arid zone waters in central Australia. These rotifer numbers in turn are low relative to well-watered billabongs of eastern Australia, where > 100 rotifer species have been recorded from single net tows in Murray-Darling billabongs (Shiel *et al.* 1998). In terms of novelty, only two of the Mandora rotifers are new (a *Brachionus* related to the cosmotropical *B. budapestinensis* Daday, and a *Cephalodella* close to the South American *C. boettgerii* Koste). The estimated 10% endemicity for the Mandora rotifers probably reflects the small sample size and perhaps incomplete collecting. It is less than the continental endemicity and the estimated 12% for Western Australia. Notably, the remaining Mandora Marsh rotifers are all cosmopolitan taxa.

Branchiopoda: Anomopoda / Ctenopoda (cladocerans)

With only five taxa identified from Mandora Marsh, the cladoceran representation in the microfauna is poor relative to other Western Australian studies (cited above) and Australia in general. Four of the five taxa occurred once, with only *Ceriodaphnia cornuta* Sars at more than one site. The three indeterminate species are probably Australian endemics. For example, the Australian form of *Moina micrura* Kurz, collected from Coolabah Claypan, has been shown by genetic analysis to be distinct from the nominate species (Petrusek *et al.* 2004) but the regional distribution of this, and other indeterminate species, remains unknown. About half of the described Australian cladocerans (~ 200 spp.) are endemic.

Other Microcrustacea

Like other microinvertebrate groups, the ostracod and copepod faunas of Mandora Marsh were relatively depauperate. More than 150 species of surface water ostracods are known from Western Australia, although fewer than half are described (Halse 2002). Up to 10 ostracod species per site were recorded from seasonal pools in semi-arid areas on the mid-west coast (Halse *et al.* 2000), and up to 12 were recorded at individual Pilbara springs (Pinder *et al.* 2010), whereas a total of only five living species were recorded from Mandora Marsh as a whole, with a maximum of three at a site. The marsh sites contained a mixture of stygal (?*Candona*, *Vestalenula*, *Metacyclops*) and epigean species, with most of the named species having widespread occurrence in Australia or beyond. Three of the four described copepod species collected are widespread in Australia (Halse *et al.* 2002; Pesce *et al.* 1996b); *Metacyclops mortoni* was the exception, having previously been collected only from deep groundwater near Cape Range on the northwest coast and the Ashburton River on the mid-west coast (Pesce *et al.* 1996a).

Oligochaeta

All species of oligochaetes recorded are widespread in Australia. *Allonais ranauana* (Bolt) frequently occurs in arid and tropical sites (including Coopers Creek, South Alligator River and Lake Gregory), but has not been recorded from southeastern or southwestern Australia. Worldwide, *A. rananana* is fairly widespread, but particularly common in Africa. *Pristina longiseta* Ehrenberg and *Dero furcata* Müller both are worldwide, cosmopolitan species (A. Pinder, Dept. Environment and Conservation, pers. comm.).

Gastropoda

Two gastropod species were recorded; the planorbid *Gyraulus* sp. and the assimineid *Assiminea* sp. nov., which is new to science. Further survey effort is required to determine whether or not it is endemic to these springs. It was present at both saline Fern Spring (5.78 g L^{-1}) and freshwater Linear Spring (0.62 g L^{-1}). *Gyraulus* sp. is probably widespread across northern Australia (W. Ponder, Australian Museum, pers. comm.) and it too was present in both saline and freshwaters.

Arrenuridae / Eylaidae / Hydrachnidae

The identified species of water mites are common across the north of Australia. It is likely that many of them parasitise adult dragonflies, providing an efficient means of distribution (Harvey 1998).

Coleoptera / Hemiptera

The hemipteran fauna of the springs was relatively rich but dams and soaks often provide good habitat for *Anisops*, of which seven species were present. The fauna contained typical northern Australian species (Lansbury 1969; Wroblewski 1970, 1972). The occurrence of *Micronecta lansburyi* Wroblewski is of interest because it has rarely been recorded. The beetle fauna consisted of at least 27 species and was substantially richer than that at many other north-western Australia wetlands (Halse *et al.* 1996, 1998; Pinder *et al.* 2010). It was comprised mostly of widespread species of Dytiscidae and Hydrophylidae (Watts 2002).

Diptera

The species of Chironomidae recorded from the Mandora sites are all cosmopolitan in distribution. *Tanytarsus barbitarsus* Freeman is a halobiont species, and was only recorded from the Salt Creek Claypan Spring (38.5 g L^{-1}). Other species of chironomid appeared restricted to freshwater sites, though some at least are known to have a range of tolerances, *e.g. Procladius paludicola* Skuse and *Chironomus* aff. *alternans* Walker. Of other dipterans that could be identified to species, the culicid *Culex bitaeniorhynchus* Giles has a generally northern distribution and is known to breed in wetlands and creeks, and is most common following wet season rains (Liehne 1991). *Culex annulirostris* Skuse is common and widely distributed across Australia.

Trichoptera / Odonata

There are many undescribed *Ecnomus* and *Oecetis* species across the north of Australia, and given the mobility of the adults, these species are likely to be common. *Triplectides ciuskus seductus* Morse & Neboiss is also common across the north of Australia (J. Dean, Victorian EPA, pers. comm.). The odonate fauna was depauperate and consisted of widespread species (Watson 1962).

Biogeography

Though the marsh was comparatively taxa-rich overall, the aquatic invertebrate fauna of individual sites was taxa-poor in comparison with other temperate and tropical wetlands in Western Australia (*e.g.* Davis *et al.* 1993; Storey *et al.* 1993; Edward *et al.* 1994; Halse *et al.* 1996, 2000, 2002; Pinder *et al.* 2004). It must, however, be noted that sampling the shallow seeps was problematic, and one-off sampling will have underestimated the richness of the Mandora wetlands given the high degree of temporal variability in Australian inland waters (Sheldon *et al.* 2002).

The insect species at the marsh were characterised by strong-flying adult stages, promoting efficient dispersal. Unsurprisingly, the macroinvertebrates of the wetlands consisted of few taxa with limited distributions or endemic to the area. The majority of endemic taxa were recorded from the microinvertebrate fauna, but richness and levels of endemism were mostly low compared to other detailed fauna surveys (Halse *et al.* 1998, 2000; Meisterfield & Tan 1998; Shiel *et al.*1998; Pinder *et al.* 2010).

Approximately 15 M yr BP, the Mandora system was part of a large drainage system known as the Wallal Palaeoriver. A gradual shift toward more arid climatic conditions caused the drainage valley to fill with alluvial and aeolian material (Watkins *et al.* 1997). By about 10 000 yr BP, the system was part of a shallow marine system. Carbon dating of peat sediments in Eil Eil Spring (Wyrwoll *et al.* 1986) suggest the wetlands that exist today formed some 7 000 years ago, and have changed little in that time. The relatively short life of the springs could further explain the low levels of endemism in the aquatic fauna.

That the Mandora area once was an estuary could also explain the presence of inland mangroves, mangrove fern, a new species of *Acentrogobius* gobiid, and the endemic assimineid gastropod. *Acentrogobius* is a poorly defined genus currently including marine and estuarine species, widespread in the Indo-west Pacific (H. Larson, NT Museum, pers. comm.). Gastropods of the genus *Assiminea* also are regarded as marine. The presence of new species of these 'marine' genera and isolated occurrences of mangrove plant species in the Mandora system probably reflects isolation at the start of the Holocene, some 9 000–10 000 yr BP (Ridpath *et al.* 1991) with subsequent genetic and morphological divergence.

Conservation and Management

Mound springs of Australia are widely recognised as having high conservation significance, particularly in arid zones, where their permanent water provides refugia for flora and fauna. They are known to support remnant populations of species with a once wider distribution, as well as new and endemic species, resulting from speciation of isolated populations. Upwelling flows originating from subterranean habitats may also bring short-range endemic and more broadly distributed stygofauna to the surface.

The Mandora system contains a variety of wetland types, with closely adjacent sites ranging in salinity from fresh to saline despite only small variations in topography. This would indicate a complex groundwater system with fresh and saline aquifers likely at different depths, feeding different parts of the system. The saline spring-wetlands are likely driven by an aquifer containing stored marine salts from pre-Holocene. However, it is currently not known if the freshwater springs are driven by deep artesian water, or unconfined near-surface groundwater recharged by local rainfall. This diversity in wetland types within the one system is partly why Mandora Marsh was listed under the Ramsar Convention (Jones 1993).

Pressure from stock and feral animals appears to be a major threat to the wetlands. Elevated nutrient levels in the two wells suggests nutrient enrichment of the shallow groundwater aquifer at these locations, which is likely, given the heavy stock usage of the bores, shallow depth to groundwater (~ 3 m below surface) and sandy nature of the soils. However, the spatial extent of any enrichment is not known, given the low density of bores for sampling. Cattle and feral camels have already adversely affected the ecological health of the mound springs. Apart from the physical damage caused by stock trampling the mounds and grazing the regenerating vegetation, the tendency of stock to remain at the springs for long periods during hot weather, is degrading the vegetation and water quality. Sampling was conducted at the end of the dry season, when wetlands in general, and particularly those in arid zones, tend to recede and evapoconcentrate. Even so, the occurrence of algal blooms in the shallow moats around Melaleuca and Saunders springs, combined with highly elevated nitrogen levels at these sites relative to others, indicates nutrient enrichment. Interestingly, Mitchell (1985) recorded highly elevated dissolved nitrate levels from mound springs in northern South Australia which was attributed to defecation by stock, and considered that stock would eventually result in the destruction of the wetlands. Halse *et al.* (2005) acknowledged that domestic stock degrade wetlands through pugging and grazing, with most damage occurring in the second half of the dry season as temperatures increase and water and fodder are scarce, with cattle camping around natural (and man-made) waterholes. But Halse *et al.* (2005) also suggested cattle may have little influence on episodic wetlands such as Mandora Lake, unless the wetlands are part of a landscape subject to widespread overgrazing. The permanently inundated mound springs of Mandora Marsh concentrate domestic stock and feral camels, as they provide a reliable water source in an arid environment, and it appears this pressure is damaging the springs.

In recognition of the damage by stock and feral animals, Saunders Spring was fenced in November 1997 by the holders of Anna Plains pastoral lease, in association with the Broome Botanical Society, using funds from the Commonwealth Natural Heritage Trust. Part of the strategy was to leave a man-made pool outside the fenced area to provide for stock. The fencing appeared to be of immediate benefit as understorey vegetation within the fenced area was visibly regenerating and the moat was well shaded, with no algal activity. However, algal growth was extreme in the pool outside the fence which was subject to heavy stock use (Storey pers. obs.). Mitchell (1985) and Halse *et al.* (2002) recommended fencing of springs to exclude stock and feral animals and so prevent eutrophication and physical damage. The increase in taxa richness at Saunders Spring between 1999 (current study) and 2008 (Daniel *et al.* 2009) suggests progressive improvement in ecological health following fencing of the site in 1997, but further sampling would be required to corroborate any causal link.

Change in hydrology is also a major threat to mound springs. To the north-east of Mandora, trials were conducted in the late 1990s to grow irrigated cotton using groundwater extracted from the La Grange sub-basin. Although these trials have not progressed, they may in future (Hale & Butcher 2009), and water extraction from the aquifers feeding the springs at Mandora would be extremely detrimental to these springs and would probably lead to their loss. Maintenance of a natural hydrological regime is seen as a critical management issue for most arid-zone wetlands (Harris 1992; Kingsford & Thomas 2004; Halse *et al.* 2005). Mitchell (1985) also identified over pumping from artesian bores as a threat to the existence of mound springs in northern South Australia, with reduced flows in some springs attributed to over pumping at adjacent bores.

The aquatic fauna of the wetlands at Mandora Marsh may not be unique, but the system is worthy of conservation as it represents one of only a few permanent arid zone wetlands and contains new and restricted species. Mitchell (1985) sampled 13 mound springs in northern South Australia and separated the aquatic invertebrate fauna into two components, the insects with good powers of dispersal and therefore a wide distribution, and non-insects, with low powers of dispersal and no drought-resistant stages in their life cycle (*i.e.* hydrobiid gastropods, phreatoicid isopods, an amphipod and a gobiid teleost). Together with some endemic ostracods, Mitchell (1985) considered these non-insect elements to be the unique mound spring faunal assemblage. The aquatic fauna of the Mandora springs and wetlands is similarly classified, with the non-insect mound spring fauna having the highest conservation value. In terms of aquatic fauna biodiversity, each wetland at Mandora is not particularly species rich, but as a heterogeneous wetland suite it does support a relatively diverse fauna. However, the highest conservation value arises when the overall ecological values of the Marsh are considered in the context of the mix of waterbird (Halse *et al.* 2005), aquatic invertebrate (this study) and plant values (Graham 1999). The assemblage values for the mound springs and Salt Creek, and the species values for Mandora Lake (Walyarta) where waterbird values are very high after flooding (Halse *et al.* 2005) make this system worthy of protection. Currently the Mandora Marsh system is part of the Eighty-mile Beach Ramsar site, with the beach and the episodically-flooded country between the beach and the Marsh regularly supporting substantial waterbird values. As such, the values of Mandora Marsh should not be viewed in isolation from those west of the Great Northern Highway (Fig. 1).

In conclusion, this study has extended our knowledge of the ecological values of Mandora Marsh, but the values are yet to be fully described and, given possible threats from future adjacent development, it is important that values are documented so that any planning process is properly informed. Subsequent to this survey, and in recognition of the conservation value of the marsh system, it is planned to revise the pastoral lease boundary of Anna Plains Station in 2015 to encompass Mandora Marsh in a nature reserve (Hale & Butcher 2009). In recognition of the threat feral camels pose to arid zone ecosystems across northern and central Australia, the Federal Government in 2009 committed $17M to the control of camels. The 2010 groundwater allocation and management plan for the La Grange subareas (Department of Water 2010) also acknowledges the need for further investigation of the water requirements of groundwater dependent ecosystems including those of Mandora Marsh. All initiatives will help protect the mound springs of Mandora Marsh.

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Appendix

Systematic listing of taxa recorded from 12 sites. Codes: BS = Bretts Spring; CC = Coolabah Claypan; EE = Eil Eil Spring; FS = Fern Spring; FW = Friday Well; LEE = Little Eil Eil Spring; LS = Linear Spring; LW = Lyngett Well; MS = Melaleuca Spring; SCS = Salt Creek Claypan Spring; SS = Saunders Spring; TS = Top Spring; core = microinvertebrates taken from pore water in a peat core; † microinvertebrates not sampled.

Taxon	BS	CC	EE	EE core	FS	FS core	FW	LEE	LEE core	LS †	LW	MS	SCS	SS	SS core	TS †	No. of occurrences
PROTISTA																	
Ciliophora			*														1
Foraminifera						*			*						*		3
RHIZOPODA																	
Arcellidae																	
Arcella cf. *discoides* Ehrenberg, 1843													*				1
Arcella sp. A			*		*				*						*		4
Arcella sp. B															*		1
Arcella sp. C		*															1
Arcella sp. D			*														1
Centropyxidae																	
Centropyxis aculeata (Ehrenberg, 1830)	*	*	*	*				*	*								6
Centropyxis constricta (Ehrenberg, 1841)								*									1
Centropyxis cf. *discoides* Penard, 1890					*												1
Centropyxis cf. *platystoma* Penard, 1890								*									1
Centropyxis sp.		*															1
Difflugiidae																	
Difflugia cf. *ventricosa* Deflandre, 1926															*		1
Difflugia sp.		*							*								2
Gromiidae																	
Pseudodifflugia sp.								*									1
Hyalospheniidae																	
Cyclopyxis cf. *kahli* (Deflandre, 1929)								*									1
ROTIFERA																	
Bdelloidea																	
Bdelloidea sp. indet. contracted [L]	*	*	*					*							*		5
Bdelloidea sp. indet. contracted [S]			*														1
Monogononta																	
Asplanchnidae																	
Asplanchna brightwelli Gosse, 1850		*															1
Brachionidae																	
Brachionus angularis Gosse, 1851		*															1
Brachionus cf. *budapestinensis* Daday, 1885		*															1
Brachionus quadridentatus Hermann, 1783		*															1
Platyias patulus (Müller, 1786)	*	*															2
Conochilidae																	
Conochilus dossuarius (Hudson, 1885)		*															1

Lecanidae												
Lecane (*Monostyla*) *bulla* Gosse, 1851	*					*						2
Lecane (*M.*) cf. *closterocerca* (Schmarda, 1859)						*						1
Lecane (*s.str.*) *flexilis* (Gosse, 1886)						*						1
Lecane (*M.*) *hamata* Stokes, 1896						*						1
Lecane (s.str.) *hastata* (Murray, 1913)						*						1
Lecane (*s.str.*) *luna* (Müller, 1776)	*			*								2
Notommatidae												
Cephalodella n. sp. (aff. *C. boettgeri* Koste, 1988)						*						1
Testudinellidae												
Testudinella patina (Hermann, 1783)		*										1
MOLLUSCA												
GASTROPODA												
Assimineidae												
Assiminea sp. nov.				*				*				2
Planorbidae												
Gyraulus sp.		*	*	*		*	*	*		*		7
ANNELIDA OLIGOCHAETA												
Allonais ranauana (Bolt, 1932)			*					*				2
Pristina longiseta Ehrenberg, 1828			*					*			*	3
Dero furcata Müller, 1774			*								*	2
Naididae												
Naididae sp. (indet.)						*						1
CRUSTACEA												
BRANCHIOPODA: Anomopoda/Ctenopoda												
Chydoridae												
Alona rectangula novaezealandiae Sars, 1904	*											1
Daphnidae												
Ceriodaphnia cornuta (s.l.) Sars, 1885	*	*	*									3
Macrotrichidae												
Macrothrix cf. *breviseta* Smirnov, 1976		*										1
Macrothrix sp. B (cf. *Neothrix*)		*										1
Moinidae												
Moina cf. *micrura* Kurz		*										1
COPEPODA												
Cyclopoidae												
Halicyclops spinifer Kiefer, 1935						*						1
Mesocyclops brooksi Pesce *et al.*, 1996		*	*	*		*						4
Metacyclops mortoni Pesce *et al.*, 1996	*											1
Cyclopoidae					*							1
Canthocamptidae												
Cletocamptus deitersi (Richard, 1897)			*	*		*	*		*			5

Taxon	BS	CC	EE	EE core	FS	FS core	FW	LEE	LEE core	LS †	LW	MS	SCS	SS	SS core	TS †	No. of occurrences
Ameiridae																	
Ameira sp.													*				1
Parastenocarididae																	
Parastenocaris sp.					*												1
OSTRACODA																	
Candonidae																	
?*Candona* sp.			*												*		2
Darwinulidae																	
Vestalenula marmonieri Rosetti & Martens, 1999			*					*									2
Cypridiae																	
Cyprinotus kimberleyensis McKenzie, 1966	*	*															2
Cypretta baylyi McKenzie, 1966			*	*				*			*						4
Cypretta sp. B		*															1
Diacypris spinosa De Deckker, 1981 (dead)													*				1
Limnocytheridae																	
Limnocythere ?dorsosicula De Deckker, 1981											*						1
SYNCARIDA																	
Parabathynellidae																	
Kimberleybathynella mandorana Cho *et al.* 2005						*											1
ARTHROPODA																	
ARACHNIDA																	
Arrenuridae																	
Arrenurus sp.	*	*	*							*							4
Arrenurus balladoniensis Halik, 1940		*															1
Arrenurus tricornutus Viets, 1984		*															1
Eylaidae																	
Eylais sp.			*		*												2
Hydrachnidae																	
Hydrachna sp.												*					1
Unionicolididae											*						1
INSECTA																	
EPHEMEROPTERA																	
Baetidae																	
Cloeon sp.	*	*								*							3
ODONATA																	
ANISOPTERA																	
Libellulidae																	
Orthetrum caledonicum (Brauer, 1865)	*																1

Trapezostigma stenoloba Watson, 1962	*					*					2
Libellulidae sp. (juvenile)		*			*	*					3
ZYGOPTERA											
Coenagriidae											
Xanthagrion erythroneurum (Selys, 1876)		*									1
HEMIPTERA											
Corixidae											
Agraptocorixa parvipunctata (Hale, 1922)							*				1
Micronecta lansburyi Wroblewski, 1972		*									1
Micronecta virgata Hale, 1922	*	*	*		*	*	*				6
Micronecta sp.		*									1
Naucoridae											
Diplonychus eques (Dufour, 1863)		*	*		*	*					4
Notonectidae											
Anisops canaliculatus Brooks, 1951									*		1
Anisops nasuta Fieber, 1851				*		*	*				3
Anisops nodulata Brooks, 1951										*	1
Anisops semita Brooks, 1951		*					*		*		3
Anisops stali Kirkaldy, 1904		*							*		2
Anisops sp. E									*		1
Anisops sp.		*	*		*				*	*	5
Pleidae											
Paraplea sp.						*					1
COLEOPTERA											
Dytiscidae											
Allodessus bistrigatus (Clark, 1862)									*		1
Allodessus sp. (larva)	*										1
Copelatus nigrolineatus Sharp, 1882							*				1
Cybister tripunctatus (Olivier, 1795)				*							1
Eretes australis (Erichson, 1842)				*					*		2
Hydaticus consanguineus Aubé, 1838				*			*		*		3
Hydaticus vittatus (Fabricius, 1775)				*					*		2
Hydaticus sp. (larva)	*										1
Hydroglyphus leai Guignot, 1939	*	*	*	*			*	*	*		7
Hydroglyphus trifasciatus Watts, 1978	*	*	*	*	*		*	*	*	*	9
Hydroglyphus trilineatus Watts, 1978					*						1
Hyphydrus elegans (Montrouzier, 1860)				*							1
Hyphydrus lyratus Swartz, 1808		*		*							2
Laccophilus sharpi Régimbart, 1889	*	*	*	*		*	*		*		7
Laccophilus sp. (larva)	*										1
Rhantaticus congestus (Klug, 1833)				*							1
Rhantus suturalis (Macleay, 1825)			*	*							2
Hydraenidae											
Octhebius sp.	*			*			*	*	*		5

Taxon	BS	CC	EE	EE core	FS	FS core	FW	LEE	LEE core	LS †	LW	MS	SCS	SS	SS core	TS †	No. of occurrences
Hydrophilidae																	
Amphiops sp. (larva)	*																1
Berosus australiae Mulsant & Rey, 1858					*							*		*			3
Berosus pulchellus Macleay, 1825					*							*	*	*			4
Berosus sp. (larva)										*							1
Coelostoma fabricii (Montrouzier, 1860)					*									*			2
Enochrus (Methydrus) malabarensis (Régimbart, 1903)	*		*		*			*		*		*	*	*			8
Enochrus (Methydrus) deserticola (Blackburn, 1896)	*											*					2
Enochrus (Methydrus) elongatus (Macleay, 1871)												*					1
Enochrus sp. (larva)			*														1
Helochares sp.	*												*				2
Helochares sp. (larva)													*				1
Paracymus sp.			*														1
Regimbartia attenuatus (Fabricius, 1801)	*		*		*			*		*		*		*		*	8
Sternolophus (Neosternolophus) marginicollis (Hope, 1841)							*										1
Hydrochidae																	
Hydrochus sp.					*												1
DIPTERA																	
Chironomidae																	
Ablabesmyia ?notabilis (Skuse, 1889)		*															1
Chironomus aff. *alternans* Walker, 1856	*		*							*							3
Dicrotendipes sp.		*															1
Paramoina sp.	*																1
Polypedilum (Pentapedilum) leei Freeman, 1961	*	*												*			3
Polypedilum nubifer (Skuse, 1889)		*															1
Procladius paludicola Skuse, 1889	*	*								*							3
Tanytarsus barbitarsis Freeman, 1961													*				1
Tanytarsus spp.	*	*			*	*		*		*		*				*	8
Ceratopogonidae		*	*		*			*					*				5
Empididae													*				1
Tabanidae			*														1
Culicidae																	
Anopheles (Cellia) sp.	*																1
Anopheles sp.													*				1
Culex annulirostris Skuse, 1889	*		*							*						*	4
Culex bitaeniorhynchus Giles, 1901			*														1
Culex sp.	*							*									2
Ephydridae			*														1
Stratiomyidae	*		*		*			*		*		*	*	*		*	9

TRICHOPTERA																	
Ecnomidae																	
Ecnomus sp.		*															1
Leptoceridae																	
Triplectides ciuskus seductus Morse & Neboiss, 1982	*	*															2
Oecetis sp. (larva)		*															1
Oecetis sp. (pupa)		*															1
Total number of taxa	*36*	*48*	*36*	*2*	*31*	*2*	*2*	*32*	*6*	*21*	*3*	*20*	*15*	*21*	*6*	*9*	*138*

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Eradication of feral cats on Rottnest Island, Western Australia

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Abstract

Domestic cats were introduced to Rottnest Island both as pets and as predators of commensal pest animals such as the Black Rat and House Mouse at various times since European settlement. Historically, the Rottnest Island Authority has attempted to rid the island of all cats since the 1960s. It was suggested that cats maybe influencing the abundance of native fauna and if left uncontrolled, the cat population was likely to increase and could result in considerable damage to ground nesting birds and heavy predation pressure on Quokkas and reptile species.

The Department of Environment and Conservation was approached by the Rottnest Island Authority to assess feral cat numbers and control options on the island and if possible to eradicate the feral cat population. A feral cat monitoring and trapping campaign was conducted in November 2001 and 2002. Four cats were trapped and no further cat activity has been observed or cats sighted by Rottnest Island staff or the general public in the eight years subsequent to this program suggesting that eradication has been successfully achieved.

Keywords cat eradication, islands, cat trapping.

Introduction

Domestic cats, *Felis catus* (Linnaeus, 1758), have been introduced to Rottnest Island at various times since European settlement (King 1985). It is likely that they were introduced both as pets and as predators of commensal pest animals such as Black rats, *Rattus rattus* (Linnaeus, 1758) and House mice, *Mus musculus* (Linnaeus, 1758). Historically, the Rottnest Island Board (now the Rottnest Island Authority) has attempted to rid the island of all cats. It was proposed by the Board in 1965, that all cats, including domestic pets, be removed from the island by October 1965. "All wild cats would be exterminated by shooting and trapping" (West Australian Newspaper 1965). Fifteen years later in July 1980, in an effort to protect the island's bird populations, all domestic cats were removed from Rottnest Island. Approximately 20 residents had domestic pet cats at this time and the Board was still working on a solution to destroy all feral cats (West Australian Newspaper 1979, 1980).

By 1985, a small population of feral cats was present on the island but its size was unknown (King 1985). There were no data to suggest that cats were implicated in the decline of any fauna on Rottnest Island however, there was the possibility that cats were influencing the abundance of native fauna (op. cit.). If the cat population was left uncontrolled, it was likely to increase and could result in considerable damage to ground nesting birds and heavy predation pressure on Quokkas, *Setonix brachyurus* (Quoy & Gaimard, 1830), and reptile species (King 1985).

Techniques available for the control of feral cats prior to the 1990s were not particularly effective or economical. As such, long-term management options for the control of the cat population on the island at this time were to: – continue the prohibition of importation and/or the keeping of domestic cats; maintain the occasional cage-trapping program; exclude cats from rubbish bins and the waste disposal site to reduce the source of supplementary food and the possibility of conducting localised baiting programs. The implementation of this on-going management strategy, excluding baiting, was reported to have reduced, but not eradicated, the feral cat population by 1997 with a small number of cats (possibly two or three) remaining (Pontre 1997). As part of the Rottnest Island Management Plan (1997–2002) control programs for feral cats were to continue to enable eradication of cats from the island (Pontre 1997).

Control of feral cats is recognised as one of the most important fauna conservation issues in Australia today and as a result, a national 'Threat Abatement Plan (TAP) for Predation by Feral Cats' has been developed (EA 1999; DEWHA 2008). Under the TAP the goal is to protect affected native species and ecological communities, and to prevent further species and ecological communities from becoming threatened. In particular, the first objective of the TAP is to: – prevent feral cats from occupying new areas in Australia and eradicate feral cats from high-conservation-value 'islands'

The Department of Environment and Conservation (DEC) has been developing control strategies for feral cats under the umbrella program 'Western Shield'. This research has led to the successful design and development of an effective trapping technique and a bait that is readily consumed by feral cats and can be used over broad-scale areas for their control. The Department was approached by the Rottnest Island Authority to assess feral cat numbers and control options on the island and if possible to eradicate the feral cat population. Researchers visited Rottnest Island in November 2001 and again in November 2002 to conduct a feral cat monitoring and trapping program. This paper describes the feral cat eradication campaign on the island.

Methods

Site Description

Rottnest Island is an 'A' Class reserve (Reserve A 16713), vested in the Rottnest Island Authority, gazetted for the purpose of public recreation. The island is located on the southern west coast of Western Australia at 32°00' S and 115°30' E. The island, an area of 1705 ha (Abbott & Burbidge 1995), lies in an approximate east-west orientation and is 11 km long and less than 5 km wide at its widest point.

Undulating old dunes, now limestone and overlain by sand, cover the greater part of the island (Playford 1983). A chain of lakes dominate the north-eastern area. Many small swamps and soaks, located in the interdunal depressions, are scattered around the eastern half of the island. The serrated coastline consists of a succession of exposed headlands and sandy bays. There are also a number of sand blowouts that support little or no vegetation (Anon. 1978; Anon. 1983). The vegetation on the island has changed dramatically since settlement and today is described as predominately a low shrubland dominated by *Acanthocarpus preissii* (Anon. 1978; Anon. 1983; Pen & Green 1983; Rippey & Rowland 1995). Other plant communities present are described in more detail in the above references.

The climate of Rottnest Island is Mediterranean, with mild winters and hot summers, with February being the warmest month with an average maximum of 26.5 °C and minimum of 18.7 °C and July the coldest month with an average maximum of 17.2 °C and minimum of 11.6 °C. The average annual rainfall is 713 mm with January being the driest month and June the wettest with mean rainfalls of 6.8 and 156 mm per month respectively (Bureau of Meteorology).

Survey of Cat Activity and Control Options

Records of cat sightings by Rottnest Island Authority staff, maintenance personnel and residents have been documented since April 2001. These records suggested that perhaps up to five feral cats were present on the island at the time this program was implemented. Prior to control measures being put into operation, extensive, intensive searches, over a period of ten days, were conducted across the island looking for evidence of cat activity. These included locations at which cat activity had been reported to island rangers. All areas of sandy substrate, including access tracks/roads, beaches, interdunal areas and sand blowouts, where cat tracks could be observed were surveyed either on-foot or from a 4WD vehicle, in 2002 an All Terrain Vehicle was also used. The surveys for cat activity in 2002 also coincided with the annual maintenance of the island's extensive firebreak network. Ripping of firebreaks assisted in the detection of cat activity by removing vegetation and breaking compacted soil, such that cat tracks could be reliably detected. The location of fresh cat activity, its extent and the distances between sites provided a focus for control effort.

King (1985) suggested that baiting might provide a suitable method to control cats on Rottnest Island although at the time, there appeared to be considerable difficulty in getting feral cats to accept baits. With the successful development of the feral cat bait (*Eradicat*®) containing the toxicant sodium monofluoroacetate (compound 1080), baiting is now recognised as the most effective method for controlling feral cats where there is no risk posed to non-target species populations (EA 1999; Algar & Burrows 2004; Algar *et al.* 2007). These baits have also been employed to successfully eradicate cats from Hermite Island in the Montebellos (Algar & Burbidge 2000; Algar *et al.* 2002) and on Faure Island in the eastern gulf of Shark Bay (Algar *et al.* 2010). On Rottnest Island however, broad-scale application of feral cat baits was discounted as a suitable control strategy for two reasons. Firstly, Quokkas were likely to consume multiple baits. Despite Quokkas being listed as highly tolerant to the toxin 1080, they have a high, yet variable LD_{50} of between 10–40 mg/kg (King 1990). The current 1080 dose rate for cat baits is 4.5 mg 1080/bait and therefore, an individual Quokka would only need to consume 2–3 baits for there to be a potential risk. Secondly, research has suggested that the optimum time to conduct baiting programs and maximise their effectiveness is under cool, dry conditions in late autumn/winter (Algar & Burrows 2004; Algar *et al.* 2007). At this time rainfall, which will cause degradation of feral cat baits is less likely to occur than during the summer months, and the abundance and activity of all prey types, in particular predator-vulnerable young mammalian prey and reptiles, is at its lowest and bait degradation due to rainfall, ants and to hot, dry weather, is significantly reduced.

The timing of the program on Rottnest Island during the early summer, non-target bait risks and the fact that only a few cats appeared to be on the island suggested that a trapping program would be a more viable control strategy.

Trapping Program

Two trapping techniques were used; cage traps were deployed in residential areas where cats would be used to scavenging food scraps. Leg-hold traps were located at sites distant from of human habitation.

In November 2001, 30 wire cage traps (60x20x20 cm) with treadle plates were located in the settlement area. Further to these, cages were also placed at the nursery (1 cage), university house – Wadjemup Hill (2 cages), ranger's house – Wadjemup Hill (2 cages) and immediately outside the waste disposal site (2 cages) (see Fig. 1). The majority of these traps were left in position over a five-day period, providing 145 trap-nights. In November 2002 five cage traps were placed at various locations around the perimeter of the waste disposal site. These traps were operated for a total of 15 trap-nights. All traps were baited with either fresh pilchards or non-toxic *Eradicat*® feral cat baits and sprayed with an ant deterrent compound (Coopex®) at a concentration of 12.5 gl^{-1} Coopex, as per the manufacturer's instructions.

Cage traps can be ineffective for trapping feral cats (Friend & Algar 1993; Lee 1994; EA. 1999). A more effective technique to trap feral cats utilises padded leg-hold traps, Victor 'Soft Catch'® traps No. 3 (Woodstream Corp., Lititz, Pa.; U.S.A.), a Felid Attracting Phonic (FAP) that produces a sound of a cat call, and a blended mixture of faeces and urine. The trapping methodology is described in detail in Algar *et al.* (2010).

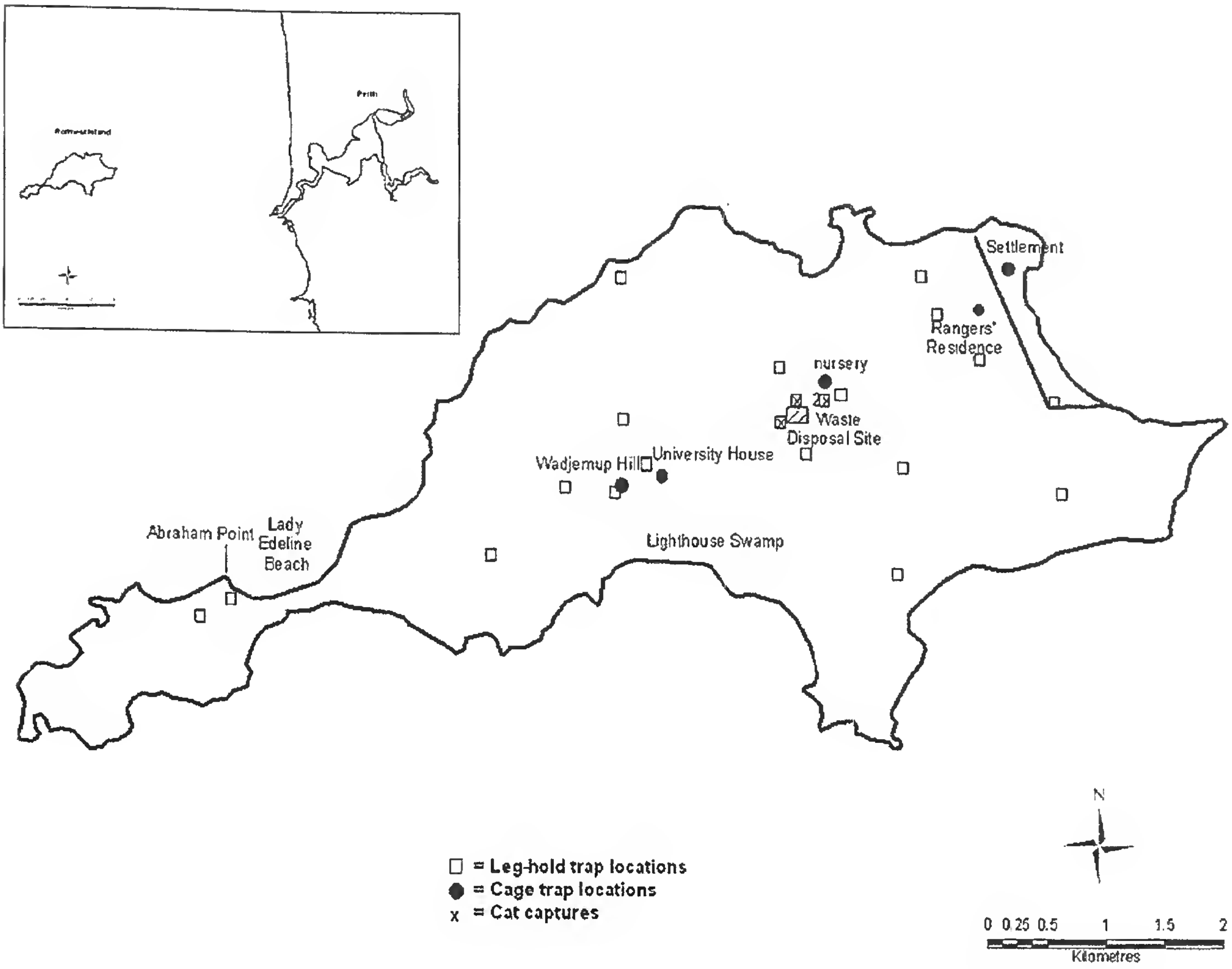

Figure 1. Leg-hold trap, cage trap and cat capture locations

To prevent the potential risk of capturing Quokkas and causing them injury, the leg-hold traps were located in areas from which Quokkas were excluded. Existing fenced rehabilitation exclosures that excluded Quokkas were used where available. Where existing exclosures were not available, or they did not exclude Quokkas, small exclosures (approximately 2x2x1 m), manufactured on-site with rabbit netting and star pickets, were employed. In 2001, 21 trap sites were located strategically around the island (see Fig. 1). All traps were left in position for a minimum of five days, providing a total of 162 trap nights. In 2002, three trap sites were located around the waste disposal site over a three day period. The traps were routinely checked at first light each day.

Necropsies and Analyses

Trapped cats were destroyed using a 0.22 calibre rifle. All animals captured were sexed and weighed; a broad estimation of age (as either kitten, juvenile or adult) was recorded using weight as a proxy for age. The pregnancy status of females was determined by examining the uterine tissue for embryos. Stomach contents were collected for diet analysis. Samples of brain, muscle, spleen, blood and faeces were collected to determine the incidence of diseases and parasites in the population that are potentially harmful to both humans and native fauna.

