Physico-chemical profile and energy fixation in Bundera Sinkhole, an anchialine remiped habitat in north-western Australia

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Abstract

Bundera Sinkhole, in arid north-western Australia, contains a community of stygofauna, including remipedes and thaumatocypridid ostracods, whose closest known affinities lie with the fauna of anchialine caves on either side of the North Atlantic. The physico-chemical profile was determined using a sonde carried by divers and by chemical analysis. The physico-chemical profile is complex, as the characteristic thermo-halocline is associated with polymodal profiles of oxygen, hydrogen sulphide and redox to a depth of 33 m. Within the upper part of the profile there was a gradient in the dissolved inorganic nitrogen (DIN) species associated with the thermo-halocline, and an accumulation of NO₃ below the thermo-halocline that is consistent with nitrification, a chemolithotrophic process. The presence of sulphide layers above the thermo-halocline, together with characteristic colour and behaviour of the associated bacteria, are consistent with sulphide-based chemoautotrophy. Stable isotope ratios for carbon and nitrogen of the fauna and autotrophic energy sources are also consistent with chemosynthesis occurring in Bundera Sinkhole. The arid climate, low ground-water flow, and small size of the cave provides the stable conditions allowing the development of a marked polymodal vertical stratification in the physico-chemical profile. The input of profuse organic matter and the penetration of the photic zone to the thermo-halocline provide the varied conditions for the development of this complex microbiological assemblage.

Introduction

Anchialine (or anchihaline) ecosystems are inland underground mixohaline waters affected by marine tides, usually with little if any surface exposure (Stock et al. 1986; Iliffe in press). Anchialine systems are noted both for their relict faunas and their high species richness (Sket 1981, 1996); at least ten new families and a new class (the Remipedia) of crustaceans have been described from anchialine systems in the last few years (GA Boxshall, Natural History Museum, London, pers. comm.). Therefore they are the object of an accelerating research effort and widespread conservation assessment as their restricted, coastal warm latitude, often karstic, setting makes them exceptionally vulnerable to anthropogenic pressures, a theme developed more fully elsewhere (Humphreys et al. 1999).

Where anchialine systems occur in drowned sinkholes (cenotes), they typically have a freshwater layer overlying seawater and in consequence have a stratified photic and physico-chemical profile. The marine influence typically consists of an hypoxic layer of seawater (ca. 33-36 g L⁻¹) beneath one or more layers of limnetic to polyhaline water (Yager et al. 1994). Below the density interface, such ecosystems support a wide range of relict life forms that comprise a community whose general structure is predictable from the Caribbean region, the Canary Islands and north-western Australia (e.g. Baltanas & Danielopol 1995; Yager 1981, 1987a, b, 1989, 1994; Yager & Schram 1986; Holsinger 1989; Danielopol 1990; Yager et al. 1994; Yager & Humphreys 1996; Danielopol et al. in press).

Remipedes have always been collected beneath a density interface (thermo-halocline) in hypoxic water of 0.1-0.22 mg L⁻¹ DO, sometimes beneath a layer of hydrogen sulphide (Yager 1994; Yager et al. 1994). The integrity of such anchialine ecosystems may depend on the maintenance of this stratification. Filamentous sulphur bacteria of the Beggiatoa-Thiothrix group can be common in anchialine caves and their hypothesised contribution to primary production by chemoautotrophy (Yager 1991a; Yager & Humphreys 1996) has been supported using stable isotope analyses (SIA: Pohlman et al. 1997).

The only deep anchialine system known in Australia, and the only continental anchialine system known in the southern hemisphere (see Iliffe in press: Fig 1), is Bundera Sinkhole in north-western Australia. This ecosystem includes the only known site for the crustacean class Remipedia in the southern hemisphere (Yager & Humphreys 1996), in an area containing the characteristic higher taxa of stygobiont crustaceans comprising thermosbaenaceans, hadziid amphipods, ostracods, cirolanid isopods, mysids, and caridean shrimp (Humphreys in press). In this arid area the surface layers are not fresh but have a salinity about half seawater rising to seawater at depth (Yager & Humphreys 1996).

This study documents the gross physico-chemical profile of the Bundera Sinkhole (temperature, salinity, dissolved oxygen, pH, redox), and the fine scale distribution at selected depths of H₂S and dissolved
inorganic nitrogen species (nitrite, nitrate and ammonium), and the stable isotope signatures ($^{13}$C and $^{15}$N) of various components.