Results

Cat Monitoring Program

In 2001, evidence of fresh cat activity other than when a cat was captured, was recorded on 11 occasions during searches. All but one of these areas of cat activity was within 2 km of the site of capture of the three cats. The track activity along Lady Edeline Beach to Abraham Point (see Fig. 1) was less than 5 km from the capture sites. This area of activity was observed only once and despite intensive searches along this and other beaches and interdunal swales in the area, no further evidence of cat activity was recorded during the program.

No evidence of cat activity was observed or reported following the capture of the three cats (see below) until April 2002. Feral cat activity was reported to island rangers at three locations during 2002, twice within the settlement and once at Lighthouse Swamp (see Fig. 1 for location) (B Daw, pers. comm.). Searches of these locations in November 2002 failed to detect the presence of feral cats. Tracks of what appeared to be a single cat were noted on three successive mornings in November 2002, that activity was confined to the waste disposal site proper and within a several hundred-metre radius.

Following capture of this cat, no further cat activity was observed. In the eight years subsequent to this program, no further cat activity has been observed or cats sighted by Rottnest Island staff or the general public (R Caccianiga pers. comm.).

Trapping Program

Three cats (2 male and 1 female) were captured in 2001 and a further cat (female) was trapped in 2002; all were caught in leg-hold traps. Examination of uterine tissue for scarring indicated that neither female cat had carried a litter in at least the previous year. Details of the cats captured, in terms of sex, weight, coat colour and age, are presented in Table 1. Captures ROT 01–03 were in rehabilitation exclosures either side of the waste disposal site, with ROT 02 and ROT 03 being trapped at the same location. ROT 04 was captured in a small exclosure, manufactured on-site located at the waste disposal site gate. The capture locations are shown in Figure 1.

The trapping program in 2001 also resulted in the capture of two non-target species: 37 Quokkas and two King Skinks, *Egernia kingii* (Gray, 1839). All but one of these captures occurred in cage traps. One Quokka was captured in a leg-hold trap, within the rehabilitation exclosure immediately north of the refuse site. The animal was released with a slight oedema to the limb held and the trap set decommissioned. In 2002, three quokkas were captured in cage traps and released without injury. A Ring-necked Pheasant, *Phasianus colchicus* (Linnaeus, 1758), and an Australian Raven, *Corvus coronoides* (Vigors & Horsfield, 1827), were captured in the same leg-hold trap on two consecutive days. Both animals received lacerations to the held limbs and were destroyed.

Of the four cats trapped, three had dietary items in their stomachs. The stomach volume and contents of captured cats are described in Table 2.

Analyses of the samples collected to determine the incidence of diseases and parasites in the population were only conducted on cats captured in 2001. Results of the analyses indicated that all three cats had been exposed to *Toxoplasmosis*, serum immunoglobulin titres are given in Table 3 (where > 1/64 is considered positive and <1/24 is considered a cross-reaction). No tissue cysts were detected in any of the cats, however the cat tapeworm (*Taenia taeniaformis*) was present in the stomach of cats ROT 02 and 04.

Table 1

Capture records of trapped cats (K = Kitten, J = Juvenile, A = Adult)

Date	Sample No.	Sex	Weight (kg)	Coat colour	Age (K/J/A)
20/11/01	ROT 01	Male	3.50	Black/white	A
22/11/01	ROT 02	Female	2.65	Black/white	A
23/11/01	ROT 03	Male	3.85	Black	A
20/11/02	ROT 04	Female	3.10	Black/white	A

Table 2

Stomach volume and contents of trapped cats

Sample No.	Approximate stomach volume (%)	Stomach content
ROT 01	10	1x *Egernia kingii*
ROT 03	60	2x *Mus musculus*, 2x *Phasianus colchicus* runners
ROT 04	70	1x *Mus musculus*, cooked chicken bones and meat

Table 3

Serum immunoglobulin titres for *Toxoplasmosis* in captured cats

Cat Number	Serum immunoglobulin titre (IgD)
ROT 01	1/256
ROT 02	1/128
ROT 03	1/128

Discussion

Results from this program and the fact that no cats have been observed or trapped since its completion suggest that cats have been successfully eradicated from Rottnest Island.

The three cats captured in 2001 all had stumpy tails, which implies a high degree of relatedness. The cat trapped by Authority staff, earlier in 2001 also had a stumpy tail (B Daw pers. comm.). The coat phenotype of the cat trapped in 2002 suggested it was a close relative of the three individuals captured during 2001 and was not likely to be a recent introduction. Inbreeding often brings about an increased frequency of homozygosity and causes a reduction in survival and reproduction (Ishida *et al.* 2000) which could explain the absence of kittens and sub-adult cats on the island. The low density of cats on Rottnest in November 2001 and the apparent close relatedness of these animals suggest that previous control efforts by the Authority were successful in diminishing the viability of the feral population. The lack of genetic input from a domestic population and the dramatic reduction of the feral population left it inbred and invigorous. Very few individuals were captured in the previous decade and a population of only four individuals was present on the island in 2001. Given the capacity of cats to produce up to eight kittens in a litter and conceive potentially twice in a year, the removal of so few individuals would not have kept pace with recruitment by a viable population.

Records of cats captured during previous trapping programs are summarised in Table 4. This shows a decline in the number of cats caught over the years. A total of 63 cats was captured during these various exercises and of these animals, 81% were trapped in the general vicinity of the waste disposal site. All four cats trapped during this campaign were also captured in the general vicinity of the waste disposal site which indicates that this area is a focus of cat activity on the island.

It is always possible that individual cats will re-establish on the island. The most likely route of reinvasion is from domestic cats straying from pleasure craft moored around the island. Evidence suggests that if any cats do stray onto the island they will eventually

Table 4

Location of cat captures on Rottnest Island. Of the total number of trapped cats those captured at the waste disposal site are indicated in parentheses

Year	No. cats captured
1986	39 (31)
1987	2 (2)
1988	3 (2)
1989	4 (3)
1992	2 (2)
1993	4 (3)
1994	5 (5)
1995	2 (2)
1996	1 (0)
2001	1 (1)

locate at the waste disposal site or the settlement where they will be able to scavenge food. Cats that do stray onto the island will be highly visible, invariably solitary and therefore easily removed. Removal of these animals should employ the trapping methodology adopted in this program. Cats are very inquisitive about other individuals in their area and this is likely to be more so if they are recent strays onto the island. The communication instincts of cats are principally reliant on audio and olfactory stimuli and the trapping technique uses lures based on these traits. As shown by this exercise, placement of traps in exclosures prevented the capture of non-target species but did not hinder access by cats. The employment of exclosures where capture of non-target species is an issue may also provide an invaluable tool in cat control strategies elsewhere.

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Do possum (*Pseudocheirus occidentalis; Trichosurus vulpecula hypoleucus*) counts vary with the time of the survey?

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Abstract

Western Ringtail Possums (*Pseudocheirus occidentalis*) are listed as conservation significant species with both State and Commonwealth governments. As a consequence any areas proposed for development that potentially support these possums are surveyed to determine their abundance. Here we report a decline in the number of *P. occidentalis* recorded for successive surveys during the same night suggesting that some individuals either retreat to areas where they are not visible or observer fatigue results in fewer possums being detected. In contrast, there was no difference in the number of Common Brushtail Possums (*Trichosurus vulpecula hypoleucus*) observed during the same surveys. This finding has important consequences for environmental consultants or researchers that are undertaking multiple surveys during a night to record local population sizes for Western Ringtail Possums in Western Australia.

Keywords: Western Australia, fauna surveys

Introduction

Western Ringtail Possums *(Pseudocheirus occidentalis)* are listed as vulnerable under the Commonwealth Environment Protection and Biodiversity Conservation (EPBC) Act (1999) and as a Schedule 1 species (Fauna that is rare or is likely to become extinct) under the Wildlife Conservation Act (1950), with the consequence that land developers that may impact on these possums are required to survey the site prior to a development to estimate the population.

Pseudocheirus occidentalis is most abundant along the coastal strip of vegetation between Yalgorup and Dunsborough (Jones *et al.* 1994b; de Tores 2008; DEWHA 2008), but scattered populations also exist throughout the south-west of Western Australia as far east as Two Peoples Bay near Albany with some isolated inland populations at Collie, Yendicaup and Moradalup (Jones *et al.* 2007). *Pseudocheirus occidentalis* is nocturnal and usually shelters by day in a drey (bird-like nest) or tree hollow. Dreys are typically located in the crown of Peppermint trees (*Agonis flexuosa)*, but may be constructed in other tree species, such as Melaleuca, Banksia, Marri, Tuart and Jarrah trees (Thompson & Thompson 2009). But they can also be found in dense sword grass, grass trees, reeds, sedges, blackberry thickets, fallen logs and disused rabbit warrens or use tree hollows as retreats (de Tores 2008; Harewood 2008; Jones *et al.* 1994a).

Because of its conservation status, all areas that support native vegetation or peppermint trees that are proposed for development in the coastal strip between Bunbury and Dunsborough should be searched for *P. occidentalis* and their dreys counted as part of the fauna assessment to support an environmental impact assessment or native vegetation clearing permit application. Being nocturnal requires that the counting of *P. occidentalis* must be done at night. Where multiple areas are to be surveyed at the same time, environmental consultants will frequently survey multiple sites in one night. Searches are typically undertaken using a head torch and walking through an area counting the number of *P. occidentalis* seen.

Lindenmayer *et al.* (2001) and Goldingay & Sharpe (2004) concluded from their review of the effectiveness of spotlighting to count arboreal mammals that this technique was being used more frequently in environmental assessments and additional research was required to determine whether spotlighting counts can be calibrated and used for population estimates.

Davey (1990) reported spotlighting to be an effective method for surveying nocturnal marsupials, however, Lindenmayer *et al.* (2001) reported that spotlighting significantly underestimated the population size for greater gliders (*Petauroides volans*). Wayne *et al.* (2005) suggested that there was variability in the different methods of counting *P. occidentalis*, and best results were obtained from scat surveys and spotlighting using a 50-W hand-held spotlight. They also noted significant differences among observers in spotlighting counts. Our experience using head torches while walking and searching trees during repeated surveys of *P. occidentalis* at the same site also indicated that counts varied, suggesting that not all *P. occidentalis* were being detected during a single survey. Wayne *et al.* (2005) reported that heavy rain, wind, cloud cover and vegetation structure affected counts of *P. occidentalis* and the Common Brushtail Possum (*T. v. hypoleucus*), however, start time after sunset did not affect detection rates for either of these species. Contrary to the data presented by Wayne *et al.* (2005), our anecdotal records from repeated surveys of the same site suggested that survey period might be affecting detection rates. Our objective for this study was to determine whether the number of *P. occidentalis* being detected varied with successive surveys on the same night. As our survey area also contained a population of *T. v. hypoleucus,* we included this species in the survey.

Methods

The study site consisted of a rectangular 5.3 ha isolated patch of Peppermint (*Agonis flexuosa*) woodland approximately four kilometres from the Busselton town centre (33.66 °S, 115.39 °E). The area outside of the isolated patch of Peppermint trees was grassed open pasture, a bitumen road and school playing fields, and a partially developed housing estate. The area contained a series of parallel black plastic irrigation pipes that were approximately 9m apart which were used as transects.

Possums were located by two observers using head torches while walking along every second irrigation pipe on two consecutive nights (22nd and 23rd December 2008). Generally, one observer would walk along the irrigation pipe and the other to the eastern side of the irrigation pipe. The search focussed on the area approximately 10–15 m either side of the irrigation pipe. The two observers were experienced in spotlighting *P. occidentalis* and were familiar with this site as it had been surveyed on multiple previous occasions.

Surveys commenced after dark at about 22:45 hr, 01:15 hr and 4:00 hr on both nights. Possums did not flee when they were detected and therefore double counting of individuals was unlikely, although possible. The location of each possum was recorded using a GPS, along with the tree species and the height of each possum in the tree. It was not practical to measure the height of the possum in a tree, so the height of each possum was estimated.

Data analysis

Multiple surveys on the same night and repeated again on a subsequent night would generally require a repeated measures approach to analysing the data. However, this is not possible in this circumstance as repeated measures ANOVA requires more subjects than repeats which we do not have for the number of observations of either species of possum. We did not record each individual possum's height in a tree for subsequent surveys, as there was no way of identifying individual possums.

We used a goodness-of-fit measure to examine the extent to which the number of observed possums deviated from the average number of possums observed for the six periods of observation. As there was a significant difference in these data, we then combined the number of observations for the first, second and third periods on each night and tested for a temporal difference in observations against the average number of observations over the entire night. A significant difference would indicate there was a temporal variation in the number of observations. A two factor ANOVA was used to test for difference in the height of possums in trees. A Tukey post-hoc test was used to examine differences between survey periods. A level $\alpha = 0.05$ was used in all analysis to determine if differences were significant.

Results

There were clear skies with almost no wind on both evenings the surveys were undertaken. The survey site has a tree canopy that ranges from 5–20m with little vegetation other than tree trunks to 3m above ground level. Visibility with head torches in locating possums was generally good.

There was a significant difference between the number of *P. occidentalis* observed over the six survey periods ($\chi = 12.4$, df = 5, $P = 0.03$) and a significant difference among the three survey periods ($\chi = 11.1$, df = 2, $P = 0.004$) indicating a significant and declining temporal difference in the number of *P. occidentalis* counted (Fig. 1). The pattern was the same for *T. v. hypoleucus* for the second to the third period but it was not true for the first to the second period (Fig. 2; $\chi = 4.8$, df = 5, $P = 0.44$).

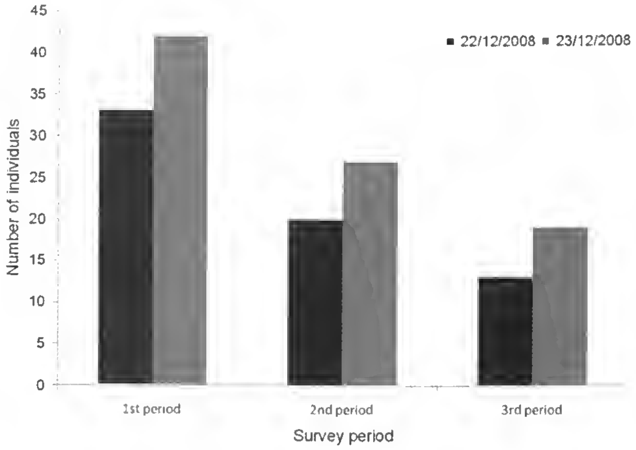

Figure 1. Number of *Pseudocheirus occidentalis* seen during three successive surveys on two nights.

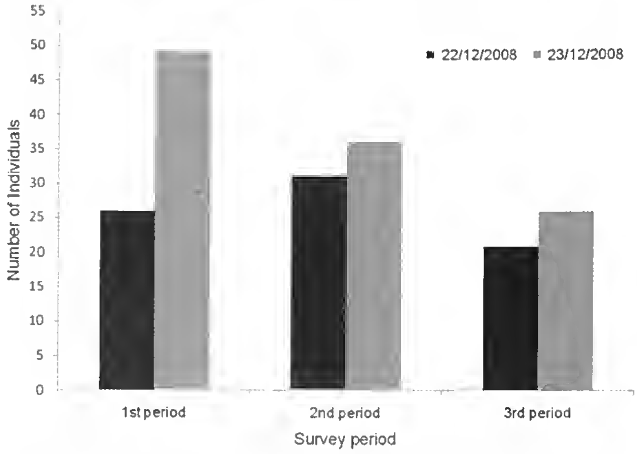

Figure 2. Number of *Trichosurus vulpecula hypoleucus* seen during three successive surveys on two nights.

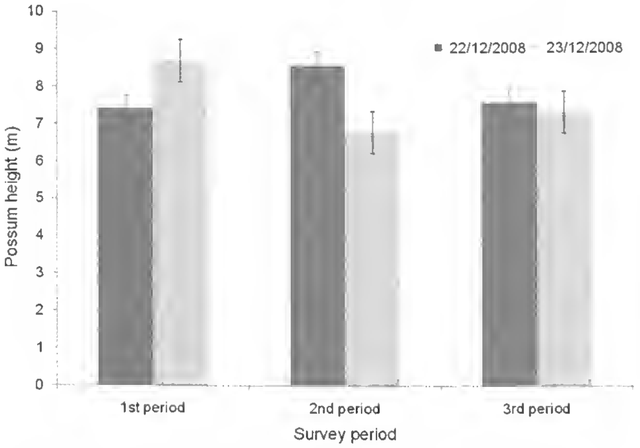

Figure 3. Height of *Pseudocheirus occidentalis* in trees during three successive surveys on two nights (1 se bars).

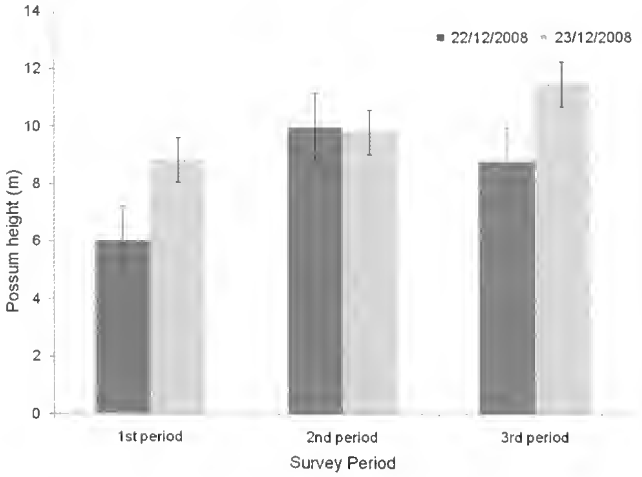

Figure 4. Height of *Trichosurus vulpecula hypoleucus* in trees during three successive surveys on two nights (1 se bars).

The estimated height of *P. occidentalis* varied significantly among survey periods ($F_{1,2}$ = 15.5, $P < 0.01$; Fig. 3) and there was a significant interaction between night and survey period ($F_{1,2}$ = 4.1, P = 0.02; Fig. 3). The post-hoc test indicated significant differences between the first and second, and first and third surveys. Similarly, for *T. v. hypoleucus*, there was significant difference in the height they were observed in the tree ($F_{1,2}$ = 127.6, $P < 0.01$; Fig. 4), a significant interaction between night and survey period and again the post-hoc test indicated significant differences between the first and second, and first and third surveys.

Discussion

Difference in the number of possums counted during successive surveys on the same night can be attributed to one of three reasons: a) possums moved out of the survey area; b) possums were not be detected as they had not emerged or have returned to their retreats, were higher in the canopy and could not be seen or had turned their heads away from the light so that their eye shine was not evident (Davey & Robinson 1986; Lindenmayer *et al.* 2001); or c) observers were less vigilant, became fatigued and tired, or changed their behaviour in some way during subsequent surveys each night and missed seeing individuals as the night progressed (Lindenmayer *et al.* 2001).

A few possums occasionally moved in and out of the study area. We know that this occurs as dead possums have been recorded on roads in the adjacent area and a night security guard for the nearby housing estate has occasionally seen possums running across the road at night. We believe the number of possums that moved out of our survey area on any one night would have been low, and it is likely that a similar number of possums moved into and out of the survey area as our long term data for the site suggested the population was relatively stable.

Lindenmayer *et al.* (1991) reported emergence times from dens varied for arboreal marsupials and differences were correlated with body mass, field and standard metabolic rate. MacLennan (1984) reported that for the first two hours after sunset, *T. vulpecula* spent most of the time in dens grooming, moving around or sitting, and feeding did not commence until the second hour after sunset. MacLennen (1984) reported *T. v. hypoleucus* mostly foraged between the fourth and eight hour after sunset and returned to their den 8.98hrs (± 0.96) after sunset. Sunset was about 19:05hr and we commenced our survey at about 21:45hr (corrected for daylight saving) which was greater than two hours after sunset. Based on the observations of MacLennan (1984) some *T. v. hypoleucus* may have still been in the den or were preparing to come out and forage. Similar data on emergence times are not available for *P. occidentalis*.

The height of possums in trees varied significantly, but they were not significantly higher in the last survey each night compared with the earlier surveys (Figs 3 and 4) suggesting that the difference in the number of individuals observed was not due to more individuals being higher in the tree canopy and more difficult to observe.

In summary, we counted more *P. occidentalis* during the first survey of the night, and this number decreased on both nights during the second and third surveys. The pattern was different for *T. v. hypoleucus*, with a decrease on both evenings between the second and third surveys, but with a difference between the two nights for the first survey. We consistently observed more possums of both species during the second night survey. There are two possible reasons for the variation in the number of possums recorded. *Pseudocheirus occidentalis* either shifted their position so that they were difficult to observe (*e.g.* moved into a tree hollow, moved higher in the canopy, looked away from the light) or observer fatigue meant that fewer possums were recorded. There is no evidence to suggest these possums moved higher in the canopy between successive surveys each night but it is possible that *P. occidentalis* returned to their retreats and could not be seen. If *T. v. hypoleucus* were slower to emerge on the second night, then both reasons outlined above could have contributed to the reduced number of possums being observed during the second and third survey periods each night. We presumed that the difference in the number of possums recorded between the two nights reflected the difference in the number of possums that were active and out of their retreats.

Whatever the reason for the lower number of *P. occidentalis* being observed during the second and third surveys each night, it is evident that observations during the early part of the evening record a higher number of individuals than those later. In addition, the number of possums observed varied significantly between the two successive nights. These findings have important implications for future *P. occidentalis* surveys that are used to record the number of individuals in a particular site or are used as part of an ongoing monitoring program. Our recommendations are that surveys should be concluded by midnight and a minimum of two surveys should be undertaken to record the population of possums in an area.

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A comprehensive classification of inland wetlands of Western Australia using the geomorphic-hydrologic approach

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Abstract

An expanded site specific classification of wetlands using landform types and water regimes as fundamental and primary criteria is presented here. The subdivision of landform types host to wetlands now includes hills, cliffs, slopes, flats, vales, channels, and basins. Further to the initial subdivision of water regimes as permanent, seasonal and intermittent inundation, or seasonal waterlogging, is now added permanent waterlogging. Combining landform types and water regimes results in 22 primary categories of wetlands. Use of landform, water and vegetation descriptors further separate types of wetlands, and it is at this lower level that attributes such as scale (size), hydrochemistry, wetland plant communities, and origin, can be emphasised. Self-emergent wetlands are differentiated from *in situ* wetland accumulations associated with convex landforms and which conform to the underlying land surface. This classification may be used as the basis for a world register and/or mapping of wetlands from arid to humid climates, and the number of classes which cover the global diversity of wetlands at the primary level is relatively small.

Keywords: inland wetlands, wetland, classification, geomorphic-hydrologic, Western Australia

Introduction

Land, in a general sense, is that part of the Earth's surface that stands above mean sea level (Hettner 1928; Jackson 1997). Landforms and other features produced by natural processes, which comprise the Earth's surface, have characteristic and repetitive shapes. Major forms include mountains, plains and plateaux; minor forms include dunes, valleys, cliffs, hills, and slopes (von Engeln 1949; Fairbridge 1968; Jackson 1997). Wet landforms are also part of the Earth's land surface. The most obvious difference between wet landforms and all others is a predominance of water in the former (Warner 2004). Because of the richness and diversity of biological processes which take place in wetlands, the wide range of wet landforms have been variously defined, delineated, described, and categorised often from a consideration of what drives their productivity. In all cases, what drives productivity is the presence of water and the modification of its chemistry through geological/geomorphic, hydrological, hydrochemical, and biotic interactions. Definitions of wetlands have attempted to include the basic three wetland attributes of geologic/geomorphic, hydrologic and biotic features and processes. Boundaries of wetlands have been delineated with respect to their geological, hydrological, and biotic properties; and descriptions of wetlands have included geological, hydrological and biological attributes and values (Cowardin *et al* 1979; Ramsar 1996; Tiner 1999; Richardson & Vepraskas 2001). It is important that wetland classifications also incorporate these three attributes.

And herein begins the problem because two of these components are more stable (*viz.*, geologic/geomorphic and hydrologic) and find a longer term expression in nature than the third (*viz.*, biologic). Therefore, for the purposes of classification and mapping, the two components that are less dynamic and changeable, ideally, should be selected at the basic level, leaving the third important component of biology to be incorporated at higher levels. What this means is that attributes of land and water should constitute the primary level of classification while biological attributes should be added at subsequent levels to incorporate more detail

A classification of wetlands at the site-specific level, using the primary attributes of land and water, accompanied by second level descriptors, was devised for south-western Australian conditions (Semeniuk 1987; Semeniuk & Semeniuk 1995). This classification has been tested throughout different climatic and biogeographic regions, geological and physiographic provinces of Western Australia, Australia, and globally, and has been found to be applicable to a large range of wetlands. Since 1995, with a larger database and terrain testing, the original wetland classifications of Semeniuk (1987) and Semeniuk & Semeniuk (1995) were expanded to incorporate additional wetland types, hydrologic regimes, and to refine some terms. These changes are formalised in this paper.

The approach, underpinning the classification presented herein, rests on the premise that wetlands are essentially "wet landforms" and lend themselves to systematic classification initially on their landform and water characteristics. In this context, some ecosystems proposed to be wetlands by other authors, are excluded. Glacial ecosystems are excluded on the basis that their environmental driver is ice, not water. Underground ecosystems, such as caves, also are excluded on the basis that they do not occur *on* the land, but are subterranean.

Classification principles, existing wetland classification methods and criteria were reviewed by Semeniuk (1987) and Semeniuk & Semeniuk (1995, 1997, 2011). It is not intended to present further reviews in this paper, but the resulting changes to the existing geomorphic-hydrologic classification presented here are the outcome of this ongoing enquiry.

Landform refers to the structure or geometry of the land and not to the resulting combination of landform and accumulated wetland sedimentary fill. In Semeniuk & Semeniuk (1995), five landform types, which host wetlands, were identified: hills, slopes, flats, channels, and basins. As landforms are intergradational, two principles were established: 1. the complete landform should be classified and not compartmentalised into its smaller parts; and 2. in the situation where basins are interconnected, each basin is defined by its central depression even though the littoral zones of the adjacent basins may adjoin.

In Semeniuk & Semeniuk (1995), four categories of water regime or hydroperiod were identified: permanently inundated, seasonally inundated, intermittently inundated, and seasonally waterlogged. For the gradations between the categories, and hydroperiods, three principles were established: 1. hydrological condition should be the prevailing one, 2. hydrological condition should be based on the presence of water rather than its absence, and 3. an area of 10% of the wetland should be used as the cut off point between lake and sumpland during periods of surface water contraction (Semeniuk 1987). An example, in which the first principle is relevant, concerns the variable expressions of permanent inundation. All three of the following conditions are included in this category: 1. areas where water covers the land surface throughout the year in all years, 2. permanently flooded areas which seasonally or intermittently contract to a central pool (but > 10% of the basin area), exposing wetland margins, and 3. areas where water covers the land surface but which are intermittently exposed. The second principle directs classification of wetlands to the period when surface or subsurface water is present. The third principle enables the researcher or land manager to distinguish between a lake and a sumpland, using as a criterion, the percentage of wetland which is inundated.

Descriptors to further classify wetlands were used for both land and water. For land they were based on geometry, size, and vegetation cover and type (Semeniuk & Semeniuk 1995; Semeniuk *et al* 1990). For water they were primarily related to water salinity and its consistency.

The comprehensive geomorphic-hydrologic classification of wetlands

In the approach to wetland classification presented here, non-emergent wetlands are separated in the first instance from self-emergent wetlands. The latter, through vertical accretion of sedimentary materials or chemical and biogenic accumulations have mounded beyond the original land surface into a new convex wetland geomorphology (Fig. 1).

Non-emergent wetlands

Non-emergent wetlands are terrain conforming, and occur in any landscape where there is sufficient water to maintain them. The initial subdivision of landforms host to wetlands from the Swan Coastal Plain (Semeniuk 1987, and augmented in Semeniuk & Semeniuk 1995) has now been expanded to seven landforms, *viz.*, basins, channels, vales, flats, slopes, cliffs and hill-tops (Fig. 2). It should be noted that the individual landforms are not, at this level, subdivided into genetic types. For example, basins have not been subdivided into groups of glacial origin, those formed by fluvial action, shoreline processes, wind deflation or deposition, isostatic uplift or those of biotic origin. Nor have basins been distinguished on the basis of size, with larger basins separated from smaller or shallower ones. In this instance, the term "basin" is only employed to differentiate it from either a vertical or horizontal flat surface, a convex or sloping surface, or an open ended concave feature. As Coleman (2003) remarks "morphological features are usually obvious". They are also relatively slow to change, and hence lend themselves to classification.

As noted earlier, the requirement to identify an entire landform feature or geomorphic element excludes subdivision of landforms into smaller land components, such as 1. the littoral and the central parts of a wet basin, 2. the transition zone between upland and lowland, 3. the top and slope of a hill, 4. the area of basin behind a lunette that may occur within a wetland, 5. the pools and riffles of a channel, or 6. "fuzzy" non-prescriptive areas supporting a plant community.

Further to the initial subdivision of water regimes, *viz.*, permanently inundated, seasonally inundated, intermittently inundated, and seasonally waterlogged (Semeniuk 1987; Semeniuk & Semeniuk 1995), is now added a fifth – permanently waterlogged.

Water regimes are important to biological processes, population densities, community composition, and overall regeneration and ecological maintenance. The water regime is also important in determining the nature of wetland fills, the types of interactions between the geology, plants and water, and the resulting wetland hydrochemistry (Semeniuk 2007) which, in their turn, also influence biological and ecological functioning. Hydroperiod and the chemistry of waters will also determine the specific type of wetland that will develop (Warner 2004). Therefore, it is particularly important to distinguish between water that is permanently available, regardless of temperature, colour, depth, salinity, and dissolved oxygen, water that is available intermittently, but in a regular, cyclic, predictable, pattern, and water that is intermittently available in an unpredictable, opportunistic, longer term regime. Thus, three categories of cycle (or period) have been recognised – permanent, seasonal and intermittent. Seasonal and intermittent are distinguished on the important basis of a recurrence every year *versus* an occurrence of inundation every 5–20 years. It is also important to emphasise that the attribute selected for classification is the availability of water. The presence of ice in a wetland, and certainly permafrost, does not denote water availability. Wetlands, in which ice alternates with melt water, would fall into the category of a seasonal water regime.

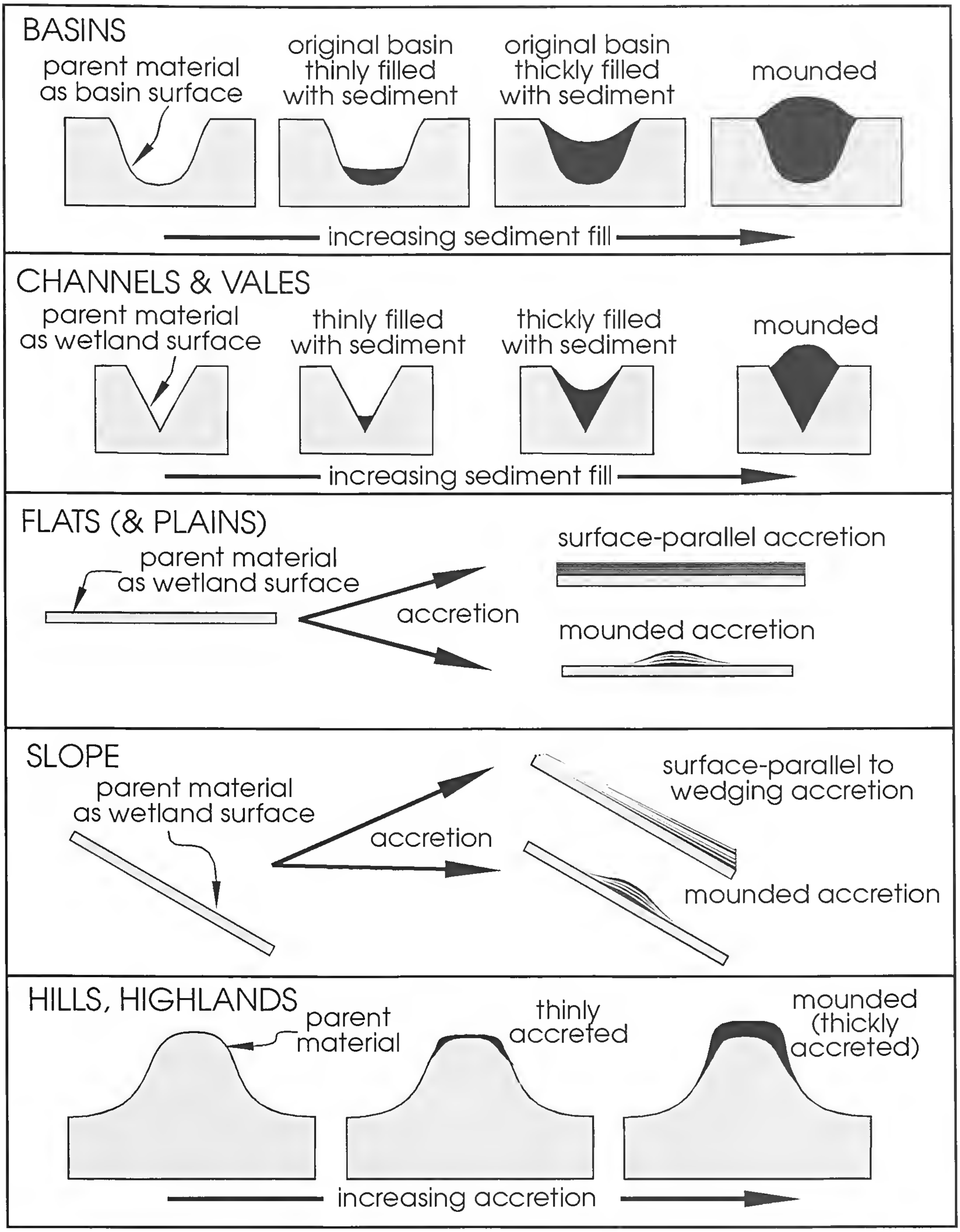

Figure 1. Stages in the accretion, filling or mounding of sediments, precipitates and biogenic deposits within and on wetlands.

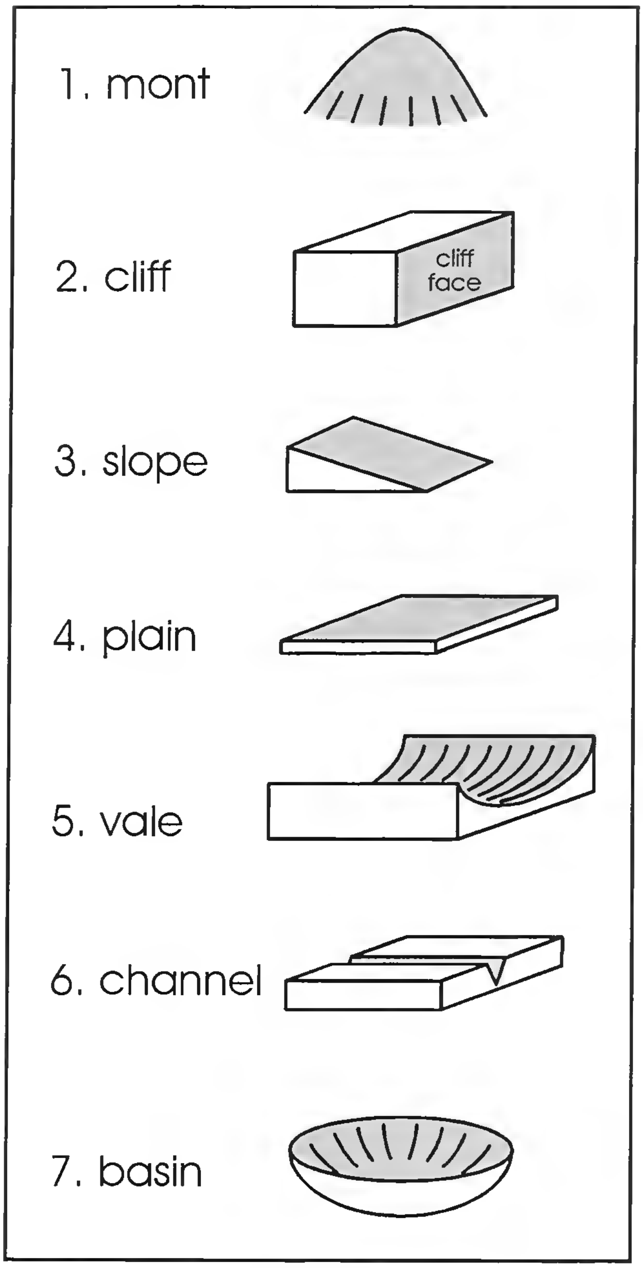

Figure 2. Seven landform types host to wetlands

Another aspect of the water regime relates to where the water resides. This distinction is made because biota have specific niches and because surface water and subsurface water are subject to different hydrological, chemical and ecological processes. Water that inundates the surface of the wetland may well also saturate the subsurface but this is not always the case. In many wetlands, in arid areas, or areas underlain by bedrock, water (as rainfall or as run-in) may be perched. Where surface water is present it is considered to be the source and driver of specific wetland functions and processes, wholly separate and different to the saturation of the subsurface sediments. These concepts are embedded in the terms inundation and waterlogging.

Definitions of terms for landscape and water regimes are provided in Tables 1 and 2.

Table 1

Types of wetland landforms

Landscape	Description/definition
basin	contained, closed depression in the landscape
channel	linear, though not necessarily straight open relatively narrow depression
vale	linear, open, relatively broad depression in the landscape, valley tract
flat (or plain)	flat or very slightly undulating terrain
slope	sloping surface
cliff	vertical or near vertical precipice; steep or overhanging face of rock
hill-top	convex upper surface of landscape

Table 2

Types of wetland water regimes

Water regime or hydroperiod	Description/definition
permanently inundated	water present above surface permanently, though its level may fluctuate
seasonally inundated	water present above surface on a seasonal basis
intermittently inundated	water present above surface intermittently, say, every 5–10 years
permanently waterlogged	water wetting or saturating the surface or near-surface permanently
seasonally waterlogged	water wetting or saturating the surface or near-surface seasonally

Vales and cliffs are the two new landform types used in the geomorphic-hydrologic classification in this paper. While the term valley describes narrow to broad u-shaped to v-shaped (straight, meandering, irregular) linear depressions, to gorges, that are often fluvial tracts of rivers, creeks, and wadis, and that range in size from tens of metres to tens of kilometres in width and several metres to a few hundred metres in relative depth, vales are small valleys. The cross-sectional form of a vale is generally smoothly u-shaped, showing no clear demarcation between the base and sides, and the development of wet flats and slopes, usually found in valleys, is minor, and discontinuous. Vales may or may not contain segments of small scale channels. Where a channel passes through a vale it is classified independently. Vales are commonly sites of saturation or waterlogging. Vales host to wetlands are underlain by soils or sediments diagenetically or pedogenically altered by waterlogging, and support wetland plant assemblages.

Cliffs are a product of rock outcrop weathering and erosion and have some affinity to slopes. There are several common locations where cliffs are present: at the coast, in the thalweg of a stream as it descends from plateau to plain, or from hanging valley to lower valley, on the edge of canyons, and in mountainous regions, where erosion is active. As coastal cliffs are a subset of coastal wetlands and this paper deals with inland wetlands, coastal cliffs are not described further here.

Cliffed sections of a stream channel, such as waterfalls along their tract, are not considered to be separate wetlands although they comprise separate habitats and components of the stream bed similar to internal pools, riffles, and point bars. Cliffs, as described here, refer to landforms associated with erosional processes of high land. They may occur in arid or humid environments. Wet cliffs are normally sites of permanent or seasonal waterlogging, due to seepage from, or between, rock strata. It is interesting to note that, similar to palusplains, the wetting of the surface may be patchy with saturated and unsaturated domains co-existing due to the conditions of water flow. As for other seasonally waterlogged landforms (palusplains, paluslopes, damplands), a thin veneer (film) of water trickling over the surface does not constitute inundation. The thin surface water is an expression of the process of seepage from a waterlogged porous, and/or granular medium. On cliffs, the hydrochemical and biological response to waterlogging is mineral precipitates or specific plants and biota colonizing the cliff surface.

The terms in Table 2, used to describe water regime or hydroperiod, correspond to recognizable states in hydrological and wetland ecological cycles, but may find various expressions in the short and longer term (Fig. 3). In the short term, the condition of 'permanent inundation' would be applied to wetlands with any depth of surface water, and in which there are considerable volume fluxes, whether seasonal or resulting from extraction. The term would be assigned to all of the following situations: constant surface water, surface water present throughout each year except in extreme drought, surface water with annually fluctuating levels, surface water ranging from 0.2 m or > 100 m. Wetlands with surface water levels which are declining or increasing gradually over a decade, but which remain inundated, would also fit into this category. However, in the longer term, for normally seasonally inundated wetlands, a single periodic decadal storm or cyclone event, resulting in a year long inundation would not shift the category of water regime to one of permanent inundation, as it is not the prevailing condition. A wetland which is dry more than 50% of the time would best be categorised as having a regime of intermittent inundation. Intermittent wetting implies that it is not part of an annual cycle or even a regular decadal cycle. Intermittent, as used here, incorporates the terms "intermittent", and "episodic" as used by Boulton & Brock (1999), and can be defined in their terms as a condition in which "annual inflow is less than the minimum annual loss in 90% of years". This means that the wetland would be "dry most of the time with rare and irregular wet phases that may persist for months" (Paijmans *et al* 1985; Boulton & Brock 1999). All unpredictable episodic rain events regardless of frequency are included in the term intermittent inundation. "Ephemeral" as defined by Boulton & Brock (1999) is not considered to be a wetland descriptor under the definition used here (Semeniuk 1987). Seasonal inundation, as the name implies, refers to a prevailing condition of surface water during the wet season for each year. During the succeeding dry period, the wetland may be dry or waterlogged.

Waterlogging, in the short term, is also likely to fluctuate seasonally with various degrees of sediment saturation. Wetlands with water tables in the upper 2 m interval are likely to experience waterlogging to various degrees, depending on sediment type and height of the zone of capillary rise. Brief intervals (days, weeks) after rainfall events wherein groundwater rises to intersect the wetland surface are incorporated into the regime of permanent and seasonal waterlogging. In the longer term, wetland waterlogging may be succeeded by drought conditions.

Permanent waterlogging is the new category of water regime in this paper. Here, inundation of the wetland would be a rare or uncommon rather than a prevailing condition. If a wetland is seasonally inundated, it may also be permanently waterlogged, however, the former presides over the latter state. Permanent waterlogging is likely to occur if the water table fluctuations are reduced and discharge rates are matched by continual recharge, *e.g.*, evaporation from the surface downward is countered by rising capillary and artesian pressure above the saturated zone.

Combining the types of landform and water regimes (or hydroperiod) results in various possible categories of wetlands (Table 3). Some categories axiomatically

Table 3

Matrix combining the land and water attributes and terms for wetland classes

	Water regime or hydroperiod				
	Permanent inundation	**Seasonal inundation**	**Intermittent inundation**	**Permanent waterlogging**	**Seasonal waterlogging**
Landform					
basin	LAKE[1]	SUMPLAND[2]	PIRAPI[4]	BASINMIRE[5]	DAMPLAND[2]
channel	RIVER[1]	CREEK[1]	WADI[1]	CHANNELMIRE	TROUGH[1]
vale				VALEMIRE[5]	PALUSVALE[5]
flat or plain		FLOODPLAIN[1]	BARLKARRA[4]	FLATMIRE[5]	PALUSPLAIN[2]
slope				SLOPEMIRE[5]	PALUSLOPE[3]
cliff				CLIFFMIRE[5]	PALUSCLIFF
hill-top				MONTMIRE[5]	PALUSMONT[3]

[1] existing term adapted for wetland terminology; [2] term coined by Semeniuk (1987); [3] term coined by Semeniuk & Semeniuk (1995); [4] terms adopted from the indigenous peoples of the Great Sandy Desert region for that type of wetland; pronounced "birabi" with the accent on the first syllable; [5] new terms coined in this paper

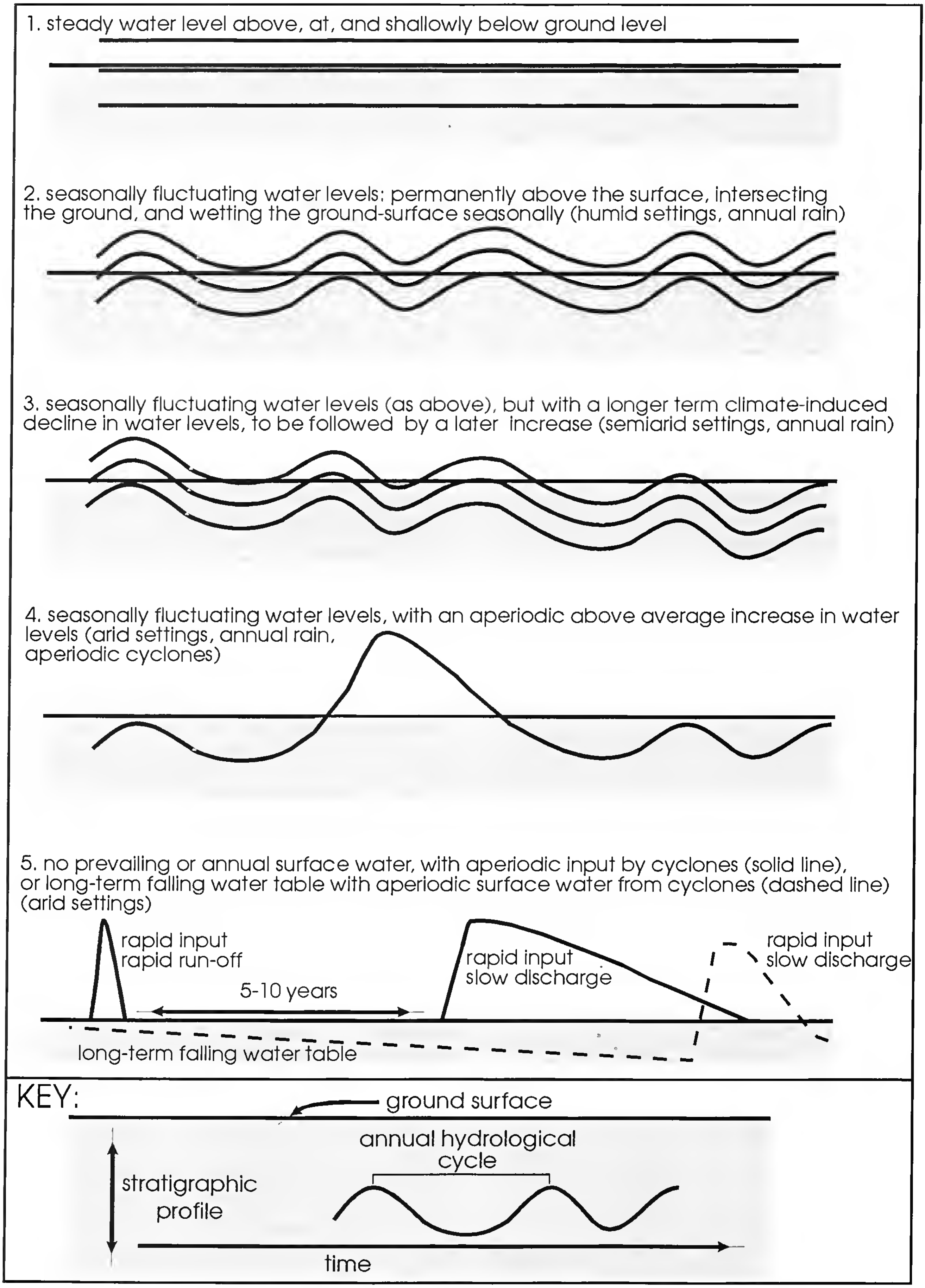

Figure 3. Typical graphs of wetland hydroperiods for surface and groundwater in a variety of hydrological settings.

are not possible (*e.g.*, permanently inundated hill-tops, or slopes), and so focusing on actual wet terrain that is realistic, there are 22 primary categories of wetlands, as follows:

Two criteria have been used to select terms for nomenclature: they must be single words, rather than multiples of words (*e.g.*, "seasonally inundated basin"), and preferably, if newly coined, the core attributes of the term should be deducible by its deconstruction (*e.g.*, palusmont, whose etymology is from Latin – *palus* and *mont*, meaning wet and marshy, and hill-top, respectively).

Existing terms have already been employed for some wetland categories (*e.g.*, lake, floodplain, river). The choice of "lake" and "river" was made because they have been used relatively consistently, in the literature and in common usage, to refer, respectively, to a permanently inundated basin and a permanently inundated channel. There has been some reluctance by scientists and wetland managers to accept these terms in their new context and constraints. Coleman (2003) expresses the view that: "Semeniuk (1987) ....creates the situation where a little brook in southern Australia is now a "river" and a major waterway in the northern area is now a creek". In practice, it is less likely to be a problem than is suggested, as waterways are not likely to be "major" very often if they are seasonal, and most creeks are likely to be small scale channels. Exceptions clearly exist, but adopting the variation in meaning results in a definition with greater precision as to landscape setting and hydrological regime. Importantly it avoids the use of a substitute term, if a new term is coined, which is likely to be even less familiar. However, we will further explore below the issues underlying these terms.

Our fundamental tenet that a wetland is a "land-and-water" feature of the Earth logically results in land and water being treated as primary attributes of the geomorphic-hydrologic classification. Primary attributes should be consistently applied within any classification. Thus, if a river is defined on attributes of landform (a channel-way) and hydrology (permanent inundation), the term "river" needs to be applied wherever a channel-way is permanently inundated, *regardless of scale*, and if a creek is defined on the attributes of landform and hydrology, the term "creek" needs to be applied wherever a channel-way is seasonally inundated, also regardless of scale.

To date, in Australia, there has been inconsistent use of terms for fluvial wetlands, often related to local usage. Large intermittently flowing waterways are variously named "river" or "creek", with the nomenclature appearing to be based on classification at a time when water was present, on size, and on historic precedent. The Gascoyne River, flowing only every 5 years (on average) is geographically termed a river. In this case it appears the term "river" is applied because it has the geomorphic form of a river, even though it does not have the hydrologic attributes of a traditional permanently flowing waterway. Similarly, many of the so-called "rivers" of the Pilbara region, regardless of their size, are surface-dry, sandy channel-ways for most of the year, and to attribute the term "river" to them, scientifically, misleadingly brackets them in the same hydrological context as permanently flowing waterways.

The choice of "floodplain" was made because *sensu stricto* its meaning is an inundated flat. The common association of the term "floodplain" with a channel is not incorporated in this context. New terms were coined by Semeniuk (1987) and Semeniuk & Semeniuk (1995) for "sumpland", "palusplain", "paluslope" and "palusmont" because existing terms such as "swamp", "fen", and "bog", carried with them meanings additional to land and water attributes (such as wetlands colonised by trees, or sustained minerotrophically, or underlain by peat) which often made the terms more parochial in application. Where terms have been coined for seasonally waterlogged terrains, the prefix "*palus*" has most often been used. New terms were coined where there were no pre-existing terms for a particular wetland type, *e.g.*, "dampland" for seasonally waterlogged basins and "barlkarra" for intermittently inundated flats (Table 3).

While previously Semeniuk & Semeniuk (1995) used the term "playa" to denote an intermittently flooded basin, its usage has since been re-considered in the light of its long term meaning in Australia, its variety of meaning across the world, and its etymology. The Spanish word, from which it has been appropriated, literally means "beach", but the term is now widely applied to dry lake beds or flats consisting of fine grained sediments infused with alkali salts (Neal & Motts 1967; Jankowski & Jacobson 1990, Briere 2000). In many other countries, there are local names for these features, *e.g.*, sabkha, takyr, kavir, alkali flats, and pan but, in each case, the term refers only to the attributes of dryness and/or saltiness. When rain filled, the depression is commonly termed a playa lake, which reverts to a playa when the water has evaporated.

The objective, in searching for a term for an intermittently flooded basin, was to emphasise the intermittent inundation of the land rather than water quality. In the classification presented in this paper, an intermittently inundated basin may be fresh water or saline, and underlain by mud or sand sized particles. We therefore propose replacement of "playa" of Semeniuk & Semeniuk (1995) with the term "pirapi", an indigenous word from the Karajarri language and people of the Great Sandy Desert region of Western Australia, referring to intermittently flooded basins that may be saline, brackish, or freshwater (Table 3). The indigenous peoples of the arid regions, in surviving in largely waterless desert environments for thousands of years, have categorised wetland environments for practical purposes, and commonly provide appropriate terms that encapsulate wetlands in terms of their permanence, intermittence, and maintenance.

For terms coined since 1987, we have attempted to have a consistency in nomenclature so that the landscape setting and hydrological regime of a wetland can be "unpacked" etymologically. Thus all permanently waterlogged wetlands end in the suffix "mire", and all seasonally waterlogged wetlands commence with the prefix "palus". The landform setting of the wetland also often is evident, *e.g.*, mont, slope, channel, and vale.

Although much peatland is permanently waterlogged, particularly in Europe, Russia and New Zealand, permanent waterlogging is also a common condition in many saline basins where the water table is near the surface underlying a salt crust. The suffix "mire", in its original meaning of "boggy, wet spongy earth", is resurrected here to be used in conjunction with a landscape type to denote the various types of permanently waterlogged terrains. It does not, in this context, denote water chemistry or water salinity, which may be acidic or alkaline, freshwater or saline. Neither, in spite of the tendency to associate "mire" with peat substrates (Gore 1983; Immirzi *et al.* 1992), does it imply the composition of the accumulated wetland sedimentary fill. Therefore it may be applied equally to any wetland whose surface sediments are aquatic, aeolian, alluvial, or sheet washed mineral and organic deposits that are permanently waterlogged.

Thus, embedded in single word terms are the attributes of landform shape and hydrologic regime, to which various descriptors can be added. For example:

- the term "lake" carries within the word the attributes of being a "basin that is permanently inundated",
- the term "basinmire" carries within the word the attributes of being a "basin that is permanently waterlogged", and
- the term "dampland" carries within the word the attributes of being a "basin that is seasonally waterlogged".

The wetland category of "springs" has not been included in this classification, although many authors and environmental managers make these wetlands a separate class because they are freshwater and important as drinking sources. "Spring" is used in the sense here of any groundwater discharge that reaches the surface, and includes permanent or seasonal flowing water, a seasonal trickle, and saturated sediments (Duguid *et al.* 2005). Springs within a basin or channel are not separated as a wetland type but viewed as a discharge mechanism contributing to the water regime. Springs, associated with slopes or mounds, that create a discrete wetland type, are named paluslope, slopemire, or mound spring. This approach mirrors in part that presented in the Ramsar Convention (Anon 1991, 1996) where springs are not categorised as wetland types.

Descriptors for non-emergent wetlands

For purposes of capturing wetland diversity, adding the adjectival descriptors will further separate types of wetlands, and it is at this level that attributes such as scale (size), hydrochemistry, wetland plant communities, and origin, can be emphasised.

Descriptors of landform

Scale is applied to individual wetlands, as a descriptor, using a fixed, defined frame of reference. For site specific wetlands, the scales of observation, or fixed frames, from Semeniuk (1987), are employed for wetland basins, slopes, and plains that tend to be equant in plan view and for wetland channels that tend to be linear (Fig. 4). Other landform attributes useful as descriptors for refining the wetland categories are shown in Figure 5. Application of landform descriptors is provided below, using a sumpland, with size, shape, and sediment as descriptors:

- mesocale sumpland
- mesocale, irregular sumpland
- mesocale, irregular, diatomaceous, sumpland

Descriptors of water

Hydrological attributes, useful as descriptors for refining various wetland categories, are: salinity, consistency of salinity, opacity and colour, specific hydrochemical characteristics, water source, water maintenance, depth, and rate of movement (Fig. 5). For instance, sumplands can be separated into four types on the basis of fresh or saline water descriptors alone:

- a freshwater stasosaline sumpland,
- a freshwater poikilohaline sumpland,
- a saline poikilohaline sumpland,
- a saline stasosaline sumpland.

Such categories will have follow-on implications for the type of biota resident in the wetland.

Descriptors of vegetation

A qualitative scheme for systematically classifying wetland vegetation was designed using the extent of vegetation cover and internal organization into plant communities (Semeniuk *et al.* 1990). The nine classes and their terms can be used as descriptors (Semeniuk *et al.* 1990). For further refinement, established structural and species dominance terms may be added.

Progressive use of descriptors

With progressive use of descriptors, various levels of wetland classification are possible, from simple high level classification (*e.g.*, as lake or sumpland), to more detailed and complex categorization, using a serial range of descriptors (Fig. 6). Reasons for categorization at the different levels range from mapping of wetlands at the regional scale (high level categorization) to comparative ecological or land assessments requiring characterization of their diversity at the local scale. An example of progressive classification is listed below:

- sumpland
- mesocale sumpland
- mesocale, subhaline sumpland
- mesocale, subhaline, diatomaceous sumpland
- mesocale, subhaline, diatomaceous, latiform, sedge covered sumpland

Self-emergent wetlands

As noted earlier, self-emergent wetlands are those that have accreted sedimentary, chemical and/or biogenic materials beyond their original geomorphic surface to develop a new wetland landscape. Progressive accumulation of autochthonous material in a basin, channel, flat, vale, or slope, may eventually result in the complete filling of the lowland and the development of a convex surface. Self-emergent wetlands should be differentiated from the accumulated *in situ* materials associated with convex wetland forms (hill tops and slopes), which conform to the underlying land surface, forming a geometric sheet or ribbon of wetland sedimentary products commensurate with the land contours. As the formation of self emergent wetlands is independent of the landform type which hosts these wetlands, they do not naturally lend themselves to the classification outlined earlier. In wetland mounds, the landform is not a fundamental component. However, such wetlands can still be categorised hydrologically.

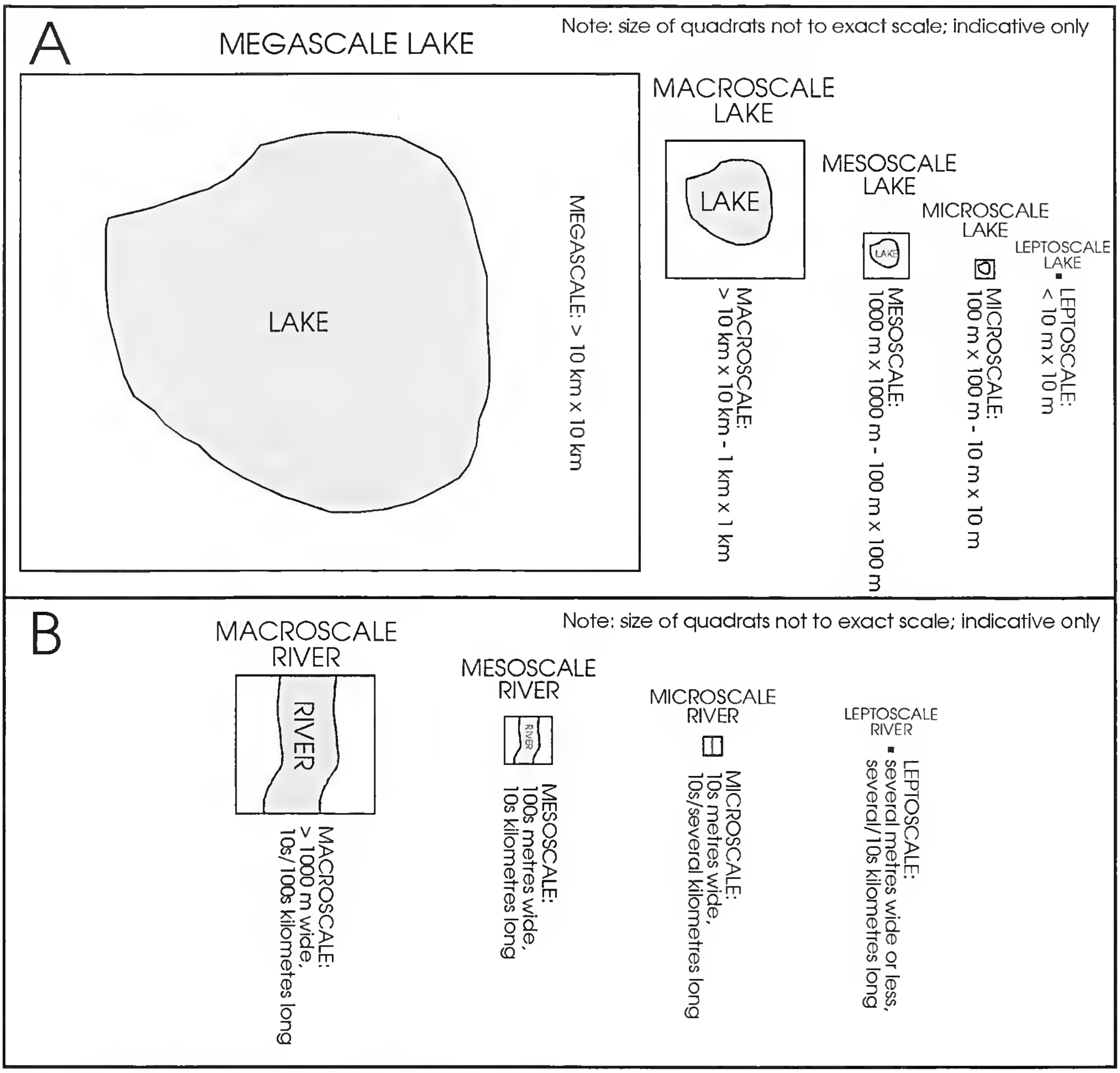

Figure 4. Scales of reference for equant and linear wetlands – plan view

Descriptors can also be applied to the various wetland mounds. For example self-emergent wetlands can be differentiated into several types, based on the composition of their accretionary material:

1 organic mound, or peat mound
2 phytolithic mound
3 diatomite mound
4 carbonate mound
5 gypsite mound
6 sinter mound
7 mud mound

Phytolithic lithology and diatomite tend to occur in layered structures, rather than as isolated homogeneous accumulations (*e.g.*, Belperio 1995; Ashley *et al.* 2002; Lynne 2007) In Western Australia, organic peat mounds occur, for instance, on the Swan Coastal Plain, at Eil Eil spring near Salt Creek (the Great Sandy Desert), at Mandora Soak, and along Geegully Creek (Gordon 2001; DEWHA 2010). Such mounds are permanently saturated, and water flows on the surface or from discrete channels. They are composed of peat, approximately 2–3 high, and some are surrounded by shallow moats. Peat and phytolithic mounds occur in a broad shallowly incised valley at Munro Springs with the permanently saturated central peat plug confined by carbonate mud under the lowlands and standing about 0.5 m above the surrounding plain.

Examples of carbonate mounds from several metres to tens of metres in diameter and up to several metres in height occur on the margins of the Great Artesian Basin, South Australia, where aquifers adjoin basement rocks, or where water escapes to the surface along fault lines and fracture zones (Boyd 1990; Mudd 2000; Harris 2002). Gypsite mounds are convex accumulations of gypsum. Examples occur in the "birridas" of Shark Bay region, and the saline basins of the Eyre Peninsula. They are formed

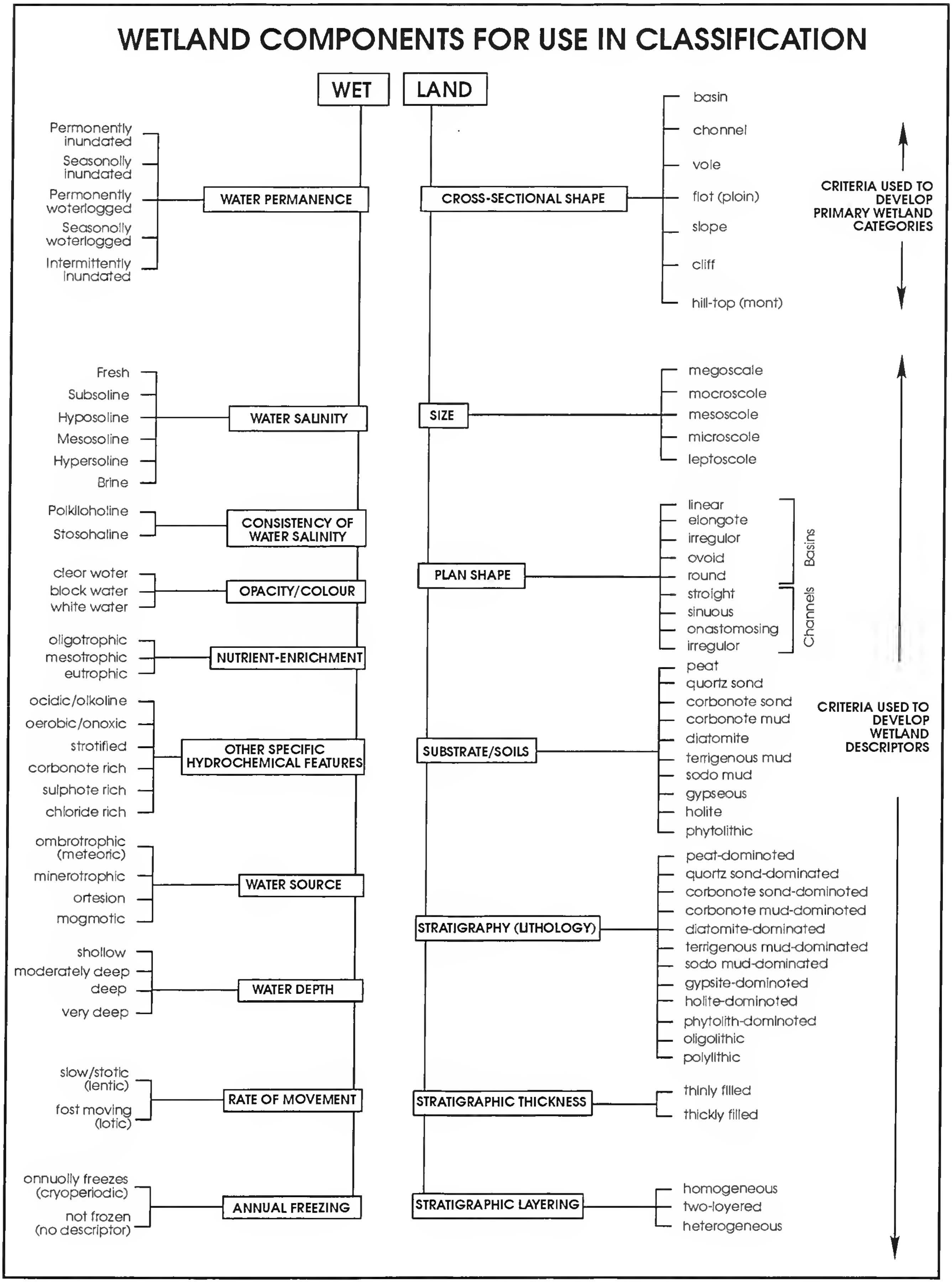

Figure 5. Range of land and water descriptors for the wetland classification. Sediment terms in part after Semeniuk & Semeniuk (2004)

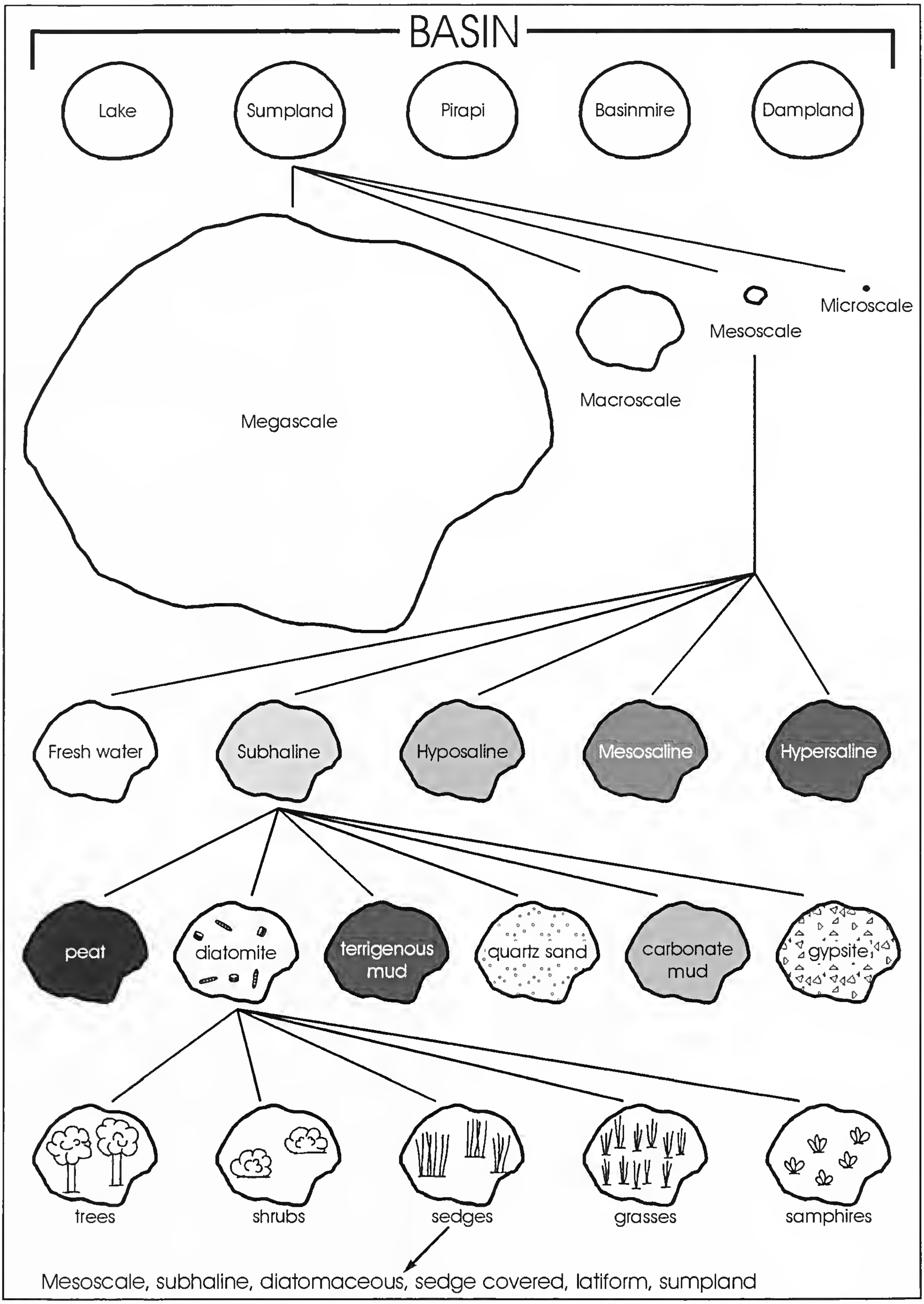

Figure 6. Use of a basin to illustrate levels of classification. Progressive levels of wetland discrimination use descriptors of scale, water salinity, wetland fill, and types of vegetation cover.

by gypsum precipitation from groundwater, causing crystal displacement, crystal accretion, and mounding of the surface. Siliceous sinter is a terrestrial hot spring deposit formed where silica-rich thermal fluids ascend to the surface and discharge in vents, pools, and outflow channels, usually in the region of volcanic activity (Inagaki *et al.* 2001; Lowe *et al.* 2001; Lynne 2007). Mud mounds are underlain by accumulations of clayey silt. Examples occur on the Liverpool Plains, and Gunnedah, New South Wales, where they overlie the Jurassic basalt. The permanently saturated mounds are flat topped, 1.2 m high and 4–8 m diameter, with ponds on the surface and intermittent flow (Acworth & Timms 2003).

Discussion and conclusions

In this discussion, the wetland geomorphic-hydrologic classification, saline wetlands, vegetation as a criterion for classifying wetland types, ecological character, and the use of the words classification and evaluation interchangeably, are explored.

Central to the geomorphic-hydrologic wetland classification is the tenet that land and water are the main determinants of wet lands and that the classification should reflect this. As a consequence, it follows that land and water features should be the primary criteria applied to every type or class of wetland. Several criticisms have arisen of this approach. Earlier in this paper, some discussion was presented regarding the ranking and importance of land and water attributes over scalar ones, and here this discussion is continued to include the use of scale to subdivide wetland types and the choice of terminology.

Stepping outside the current paradigm of usage of fluvial wetland terms, in this paper (and following Semeniuk 1987, Semeniuk & Semeniuk 1995), all permanently flowing channel-ways are classified as "rivers". Variation in size of rivers is denoted by a descriptor. Several critics of the wetland classification overlook the usefulness of appropriate adjectival descriptors. With a simple adjectival addition, a "brook" in South Australia can be classified in a straight forward way as a leptoscale river, a channel in southern Western Australia can be classified as a mesoscale river, and the Amazon River in South America as a megascale river – in all cases the term "river" denotes a permanently flowing waterway, a feature in common with each of the examples, and the scale descriptor denotes the size of the waterway. Similarly, a seasonally flowing channel-way in the Pilbara region is classified as a macroscale creek, which is hydrologically distinguishable by the nomenclature from a river.

Where channels vary in width from narrow to broader waterways, along their course, depending on the terrain they are traversing, the classification may be applied either to the whole system or to each segment. The Walpole River in Western Australia, for instance, is classified as a leptoscale river, but along its length, it varies in width from macroscale to leptoscale.

Thus, without the coining of new terms (for reasons outlined above), the terms "river" and "creek" (*sensu* Semeniuk 1987), with scale adjectival descriptors, firmly address wetland channel-ways that are permanently inundated *versus* those that are seasonally inundated.

Ponds, lakes and inland seas present another example where a wetland geomorphic-hydrologic type transcends scale but where a descriptor can be used to separate types of permanently inundated basins. Ponds and lakes are perceived as smaller and larger permanently inundated basins where scale is used as a *primary determinant* for classification and nomenclature (Cowardin *et al.* 1979). Although the rationale for this separation appears to be based on water depth and plant response, these size class distinctions have not been extended to other wetland types. Also there are permanently inundated wetland types that do not conform to Cowardin *et al.* 1979, being small but deep, or large but very shallow. If size categories were to be extended to other wetland types in the classifications adopting this approach, the number of wetland classes would increase significantly. We contend that landform (in this case, the basin itself) and hydrology (the permanency of inundation or waterlogging) should consistently be used as the primary determinants, and that small scale basins should be separated from larger scale basins by a descriptor.

The wetland classification, presented here, transgresses another widely held view about wetlands, in this case, "salt lakes". "Salt lakes" occur over 50% of Western Australia, and therefore constitute a significant proportion of the State's wetlands to be classified. As such, they merit some further discussion. The term "salt lake" has been applied to a variety of wet and dry habitats: samphire flats, bare areas, clay pans, sand basins, rock basins, lagoons, playas, playa lakes, and saline lakes (Bayley & Williams 1971; De Dekker 1983, 1988; Proctor 1990; Schubel & Lowe 1997; Yechieli & Wood 2002; Boggs *et al.* 2006; Lichvar *et al.* 2006; Gregory 2007; Shaw & Bryant 2011), although the arid zone contains few true lakes (perennially inundated basins), and those few invariably have inflowing rivers rising in fringing uplands, or distant humid areas (*e.g.*, the Caspian Sea, Dead Sea, Great Salt Lake and Lake Aral; Yechieli & Wood 2002; Timms 2006; Shaw & Bryant 2011).

The discussion on "salt lakes" that follows is focused on basins in order to be succinct. Arid zone basins owe their origin to the same broad spectrum of basin forming geomorphic processes as basins in humid zones, *viz.*, tectonic forces, meteor impacts, volcanic crater development, and biogenic, weathering, erosional, fluvial, depositional, and obstruction processes (Hutchinson 1957; Mabbutt 1977; Goudie & Wells 1995). Arid zone wet basins contain a variety of sedimentary deposits and geochemical environments (Mabbutt 1977; Goudie & Wells 1995; Reynolds *et al.* 2007; Shaw & Bryant 2011). Contrary to implications in the use of the term "salt lake" (and particularly the epithet "lake"), hydrological regimes for such designated bodies span conditions of permanent inundation, wet more than 75% of the time to dry more than 75% of the time with a negative water balance (Briere 2000, Timms 2006; Boggs *et al.* 2007; Castaneda & Garcia-Vera 2008; Shaw & Bryant 2011). The type of hydrological input to the basins may be 1. direct precipitation which becomes perched or infiltrates to recharge the water table, 2. inflowing groundwater 3. inflowing surface water, 4. saturation by a stable near surface water table, 5. inundation by a fluctuating near surface water table, or 6. combinations of the above.

Not all "salt lakes" contain salt. Evaporite mineral accumulation is dependent on the relationship between the position of the water table, and the role of groundwater in recharge, throughflow, or discharge, as illustrated in Rosen (1994) and Reynolds *et al.* (2007), and the type of evaporite mineral accumulation is dependent on salinity. Clay floored wetlands are characteristic of regimes with low groundwater input or where the surface lies above the influence of the zone of capillary rise (Neal 1969; Rosen 1994). Where the water table lies close to but does not intersect the basin floor, the groundwater salinity, density, and hydraulic conductivity of the aquifer, in combination with evaporative concentration, will determine the overall wetland salinity and usually results in salt precipitation (Bowler 1986), giving rise to the impression of ubiquitous "salt lakes" underlain by gypsum-dominated precipitates, or halite precipitates.