**Methods**

**Bundera Sinkhole**

The flooded sinkhole is located on the Cape Range peninsula in north-western Australia and is situated 1.7 km inland from the Indian Ocean. The cave has a single entrance from which a flooded passage, inclined at ca. $30^\circ$ from the horizontal, extends about 70 m (Fig 1) to a maximum depth penetrated by divers of 33 m. The sinkhole is situated in the middle of a flat, 4.5 km wide coastal plain and the water table lies ca. 5 m below the surrounding surface. The depth to water and the small area of the sinkhole (ca. 10 x 20 m) will contribute to the stability of the water profile by shielding it from the often strong winds.

Characteristic of anchialine systems, the water level is influenced by the semi-diurnal marine tides of the area and which have an amplitude ca. 16% of the ocean tides (Humphreys et al. 1999). The tidal influence also affects the sinkhole in other ways; at high water the algal mats are submerged and the water surface is clear, but at low water the algae are emergent and, by spreading over the surface, greatly restrict light penetration.

A number of obligate subterranean species occur in the sinkhole below the thermo-halocline and are known only from this site. They include *Lasionectes exleyi* Yager & Humphreys (Remipedia), *Danielopolina* sp nov Danielopol et al. in press (Ostracoda: Halocyprida;
Thaumatocyprididae), *Liangoceradocus branchialis* Bradbury & Williams (Crustacea: Hadziidae), as well as the more widespread species *Stygiocharis stylifer* Hlothuis (Decapoda: Natantia: Atyidae) and *Milyeringa veritas* Whitely (Pericormises: Eleotridae). Above the thermohalocline are found the algae *Rhizoclonium tortuosum* (Dilliw) Kuetz (Chlorophyta: Cladophoraceae) and *Lamprothamnium papulosum* (Wallr) J Gr (Charophyta: Characeae), and the invertebrates *?Spionidae* (Annelida: Polychaeta), *Halicyclops* sp nov (Copepoda: Cyclopidae), *Phlyctenopora* sp Brady (Ostracoda: Cypridaceae), *Irravidia* sp (Mollusca: Irravidiidae), water striiders *Limnogonus* (L) fossarum gilguy Andersen & Weir (Gerridae: Hemiptera) and *Kiefferulus intertinctus* Skuse (Chironomidae, Diptera).

**Climate**

The climate of the Cape Range peninsula is hot (mean daily temperature of ca. 27 °C) and arid and the annual evaporation (3219 mm) greatly exceeds the sparse precipitation (median 280 mm). Vegetation in the immediate vicinity of the sinkhole comprises predominantly hummock grassland and occasional shrubs.

**Diving**

The cave is thought to have been dived on only six sessions covering the years 1991 to 1998. Diving has mostly been conducted using SCUBA gear typical of that used for cave diving with built in redundancy. The current samples were collected using rebreathing gear used for cave diving with built in redundancy. The sessions covering the years 1991 to 1998. Diving has been conducted using SCUBA gear typical of that used for cave diving with built in redundancy. The daily temperature of 27 °C and arid and the annual evaporation (3219 mm) greatly exceeds the sparse precipitation (median 280 mm). Vegetation in the immediate vicinity of the sinkhole comprises predominantly hummock grassland and occasional shrubs.

**Sonde**

Vertical profiles of depth (m), temperature (°C), dissolved oxygen (DO, mg L⁻¹), redox (mV), pH, and salinity (mg L⁻¹ TDS) were made using a 44 mm diameter sonde (Datasonde3 Multiparameter Water Quality Datalogger DS3, Hydrolab Corporation, Austin, Texas) in unthetered mode. During the first dive the sonde was pushed (sensors foremost) by the lead diver to record the undisturbed water column and sampling every second. The probes were calibrated against appropriate standards traceable to international standards. A LoFlow membrane was used on the DO probe without a stirrer. DO readings were calibrated to a salinity (TDS) of 18 g L⁻¹ and not further corrected. Redox values were measured with a silver-silver chloride reference electrode and the readings offset to be based on the standard hydrogen electrode using the European convention (reducing environment negative). The data were transferred to a computer at the end of the dive.

**Sample collection**

Debris, sediments, animals and plants were collected from the immediate environment and at various depth in the sinkhole. Divers collected fauna and organic detritus from the sinkhole using towed plankton nets and handheld suction devices, nets and bottles. Sediment and organic samples were collected into vials or polyethylene bags for transport to the surface. Terrestrial vegetation samples were stored in polyethylene bags. Organic samples for SIA analysis were frozen until dried in an oven at 45 °C and ground under liquid nitrogen. Chemical analysis was conducted on water samples collected through a range of depths determined from the redox and DO profiles measured during the first dive. The water samples were collected using a 50 mL syringe at predetermined depths measured with a gauge.