The aim of the present wetland classification for "salt lakes" is to separate out basins from flats, (ignoring at this primary level, their origin, sedimentary fill composition, and stratigraphy, position in the landscape, underlying geology, and scale), and to incorporate the hydrological regimes which are currently amalgamated under the term "salt lakes". In terms of water regime, "salt lakes" range from truly perennial shallow to deep water lakes to perennially dry basins (non-wetlands). In terms of the classification herein, "salt lakes" would be categorised as five different types of wetlands: saline lakes, saline sumplands, saline damplands, saline pirapis, saline basinmires, and one saline non-wetland basin – a playa. It is evident from this example that the diversity of wetland types, from a hydrologic perspective, is increased. The perched-freshwater wetland basins that occur amidst clusters of "salt lakes" in arid and semi-arid regions and are episodic, ephemeral, essentially closed systems, with fresh surface water that becomes brackish over time as evaporation decreases water volume and increases ionic concentrations, would be classified as freshwater poikilohaline pirapi. This designation potentially signals a wetland with different origin, different processes and functions.

It has been argued that in hierarchical wetland classifications, wetlands with very similar vegetation may be assigned to different classes on the basis of an attribute such as landform (Barson & Williams 1991). This perspective is underpinned by a practical consideration, that for most researchers, structural plant community types (heaths, grasslands, sedgelands, woodlands) and dominant species *within a region* are easily recognised, and can be used to identify wetland "types", while different substrates, hydrology, chemistry, and, even in some cases, landforms are not. There are three problems with this approach. Firstly, it suits regional projects rather than global ones. Secondly, it assumes that plant species and communities are surrogates for wetland hydrological regimes whereas, in reality, plant distributions are determined by numerous factors. Thirdly, it is underpinned by the notion that it is more important to link together wetlands with the same plant communities than it is to address the overall functioning of a wetland. Employing this perspective would result in superficial links between wetlands, particularly where there is a limited species pool. The corollary is that wetlands from different regions of the world would be separated from the main wetland groups, giving the incorrect impression that it is the wetlands that are diverse instead of the species pool being different. In Australia, this approach would result in permanently and seasonally inundated wetland basins, rivers, creeks, and floodplains all being classified as the same wetland type (*e.g.*, *Melaleuca* swamp) because they are all colonised by *Melaleuca rhaphiophylla*, or floodplains, creeks, wadis, palusplains, intermittently inundated basins, sumplands, estuarine flats and deltas, all being classified similarly because they are colonised by *Tecticornia halocnemoides*. The selection of more uniform and fundamental attributes could result in producing a platform on which global wetlands are comparable.

Another significant feature of the use of wetland classification relates to "ecological character" as defined in Resolution VII.10 of the Ramsar Convention. The values inherent in key environmental attributes determine a wetland's "ecological character". These key attributes include landform, substrate, scale and spatial arrangement, size, water regime, source and depth of water, salinity, and vegetation types and species. It is considered that if the wetland types distinguished in a classification do not have distinctive "ecological character", they will not be useful (Duguid *et al.* 2005). The following is a brief account of how the geomorphic-hydrologic classification, presented here, fulfils that objective.

Ecology is described as the study of relationships between organisms and their environment, including the study of communities, patterns of life, natural cycles, biogeography and population changes. The "relationship between organisms and their environment" is a complex one reaching back through a hierarchy of causes and effects from small to large scale. The landform host to the wetland is an important part of the wetland environment and determines many of the wetland functions that occur, such as the nature of sedimentary, hydrological and evolutionary processes. "Communities, relationships of organisms to each other, biogeography and population changes" directly respond to changes in landform size, landform aspect, height, structure, geometry, and setting. Landform also indirectly influences ecological response through its effects on water depth and water chemistry.

This can be expressed in myriad ways but a simple example is that of open *versus* closed systems. Below, the specific landforms selected for the wetland classification are listed in Table 4 beside corresponding sedimentary and hydrological wetland features and processes. Hydrochemistry would normally be included in Table 4 as a major link between landform and biota, however, it has not been included because it is so variable (for a given wetland it can include: neutral, acidic or alkaline rich waters and sediments, freshwater to hypersaline, waters that are iron rich, carbonate rich, dominated by sodium, calcium, potassium or magnesium, nitrogen rich, or phosphorous rich or poor, amongst others).

Table 4 shows that there are diverse processes operating in the various wetlands and that the number of these increase for the (relatively closed) basin wetland types. One of the most important controls that landform can exert on the ecological character of wetlands is water depth. Other features include closed *versus* open hydrological systems, *in situ versus* allochthonous sediment accumulation, and wetland fill composition,

Table 4

Range of attributes which commonly occur in each wet landform type

Landform type	**Hydrological processes maintaining the wetland**	**Water regime, duration, depth of water**	**Sedimentological, pedogenic, diagenetic, or biogenic processes**	**Wetland fill or underlying wetland substrate**
Hill top	1. Direct precipitation 2. Upwelling 3. Perching	Waterlogging Permanent or seasonal Shallow < 1 m	1. Weathering 2. Aeolian deposition	1. Soils 2. Saprolite 3. Blanket peat
Cliff	1. Runoff 2. Seepage	Waterlogging Permanent or seasonal Shallow < 1 m	1. Weathering 2. Biogenic accretion	1. Mineral deposits 2. Organic matter
Slope	1. Direct precipitation 2. Seepage 3. Surface flow	Waterlogging Permanent or seasonal Shallow < 1 m	1. Colluvial processes 2. *in situ* accumulation	1. Mineral deposits 2. Organic matter
Vale	1. Direct precipitation 2. Seepage 3. Groundwater	Waterlogging Permanent or seasonal Shallow < 1 m	1. Colluvial processes 2. *in situ* accumulation 3. Aeolian deposition	1. Organic matter 2. Quartz sand
Channel	1. Direct precipitation 2. Surface flow 3. Groundwater 4. Seepage	Inundation Permanent, seasonal, intermittent Waterlogging Permanent, seasonal Shallow < 1 m to deep	1. Fluvial erosion, transport, deposition 2. *in situ* accumulation	1. Clay, silt 2. Quartz sand 3. Gravel
Flat	1. Direct precipitation 2. Perching 3. Groundwater 4. Surface flow 5. Upwelling	Inundation Seasonal, intermittent Waterlogging Permanent, seasonal Shallow < 1 m	1. Fluvial deposition 2. *in situ* accumulation 3. Aeolian deposition 4. Diagenesis	1. Clay, silt 2. Quartz sand 3. Gravel
Basin	1. Direct precipitation 2. Springs or seepage 3. Groundwater 4. Perching 5. Discharge into basin 6. Sheet wash	Inundation Permanent, seasonal, intermittent Waterlogging Permanent, seasonal Shallow < 1 m to very deep > 100 m	1. *in situ* accumulation 2. Sheet wash 3. Aeolian deposition 4. Diagenesis 5. Biogenesis	1. Clay, silt 2. Quartz sand 3. Organic matter 4. Diatomite 5. Carbonate mud 6. Sponge spicules 7. Phytoliths 8. Gypsite 9. Evaporites

which vary between basins, flats, channels, slopes and vales. The water regime also is a prime determinant of ecological processes and interactions in the wetland. This is widely recognised and is important enough to be dealt with directly rather than through the use of plant species or plant community surrogates for hydrological conditions. The pattern of inundation, waterlogging, and drying, is a major influence on wetland biota populations and life cycles. Inundation and waterlogging are also strongly linked to, or influence water depth, water temperature, water salinity, and water chemistry, all of which have effects on community composition, distribution and viability.

Focusing upon landform and hydrology at the primary level of the classification sets the stage for further partitioning of wetlands based on attributes which affect biota at the scale of the individual wetland, individual sediment layer, water sediment interface, or rhizome layer. Variations in substrate or water chemistry in the subset of "sumplands" for instance, become more meaningful than a broad documentation of substrate across all types of wetlands.

As Figure 6 suggests, when the fundamental classification is augmented by just four descriptors, scale, salinity, substrate and vegetation, hundreds of different sumplands can be differentiated, which would underpin the different types of "ecological character".

From the recent literature, there appears to be a growing trend to perceive, and to use, classification as a tool, or a surrogate, for evaluation. The terms evaluation and classification are increasingly being used interchangeably (Brinson 1993, 1996, 2011; Millennium Ecosystem Assessment 2005). Classification, for purposes of discriminating wetland functions or utility, immediately brings into consideration value judgements of wetlands. In this paper, this approach is not followed because, in our experience, classification and evaluation have entirely different objectives and independent methods. The aim of classification is simply to group objects on the basis of common features, and one of its most practical applications is mapping.

This classification may be used to provide a world register and/or map of wetlands. From arid to humid climates wherever there are wet basins, hills, slopes, flats and channels, these can be categorised using landform and water regime attributes. Effort has gone into ensuring that the class terms are non-genetic and not dependent on local knowledge or on names of plant species. The terms are single words and many of them can be deconstructed into land and water descriptions. Finally, the number of classes which cover the global diversity of wetlands at the primary classification level is relatively small. It should be noted that at the primary level of classification this approach does not differentiate between a peat-filled basin that is permanently waterlogged by freshwater and a salt-encrusted basin that is permanently waterlogged by saline water. Both are classed as pirapi at the primary level. Their differences are brought out through the use of descriptors such as hydrochemistry, scale, stratigraphy, and biotic response.

As the classification scheme outlined above is based on landscape geometry and prevailing water regime, care is needed to assign categories where gradational boundaries exist between classes. Although most landforms can be easily assigned to one category or another, basins and slopes can grade into plains, and river channels can grade into lakes. This type of gradation is often linked to the stage of evolution of the landscape. In this context, both scale and setting are useful guides to choosing one or the other. The same situation can occur for water regime and hydroperiod and, here, the concept of prevailing conditions is useful. In a basin which is seasonally inundated for 7 out of 10 years, it is better for ecological purposes to focus on the prevailing condition rather than the exception and so this basin would be classified as a sumpland. Semeniuk & Semeniuk (1995) argue for simplifying the various categories of water regime into a few, so that classification becomes practical without reflecting every nuance of natural variation at the primary level, and this document continues this theme, presenting a simplified case of five water regimes. The objective of the classification is to provide broad classes of categories at the primary level, and use the descriptors to add details.

Wetlands may change over time and, in any classification, their dynamic nature may need to be addressed. Changes may occur in response to natural or anthropogenic influences. Natural climatic change, for instance, can determine both short and longer term responses of wetlands. These responses are effected through changes in hydrological and sedimentological patterns, hydrochemistry, and ecological and biological responses, and it is these which may be used to corroborate the classification in doubtful cases. Hydrological changes relating to water depth and wetland boundaries tend to be rapid and sometimes annual. Biological changes can be rapid and seasonal (aquatic invertebrates, rhizomatous plants) or slower and decadal (for established wetland species). Changes to wetland sediments tend to be in response to longer term changes in prevailing conditions. When it can be demonstrated that natural climatic change or anthropologic changes have altered the prevailing wetland hydroperiod in the longer term, as opposed to a short term fluctuation, then the classification of a given wetland will need to change.

Change due to anthropogenic causes is likely to be more rapid than those induced by natural climate change. The most important anthropogenic alterations are land clearing, water level perturbations (through water abstraction and drainage), and contamination. Each of these can radically alter the internal stasis and interactions that occur between soils, plants and water and, therefore, the type of wetland which results. A wetland classification should be such that these changes can be simply incorporated into the existing structure. This can transpire when the primary level of classification contains few but fundamental criteria.

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Comparison of biomarker responses following one dose of benzo-a-pyrene administered to three native Australian fish species

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Abstract

The Australian native fish pink snapper (*Pagrus auratus* Forster) is currently used as a bioindicator species for laboratory and field studies, but is often unavailable from hatcheries, or collected in limited numbers in the field. Consequently, mulloway (*Argyrosomus hololepidotus* Lacépède) and barramundi (*Lates calcarifer* Bloch), two Australian native fish species, were tested in an exploratory study as potential bioindicator surrogates to pink snapper. Experimental fish were i.p. injected with benzo(a)pyrene (BaP), a well known biomarker inducer in fish, at a dose of 1.0 µg/g of fish. Physiological indices i.e. condition factor (CF) and liver somatic index (LSI) and a suite of biomarkers including ethoxyresorufin-O-deethylase (EROD) activity, biliary metabolites, serum sorbitol dehydrogenase (SDH), DNA damage (Comet assay) and heat shock proteins HSP 70 were explored in the three test species. Mulloway and barramundi showed a higher response in biliary metabolite levels than pink snapper, while pink snapper showed a higher EROD induction potential relative to mulloway and barramundi. Mulloway appeared to be sensitive to hepatotoxicants, as the chemical injury sustained by the liver resulted in the release of SDH in the bloodstream of this species. All three species were significantly responsive to DNA damage. When injected with BaP, the three species showed similar response for CF, LSI and HSP 70. Initial results indicate that mulloway and barramundi are suitable surrogate bioindicator species for pink snapper in relation to exposure to BaP.

Keywords: Pink snapper, Mulloway, Barramundi, BaP, Fish biomarkers, EROD, SDH, Bile metabolites, DNA damage, Comet assay, HSP 70

Inroduction

Pink snapper (*Paragus aratus* Forster 1801) is found in warm temperate to sub-tropical waters of southern Australia (Paulin 1990), and is one of the most popular commercial and recreational fish in this region. Pink snapper may reach a maximum length of 1.3 m and weight 19.5 kg (Hutchins & Thompson 1983). Juvenile pink snapper is currently used as biological tool for evaluation of biotic response to marine pollution (Gagnon & Rawson 2009; Ranaldi & Gagnon 2010). The biochemical responsiveness of pink snapper to the common pollutants pentachlorophenate (PCP) and polychlorinated biphenyls (PCBs) has been demonstrated (Tugiyono & Gagnon 2001), validating the suitability of this species as a bioindicator of environmental contamination. Limitations of this species include its extremely slow growth rate and the unreliable availability of juveniles from hatcheries. Its biology is also lacking in information, with the biology of juvenile fish mostly unknown. When pink snapper is unavailable, a surrogate species needs to be identified. Therefore, mulloway (*Argyrosomus hololepidotus* Lacepède 1801) and barramundi (*Lates calcarifer* Bloch 1790), both native to Australia, were identified as potential alternative bioindicator species.

Mulloway occur mainly in coastal embayment and estuaries, but also occur off ocean beaches and on inshore reefs to depths of about 100 m. Small mulloway tend to remain in the more saline estuaries. These fish are effective predators, feeding upon whatever prey is available in their particular habitat (DPI & F 2003). Mulloway is available all the year round from the field, or from hatcheries where it is grown for aquaculture ventures.

Barramundi is among the largest and most important commercial fishes in Australia (Allen 1997). Barramundi are large predators which can grow up to 60 kg and 180 cm in length. Barramundi are protandric hermaphrodites, reproducing as males between 2 and 6 years of age, after which they reproduce as females (Davis 1986). This species has a very extensive range in tropical and semi-tropical areas of Northern Australia, Papua New Guinea and Asia, and has potential to be used as a biomonitor of environmental pollution in the natural water bodies of the region (Mercurio *et al.* 2004; Codi King *et al.* 2005; Humphrey *et al.* 2007).

Polycyclic aromatic hydrocarbons (PAHs) are an important environmental threat. Some PAHs have well documented toxic, mutagenic and carcinogenic potentials that make them priority pollutants (Naes *et al.* 1999). Benzo-a-pyrene (BaP) is a well known inducer of biochemical reactions in fish, *e.g.* ethoxyresoryfin-*O*-deethylase (EROD) activity (Ramsak *et al.* 2007; Yun *et al.* 2008) and is used as a common standard to trigger biological responses under laboratory conditions. In addition, BaP is an ubiquitous anthropogenic contaminant found in urban and marine environments. Several studies have exposed fish to BaP either via the water column or through intra-peritoneal (i.p.) injection to the animal in order to measure the cellular response of the animal to the chemical (Lemaire-Gony 1995; Bonacci *et al.* 2003; Ramsak *et al.* 2007). In exploratory studies, i.p. injections are often the preferred route of exposure as it ensures that all organisms receive the same dose of toxicant, which reduces variability of the measured parameters. BaP at dose of 1.0 µg/g of fish has been shown to be high enough to trigger the biochemical responses in fish tissues with no mortality (Bakhtyar & Gagnon 2009). However, several studies have also administered much higher doses of BaP through i.p. injections in a variety of species without any mortality. For example; the juvenile European sea bass *Dicentrarchus labrax* (Lemaire-Gony 1995), the European eel *Anguilla anguilla* (Bonacci *et al.* 2003), the juvenile Arctic charr *Salvelinus alpinus* (Padrós *et al.* 2003), as well as black gobies *Gobius niger* (Ramsak *et al.* 2007), and the juvenile gilthead sea bream *Sparus auratus* (Banni *et al.* 2009) all have been the subject of biomarker studies using i.p. BaP injections.

Under laboratory conditions, juvenile pink snapper, mulloway and barramundi were injected with BaP in order to investigate their biochemical responsiveness to this model contaminant. A suite of physiological responses (condition factor: CF, liver somatic index: LSI) and biochemical markers specific to organic compounds *i.e.* EROD activity, biliary metabolites, biomarkers of general stress such as serum sorbitol dehydrogenase (SDH), heat shock proteins (HSP 70) and DNA damage (Comet assay) were measured in order to assess the potential of each species as a suitable alternative to pink snapper.

Material and Methods

Fish Maintenance

Juvenile pink snapper, mulloway and barramundi were obtained from a local hatchery (Fremantle TAFE, WA). Sexually immature fish were purposely selected for the current study as the presence of steroid hormones may influence some biomarker responses, *e.g.* EROD activity (Gagnon *et al.* 1994).

The basic procedures for tank setup followed the standard handling and water renewals from the US Environmental Protection Agency EPA 821/R-02-012 (2002). Aeration was constant in order to maintain close to 100 % saturation of oxygen in the aquarium water at all times. The seawater (salinity 29–36 ppt, pH 7.1–7.8 and temperature 20.0–22.0°C), used for the daily 50 % water change was collected from the hatchery site where the fish were obtained, so that laboratory seawater had the same physicochemical characteristics as the water in which the fish were reared. The seawater salinity was different for the barramundi exposure as this experiment was conducted at a separate time to other fish exposures.

Eight (8) fish per treatment (4 fish per aquarium, in duplicate) for pink snapper and mulloway were kept in 100 L aquaria. However, due to large size of barramundi, six (6) fish per treatment (3 fish per aquarium only, in duplicate) were kept in 100 L aquaria. Water physicochemistry (salinity, pH and temperature) was measured daily to ensure stable physicochemical parameters. Fish were fed once a day at a maintenance ratio of 1% body weight per day during the acclimation (7 days) and experimental (4 days) periods (Palace *et al.* 1996). The food pellets consisted of a commercially available fish food (Sketting pellet type Nova ME, 45% protein, 20% lipid, 18% carbohydrates, 8% moisture).

Fish Exposure

Negative control groups received no treatment as previous laboratory trials have shown that corn oil injection or anaesthesia with MS222 (3-aminobenzoic acid ethyl ester) does not alter biomarker responses (Webb & Gagnon 2002a). All fish were anaesthetised with MS222 prior to being injected with BaP diluted in 1 mL of corn oil at a dose of 1.0 µg /g fish.

The BaP dose was selected according to previous experience (Webb *et al.* 2008) using native Australian species, where EROD activity was successfully induced to significant levels. In addition, published literature reporting EROD activity induction in other fish species did lead to the selection of a dose of 1.0 µg BaP/g fish which was intraperitoneally injected to trigger biochemical responses. All fish were killed 4 days post-injection as the biomarker response has been shown to be maximal 3 to 4 days following BaP injection (Gagnon & Holdway 1998; Au *et al.* 1999; Webb *et al.* 2008).

At the end of the exposure period, blood was collected from the dorsal vein using a vacutainer. A portion of the blood was immediately used for the Comet assay while another portion was centrifuged at 3000 *g*, at 4°C for 10 minutes and supernatant serum was collected. Fish were killed by the method known as *Iki jime* (spike through the brain). Total weight and liver weight (g) were recorded on a Sartorius Basic B3100P electronic balance, and total, fork and standard lengths were measured (cm). The gills, liver and bile were collected and immediately frozen in liquid nitrogen. All samples were stored in freezer at -80°C until analysed for biomarkers.

Fish Biomarkers

Physiological indices *i.e.* condition factor (CF) and liver somatic index (LSI) and a suite of biomarkers including EROD activity, biliary metabolites, serum SDH, DNA damage (Comet assay) and heat shock proteins HSP 70 were explored in the three test species. This suite of biomarkers of exposure and effects are relevant to urban and industrial contamination encountered in many ecotoxicological studies.

CF was calculated according to the equation 100 x (carcass weight / standard length^3), while the LSI was calculated as 100 x (liver weight / carcass weight). Carcass weight, rather than total body weight was used as it eliminates variation due to stomach content and lipid reserves.

Ethoxyresorufin- O-deethylase (EROD) Activity

Hepatic EROD activity was analysed in liver samples. Fish livers were weighed and homogenized at a ratio of 1 part of liver with 4 parts of 0.02 M HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; pH 7.5). Homogenates were centrifuged at 9000 g for 20 minutes at 4°C and the S9 microsomal fractions were collected. All samples were prepared in duplicate according to the fluorimetric assay described in Hodson *et al.* (1991) which included a standard curve with resorufin concentrations varying from 0 to 425 pmol resorufin / mL. The reaction was initiated by adding ethoxyresorufin and terminated by the addition of analytical grade methanol to stop the reaction and precipitate proteins. Resorufin standards and samples were centrifuged at 3000 *g* for 10 minutes, and the fluorescence of the supernatant read on a PerkinElmer LS-45 luminescence fluorimeter at ex/em 530/585 nm slit 10/10. The resorufin concentrations in the samples were calculated from a standard regression after correction of measurements for blank fluorescence. Proteins concentrations in microsomes were analysed according to Lowry *et al.* (1951) using bovine serum albumin as a standard (Sigma Chemicals, Australia), on a Pharmacia UV-Visible Spectrophotometer at wavelength 540 nm. The enzyme activity was normalized to total protein contents and is reported as pmol of resorufin produced per min per mg protein (pmol mg protein^{-1}min^{-1}).

Biliary Metabolites

Naphthalene, pyrene and BaP-type metabolites were determined by fixed fluorescence (FF) measurement (Lin *et al.* 1996). It was not expected to detect high levels of fluorescence at the naphthalene wavelength however, this parameter was included in the investigation in order to insure there was no significant background fluorescence at this wavelength, for any of the fish species. Bile was diluted with 50/50 HPLC grade methanol/double distilled H_2O with a ratio of 1:2000 for the naphthalene-type metabolites. Fluorescent readings were performed against a 1-naphthol standard curve at excitation/emission 290/335 nm slit 10/10. Bile samples for pyrene and BaP-type metabolites were diluted 1:500 for negative control groups. However, experimental groups were diluted with a ratio of 1:2000 for the pyrene-type metabolites and up to 1:50000 for BaP-type metabolites in order for the fluorometric measurements to fall within their respective standards range. Metabolites fluorescing at the pyrene and BaP wavelengths were measured using 1-hydroxy pyrene as a reference standard at ex/em 340/380 nm and 380/430 nm for pyrene and BaP wavelengths, respectively.

To determine protein concentrations in bile, the bile samples were diluted 40 times with double DI H_2O and samples were prepared and incubated according to the method described in Lowry *et al.* (1951). Sample absorbances were determined at wavelengths 540 nm by using a Pharmacia Biochrom 4060 spectrophotometer.

Metabolites fluorescing at the naphthalene wavelength are expressed in mg of 1-naphthol fluorescence units equivalent per mg biliary protein, and those fluorescing at the pyrene and BaP wavelengths are reported in µg of 1-OH pyrene fluorescence units equivalent per mg biliary protein.

Serum Sorbitol dehydrogenase (SDH) activity

Fresh whole blood samples were chilled on ice immediately after collection and were centrifuged at 3000 *g* for 10 minutes. The serum was collected and immediately frozen at -80°C until analysed. Serum samples for serum SDH activity were diluted (1:9) with a solution of ß-NADH/0.1M Tris Buffer pH 7.5. Samples were incubated for 10 min at room temperature to allow the reaction of keto-acids in the serum. Enzymatic activity was initiated by the addition of fructose and the SDH activity was determined by the reduction in light absorbance at 340 nm over time (Dixon *et al.* 1987) on a LKB Biochrome 4060 spectrophotometer. Results are reported in milli-International Units (mU).

DNA damage (Comet Assay)

DNA damage was determined by the single cell gel electrophoresis (Comet assay, Trevigen, USA) according to the method adapted from Singh *et al.* (1988). Fresh blood samples (2.5–3.5 µL) were mixed with chilled PBS/DMSO solution, and the mixture added in low melting agarose (LMA) in a ratio 1:5. Sample/LMA mixture was transferred onto the two wells of a comet slide (Trevigen USA). The slides were immersed overnight in chilled lysis solution (pH 10). Alkaline horizontal electrophoresis was carried out for 20 min at 25 V and 300 mA. Slides were subsequently rinsed with DI water and submersed in chilled 80% ethanol. The slides were fixed and stained by using fixation solutions and stains provided with the kit.

Comet images (~20 per sample) were taken in greyscale on a digital microscope and stored electronically. Individual cells (~100 per sample) were quantified for DNA damage using the publicly available image analysis "CASP" programme that measures the silver stained comet parameters. The integrity of the DNA molecule was expressed as the "tail moment"; high DNA damage would lead to a high 'tail' in the micro-chromatographic process and consequently, a high tail moment represents a high DNA damage (Konca *et al.* 2003).

Heat Shock Proteins (HSP 70)

Gills were used for the determination of HSP 70 as several laboratories and field experiments have informed on the relative levels of HSP 70 present in pink snapper gills under varied conditions (Webb & Gagnon 2009). Gill samples were thawed on ice, weighed and homogenised using phenyl-methyl sulphonyl fluoride (PMSF) homogenization buffer in the ratio of 1:4 w/v. Homogenate were centrifuged at 12000 *g* at 4°C for 98 min using a Heidolph DIAX 900 homogeniser and the supernatant were collected in aliquot and stored at -80°C until analysed for heat shock proteins (HSP 70). Positive laboratory controls (heat-shocked pink snapper) were an integral part of the measurements. Concentrations of protein were determined according to Lowry *et al.*, (1951) as described in Webb & Gagnon, (2009). The supernatant containing 40 μg protein contents was mixed with Laemmli sample buffer (Bio-Rad Laboratories Pty Ltd) in a ratio 1:2, then heated at 95°C for 4 min in a waterbath. The samples were loaded in duplicate in 12% Tris Glyscine iGels wells with heat shocked standardised control samples in two outermost wells.

The gels were run for electrophoresis (225 V, 120 mA, 40 min) followed by Western transfer. The blots were blocked with 5% skim milk dissolved in Tween phosphate buffered saline and probed overnight with monoclonal (mouse) heat shock protein 70 antibody and then incubated for 2 hrs with a secondary antibody, a goat anti-mouse IgG peroxidise conjugated (Progen Biosience Australia). The blots were incubated with a chemiluminescent substrate working solution (Progen Bioscience) for 1.5 min in a dark room and images were developed on X-Ray film using 245S X-ray Processor. The images were analysed by NIH public domain image program (http://rsb.info.nih.gov/nih-image) for quantification of protein density. Heat shock protein levels are reported in pixel/μg proteins.

Data Analysis

The analysis was performed using SPSS for Windows version 16. All data were log transformed to achieve normality of distribution and homoscedasticity of variances. T-tests were used to compare between duplicates of the same treatment for example, between the two control aquaria of a species. Statistical comparisons (t-tests) of duplicate aquariums confirmed that there was no differences ($p > 0.05$ in all cases) between duplicates and consequently, the two aquariums for the same treatment were pooled for further statistical comparisons. T-tests were used to compare experimental groups to their respective negative control groups. Data was presented as means ± standard error (SEM).

Results

The physicochemical water parameters, salinity, pH and temperature, were stable throughout the acclimation and experimental (total 11 days) periods (Table 1). Fish were feeding well and were in good physical condition throughout the experiment.

The total weight and length of pink snapper and barramundi differed slightly between treatment groups (for total weight and total length p = 0.013, 0.001 and 0.048, 0.03 for pink snapper and barramundi, respectively) however, it is not expected that these differences would affect the selected biomarkers. CF was not significantly different between the treatment groups and their respective negative control groups (p = 0.57, 0.20 and 0.23 for pink snapper, mulloway and barramundi respectively). Similarly, LSI remained unchanged by exposure to BaP. None of the experimental group showed a significantly higher LSI relative to the negative control group (p = 0.62, 0.36 and 0.65 for pink snapper, mulloway and barramundi respectively; Table 2).

EROD activity was significantly induced in injected pink snapper and barramundi groups relative to their respective control groups. A significant difference (p = 0.001) was found between BaP injected pink snapper and the negative control pink snapper group. Similarly, BaP injected barramundi group was found significantly different (p = 0.005) from its non injected control group. However, injection of mulloway with 1.0 μg BaP per g of

Table 1

Summary of water parameters (means ± SEM) measured during acclimation and experimental periods.

Treatment	Salinity (ppt) Acclimation	Salinity (ppt) Treatment	pH Acclimation	pH Treatment	Temp (°C) Acclimation	Temp (°C) Treatment
Pink snapper Neg Ctrl	29.8 ± 0.26	30.2 ± 0.03	7.77 ± 0.03	7.71 ± 0.01	22.5 ± 0.13	21.8 ± 0.04
Pink snapper BaP injected	30.0 ± 0.05	30.4 ± 0.22	7.69 ± 0.02	7.70 ± 0.15	21.8 ± 0.45	22.1 ± 0.38
Mulloway Neg Ctrl	29.9 ± 0.21	30.0 ± 0.02	7.79 ± 0.03	7.76 ± 0.07	22.6 ± 0.23	21.7 ± 0.48
Mulloway BaP injected	29.7 ± 0.32	29.8 ± 0.17	7.65 ± 0.33	7.72 ± 0.13	22.0 ± 0.14	22.1 ± 0.59
Barramundi Neg Ctrl	36.9 ± 0.61	35.1 ± 0.32	7.55 ± 0.13	7.66 ± 0.16	20.9 ± 0.14	21.3 ± 0.23
Barramundi BaP injected	35.8 ± 0.21	36.9 ± 0.08	7.63 ± 0.22	7.58 ± 0.09	21.1 ± 0.33	20.3 ± 0.43

Table 2

Total weights, lengths and physiological indices (means ± SEM) of negative control and BaP-injected fish.

Treatment	Numbers (N)	Total Weight (g)	Total Length (cm)	Condition Factor (CF)	Liver Somatic Index (LSI)
Pink snapper Neg Ctrl	8	122 ± 24.7	17.3 ± 0.94	3.3 ± 0.24	1.27 ± 0.21
Pink snapper BaP injected	8	211 ± 17.6	22.6 ± 0.67	3.1 ± 0.09	1.12 ± 0.12
Mulloway Neg Ctrl	8	116 ± 04.5	22.9 ± 0.33	1.15 ± 0.07	1.23 ± 0.13
Mulloway BaP injected	9	125 ± 05.9	23.8 ± 0.42	1.25 ± 0.02	1.07 ± 0.12
Barramundi Neg Ctrl	6	408 ± 13.8	32.4 ± 0.37	1.35 ± 0.13	0.95 ± 0.06
Barramundi BaP injected	6	367 ± 12.0	31.0 ± 0.39	1.54 ± 0.03	0.89 ± 0.14

fish did not result in a significant EROD induction (p = 0.38; Fig. 1).

None of the species tested showed significant fluorescence at the naphthalene wavelength (p = 0.89, 0.64 and 0.07 for pink snapper, mulloway and barramundi respectively; Fig. 2A). Similarly, pink snapper and barramundi did not respond to the injection as 4 days post-injection, these two fish species did not produce significantly higher levels (p = 0.28 and 0.198 for pink snapper and barramundi respectively) of pyrene-type metabolites relative to their respective control groups. The injected mulloway, however, did produce significantly higher (p = 0.047) levels of pyrene-type metabolites compared to the mulloway negative control group (Fig. 2B). In all three species of fish, the BaP injected groups responded to the treatment with a significant (p < 0.001 in all cases) accumulation of metabolites fluorescing at the BaP wavelength, compared to their respective control groups (Fig. 2C).

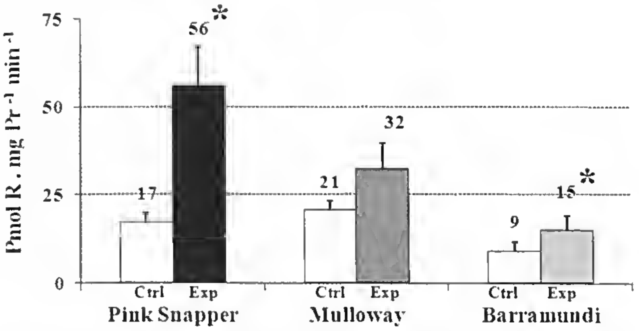

Figure 1. Hepatic EROD activity (pmol resorufin/mg protein/ min) in liver of juvenile pink snapper, mulloway and barramundi injected with BaP. *indicates a significant difference (p < 0.05) from their negative control groups.

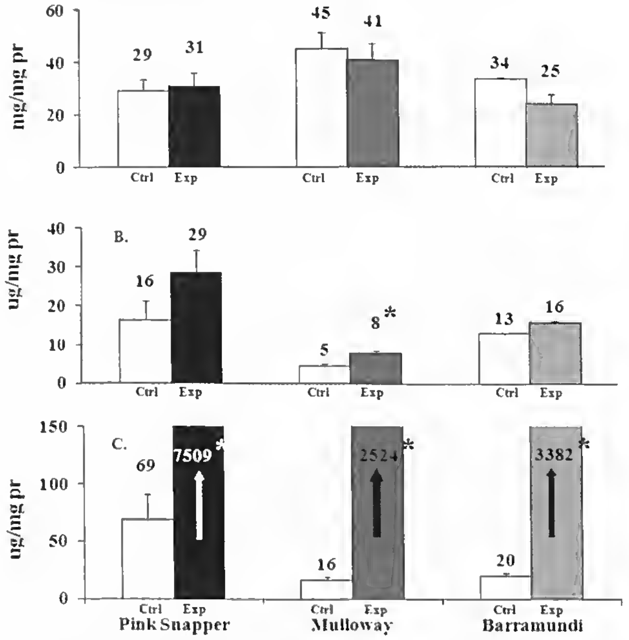

Figure 2. Biliary metabolite levels measured in pink snapper, mulloway and barramundi. BaP was injected to ascertain biochemical responsiveness and induce the presence of BaP metabolites at high levels. **A.** Naphthalene-type (mg/mg pr); **B.** Pyrene-type (µg/mg pr); and **C.** BaP-type (µg/mg pr). *For each species, indicates a significant difference (p < 0.05) from the negative control group of that species.

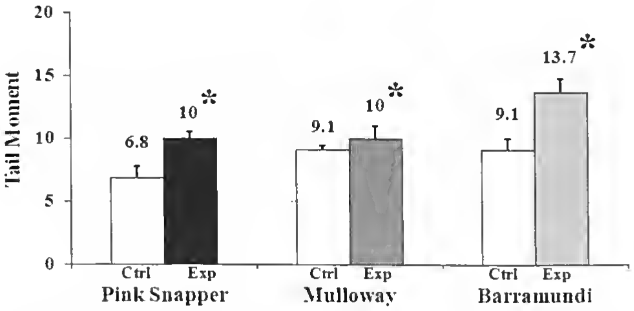

Figure 3. Serum SDH activity (mU) measured in three species of fish injected with BaP. *indicates a significant difference (p < 0.05) from negative control fish.

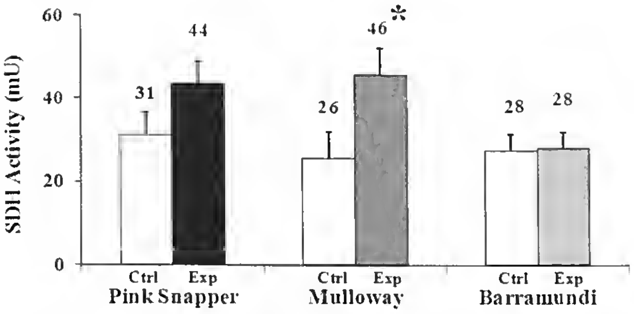

Figure 4. DNA damage (tail moment) measured in the blood of pink snapper, mulloway and barramundi injected with BaP. *Indicates a significant difference (p < 0.05) from negative control fish.

No liver damage was induced by the 4 day BaP treatment in pink snapper and barramundi, as evidenced by the lack of increase in SDH activity in the treated groups relative to the control groups (p = 0.11 and 0.99 for pink snapper and barramundi respectively). In mulloway, however, the liver SDH was released in the bloodstream and resulted in a significantly higher (p = 0.008) SDH activity in the serum of this fish species (Fig. 3).

Following injection with BaP, all three species showed a significantly higher DNA damage compared to their control groups (p < 0.001 in all cases) (Fig. 4). While the incidence of DNA damage increased by 10% in mulloway injected with BaP, the incidence of DNA damage increased by 47% and 51% in pink snapper and barramundi, respectively.

For pink snapper and mulloway, no statistical difference (p = 0.49 and 0.78, respectively) were detected in HSP 70 between the BaP treated groups (mean ± SEM 489 ± 72 and 546 ± 106 pixel µg protein[1] for pink snapper and mulloway respectively) and their respective control groups (mean ± SEM 406 ± 48 and 553 ± 114 pixel µg protein[1] for pink snapper and mulloway respectively). HSP 70 results for barramundi are not available due to the loss of samples during a power outrage.

Discussion

The unchanged response of physiological parameters such as LSI and CF following injection by BaP suggest the good physical condition of fish in all groups. These physiological parameters were not expected to undergo large variations as the fish were sacrificed only 96-hours post-injection.

BaP injections to pink snapper, mulloway, and barramundi caused metabolic perturbations in the three species exposed under laboratory conditions. Among the three species tested, pink snapper demonstrated significant response to BaP injection by increasing EROD induction 3-fold relative to its control group whereas, mulloway and barramundi showed lesser induction potential of 1.5 and 1.7 fold respectively, over their respective controls. However, barramundi demonstrated EROD activity by factor of 20 over controls when injected with β-naphthoflavone (Mercurio *et al.* 2004). Therefore, it is possible that the magnitude of the induction in barramundi might be greater than that achieved in our experiment (Whyte *et al.* 2000). The low EROD activity induction in mulloway and barramundi is well below the induction potential observed in other bioindicator species. For example, the EROD activity of Atlantic salmon (*Salmo salar*) can be induced over 50 times when injected to PCB126 under laboratory conditions (Smith and Gagnon, 2000), or the PCB-exposed shorthorn sculpin (*Myoxocephalus scorpius*) which had a 50-fold EROD induction relative to control fish under field conditions (Kuzyk *et al.* 2005). The most sensitive of the Australasian native species tested for EROD induction appears to be the sand flathead (*Platycephalus bassensis*) with an EROD activity of 13-fold over control fish in a field situation (Smith and Gagnon, 2000). Sand flathead are however not grown in aquaculture, and not abundant in many environments which generate the need to use alternative species. Whereas, the other tested Australasian fish species such as sixspine leatherjacket (*Meuschenia freycineti)* and bluethroat wrasse (*Notolabrus tetricus)* have demonstrated only 2.1 and 1.3-fold EROD activity, respectively (Smith & Gagnon 2000). Amongst the species tested in the present study, pink snapper remains the most responsive species where EROD activity can be used as a sensitive biomarker of exposure to PAHs.

When mulloway and barramundi remain the most suitable or available bioindicator species, the number of fish to be collected in a field study might need to be increased over the recommended minimum of 12 fish per site (Hodson *et al.* 1991) in order to achieve acceptable statistical probability of detecting an inter-site difference. Studies show high variability of EROD activity in field studies relative to variability in lab studies (Abrahamson *et al.* 2008). Low EROD induction potentially also means that a field study needs to carefully consider the biology of the species to be collected in order to avoid confounding variables such as water temperature, stress, species difference and reproductive status, the later potentially altering EROD activity in female fish (Hodson *et al.* 1991; Goksoyr & Forlin 1992; Gagnon *et al.* 1994; Whyte *et al.* 2000; Codi *et al.* 2005; Humphrey *et al.* 2007; Kirby *et al.* 2007; Ramsak *et al.* 2007). Under field conditions, chemical analysis would also need to be carried out on the water and sediments to determine what bioavailable chemicals are present and in which concentrations, information which would complement the observed EROD induction in field-collected fish.

Not surprisingly, none of the treated groups demonstrated increased fluorescence at the naphthalene wavelength. The background fluorescence levels at the naphthalene wavelength needed nevertheless to be measured to insure that no interference would be occurring in the absence of naphthalene. The pyrene-type metabolites varied very little in the bile of all species tested. Large variations were observed in the BaP biliary metabolites of all fish species. Despite of the similar BaP dose (1.0 μg/g wet weight of fish) received by all species, the biliary metabolites of the BaP-type showed the highest response in mulloway and barramundi relative to their control groups, with BaP metabolite levels of 109-, 159- and 169-times higher than control fish for pink snapper, mulloway and barramundi respectively.

Under field conditions where fish are usually exposed to low levels petroleum contaminants, the biliary metabolite levels are not likely to be as high as in the present laboratory study. For example, sand flathead collected from the highly PAH-affected Hobson Bay (Melbourne, Victoria) showed a 4.7-times higher naphthalene levels than a reference site, while the same fish had 8.2 times the BaP-type metabolites, relative to the reference fish (Gagnon & Holdway 2002). Similarly, black bream *Acanthopagrus butcheri* collected in an urban estuary showed 4.8- and 4.4-times levels of naphthalene and BaP biliary metabolites at impacted sites relative to a reference site (Webb & Gagnon 2002b). Despite the lower response observed under field conditions, PAH biliary metabolites remains one of the most sensitive biomarker of exposure to petroleum compounds, and can provide evidence of contaminants bioavailability when chemical analysis or other biomarkers are not indicative of exposure or effects. This is supported by previous studies demonstrating that biliary PAHs show better contamination gradients than EROD activity (Hahn *et al.* 1996; Boleas *et al.* 1998; Richardson *et al.* 2001; Yun *et al.* 2008). Under extreme exposure to petroleum compounds such as an accidental oil spill, fish biliary metabolites could potentially be measured at much higher levels than under commonly occurring field conditions.

It is recognised that the time-course induction of EROD activity and of biliary metabolite induction might differ amongst the fish species. The experimental design, with a similar dose and holding conditions for all fish species *e.g.* similar water temperatures, reduced the variability potentially attributed to differing time-course events. It is therefore believed that the time-course of EROD induction and biliary metabolite excretion is comparable between species.

No liver damage resulted at 4 day post BaP treatment in pink snapper and barramundi, as evidenced by the lack of increase in serum SDH activity in the treated groups relative to their control groups. In mulloway, however, the presence of SDH activity in the bloodstream indicates the liver damage in this group (Ozretic & Krajnovic-Ozretic 1993). It has been demonstrated that SDH activity measured in the bloodstream is an early indicator of hepatocellular injury, and that serum SDH activity is detectable before physiological and histological changes are evident (Webb & Gagnon 2007). In the present study, mulloway's LSI remained unchanged probably due to the short time period allocated between injection and termination of the experiment. In addition, it can be concluded that the measured EROD activity levels in pink snapper and barramundi were a true response not influenced by hepatocellular injuries, and that the EROD activity of mulloway might have been higher would the liver integrity have been preserved in this species. Fish livers with cellular injuries related to xenobiotic exposure are less capable of EROD induction than are non injured liver (Holdway *et al.* 1994). Mulloway appears to be the most sensitive of the species tested regarding hepatocellular toxicants.

DNA integrity was affected in all three species of fish, as seen by consistent increases in DNA damage following injection with BaP. While pink snapper and barramundi had an increase of 47% and 51% respectively in the levels of DNA damage, mulloway had a modest 10% increase showing that this former species is less sensitive to genotoxicants than pink snapper or barramundi. DNA can be affected by a variety of contaminants, especially PAHs (van der Oost *et al.* 2003), and while biliary metabolites are indicative of exposure to PAHs, DNA damage informs on biologically relevant effects experienced by the organisms.

For all species, levels of heat shock proteins HSP 70 measured in the gills remained unchanged following the 4-day treatment with BaP, which is an expected result given the short timeframe between the BaP injection and termination of the experiment. HSP 70 is also a highly variable biomarker under field conditions as several abiotic factors including exposure to heat, UV radiations, chemicals, toxic metals, poisons, air pollutants, injuries and diseases (Ali *et al.* 2003; Yoshimi *et al.* 2009) have the potential to influence HSP 70 levels making interpretation of this biomarker difficult to ascertain (Webb & Gagnon 2009).

Conclusions

Measurement of a suite of biochemical markers of exposure and effects on three species of fish indicated that mulloway and barramundi could be used as surrogate species to pink snapper for assessing the bioavailability and effects of urban and marine pollutants such as PAHs but further research is required if different classes of chemicals are to be assessed. Pink snapper showed high EROD induction potential and would be the most sensitive bioindicator if the contaminant of concern is an EROD inducer. However, mulloway and barramundi would be good alternative bioindicator species if biliary metabolites are measured but mulloway seems to be the most sensitive species to hepatotoxicants and barramundi would be the preferred bioindicator if genotoxic contaminants are present. Overall, the choice of bioindicator species will depend on the availability and ease of capture, the contaminant present in the environment and the biomarker measured. This study provides a relative responsiveness of common Australasian fish species in order to facilitate bioindicator selection.

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A new unusual Osteichthyan fish from the Gogo Formation, Western Australia

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Abstract

A unique specimen of a braincase and part of the skeleton of an osteichthyan has been etched from limestone in the Gogo Formation (Frasnian) in the Canning Basin, Western Australia. This has been reconstructed using the standard techniques for interpreting nerves and vascular systems, and an abbreviated reconstruction of the main skeletal features, nerves and vascular structures has been presented. This indicates that the pattern falls outside the range of currently known osteichthyan genera, and we consider that it represents a new pattern of structure evolved from a massive change in the genotype, probably by genomic regulatory systems. Interpretation of the bony skeleton is described using standard observational techniques and serial tomography. The dorsal skull roof consists of a posterior undivided plate with a pointed anterior projection. Ventral to this is another internal support plate attached to the dorsal plate posteriorly and laterally, and medially by vertical stubs. Orbits occur in dorso-anterior position. Strong median ridge ventrally runs anteriorly, and it joins an internasal cartilage. Antero-lateral to the braincase is a large cavity thought to be filled with cartilage in life, and lateral to that and separated from it by a bony sheet, is another cartilage filled space. All these skeletal features are diagnostic of the character of a new group of organisms.

Keywords: Devonian, dermal structure, braincase, cranial nerves, genomic regulatory systems, Osteichthyan patterns.

Introduction

This specimen was found in the Kimberley Region, Western Australia, in the Frasnian Gogo Formation (Fig. 1). The specimen was encased in a small nodule of limestone, and it was etched in acetic acid to expose the remaining bone. The eroded specimen has lost some of the dermal plates, but a well preserved the supraorbital plate and two plates adjacent to it were found in the same nodule. No lower jaw was preserved. The etching has partly destroyed much of the fine bone structure, particularly the thin layers of perichondral bone. Tomography has enabled us to reconstruct some of the missing bone, and we can observe some of the otherwise concealed endocranial bones.

The point in describing this incomplete specimen is that it is so different from other known osteichthyan genera and that it represents a new kind of biological organization in the Devonian, a Period when new osteichthyan patterns were abundant. We have previously described the importance of genomic regulation in the development of new organizations (Campbell & Barwick 2006), and this material is another example of new patterns illustrating the main point of that paper.

Only one incomplete specimen of this form has been found, among the hundreds of etched specimens from this locality. Further searches by ourselves and by other palaeontologists have failed to discover even fragments of the surface ornament. The outstanding state of preservation of the Gogo material, and the failure to discover comparable species elsewhere, indicates that it is a rare species and probably had a limited time and geographical range of occurrence.

We have reconstructed the pattern of some the nervous and vascular systems of the specimens and have represented this incomplete outline without the skeleton. In order to locate these structures for the reader we have indicated the position of some foramina through which the canals pass (Fig. 2). In doing this we have indicated the main factors in our orientation of the specimen. Of course this orientation may be criticised, but the observations of the specimen have to be interpreted in acceptable biological terms. For example, it would be difficult to consider this structure as an eroded braincase of a dipnoan in the light of structures described below.

Orientation of Specimen

In the absence of the pattern of dermal bones, endocranial structures have been the bases for orienting the organism. The lateral walls of the neurocranium are present on both sides of the specimen, and these enclose the braincase in the axis of the structure. We determine the posterior of the specimen because it has the outline of a braincase preserved in thin bone running internally, and having openings into the braincase from the external wall of the neurocranium. What is more there is no sign of a braincase at the anterior of the specimen as we

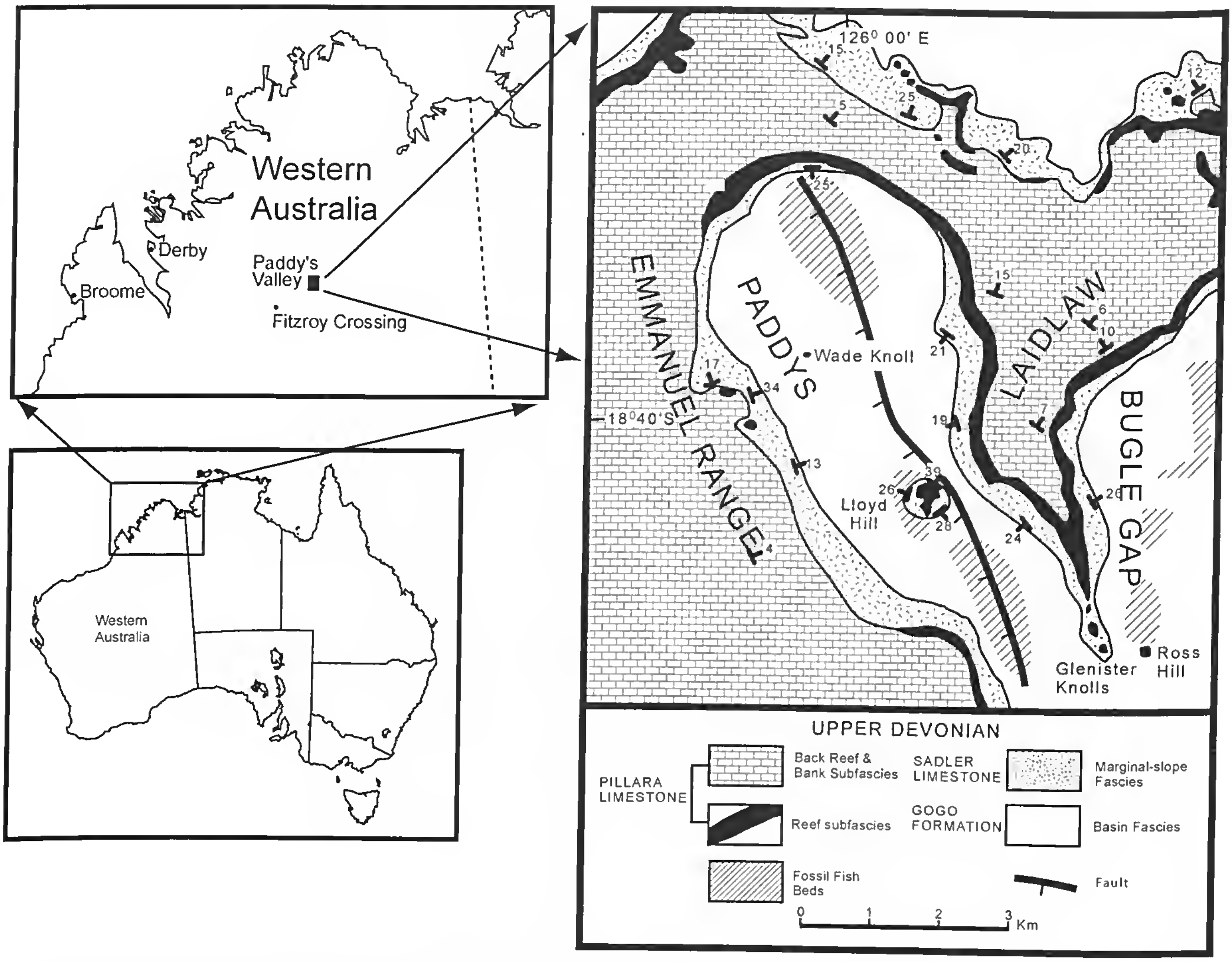

Figure 1. Map showing the position of the Gogo Formation in Western Australia, and the site of the collecting locality. Diagram modified from Andrews *et al*, 2006.

would expected if the specimen were oriented in the reverse direction to what we have proposed. Given this orientation the braincase must be dorsal to the attachment of the notochord, but the specimen is strongly weathered ventral to the braincase and the notochordal attachment has been lost. This orientation indicates not only the posterior end of the specimen but also the dorsal/ventral sides.

The neurocranial walls on each side form relatively robust structures that run almost the length of the specimen. At its posterior end it is eroded and what we consider to have been the lateral commissure has been lost, together with the articulation of the mandible. This would have been a final guide to the orientation had they been present.

This orientation of the specimen also provides the opportunity to recognize the cranial nerves N VIII, N VII, N V and N II in their correct positions. Also although not observed on the external surface, tomography shows the nerves N IX running from the posterior of the braincase in the appropriate position.

Anterior to what we determine as the nerve N VII is a large lateral extension of the neurocranium that houses the largest foramen in the wall (Figs 7, 12, 27). This foramen enters a canal that runs within the neurocranial wall into a branching system of canals that do NOT join the braincase, and must be part of the vascular system. Entering the foramen from a posterior position are two grooves that would have been for the jugular vein and the orbital artery. The size and shape of the foramen and the position of the canals, supports the orientation of the organism as suggested.

Near the proposed anterior end of the preserved specimen there is a gap for the orbit (Fig. 3) and a space for the olfactory capsule (Fig. 14). Running towards the nasal capsule are perichondrally lined tubes that divide anteriorly and terminate in the olfactory capsule or in the lateral walls of the neurocranium (Figs 14, 23). All the above features confirm the complete orientation of the specimen as we have described it below.

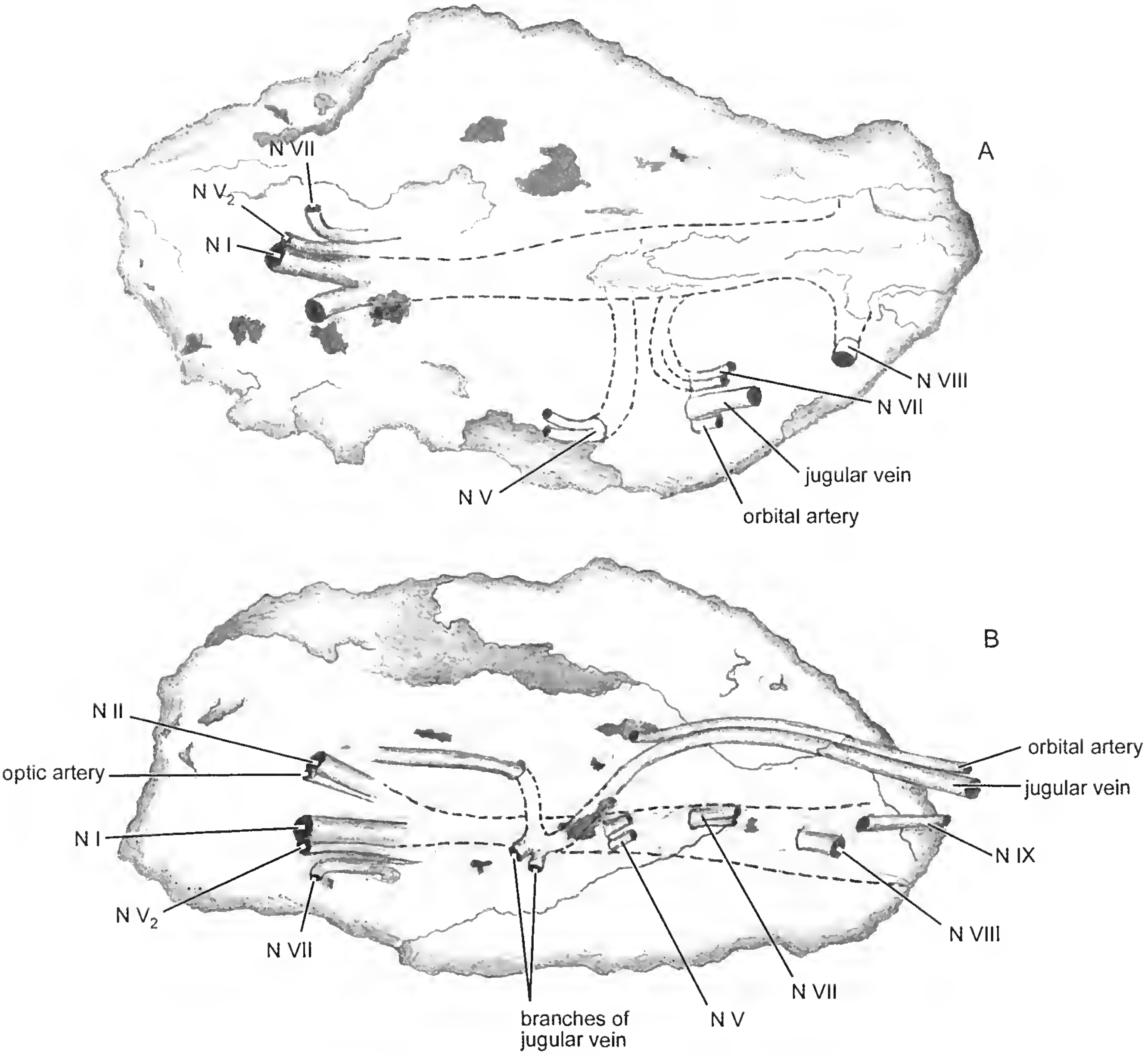

Figure 2. Partial reconstruction diagrams of the braincase of the specimens showing positions of the position of the main nerves and the jugular veins . These give the main position of these structures as a guide to the details set out in the descriptions below. (A) A ventral view of the outline of the specimen with the braincase slightly darkened and the outline of the ossified wall shown as a dotted line. Nerves N V and N VII shown as paired structures because each emerges through two foramina on the specimen. (B) Reconstructed lateral view of the specimen with the braincase slightly darkened. Dotted lines showing jugular vein and the orbital artery from impressions on the neurocranial wall, and the internal course of the jugular outlined from tomography. The internal course of the orbital artery not shown. Course of the optic nerve outlined from tomography but the internal course of the optic artery not shown. Only the left olfactory nerve shown.

Terminology

sub-dermal plate: Large posterior dermal coarse textured plate with thin covering of fine textured bone;

plate 11: Similar to sub-dermal plate separated from it by a strong suture;

internal support plate: Large plate beneath the sub-dermal plate that it joins posteriorly and laterally, but is joined to it medially and anteriorly by bony struts;

triangular plate: Median dorsal plate with triangular outline and carrying small struts, bearing a thin covering plate that was removed during preparation;

centro-lateral cartilaginous space: Open space lateral to the braincase thought to be filled with cartilage in life, but carrying veins and orbital arteries; and

lateral cartilaginous space: Smaller open space lateral to the median cartilaginous space and separated from it by a neurocranial wall, and carrying veins and orbital arteries.

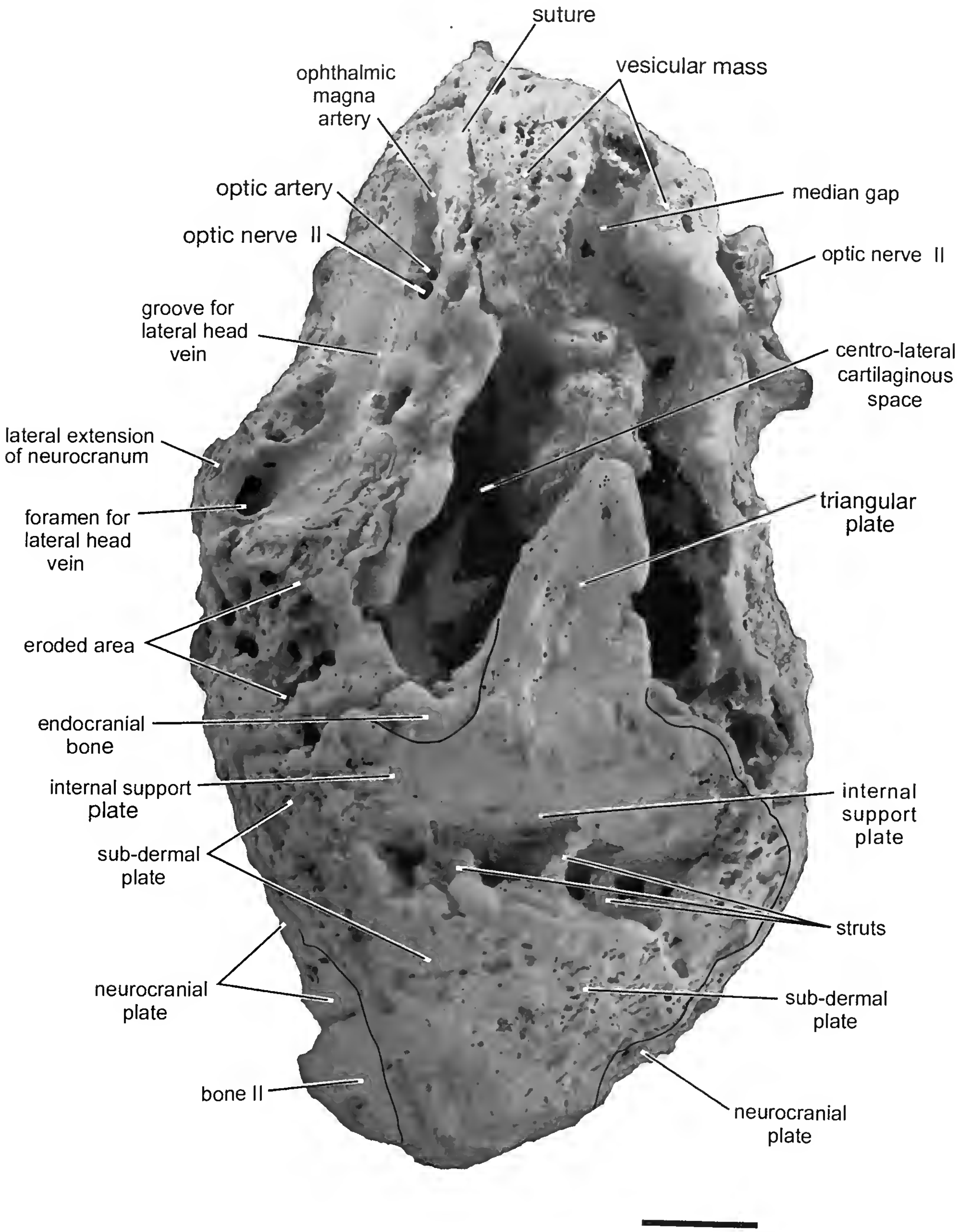

Figure 3. Dorsal view of whole specimen illustrating all the major features mentioned in the interpretation in the text. Outline of suture bounding the sub-dermal plate and anteriorly the internal support plate, in a solid inked line. Scale = 10mm.

Systematic Palaeontology

In the absence of a pattern of dermal bones and of a palate, we have been uneasy in assigning a formal taxonomic name to this isolated specimen. However, further reference to the specimen by ourselves and any other authors would find it convenient to have a formal taxonomic name.

Genus *Cainocara* n. gen.

Type species: *Cainocara enigma* n, sp.

Etymology: kainos (Gr) new: kara (Gr) head: enigma (L) obscure

Type specimen: ANU 46543 from Paddy's Valley, Gogo Formation, Western Australia.

Location of specimen: ANU = Australian National University.

Description:

Postero-dorsal surface: The exterior of the skull roof is not preserved, but the surface of the specimen is made of a sub-dermal plate (Fig. 3). This plate has patches of very fine grained bone on its surface, and this is bound into the underlying coarse bone (Fig. 12B). This fine bone we regard as the preserved remains of the original dermal surface. The suggestion that the sub-dermal plate is endocranium is not supported by the specimen. A lateral extension of the neurocranial bone curves anteriorly towards the triangular plate (Figs 3, 4). The triangular plate as is shown on Figures 24, 25, is made of the internal support plate (see below), and this implies that a layer of the sub-dermal plate originally covered the internal support plate on the triangular plate. This extends anteriorly beyond the mid-length of the specimen; the anterior end of the plate was lost during preparation.

Ventral to this large sub-dermal plate is the internal support plate (Figs 5, 6) that meets the triangular plate anteriorly at an angle of ca. 20°. The triangular plate is shown by tomography to be an extension of the internal support plate. Tomography illustrates that a canal runs ventrally off the triangular plate in a posterolateral direction (Figs 4, 25), and it seems to open into the large internal space that forms the edge of the perichondral inner lining of the centro-lateral cartilaginous space. Posteriorly and laterally the internal support plate joins the sub-dermal plate. As shown on Figure 5B, the internal

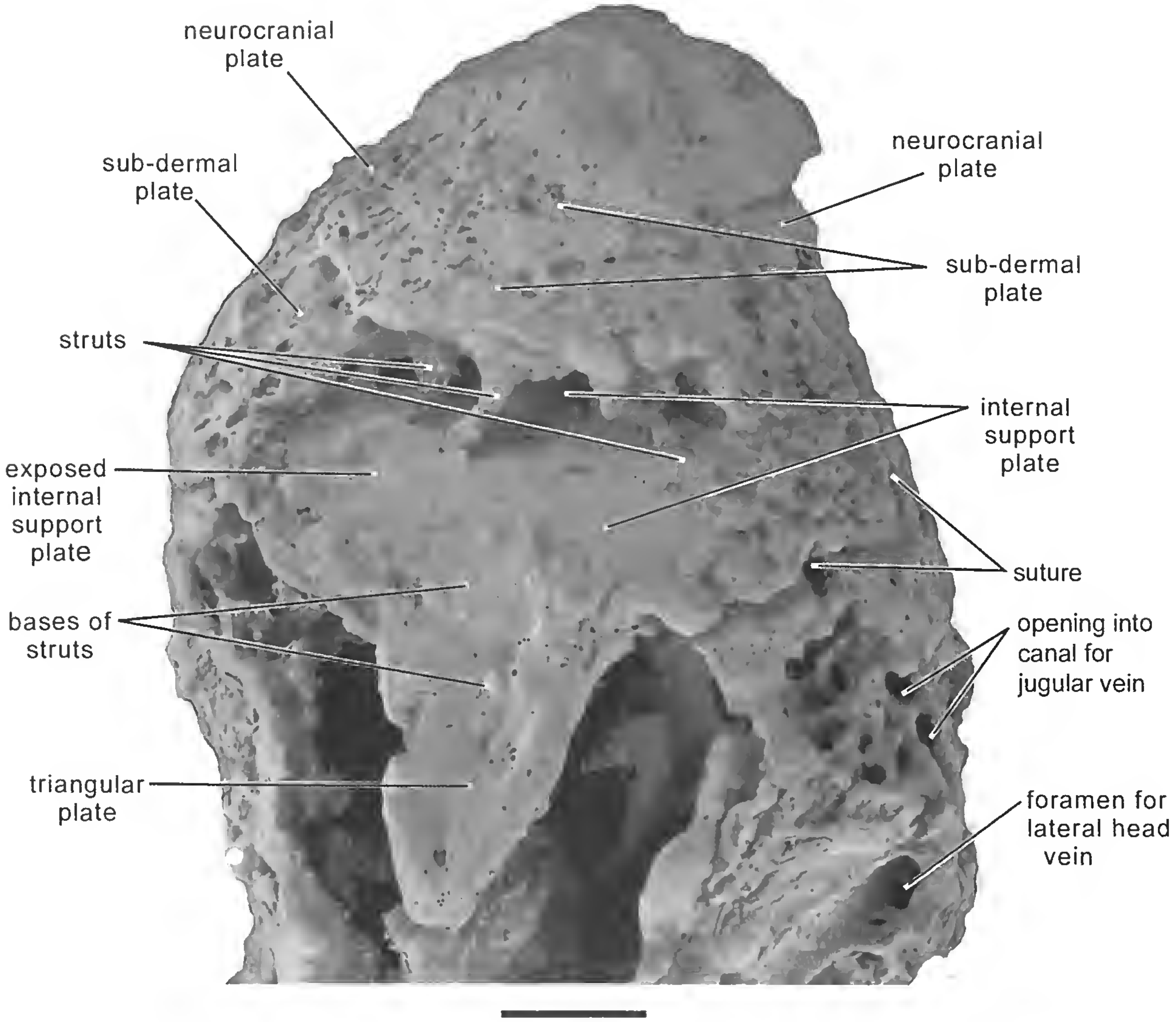

Figure 4. Slightly tilted and slightly modified dorsal view of posterior of specimen showing details of the dorsal structures more clearly than on Figure 3. Foramen in triangular plate leading to canal. Scale = 10mm.

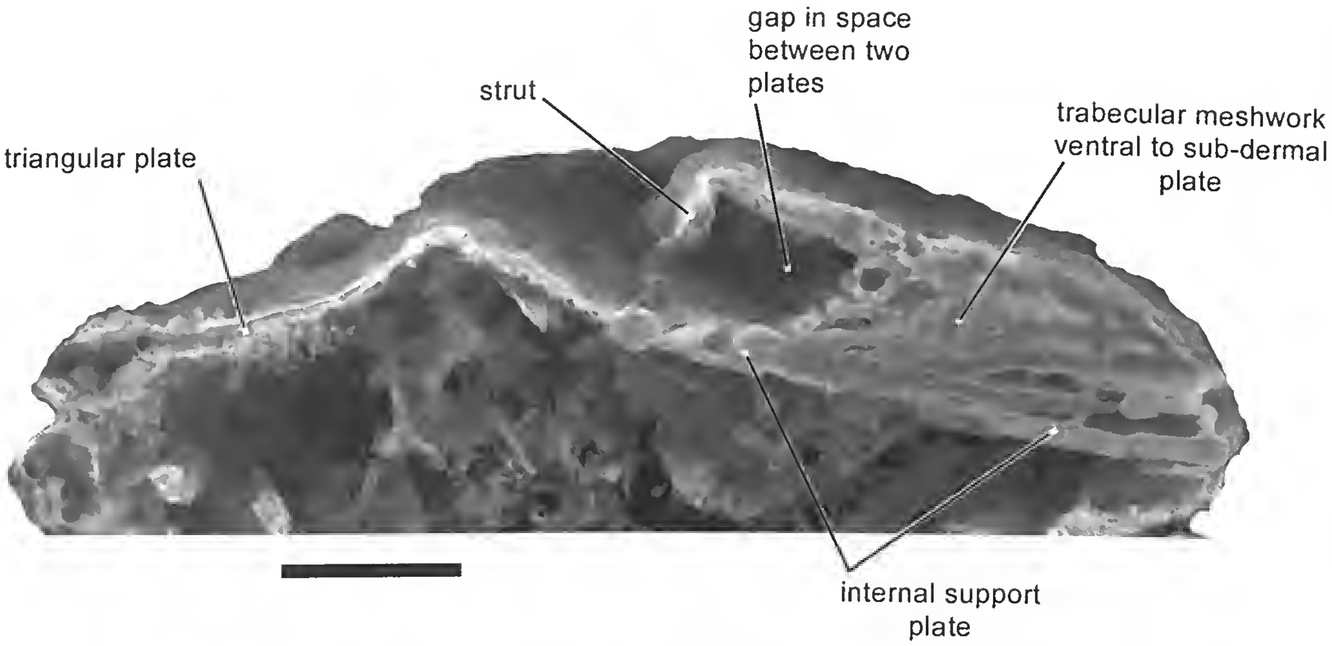

Figure 5. Thick tomographic section through the posterior part of the specimen showing the position of the internal support plate, and its supports to the sub-dermal plate. Scale = 10mm.

support plate continues towards the posterior extremity, but as shown on Figure 7B it does not reach the limits of the specimen. The trabecular meshwork lies directly on the neurocranium.

X-rays show that the internal support plate is made of fine-grained bone easily distinguished from the sub-dermal plate. Posteriorly these two plates are joined by a trabecular meshwork of plates and anteriorly by a series of bony struts (Figs 5, 6B, 13, 24, 25). The braincase lies three or four mm ventral to the internal support plate.

In the posterior view of the specimen the braincase is separated from the neurocranial walls by a small gap that we interpret as occupied by cartilage (Fig. 6A). This also would have occupied the space around the lateral and ventral parts of the bones. The walls of the braincase are preserved as a thin layer of bone. The posterior extremity of the braincase is not preserved, but the position of the neurocranial walls indicate that only a small amount of the braincase is missing. The thickness of the posterior part of the braincase indicates that it carried the dorsal paired canals described below.

The posterior of the specimen (Fig. 7A) has a fragment of another dermal plate labelled bone II. Its surface is also made of the fine grained bone such as occurs in patches on the sub-dermal plate, and its base in made of a layer of coarse bone. It is part of the dermal skeleton, and details of its surface are shown on Figure 12B. Two posteriorly-viewed images of tomographic sections (Figs 7B, C) through the posterior of the specimen and through the jugular foramen are significant. The internal support plate (Fig. 7B) lies directly on the neurocranial bone, and the trabecular network filling the internal space. Figure 7C is a section through the jugular foramina the right side retains some of the internal structure that will be dealt with in a subsequent section.

Bones of the Circumorbital Fragment

This section describes the only isolated dermal bone preserved, and it was found with the skeleton in the limestone nodule. It has the edge of the orbit preserved (Fig. 8B) and attached to it are two other dermal bones that have not been identified. The orbital fragment has no lateral line and it is therefore not an infraorbital. Being a supraorbital we have assumed that the pattern of ornament on these and the adjacent bones represents the pattern of ornament of the skull roof.

One end of the supraorbital plate thickens considerably, but the other end has lost some of the internal bone. Cross section of the thicker bone (Fig. 8A) shows three distinct layers: (a) the outer layer has about the same or slightly greater thickness as the inner layer, and it is composed finely perforated bone. No large trabecules like those of the median layer are present. Surface bosses range from 0.2 mm to 1.2 mm in diameter. Sections of the bosses show that they range in height from 50–200µ; (b) the median layer is much thicker and consists of trabecular bone. Its contact with the inner layer is clear. Thin sections show large osteocytes. In addition to the osteocyte spaces there are linear slits (Fig. 8A) that suggest the presence of blood vessels; and (c) the inner layer is of fine porous grained bones.

The structure of the external bosses is best examined in direct images from the exterior, especially in bosses that have been just forming or are partly resorbed (Fig. 10 B). As shown on Figures 8C, 10A, B, the bosses overlap the outer layer of the bone. Bosses are composed of several sheets of superimposed layers. Perforations, as shown on Figure 9B, are common, but other layers are less perforate. Apparently each layer of the boss contains both types of tissue. As shown in Figs 8C, 9B, 10B, these layers are not continuous over the whole surface of the boss, but occur in patches. Fig. 9B shows the upper layers of

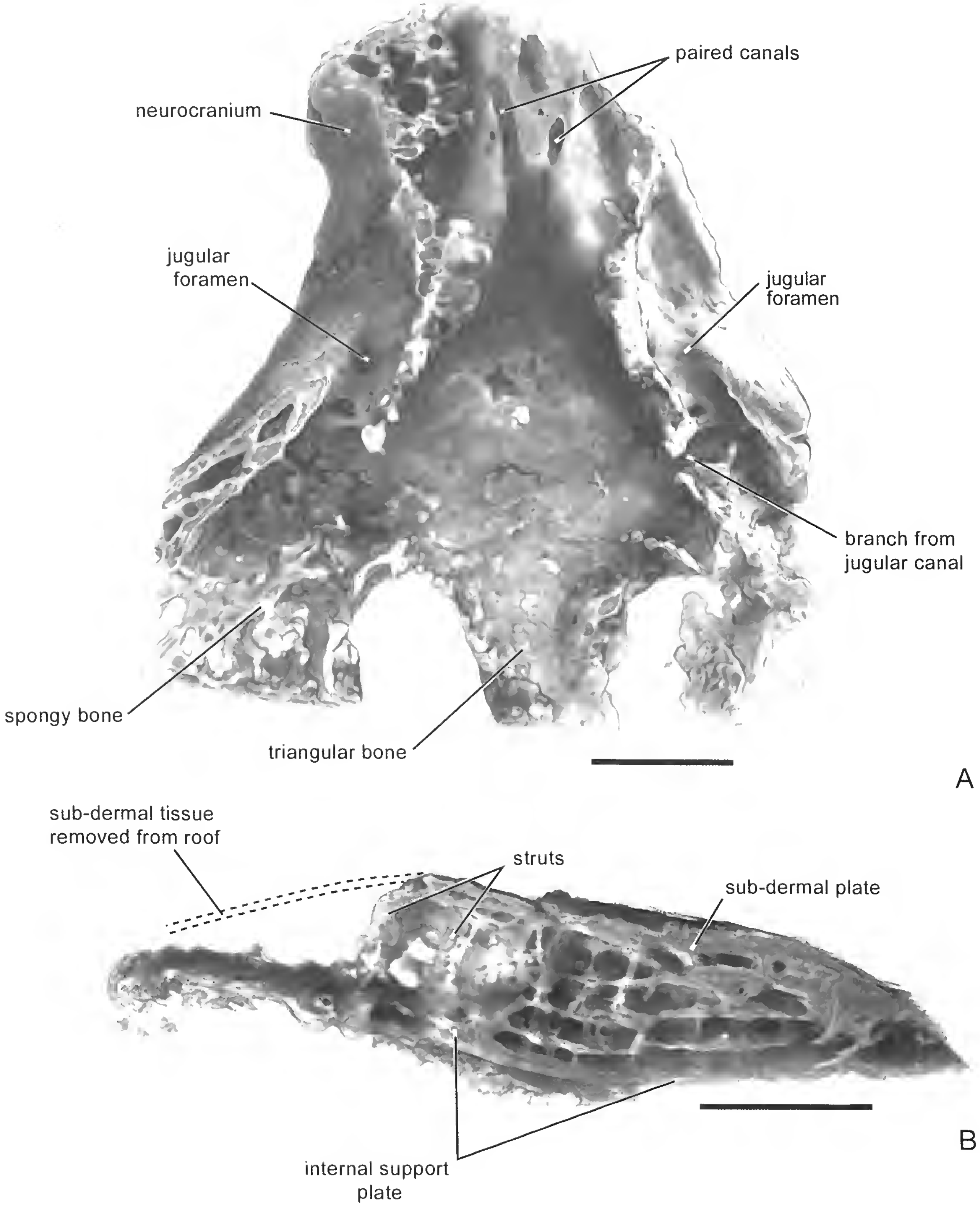

Figure 6. A. Dorsal view of a thick tomographic image of an oblique section cut dorsal to the braincase posteriorly, through the jugular vein, and the triangular plate anteriorly. Dorsal part of the organism removed. A pair of canals reach the posterior edge of the specimen. They must arise from the top of the braincase which lies ventral to this section. They could be for endolymphatic ducts or alternately nerve N X. Neurocranial bone shown posteriorly and anteriorly as spongy bone. B. A thick tomographic section through the posterior part of the specimen with the sub-dermal plate lying on the coarse bony tissue. Posterior part of the internal support plate missing. Anteriorly the subdermal plate was removed during preparation, and its position on the diagram is shown by dotted lines. The area marked as struts also shows the thickening of the bone on the other side of the specimen (see also Fig .5). Scale = 10mm.

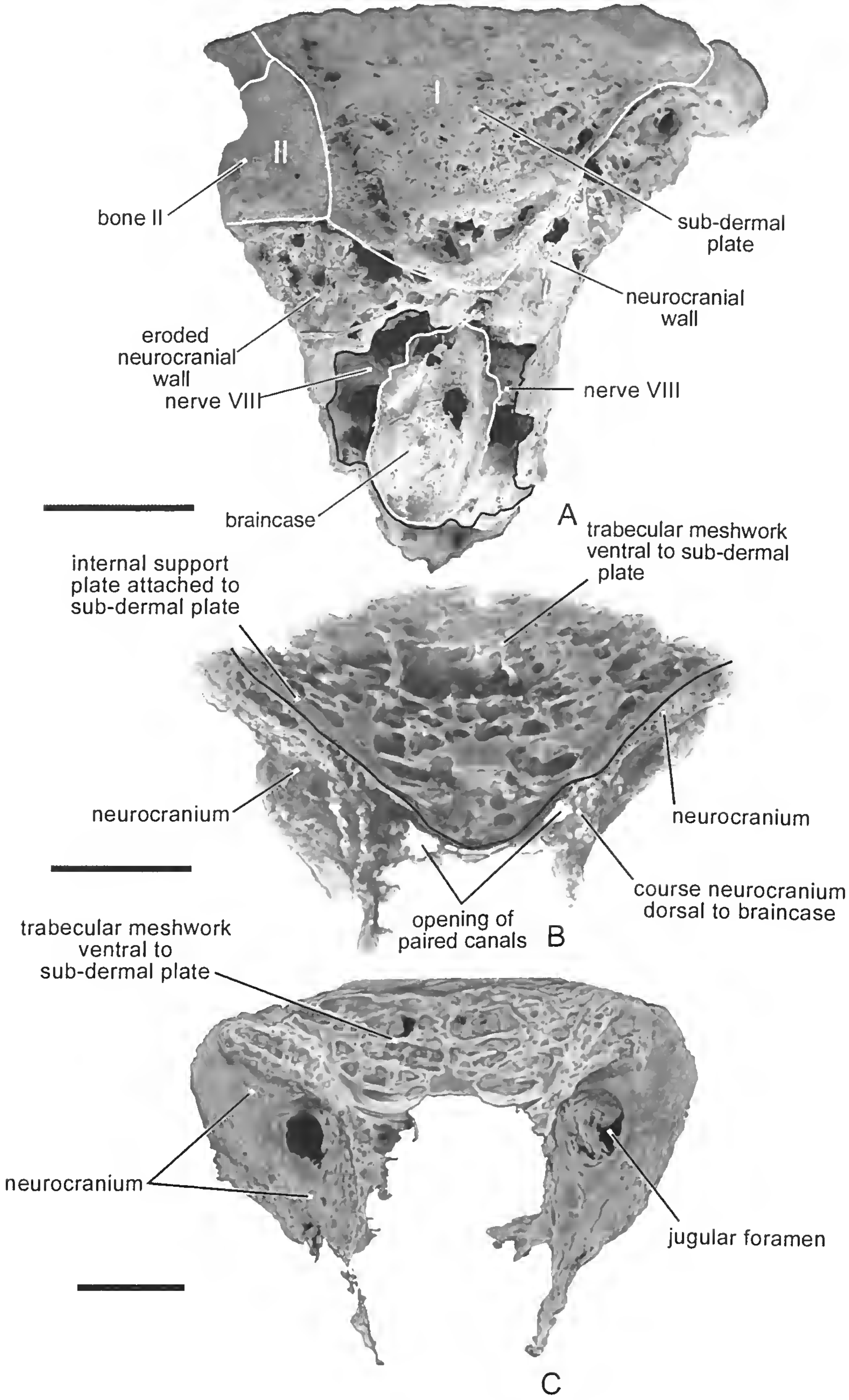

Figure 7. A. Modified image of photograph of posterior of specimen. Right side of specimen more deeply eroded than the left. Sutures bounding the sub-dermal plate, and the neurocranial wall are outlined by a white line. Space around the braincase wall left for cartilaginous material left blank. Canals carrying nerve N VIII present on the two sides. B. Thick tomographic image of a continuous section across the posterior part of the specimen, showing the internal support plate attached to the neurocranial wall but posteriorly disappearing. Suture bounding the neurocranial plate emphasised by a blackened line. Trabecular meshwork between the internal support plate and the sub-dermal plate clear, but exposing a large median gap. C Transverse thick topographic image of continuous section taken posterior to the jugular foramen, and therefore anterior to image in Fig B. Scale = 10mm.

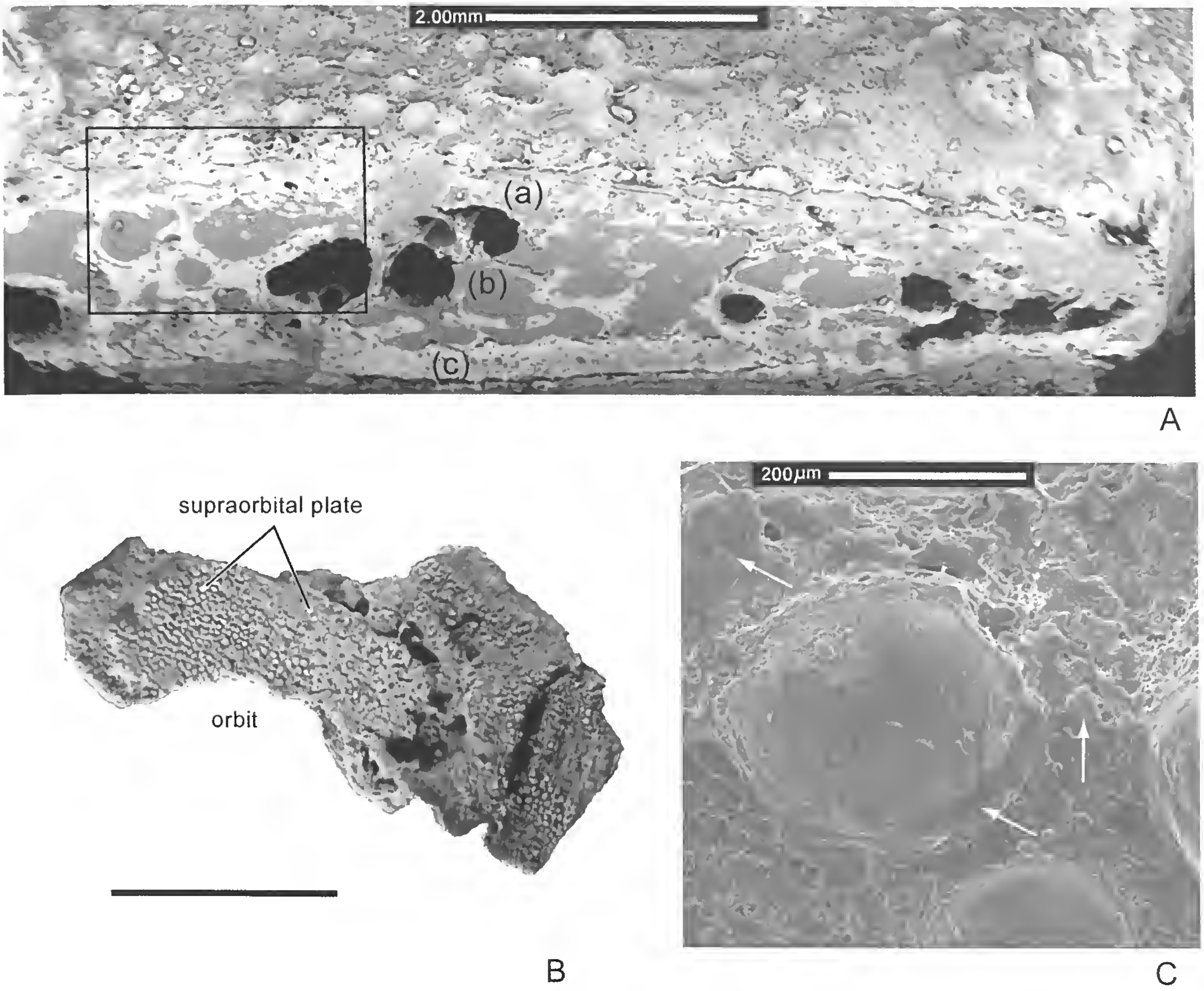

Figure 8. A. Cross section of the bone adjacent to the supraorbital plate as shown in Fig. 8 B. Top surface with the bosses still in position. The outer layer shown by (a); the coarse middle layer by (b); and the inner layer by (c). B. The isolated fragment of the supraorbital bone and the surrounding bones. Bosses on the surface similar on all plates. C. An isolated boss showing the areas between the bosses largely overlapped by layers developed from the first layers of tissue forming the boss (white arrows). Basal bone. largely obscured. Scales as shown.

bone with outgrowing edges. As shown in Fig. 10B where a boss has been partially destroyed, up to 7 numbered layers of sheets form a sequence. This kind of structure has not been observed previously in any osteichthyans.

Elsewhere the surface has a continuous layer of tissue of boss-like tissue (Fig. 11A). As has been shown above (Fig. 11B), the margins of some layers cover the bones between the isolated bosses. In some places there is even connection between the layers of tissue between the bosses (Fig. 11A). The interpretation of these structures remains problematic.

The mode of deposition of the layers on the tubercles is significant. The first deposited layers are those at the base of the boss and these must continue to expand during the growth of the animal. Note that it is these early layers that are the most extensive. Subsequent layers are deposited on these early layers and are progressively more restricted. Their margins would have been covered with epidermal tissue and so their margins would have continued to grow. This is shown on the enlarged margin on Figure 9B in which the growing edges of the layers were exposed.

So far as we are aware, this kind of depositional pattern is not known in any known primitive fish.

Endocranial Structures

The neurocranium wall consists of a single unit, and tomographic sections show that it is made of moderately thick bone. These two pieces of evidence are vital in understanding the assignment to a higher group. The neurocranial walls are extensive on the left side of the specimen, but they were lost anteriorly on the right side by erosion.

Lateral Walls of the Neurocranium: There is a large space between the outline of the braincase and the neurocranium. In Early Devonian dipnoans this gap is

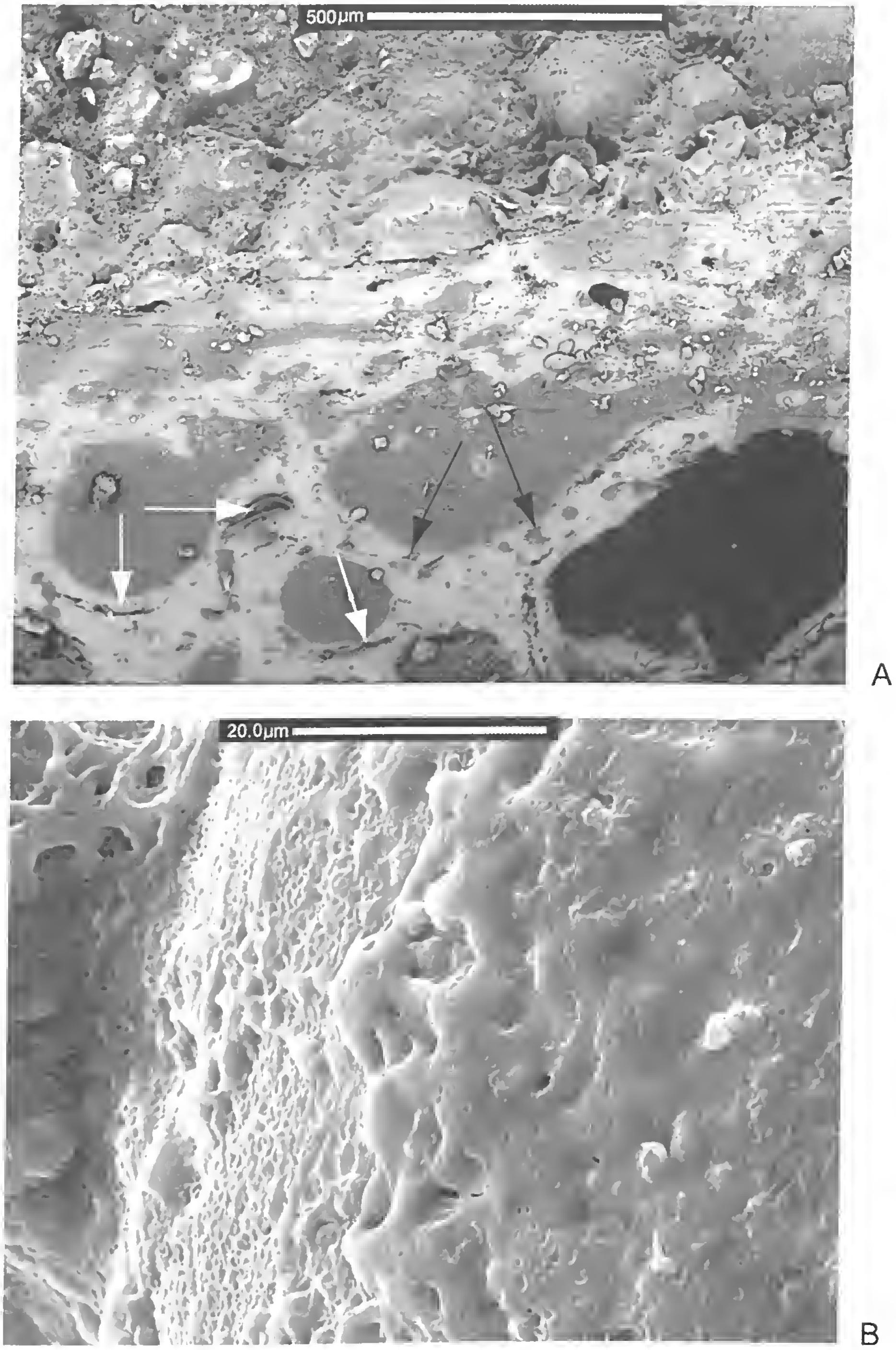

Figure 9. A. An enlargement of the area marked on Fig. 8 A. Bosses shown at top of figure. Osteocyte spaces (black arrows) in bone around the open spaces. Small slit like spaces (white arrows) possibly indicating blood vessels. B. Enlarged margin of a boss showing the edges of the superimposed layers and the basal bone on left side of image. Note the very fine pores in the most superficial layer and pores in the margins of the deeper layers. Scales as shown.

very narrow, but *Chirodipterus* from the Late Devonian has a much larger gap.

On each side of the specimen is a foramen that is up to 3.5 mm in diameter, and passes through the neurocranial walls and enters the braincase via ossified canals. The size and position of the foramen and its orientation indicates that it had a major function. This suggests that it could have been for the acoustic nerve N VIII leading out to the semi-circular canals. Obviously this implies that the labyrinth space and the semicircular canals were not connected directly into the braincase as is normal for osteichthyans, and this could be a major objection to our proposal that the organism is an osteichthyan. There is no doubt however that the ossification of the walls of the canal from this foramen clearly enters the braincase (see also tomographic sections in Figs 24A, B). The position of nerve N IX is now interpreted as running posteriorly to the edge of the specimen (Figs 12, 25B).

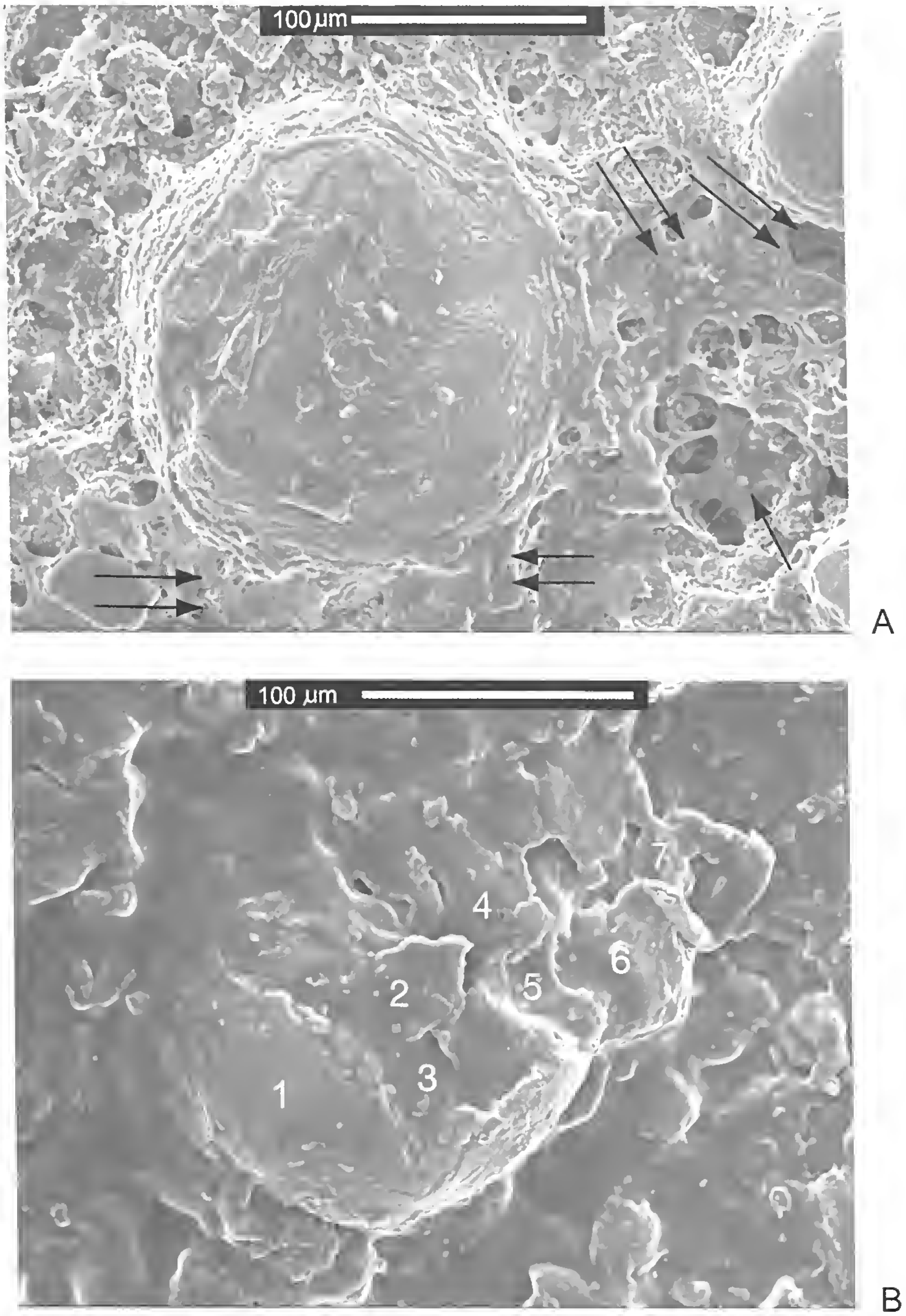

Figure 10. A. Isolated boss showing the incomplete layers on the surface, successive deeper layers, the more widespread lower layers with double arrows indicating the extensive basal layer, and a single arrow at the exposed basal bone layer. B. An isolated boss that was partly destroyed and shows the superimposed layers 1–7. The sequence interpreted from overlaps. Scales as shown.

Tomographic section in Figure 6A, as well as showing the jugular veins in section also shows a pair of canals running posteriorly. These must originate from the top of the braincase as shown in the explanation of the figure, and they run to the posterior edge of the specimen (Fig. 7B). These may be for ductus endolymphaticus or alternately for nerve N X. For the present we give them the labels "paired canals". On each side of the specimen, 15mm anterior to nerve N VIII, the neurocranial wall contains an oval depression in the floor of which are two foramina. The grooves run internally in an antero-ventral direction from these foramina and we conclude that they enter the braincase (see tomographic section 25A, B). In living vertebrates the facial nerve N VII leaves the braincase to form the hyomandibular, palatine and buccal nerves. In our specimen, these two foramina open in a postero-lateral direction and are appropriately placed to contain branches of nerve N VII (Fig. 12A).

The surface of the specimen dorsal to nerve N VII there are small foramina that are difficult to trace internally but they do not appear on the tomographic sections as entering the braincase. We interpret them as nutrient foramina.

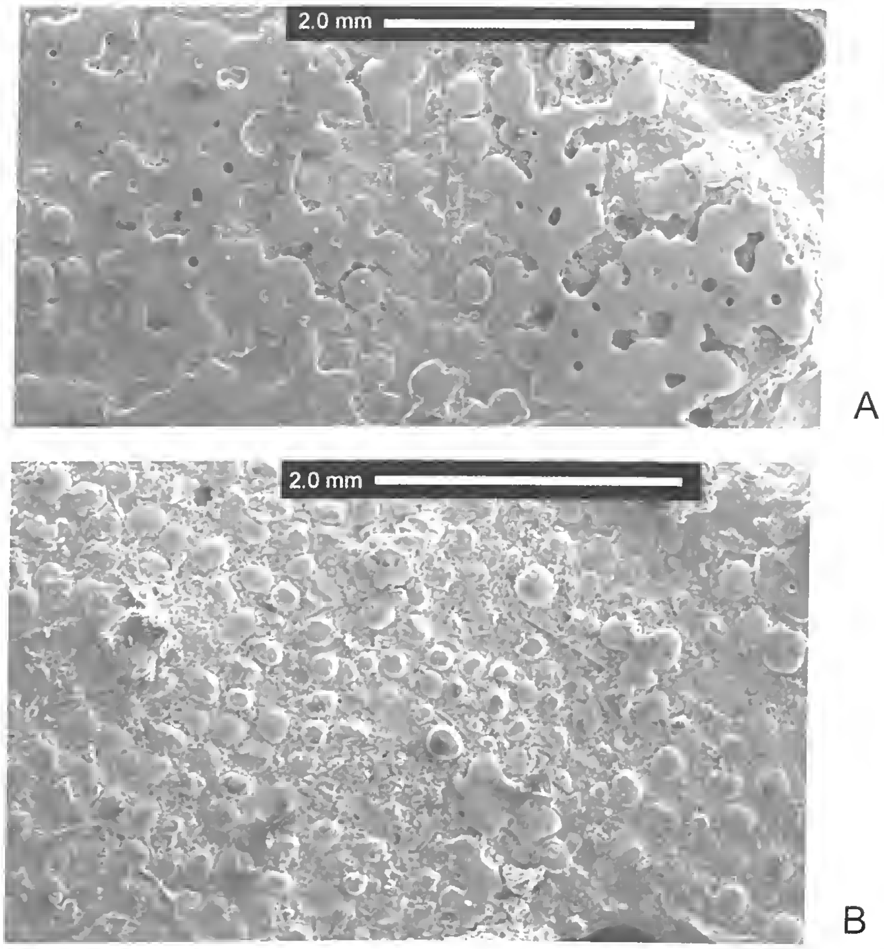

Figure 11. A and B. Two views of the surface area on the supraorbital plate. A. Has the bosses joined. B. Has the bosses separated. Scales as shown.

Dorsal to the foramen for nerve N VII, the neurocranial wall forms an even surface, and dorsally it turns laterally and runs anterolateral for 25mm to form a lateral process (Fig. 2). This is a larger and more posterior extension of the neurocranial wall than is found in any other osteichthyan. At the posterior end of this lateral extension is the largest foramen in the neurocranial wall shown on (Figs 6A, 7C, 12A and tomographically on Fig. 24A). Two shallow grooves in the neurocranial wall enter this foramen from the posterior direction. On the dorsal side is a smooth surface that runs into the foramen (the orbital artery), and on the ventral side is a larger groove within which are tiny openings that enter the wall (the jugular vein). This latter groove can be traced posteriorly for a short distance where it apparently turns laterally. Presumably these two canals enter the preserved specimen from a postero-ventral position, having entered the region through the bone that forms the unpreserved lateral commissure of the specimen. We have named this large foramen as the 'jugular foramen'. In other Devonian dipnoans the jugular vein runs lateral to the neurocranium, but in this new organism it definitely runs medial to the neurocranium mainly because of the large posterior extension of the neurocranial wall. This is just another difference from dipnoans.

Both the jugular vein and the orbital artery are visible, after entering the large foramen the internal walls of (Fig. 24 A). Both canals turn laterally, and the jugular turns towards the lateral neurocranial process. Its course can be followed tomographically (Fig. 26A, B). It forms a large U-shaped outline, and it passes anteriorly into a foramen 3mm wide for the lateral head vein (Fig. 26). There its presence can be observed on the specimen, and it bends anteriorly to a large foramen that we interpret as the foramen for the lateral head vein (Fig. 17). A furrow continues anteriorly along the dorsal surface, and this is described below.

Where the jugular canal turns dorso-laterally towards the lateral neurocranial process, a number of foramina open ventrally out of the canal (Fig. 26A), and these open into the Lateral Cartilaginous Space.

Tomography indicates that ventral to the jugular canal; a separate canal runs anteriorly and separates into an isolated canal that passes to the lateral wall of the specimen lateral to the Lateral Cartilaginous Space. This is labelled as the anterior orbital artery (Figs 17, 18, 27B, C). Another large opening lies posterior to lateral neurocranial process (Figs 17, 18). A notch in its anterior edge continues into the neurocranial wall indicating that the canal passes ventrally from it. Posterior to the openings on the dorsal surface an expanding canal runs postero-ventrally towards the jugular foramen (Figs 17, 18), but as this cannot be part of the jugular system, it can only be part of the branch of the posterior orbital artery

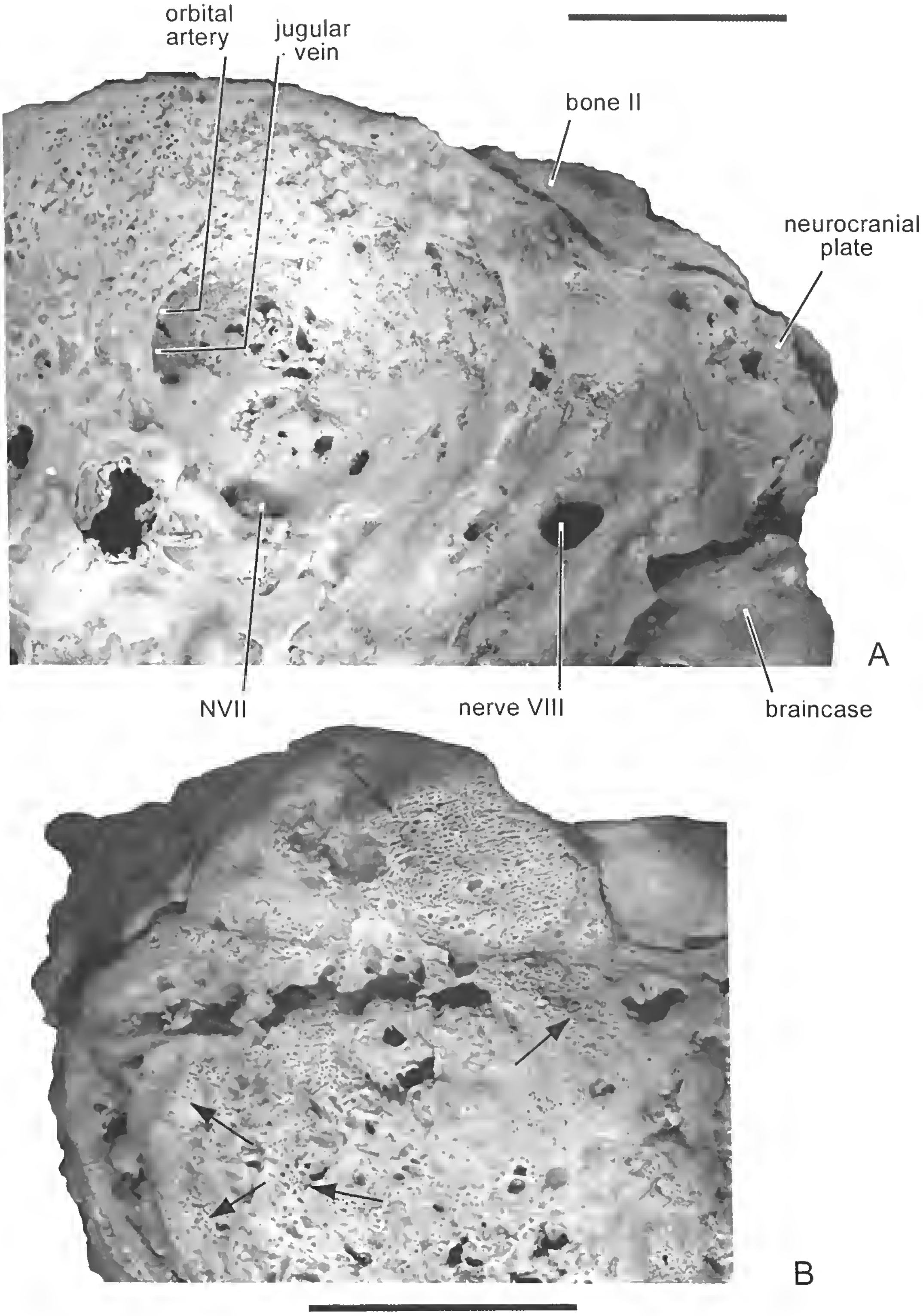

Figure 12. A. Lateral view of the posterior half of the specimen. The large jugular foramen is shown in lateral view, with entrances for the jugular and orbital artery. The latter runs posteriorly in a shallow groove. Foramina for the cranial nerves to the braincase lie in the neurocranial plate. The base of bone II surmounting the eroded neurocranial wall. Nerve N VII is in a depression with two foramina clearly exposed. B. Enlarged dorsal surface, with fine bone on surface of bone II at the top of the figureand on the adjacent surface of subdermal bone (black arrows). Scales = 10mm.

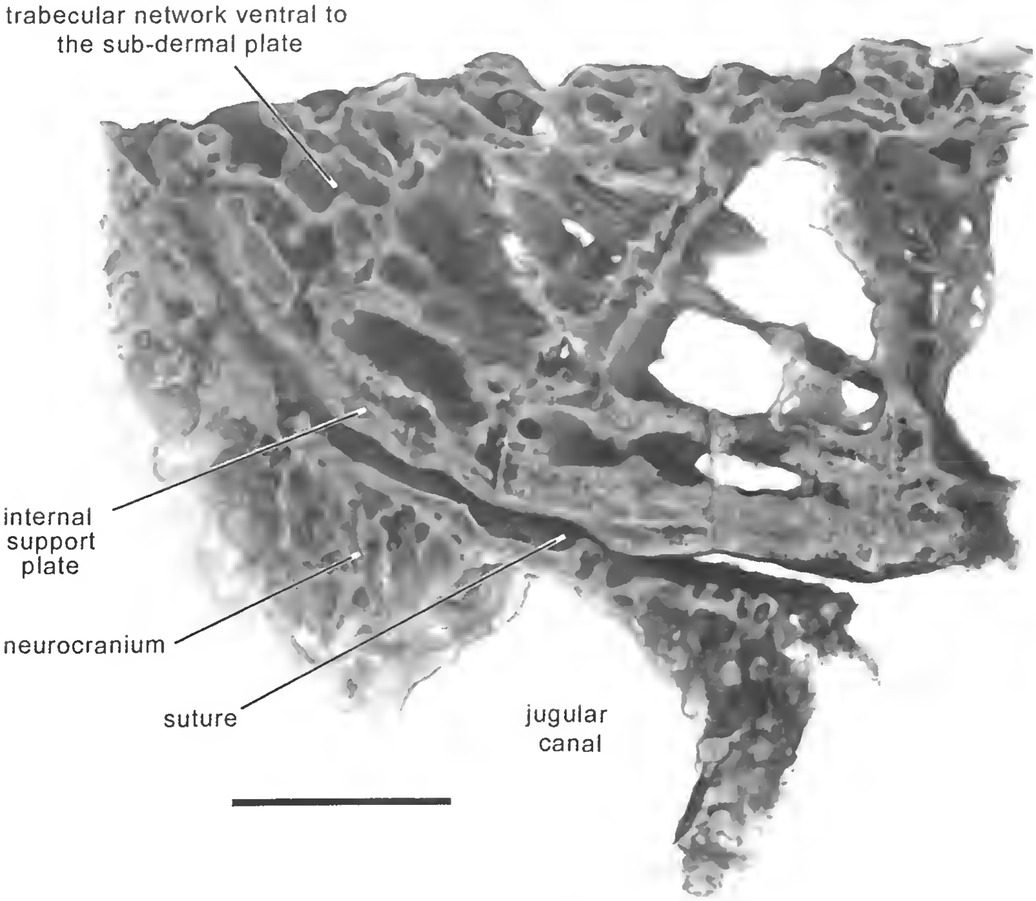

Figure 13. Tomographic image of a thick section cut across the dorsal side and across the roof of the jugular canal. The strong suture between the internal support plate and the neurocranial plate. The coarse tissue and a large gap dorsal to internal support plate. Scale = 10mm.

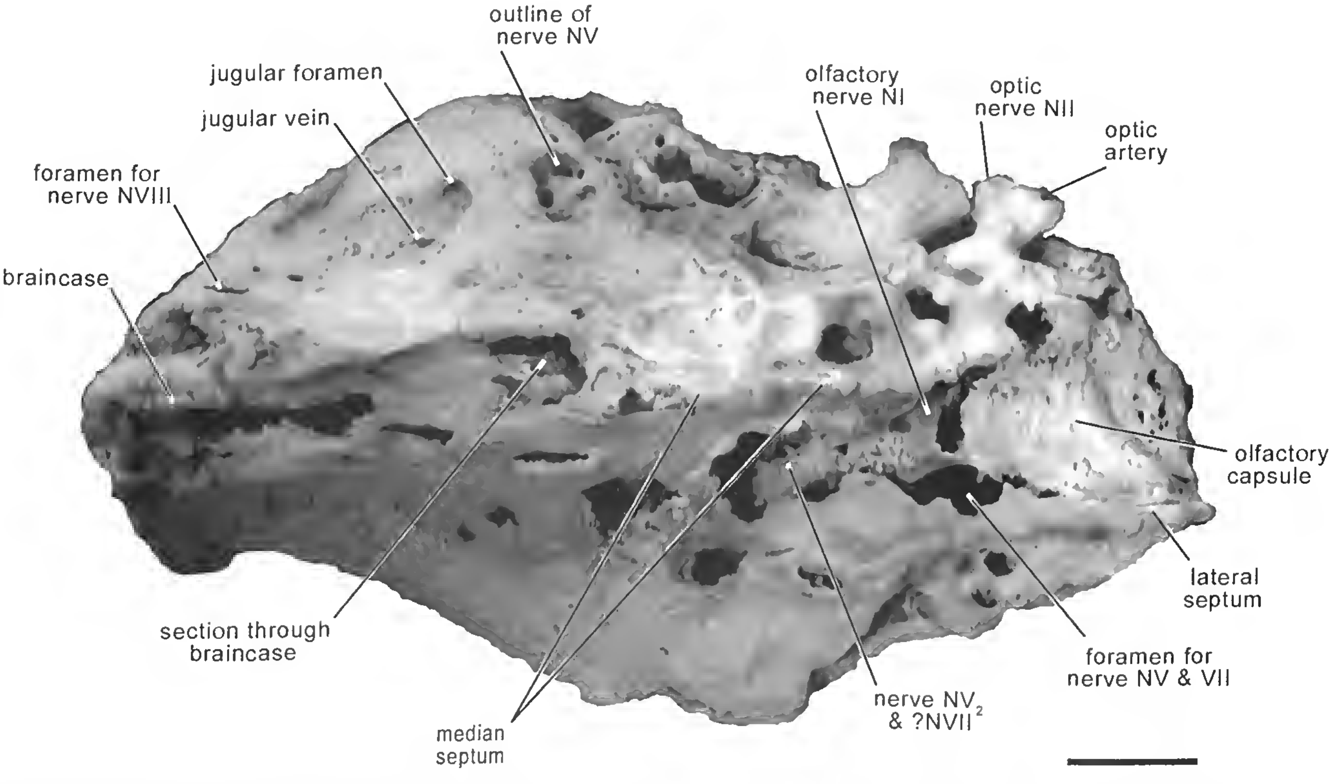

Figure 14. A. Ventral view of whole specimen. Entrance of olfactory nerve to olfactory capsule broken. Dark line across the nasal capsule indicates the anterior edge of the capsule, with the external nostril to the top right. Scale = 10mm.

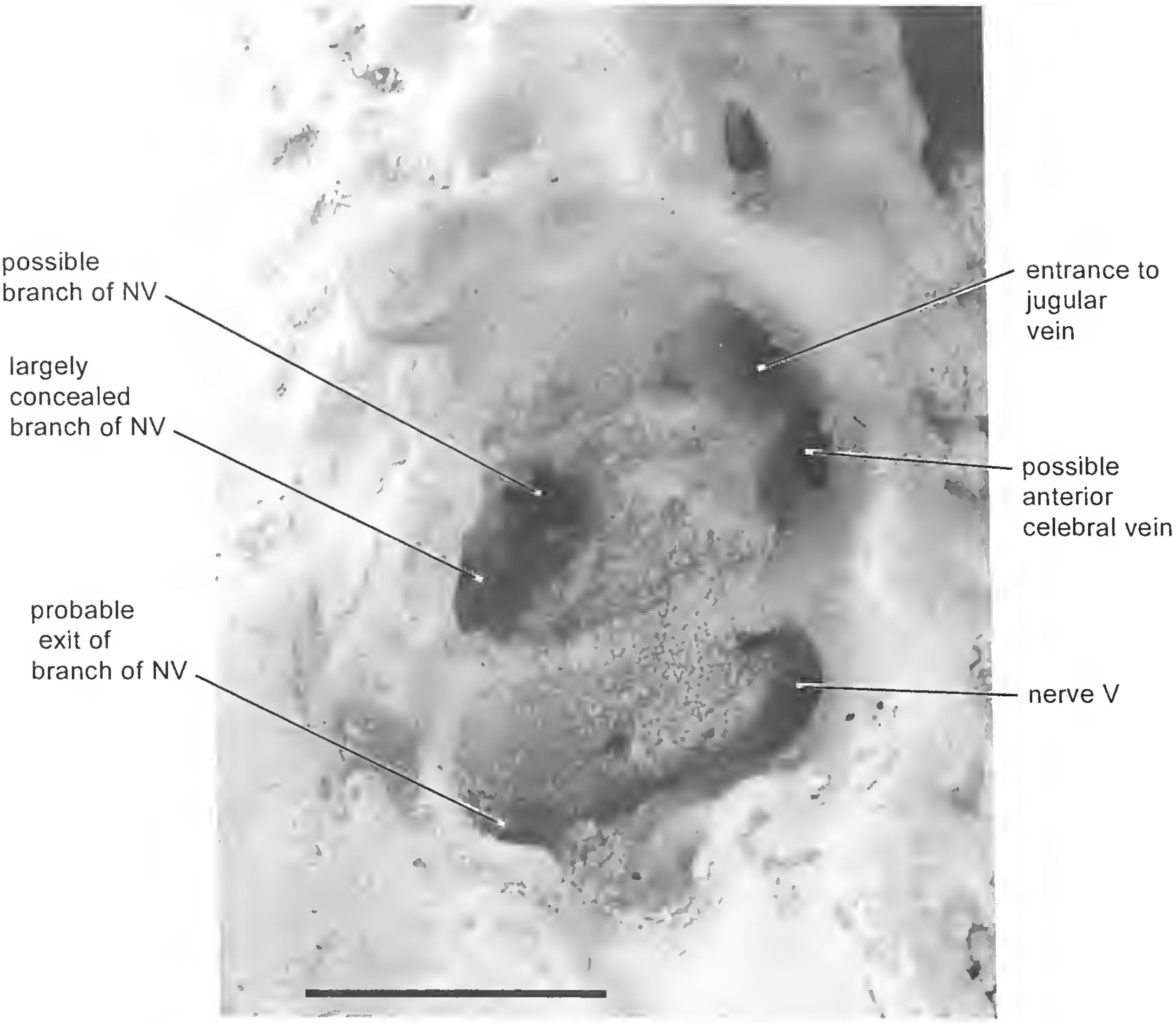

Figure 15. Diagrammatic enlargement of the gap around the space for nerve N V as shown on Fig. 16. The area is surrounded by a rim of bone, and the foramina are placed within this rim. Some of these are emphasised. The two foramina labelled nerve N V (the ventral one partly concealed by the surrounding rim) open into deep canals that run into the braincase. The dorsal of these two canals has a branch labelled possible branch of nerve N V, and it runs dorsally but its function remains unknown. Dorsally a foramen runs to the jugular area, and a lateral branch connected with this foramen is labelled cerebral vein. The bottom right has a foramen labelled nerve N and this may be a trigeminous branch. Scale = 10 mm.

system. Tomography of this area is not clear, but canals inside the jugular foramen open appropriately to permit the orbital artery to pass out in appropriate directions.

On the dorsal surface an eroded area is indicated on Figure 17. Sections indicate that the trabeculae within this space do not make a connected series, and we consider that this mass forms a supporting series of plates.

Anterior to the jugular foramen, the left side of the specimen has a single opening, but the right side has the wall slightly eroded and exposes an intricate structure. In the base of a largely rounded depressed space, is a complex arrangement of foramina for both nerves and veins (Figs 14, 15). On the posterior side there are two foramina each of which is surrounded by a slight ridge. Foramina occur posteriorly, and these run postero-ventrally towards the braincase. Its course as it approaches the braincase is shown on typographical sections (Figs 24A, B). We interpret these as carrying trigeminus nerve N V. Just dorsal to the top foramen for nerve N V is a second foramen that cannot be traced internally. It is labelled on Figure 15 as another branch of nerve N V. At the dorsal extremity of the large depression is a foramen that opens dorsally for a connection with the jugular vein. Just ventral to that foramen is a second foramen the runs anteriorly into the centro-lateral cartilaginous space. We interpret this as carrying a vein, possibly the anterior cerebral vein (Fig. 15). At the anterior edge of the main depression is another foramen that runs antero-ventrally into the broken area around the cartilaginous space. Posteriorly this foramen is by a furrow to the ventral foramen previously identified as for the nerve N V. Therefore we consider that a branch of trigeminous nerve N V passes into the centro-lateral cartilaginous space. Details of this structure are shown diagrammatically drawn from photographs (Fig. 15), but with some of the foramina emphasised because they were covered with the raised rim around the whole structure.

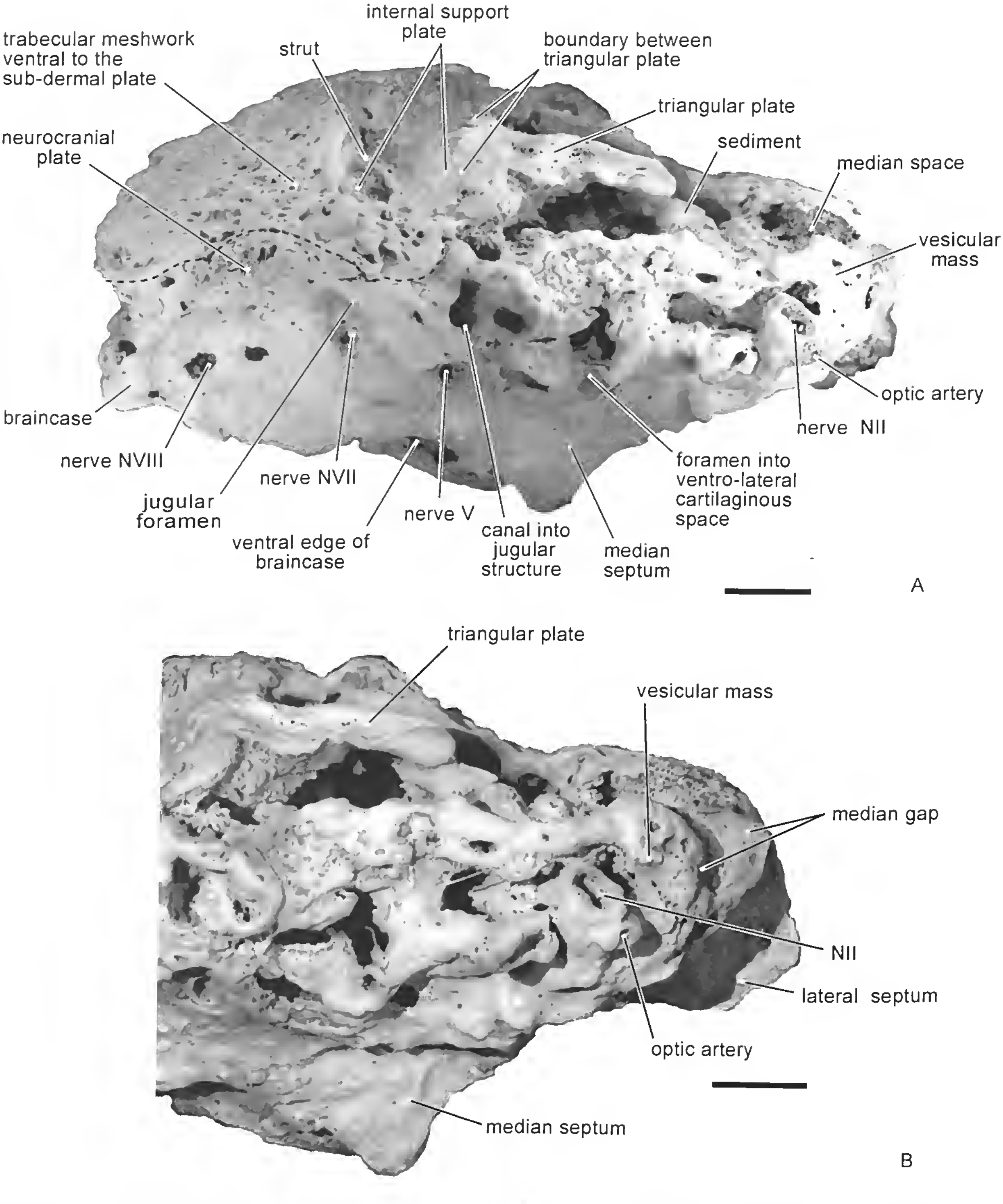

Figure 16. A. Right dorso-lateral view of whole specimen. Junction between the trabecular meshwork ventral to the sub-dermal plate and the neurocranium shown by the dotted line. B. Eroded anterior end of specimen. Note the photographic shading in the anterior part of the image tends to obscure the main structures. Scale = 10mm.

The Anterior and Median Neurocranial Walls: The left side of the specimen is better preserved than the eroded right side (Fig. 17). The anterior surface can be exposed in dorsal view, and the ventral surface has to be observed in lateral view. Both sides have been viewed by tomographic sections.

In the axis of the specimen, only part of the neurocranial wall has been preserved, but the relics show that the left and right neurocranial walls become confluent and form a solid median septum (Fig. 14; also tomographic on Figs 27–28). The median septum is cut away anteriorly in an arc. The septum is slightly deformed, and in this position it forms a small internasal cartilaginous septum. The posterior part of the median septum is eroded away, and the walls of the braincase are preserved near the posterior end of the specimen between the two sides of the neurocranial walls (Fig. 14).

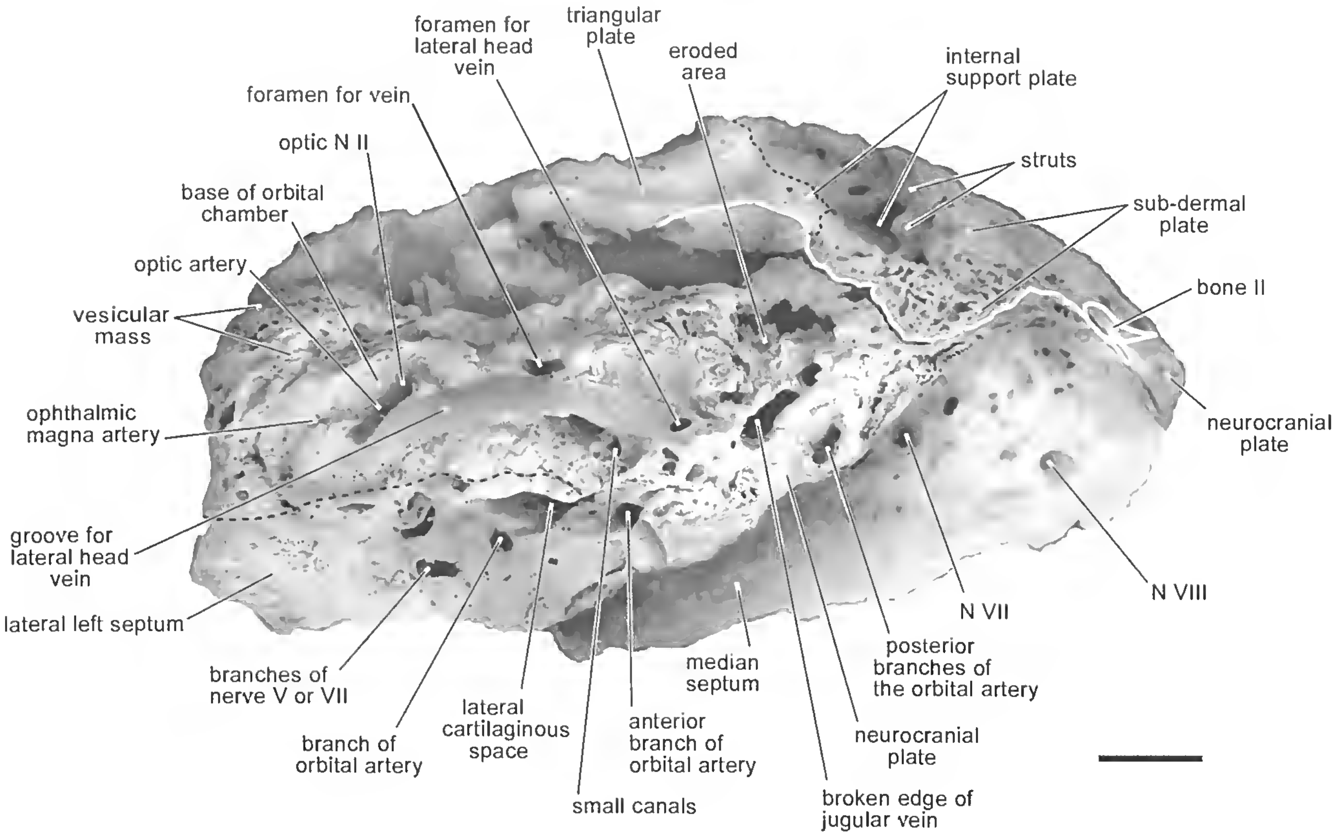

Figure 17. Slightly dorso-lateral, view of the left side of the specimen. (Anterior to the left.) The sharp boundary between the dorsal side and ventral side of the anterior section marked with a dotted line. Strong white line.marks boundary of dorsal plates. Bone II is just a small bone and its dorsal edge is concealed in the photograph. Scale = 10mm.

The preservation of the braincase is too poor to permit description.

Dorsal Anterior Walls of Neurocranium: The left side of the specimen is moderately well preserved. Entering the dorsal surface posteriorly is a large foramen (Figs 3, 14) that can be traced through an internal canal to the jugular foramen. Part of the eroded dorsal side of this canal can be seen on (Fig. 17). We regard this as carrying the jugular vein to the lateral head vein. As shown on Figure 26, the jugular vein runs into a foramen that opens on the exposed dorsal surface as for the Lateral Head Vein. This vein runs in a. groove that runs anteriorly leaving a clear passage for the vein that must have been covered with soft tissue in life. It is 3–4mm in diameter. The dominant feature on the dorsal surface is the groove for the lateral head vein and this runs to within 18mm of the edge of the specimen where it meets a slight ridge (Fig. 17).

Towards the end of the of the lateral head vein there is a deep cavity (Figs 17, 28A), within which there are two foramina. The posterior foramen is much larger and it runs poster-medially towards the braincase. Part of its internal course can be traced on Figure 28B, C. We interpret this as the optic nerve N II. The smaller anterior foramen carried the optic artery. We consider that the optic space occupied a large area that has been eroded. The base of the chamber still retains the groove for the ophthalmic magna artery (Figs 3, 17). Although the right side of the specimen was eroded, the position of the optic nerve N II is well preserved (Fig. 16). One difficulty with this interpretation is that the orbit is placed in a dorsal position, well forward from the nerve N V. The position of nerve N VI cannot be identified.

Another foramen on the median side of the groove for lateral head vein enters a canal that runs more or less ventrally into the median lateral cartilaginous space (Fig. 17). We consider that it probably contained a vein. Lateral to the foramen for the lateral head vein four small foramina that open into small canals (labelled small canals Fig. 17). These are connected with the foramina on the dorsal edge of the ventral side of the anterior surface as described in the next section of the text.

Ventral Side of the Anterior Surface: The ventral edge of the dorsal anterior face is sharp ridge, and is shown by a dotted line on Figures 17 and 18; and tomographically on Figure 27. Ventral to this ridge the surface drops into a slightly concave face. This is preserved on the left side of the specimen. The posterior part of this ventral surface is occupied by a large cavity that must have been filled with cartilage (Figs 18, 27B, C). The surrounding layer makes a complete tube (Fig. 18) that must have contained cartilage. We refer to this opening as the Lateral Cartilaginous Space, (and abbreviated to LCS). The median face of the cartilaginous surface would have continued anteriorly into a concave surface on the posterior part of the lateral septum. This concave space contains four grooves and these terminate in foramina that are the openings of canals in the interior structures (Fig. 18). The complex pattern of these grooves will now be considered in detail.

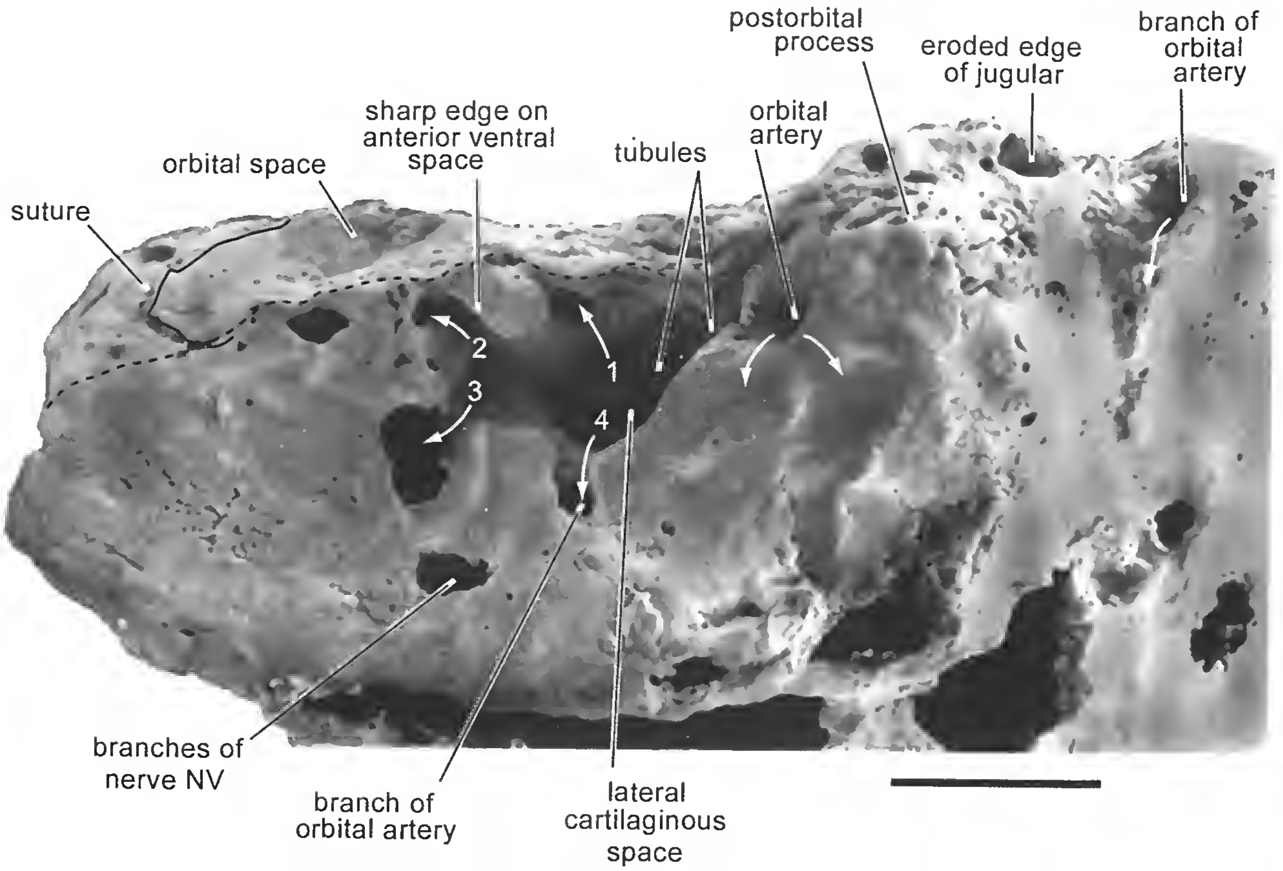

Figure 18. Ventro-lateral view of antero-lateral part of the specimen. Boundaries between dorsal and ventral faces shown by a dotted dark line. Details of connections between foramina and internal structures outlined in the text. Lateral extension of neurocranium is a rough rounded mass. Position of canals running from the anterior groove from the lateral cartilaginous space, are shown by white arrows numbered 1–4. Scale = 10mm.

The two dorsal grooves, labelled as 1 and 2 (Figs 18, 27C, 28A) bend postero-dorsally, and run in canals towards the lateral head vein. They connect with small foramina that open on the outer side of the lateral head vein. Dorsal foramen 2 also has a small canal that runs anteriorly, and it then passes into another foramen labelled as branches from dorsal foramen 2 on Figure 27C. This presumably acts as an exit of a vascular structure, but it also has a small anterior canal that runs internally but its course cannot be traced. The anterior foramen labelled 3 on Figures 18 and 27C issues into two main canals. The dorsal one is large and runs postero-dorsally towards foramen 1, but fracturing of the canal makes it impossible to trace its course. The ventral foramen in the same foramen opens into a canal that has been destroyed. The ventral branch 4 is very short, and although the canal leading from it is broken, a light shining down it shows that it merges into the side wall of the Lateral Cartilaginous Space (LCS). We consider that it carried a branch of the orbital artery (Figs 18, 27C).

The most ventral canal on the ventro-lateral surface opens through a foramen that internally connects with a canal that bends posteriorly sharply and runs posteriorly till it joins with the canal containing nerve N V_2. It contains the nerve ramus ophthalmicus superficialis, N VII, and its position is shown on Figure 23.

The Internal Structure of the Lateral Cartilaginous Space (LCS): The internal structure is lined with perichondral material that is poorly preserved. However enough remains to distinguish the following features. Postero-dorsally is a cavity that seems to open into the floor of the Centro-Lateral cartilaginous Space. It probably contains a branch of the jugular vein. Just ventral to this opening is a small rounded foramen joining a short canal running posteriorly. Its course could not be determined, but it is appropriately placed to carry a branch of the orbital artery. And finally there is another foramen described above as connecting with the canal 4 on the ventral face of the lateral septum. Summarily, we consider that the LCS carried extensions of the jugular vein and the orbital artery into the soft tissue occupying the lateral face of the head. Obviously, this soft tissue has been lost during preservation.

Centro-Lateral Cartilaginous Space (CLCS): This is partly preserved on the left side, but was eroded on the right side of the specimen. The perichondral surface is very poorly preserved, but Figure 19 shows the relics. The posterior wall is well preserved. It is entered by a foramen from the dorsal side (single black arrow in Figs 19A, B), but the canal is broken off prior to its entry. We consider that it must have been derived from the jugular vein that sends a canal to this region. Running ventrally from this foramen is a broad shallow groove that drops suddenly as shown by two posteriorly directed black arrows in Figures 19A, B. The lateral face forms a surface that rises to join the more dorsal skeleton, and more

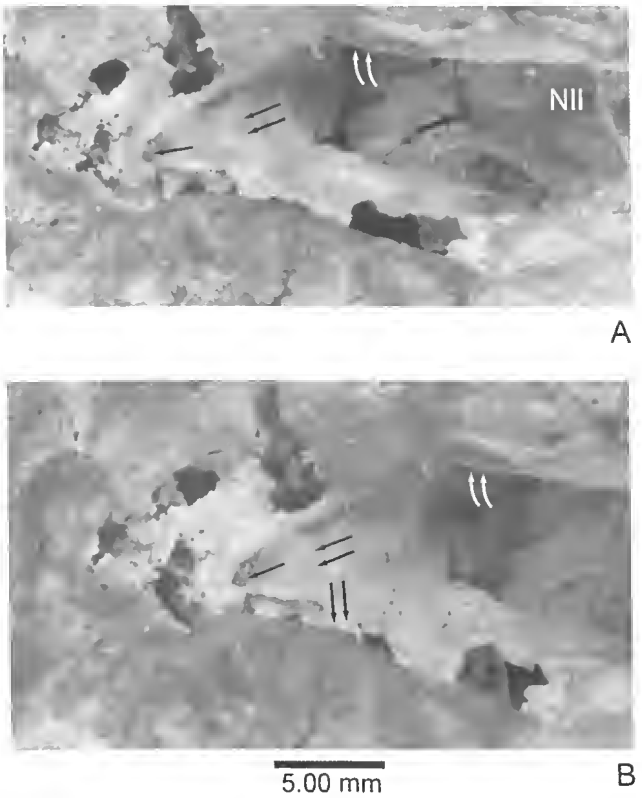

Figure 19. Two photographic views into the posterior cavity containing the centro-lateral cartilaginous space. Anterior of specimen to the right side. Much of the space is excluded by the lateral walls. A. Shows the posterior wall with the posterior foramina with a single black arrow, a furrow standing steeply with two black arrows indicating rapid descent to the broken up floor of the cavity. The deep lateral cavity running towards the lateral cartilaginous cavity is marled with two white arrows, and a single anterior black arrow runs into an anterior space. The approximate position of the canal carrying the nerve N II is marked. B. Anterior face tilted upwards, and the medial face is better exposed. Posterior face more clearly with the foramen with a single black arrow. A pouch running medially with two white arrows as on A. The two arrows medially directed mark a foramen. Some more details are mentioned in the text, but they could not be photographically illustrated because of the cover of the external bone. Scale = 5.00mm.

ventrally it turns laterally to form a pouch that runs into the Lateral Cartilaginous Space, shown by two white arrows in Figures 19A, B. Anterior to that pouch are some foramina whose function is not known.

On the median face of the groove beneath the triangular median dorsal plate, the fragment of the wall stands vertically, but it is broken off. Presumably it reached the edge of the triangular plate. It has a foramen that runs medially, but only the basal part remains (shown by two laterally directed black arrows in Figs 19A, B).

The ventral part of the CLCS is very complex, and it is partly destroyed as is shown on the Figure 19A. Near the point marked, the nerve N II enters the system and the passes to the braincase. The floor of this cavity runs ventrally beneath a transverse optic nerve N II, but its outline cannot be traced.

We conclude that the CLCS was a region within which the jugular veins diversified to serve the anterior parts of the specimens, and across which the optic nerve N II passes. Details of the distributions of the various elements require the discovery of better specimens.

The Palate

The specimen has no organised palate preserved. Several fragments of what may have been the broken up fragments of the palate were loose in the etched surface in tadly concave, and it is transversely finely ridged. Antero-medially a break continues across the roof and the unridged surface is more inclined dorsally (Figs 14, 21). Part of this surface has perforations similar to those in the overlying bone. Lateral to that the roof of the capsule reaches the broken edge of the specimen. We interpret that this surface approached the external nostril. The lateral edge of the capsule is bordered by the lateral position near the anterior end of the median septum. Each item consists of a small structure 2–3 mm across. The buccal surface of each unit has a number of irregular depressions on which there was no pattern of radial lines. The surface also has a number of small pits, and where they are worn away fine grooves are present running oblique to the surface (Fig. 20B). SEMs of the longitudinal surfaces show that the body of the structure is composed of parallel canals oriented in different directions (Fig. 20A). The mechanism by which these were deposited remains unknown.

Similar blocks of material have been described from *Cheirolepis trailli* (Pearson & Westoll 1979, Fig. 8), and each block has a radiation centre. Such centres are not present on our material. Unfortunately the internal structure of the *C. trailli* has not been described.

Exposed Anterior Face of the Specimen: The anterior end of the specimen was eroded and none of the rostral region is preserved. In dorsal and anterior views, the left side the residual material is well preserved, and it is made of vesicular bone separating multiple spaces. This is well shown in anterior drawing in Figure 21; and also on thick tomography sections in Figures 27A, B. A thick section is imaged by tomography (Figs 22A, B). These sections also show some sedimentary material that was not etched, and it is shown up on the image coloured in transparent green. Branching canals are present on both sides of the specimen, but they are not generally interconnected. There is no evidence that the canals carried nerve tissue such as those found in other osteichthyans where nerves N V and N VII are well known. On the exposed anterior of the eroded surface they appear as a number of ovate spaces that are separated by strong walls. This also supports the view that the tissue is not nerve carrying.

These structures are in contact with the dorsal side of the olfactory capsule. They could be considered as a support structure for the large olfactory capsule, but the size and complexity seems excessive for such a function. They may support the rostral region of the organism. The outer edge of this vesicular mass on the anatomical left side is marked by a suture that runs antero-laterally and separates the mass from the tissue that contains the optic nerve (Figs 3, 18). Details of the bone structure are illustrated on the tomographic sections.

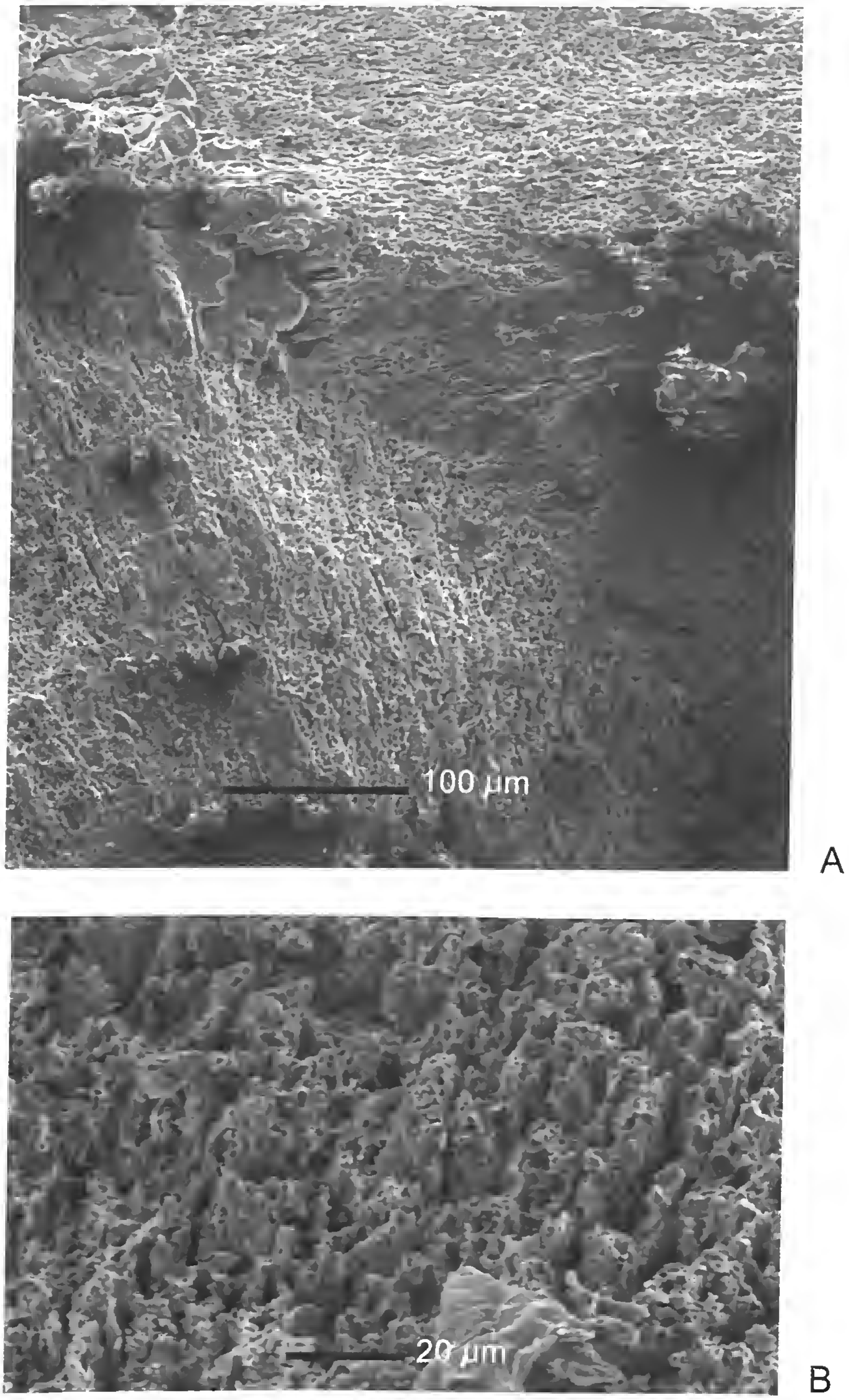

Figure 20. Scanning electron microscope image of a fragment of what is interpreted as a piece of the broken up palate. (Details see text). A. Shows a lateral view with the surface in top left hand corner, and the linear arrangement of the tissue in different orientation. B. Dorsal view of the surface with the fine pores where the tubules shown in Fig. A meet the surface. Scales as shown.

Olfactory Capsule: A coloured image of the olfactory capsule supported by the trabecular bone is shown on Figures 27A and B, and also the structure of the lateral septum. Although the preserved surface is larger than one would expect, the following points indicate why we have interpreted this structure in this way: (a) the slightly displaced olfactory nerve N I appears posteriorly in our interpretation along with a branch of nerve NV_2; (b) the position and branching of the perichondrial canals for NV_2 and N VII posterior to the surface, is what one would expect posterior to the olfactory capsule; and (c) the position of the braincase as determined from the orbital nerve N II, is appropriate.

The roof is concave, and it is transversely finely ridged. Antero-medially a break continues across the roof and the unridged surface is more inclined dorsally (Figs 14, 21). Part of this surface has perforations similar to those in the overlying bone. Lateral to that the roof of

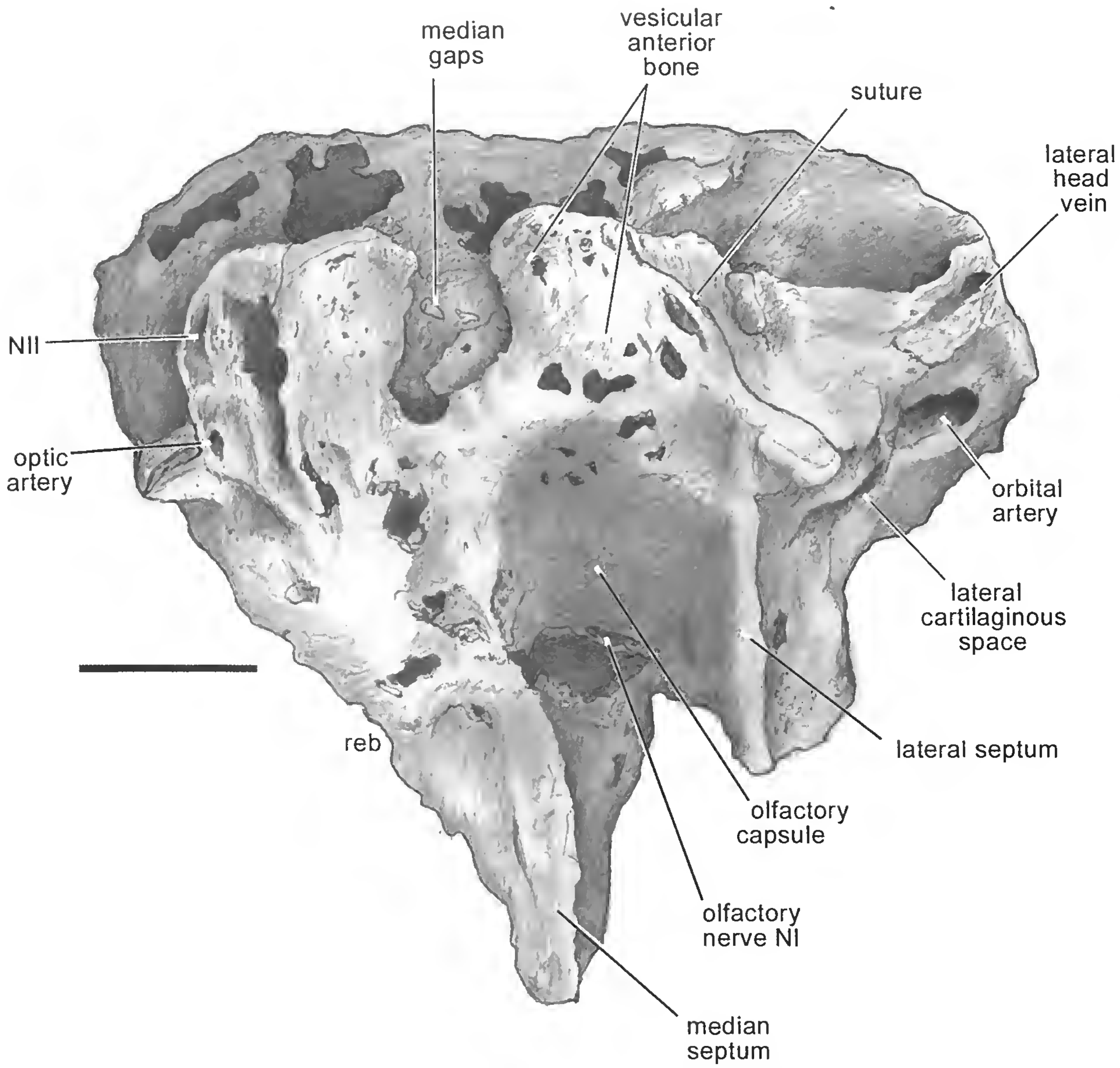

Figure 21. Drawing of the anterior view of whole specimen showing the highly eroded left side and the less eroded right side. Erosion has removed the right olfactory capsule. Anterior view of the left olfactory capsule with the end of the olfactory nerve at its posterior. Anterior end of the nasal capsule slightly eroded exposing the vesicular mass. Median septum is well posterior of the anterior edge of the specimen. Lateral cartilaginous space is compressed in this image. Compare with Figure 27 A, B. Scale = 10mm.

the capsule reaches the broken edge of the specimen. We interpret that this surface approached the external nostril. The lateral edge of the capsule is bordered by the lateral septum, and medially there is the relic of cartilaginous internasal ridge.

The position of the olfactory nerve N I is critical for this interpretation. The tubular skeleton posterior to the nasal cavity is broken away, and to save the structures their edge is attached by glue to the back of the capsule. The tubules are very delicate and slight movement will cause them to be disrupted. A small amount of distortion has occurred, and in the following we have allowed for this. A large canal is preserved entering the olfactory chamber from a postero-dorsal (probably a posterior direction before distortion Figs 14, 23). This canal runs posteriorly for about 1 cm runs toward the braincase, and although partly broken off, its extension is visible. We interpret this as carrying the olfactory nerve N I (Figs 14, 23).

Adjacent to the olfactory canal is a somewhat narrower perichondral canal, and it runs postero-dorsally for *ca*.1.5 cm from its separation from the canal carrying the olfactory nerve. Clearly it enters into the olfactory capsule, a feature of the nerve maxillaris N V_2.

Posteriorly this N V_2 branch joins a second branch that runs dorso-laterally to a point just posterior to the olfactory capsule. There it turns sharply laterally and opens through the wall of the lateral septum. This nerve must have served the antero-lateral parts of the rostral region, and we interpret it as carrying a branch of the ophthalmicus superficialis N VII. At the very posterior end of the system where these canals join they make a single unit that is broken across. The junction with other structures is lost. We assume that it runs anteriorly from both the facialis and the profundus nerves as sometimes occurs in holodipterans.

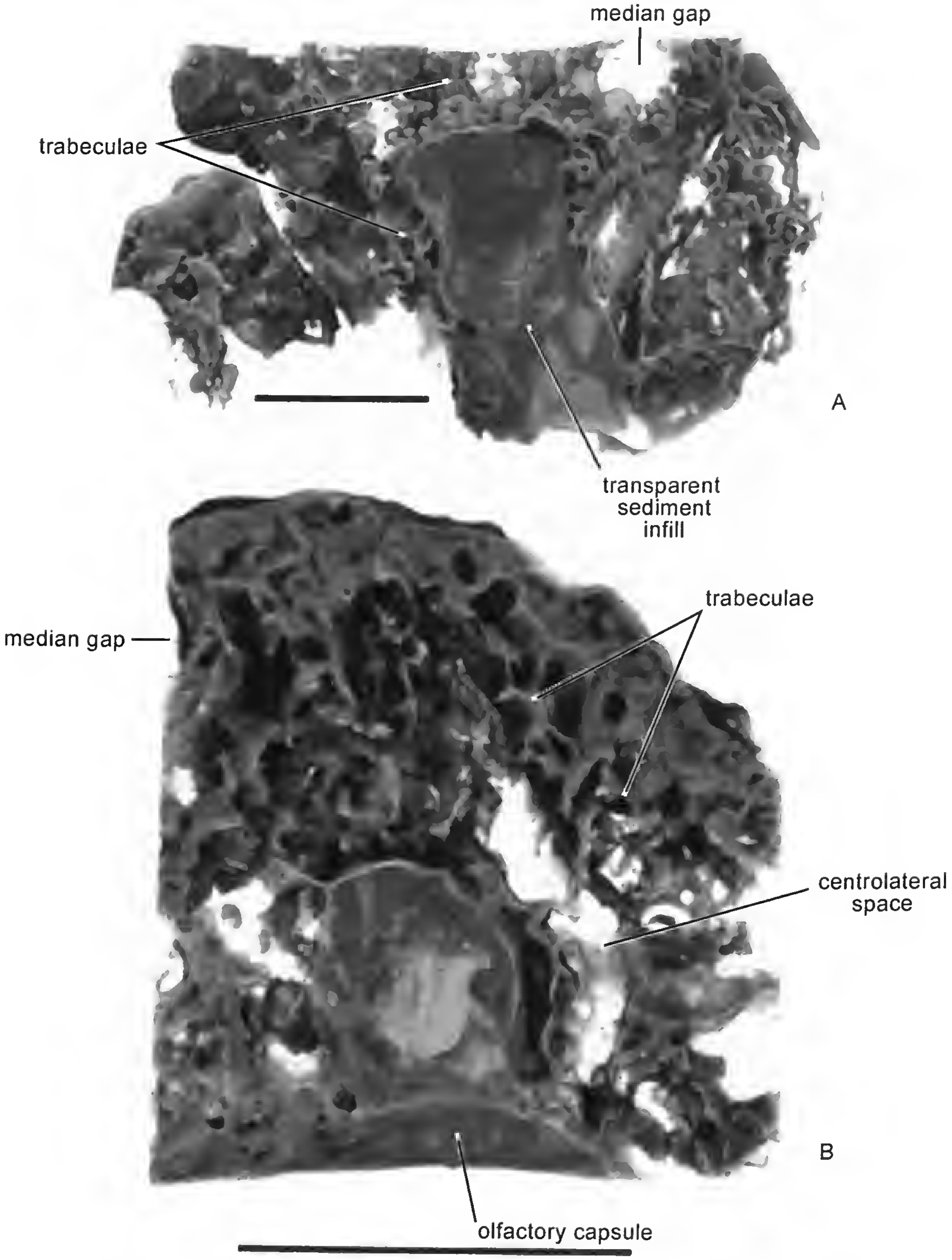

Figure 22. Tomographic sections through the anterior part of the specimen. Sediment remains transparently coloured in the axial regions, and their boundaries shown by hard lines. A. The right side of the image has irregular patterns of trabeculae without tubules. B. Right anterior transverse tomographic section through the morphological right side of the specimen, crest of the olfactory capsule ventrally, and median gap laterally. Trabeculae do not show signs of formation of canals. Large gap in trabeculae on right side of image are openings into the centro-lateral cartilaginous space. Scale = 10mm.

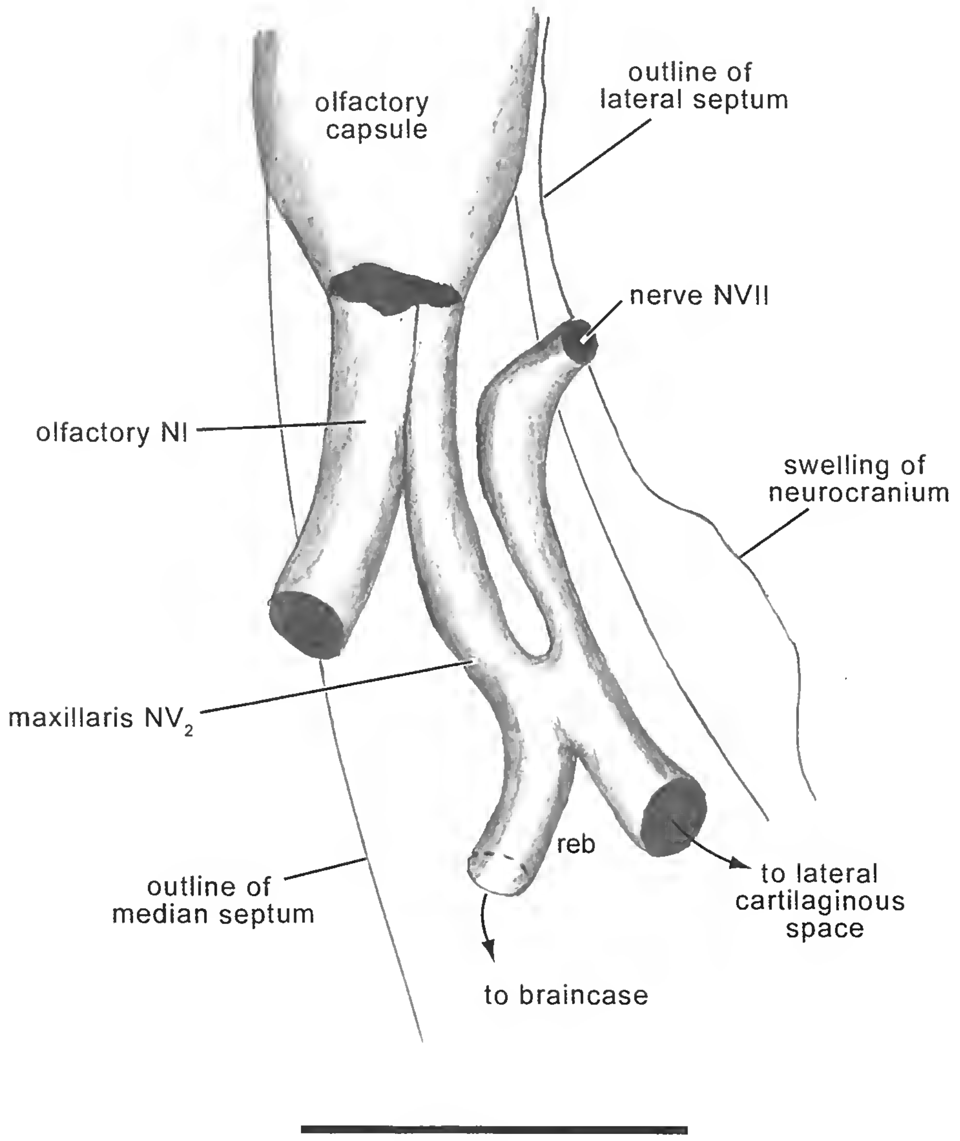

Figure 23. Reconstruction of the pattern of the nervous system posterior to the olfactory capsule. The system is very delicate and has been slightly moved from its original position. Interpretation is given in the text. Scale = 10mm.

Relationships

The relationships of this organism can be tested against a number of critical points. For this reason we consider the each major group as a separate item.

The total format, including the absence of a platybasic skull, and the position of the orbits, and the structure of the jugular drainage system, all indicate that this specimen is not an Agnathan (Wang 1991; Forey & Janvier 1994; Janvier 1996).

Among the gnathostomes, it has strong bony structures not found in chondrichthyans. The remains of the skull roof with the elongate sub-dermal plate may be suggestive of a placoderm arrangement, and the arrangement of the nerve N VIII and the lack of a labyrinth cavity attached to the margins of the braincase, and the anterodorsal position of the orbits are suggestive of a placoderm relationship. (Young 1979, 1986). However the absence of an internal plate beneath the sub-dermal bones, and the arrangement of the endocranium suggest that the similarities are the result of convergence.

The suggestion that it may be related to the acanthodians seems to be contradicted by the size and strength of the undivided dorsal sub-dermal plate and its extension to the midline, the presence of an internal support plate, the length and strength of the median septum, the size and position of the jugular veins and the lateral head vein and the structure and position of the supraorbital plates. It has been suggested that the scales of acanthodians have an epidermally deposited sequence of layers and no dentine internally. This similarity is due to convergence. Consequently we are left with the Osteichthyians.

The fine surface structure of the supraorbital bones are unlike any actinopterygians from the Middle to

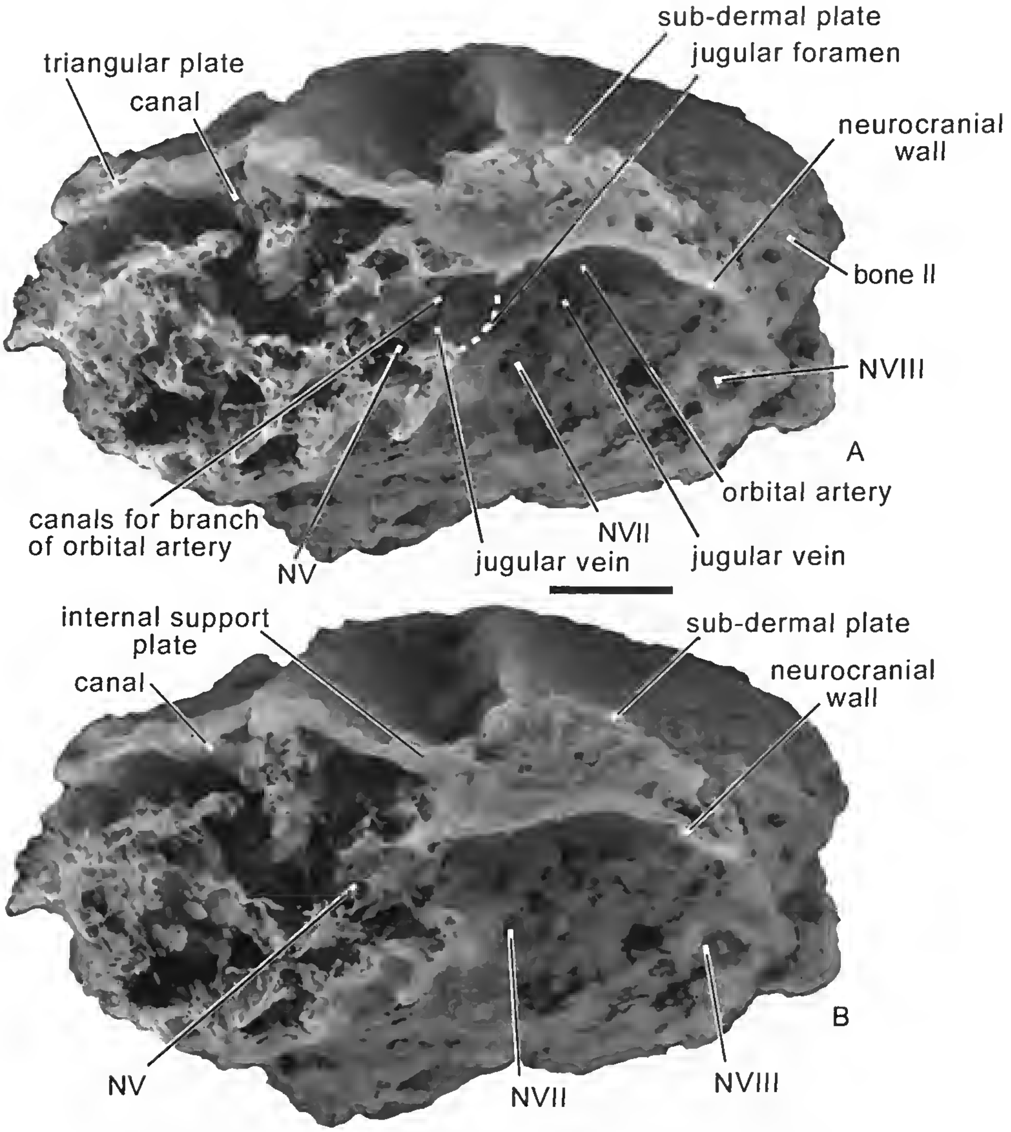

Figure 24. A. B. Two thick tomographic images of sections from the posterior part of the specimen. A. Is the more lateral section, with the opening for the jugular foramen outlined in a white dotted line. Images of nerves N VII and NV as they pass towards the braincase. The sub-dermal plate varies in thickness because of weathering. Sub-dermal plate coloured blue; internal support plate green; neurocranial wall red. Space between the sub-dermal and internal support plate filled with trabecular meshwork. B. More median slice with the positions of nerves N VIII, N VII and N V with clear. Scale = 10mm

Late Palaeozoic (Pearson & Westoll 1979; Reif 1982 p.314; Gardiner 1984; Basden & Young 2001; Long *et al.* 2008). Our specimen has distinctive bone structure, and surface rounded bosses that have no ganoin, and they are composed of sheets of material that have been deposited **not** (our emphasis) from dentine-like structures, but from the epidermis. None of these features is found in actinopterygians. Other significant features include the large median septum formed from the neurocranial wall; the large lateral septum connecting with the neurocranial wall and running laterally to the nasal capsule; the sub-dermal plate ventral to the dermal roof and also forming the outline on which the dorsal dermal plates were formed; and the large cartilaginous spaces in the median and lateral positions. In summary it appears that no unambiguous characters indicate an actinopterygian affinity.

So what of the sarcopterygians? The solid neurocranial walls are similar to some sarcopterygian patterns. The absence of an intracranial joint indicates that it is not a member of the Osteolepiforms, Rhizodontiforms, Actinistia, Panderichthyida or the Onychodontiforms, but such a feature is found in the Dipnoi. Although no tooth plates are present in our specimen, the structure of the median septum, the lateral septum, the large nasal capsule, the arrangement of the jugulars, the orientation of the remaining dermal bones, and the large cartilaginous spaces, indicate that it is not a dipnoan. The same may be said of the complex of bone ventral to the posterior sub-dermal bone and the structure of the internal support plate. The bosses on supraorbital bones and its surrounding bones are distinctive, and they are unlike the cosmine structure of the Devonian dipnoans. Clearly our organism is not a member of a sarcopterygian

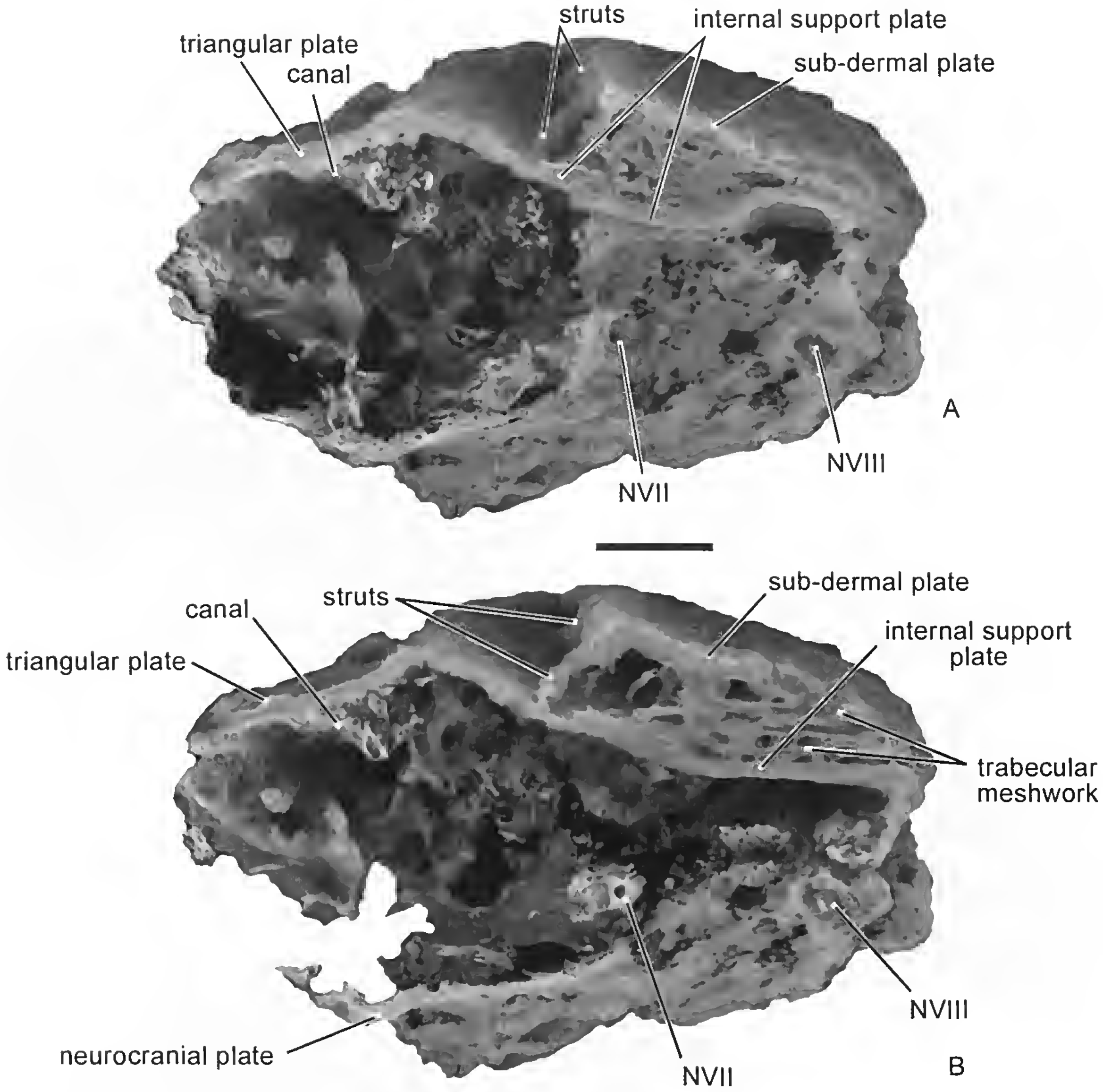

Figure 25. A. B. Two tomographic images cutting more deeply toward the braincase. and completing the series shown in Figure 24. Posterior to the struts the trabecular meshwork decreases in size indicating that the trabecular meshwork is strongest laterally. A. The section passes through the jugular foramen, and the nerve N VIII observed. posteriorly. B. The section is more median than in section A. The trabecular meshwork ventral to the sub-dermal plate is weaker medially in B than in A, indicating that the meshwork is stronger laterally. The presence of nerve N VII, and the canal from the triangular plate opening into a canal, are well outlined. Scale as in Fig. 24.

group as the subdivisions of that group are at present understood.

Despite this conclusion, we consider that the specimen indicates a new design of organisms, and should be placed ultimately in a new higher taxonomic status. In the absence of the dermal pattern of bones, the absence of a palate, and the lack of a mandible, it is impossible to establish such a higher taxon. This will await the discovery of more specimens. What then is the point of describing such an incomplete specimen as some reviewers have suggested?

Significance of discovery

Earth scientists naturally turn to environmental factors in attempting to understand the bases of major biological disparity and diversity. These usually consider such points as the abundance of oxygen in both the atmosphere and the oceans, the appearance of glaciation, unsettling of the carbon cycle, or even movement of continental masses and the evolution of continental shelves. Although any biological change has to be accepted by the environment to be successful, the origin of the changes must depend on the possibility of major changes in the genome. Such changes must affect the genomic stability, and produce the possibility of several new designs that may have short or long term possibilities that restore stability. In what follows in this text we examine some of these possibilities

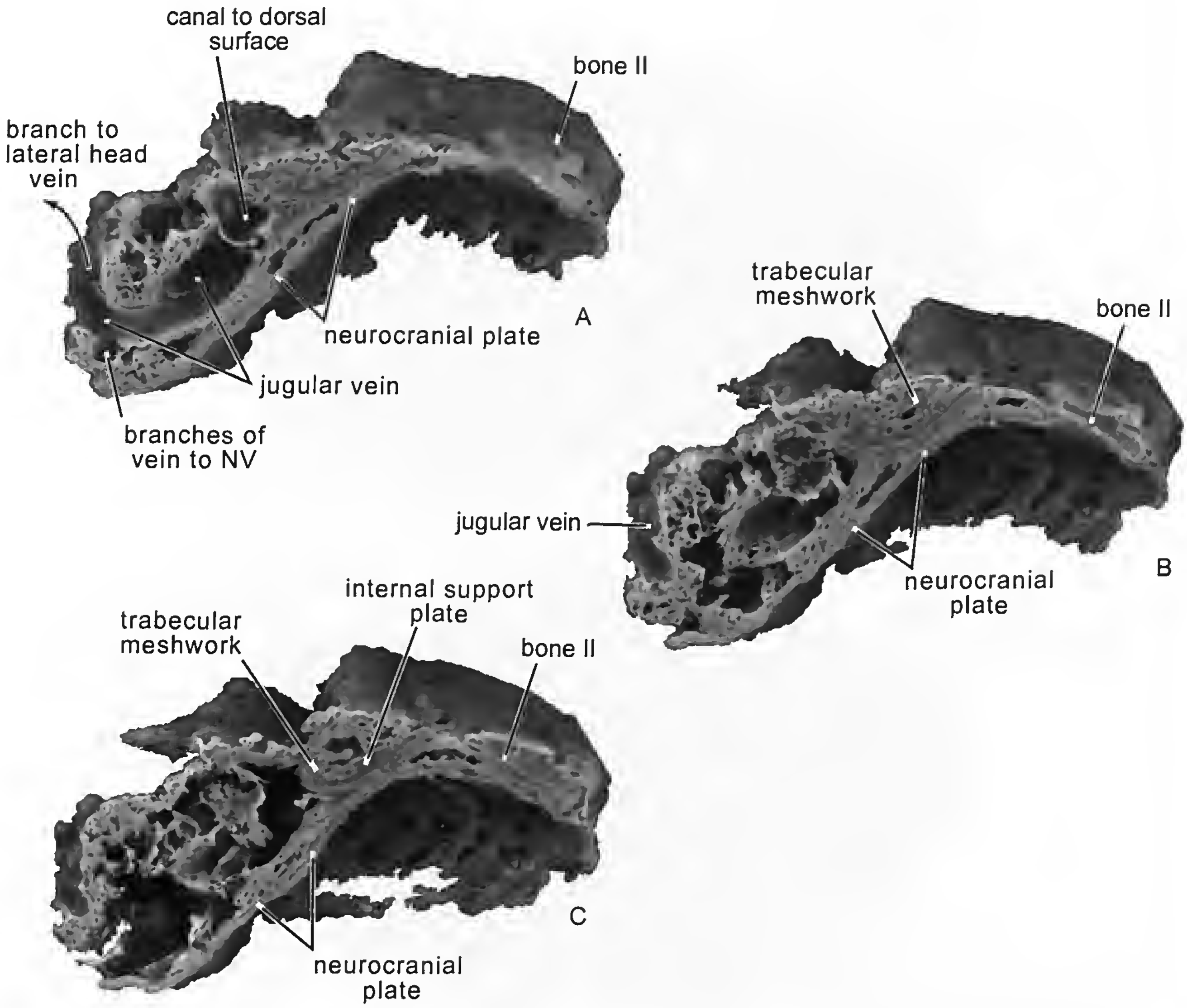

Figure 26. A. B & C. Three slightly oblique thick tomographic sections cut through the marginal part of the specimen, lateral to the jugular foramen and posteriorly through bone II. A. Has the anterior part of the jugular vein with its anterior end giving rise to a canal leading to the foramen for the lateral head vein. B and C. Two more sections cut more medially. The deeper the section the larger amount of trabecular meshwork displayed. Scale = 10mm

Most workers now accept that major changes in phenotypic patterns must take place by some process other than simple Darwinian speciation. One major process is the genomic regulatory systems, and this has been recently summarised by a geneticist Davidson (2001), in a major book on this subject. On page 13 of this work he refers to changes in patterns "to the developmental process by which special domains of unspecified cells are assigned 'regulatory states', thus ultimately creating fields of cells that will give rise to the diverse parts of a structure, an organ, an organism". A general review of the current situation is given by the geneticist Jones (2010) in an article that deals with biodiversity, natural selection and random change. We also point to the work of Carroll (2000) and Carroll *et al.* (2005).

Campbell & Barwick (2006) have discussed this point with the sarcopterygian group, Onychodontiformes, which evolved early in the Devonian and were extinct by the end of that same Period. The ancestors of this group, like those of the dipnoans, remain unknown. For discussion of the lack of ancestral groups see (Valentine 2006; Marshall & Valentine 2010). Further the importance of difficulties in identifying homologous characters in setting up cladistic analyses in attempting to understand the processes of deep evolution been discussed by Telford & Budd (2003).

Our attention to this process was first stimulated by the work of Miklos (1993) who attempted to understand what was happening with the first arrival of dipnoans in the Early Devonian. Despite extensive searching no dipnoans have been found in pre-Devonian rocks. In his first article on this subject, he sets out his main point in a summary where he commented that "The origin of body plans is the essence of metazoan evolution, **not** (our emphasis) the origin of species." Among many

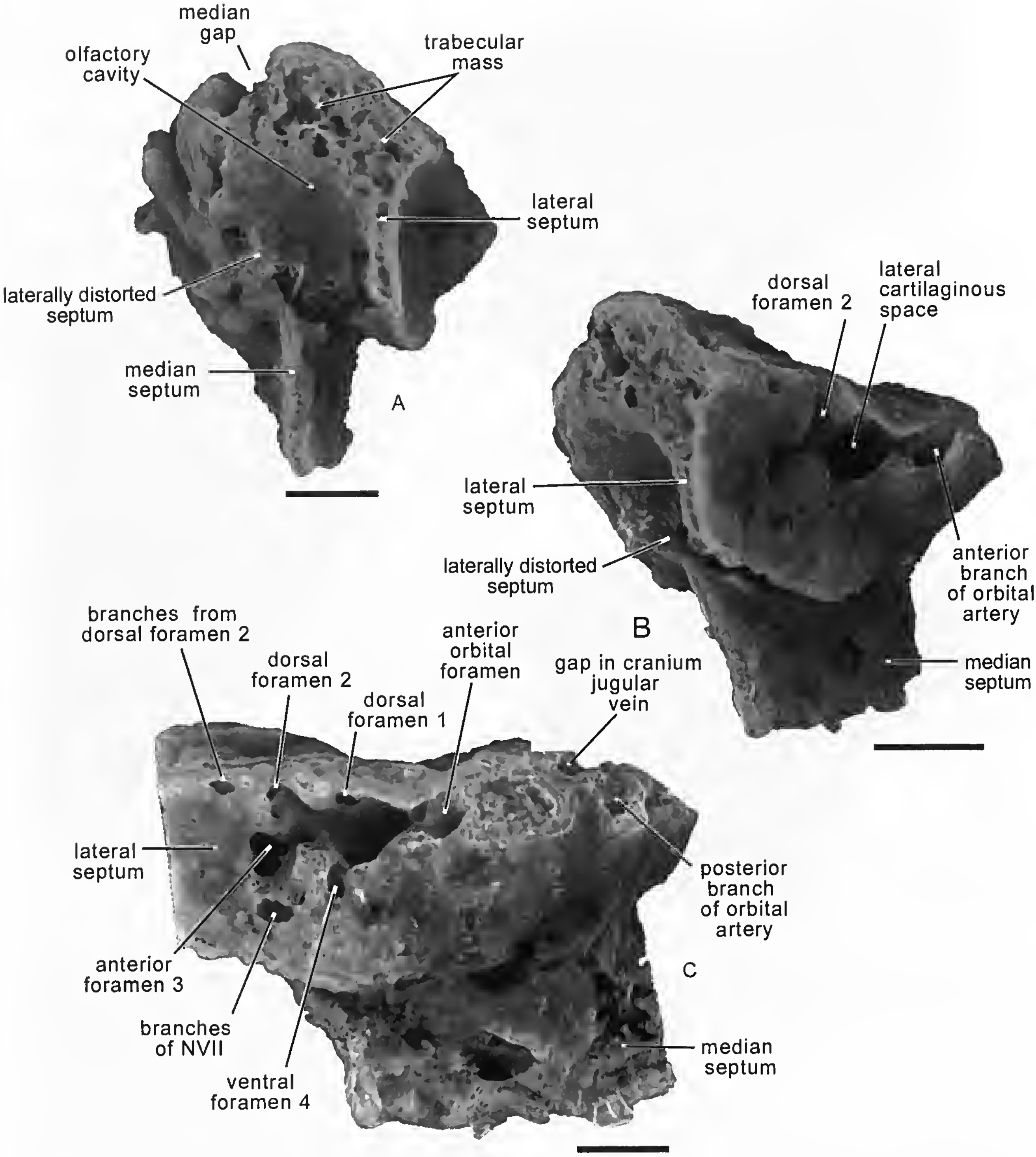

Figure 27. A–C Series of thick tomographic combination of sections. Sections through the neurocranial bones shown coloured red on all three figures. Ossified end of median septum situated posterior to the rest of the exposure. (Compare with Fig. 18). A. Is a section that cuts through the two septa. B. Sections from a more oblique view. The size of the lateral cartilaginous space is well shown, and it connects posteriorly into the anterior branch of the orbital artery. C. An even more oblique view including structures of the postorbital process with the posterior branch of the orbital artery as well as the ventral surface. (Compare with Fig. 18). Scale = 10mm.

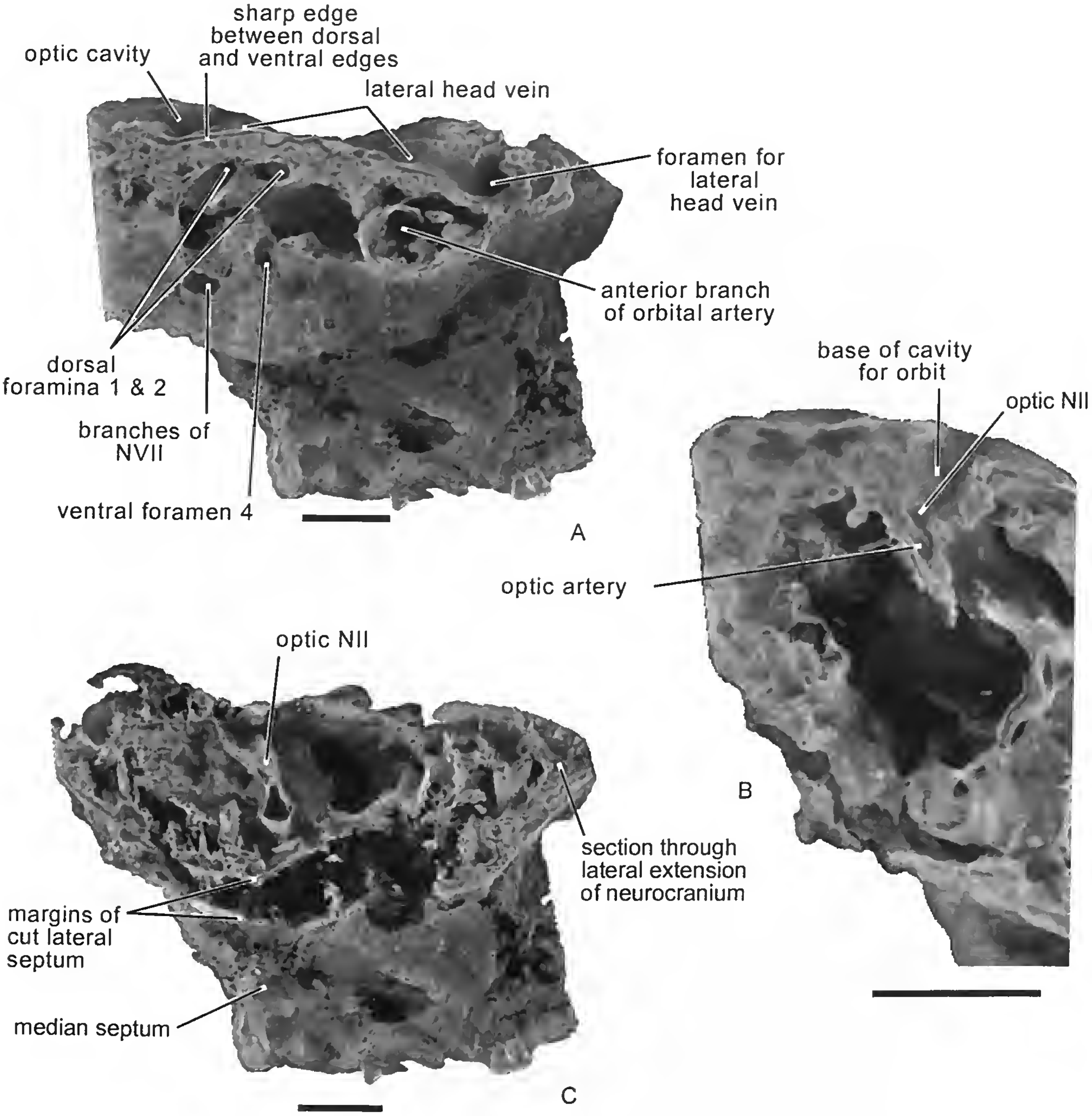

Figure 28. A. Thick tomographic combination of sections oriented to show the marked by edge of the anterior ventral surface. The edge of neurocranium coloured red. The position of the lateral head vein largely exposed dorsally along with the part of the optic cavity. Dorsal foramina 2 connected with the anterior foramen. (Compare with Fig. 18). B. An enlarged section through the base of the optic cavity with the canal for the optic artery and the base of the optic nerve N II. C. A much deeper oblique section that cuts through the lateral septum and shows the course of the optic nerve N II. Scales A–C = 10mm.

other significant comments he concluded that (p. 15) "little emphasis has been placed by evolutionists on the changes to the properties of regulatory elements in prokaryotes and eukaryotes, although there are some very thoughtful evaluations of this topic, including the enormous implications for development constraints ... it has not yet been modified to take into consideration the morphological changes that occur via the non-genic regulatory control regions in the genome." New genotypes will develop the possibility of new phenotypic patterns. A popular account of such phenotypic changes has recently been published by geneticist-ecologist, Bennett (2010), in which he refers to the chaotic course of evolution, and he points out that the non-linear pattern of macroevolution is vital. The use of cladistics in tracing macro-evolutionary patterns is really asking the wrong question.

The possibility of major changes in the phenotype is now being explored from several points of view. Dover (1992) commented that "*De novo* combinations can spread under their own steam as a consequence of the instability of the genome". Among many other points Carroll *et al.* (2005, p.185) comments that ".... evolution of new regulatory lineages --- between signalling pathways and target genes, transcriptional regulators and structural genes, and so on --- has created new regulatory circuits that have shaped the development of myriad functionally important structures. These regulatory circuits also serve as the foundation of further diversification." Taft *et al.* (2007, p 297) in their discussion of the relationship between non-protein coding DNA and eukaryotic complexity, reached the conclusion that "non-protein-coding sequences that scale consistently with the developmental complexity, indicates that in addition to important innovations in proteins involved in developmental regulation and cell signalling, most of which were in place at the base of metazoan radiation, the expansion of the cis-and trans-acting regulatory architecture has been a critical factor at the evolution of the more developmentally complex organisms".

But it is not only regulatory changes in the DNA that are significant. Work on the RNA summarised by Dinger *et al.* (2008) has concluded that changes in RNA can produce changes in their function and the separation into protein-coding and non protein-coding RNA, and these are not readily distinguished. They also comment that "the very existence of such bifunctional RNAs challenges the assumption that the RNA world can be parsed between mutually exclusive protein-coding and non-coding categories." They end their conclusions with the statement that it is likely that "RNA is a molecular multi-tasker, whose roles can simultaneously bridge both the protein-coding and non-coding domains, and not only have more than one embedded function but also produce multiple products".

Along another line of approach, work by Stephen *et al.* (2008) on the highly conserved non-coding sequences, many of which are regulatory, appear with the development of tetrapods in the Late Devonian, our stratigraphic period of concern. The same article also concludes that the process of exaptation (Gould & Vbra 1982) is important in understanding the production of new genomic structures that do not produce immediate changes in the morphologic designs. Thus a genetic change may take place and be retained in the genotype but its phenotypic expression may take place later depending on the arrival of suitable condition for its expression (Marshall 1995; Shubin & Marshall 2000). If the conclusion of Taft *et al.* (2007) that most of the greatest major regulatory changes (for example the beginning of Phyla) were present at the beginning of metazoan radiation, one would expect that subsequent more minor changes would have been most abundant during the early and middle Palaeozoic. By that time most of the earlier changes would have established themselves, and subsequent changes would have expanded the organisms into the gaps after the older morphological changes at the phylum level took place.

This brings us to the point in the Early Devonian where major changes in the phenotypes of vertebrate phenotypes occurred. Not all the changes took place over a short geological time, and many of them left few descendants. But instability in the genomes did produce an explosion in sarcopterygians, where each of these groups produced a multiplicity of new types. For example, the dipnoans that first appeared in the Early Devonian, produced at least 30 new genera in the Devonian Period, and these included several new types of feeding mechanisms. In the later Palaeozoic many of these new types of feeding were lost, and the number of new designs was reduced, and with only one or two exceptions they used one type of grinding tooth plates inherited from the previous period. We consider that *Cainocara* represents perhaps a later example of the instability of the genome that produced the other osteolepiforms earlier in the Devonian.

The dating of the Gogo Formation is relatively secure. In terms of the conodont zones it is considered to be early Frasnian, well before the Frasnian/Famennian extinction. From our point of view the discovery of this new genus is an example of a distinctive phenotypic form that apparently occurs in a limited area. From the above discussion we consider that it arose by rapid modification of the genome making changes that were for a time produced exaptation. Similar morphological changes that were expressed all through the Devonian as conditions were appropriate for their expression.

Conclusion

Although this specimen is incomplete, we have enough material on which to conclude that it does not belong to any known described major systematic Order. Further searches in the field and in the laboratory have not found any other specimen that can be assigned to the same species. No evidence has been found of even traces of similar surface structures. This is a significant discovery in the light of recent work on the origin of new morphological designs that resulted from the genetic regulation.

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