**Chemical analyses**

Total acid soluble sulphides (as mg L⁻¹ S; referred to as H₂S in the text) were determined on site using a Chemetrics test kit (CHEMets sulphide R-9510, range 0-1 and 1-10 ppm). Nitrate, nitrate and ammonium nitrogen were determined within seven days by an analytical laboratory (Chemistry Centre, Perth) of samples frozen in the field. The standard methods used (Anon 1995) were: nitrogen, ammonium fraction 4500-NH₃ G automated phenate method; nitrogen, nitrite fraction, 4500-NO₂⁻ B colorometric method; nitrogen, nitrate + nitrate fraction, 4500-NO₃⁻ F automated cadmium reduction method. Throughout square parentheses are used to denote concentration e.g. [H₂S] is hydrogen sulphide concentration.

**Stable isotope analysis**

Organismal metabolism utilizes the isotopes of an element differentially. Such ‘fractionation’ of the isotopes of nitrogen (¹⁵N/¹⁴N) and carbon (¹³C/¹²C) occurs in predictable ways such that consumers are typically enriched (are ‘heavier’) by about 3-5 ‰ in ¹⁵N and 1-2 ‰ in ¹³C relative to their food source (Ehleringer et al. 1986). Hence, stable isotope analysis (SIA) of nitrogen and carbon is a powerful tool to examine food webs (Peterson & Fry 1987; Lajtha & Michener 1994) and technical advances have recently allowed it to become a routine method in ecosystem and other studies.

Ecological studies express the isotopic composition in terms of δ values which are the parts per thousand differences from a standard:

\[ \delta X = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 10^3, \]

where X is ¹³C or ¹⁵N and R is the corresponding ratio ¹³C/¹²C or ¹⁵N/¹⁴N and the standards respectively are the Pee Dee Belemnite and nitrogen gas in the atmosphere (Peterson & Fry 1987).

SIA was conducted on plants and animals sampled both above the thermo-halocline (mollusc *Irravidia* sp, blind cave fish *Milyeringa*, girrids and tubificid worm, and in the deep cave below the thermo-halocline and layers of hydrogen sulphide (atyid shrimp *Stygicaris*, *Milyeringa*) and the algae *Rhizoclonium tortuosum* (Dilliw) Kuetz (Chlorophyta: Cladophoraceae) and *Lamprothamnium papulosum* (Wallr) J Gr (Charophyta: Characeae). In addition, plants sampled from the immediate area included *Acacia* phylloides and the hummock grass, *Triodia pungens*.

Total carbon and nitrogen and the fractionation of ¹³C/¹²C and ¹⁵N/¹⁴N and ¹³C/¹²C determinations were made by the stable isotope facility, Centre for Catchment and In-Stream Research, Griffith University, Queensland, using an automated Dumas combustion elemental analyser (‘Roboprep’, Europa Scientific, Crewe, UK) interfaced directly with a triple collector mass spectrometer (‘Tracermass’, Europa Scientific). The precision of the
mass spectrometer, derived from the standard deviation of results of a replicated series of standards within a given sample run, are 0.5 delta units for nitrogen and 0.3 delta units for carbon (pers. comm., M Sutton, Griffith University).

Results and Discussion

Here the physicochemical profile of the water in the sinkhole is described together with aspects of the associated microbiological community. The low oxygen levels and the stable isotope signatures of the biota are discussed as to the evidence they provide supporting chemoautotrophic energy fixation in the sinkhole. Finally the characteristics of Bundera Sinkhole are considered in relation to comparable systems elsewhere.

Physico-chemical profile

The water column exhibited strong physico-chemical stratification with the different parameters exhibiting different types of stratification.

**Temperature and salinity.** There was a sharp thermocline at ca. 7.5 m where the temperature increased from about 21.0 to 26.5 °C, remaining close to this temperature to the depth of the cave (Fig 2A). The upper part of the profile can be in direct sunlight as it lies within the open part of the sinkhole (Fig 1). Under conditions of optimal visibility ambient day light penetrates to a depth of ca. 15 m and the entrance can be seen from a depth of ca. 25 m (Fig 1).

Surface salinity was ca. 19 g L⁻¹ down to a marked halocline at 7.5 m (Fig 2A), below which the salinity increased to ca. 24 g L⁻¹ and from which level salinity increased steadily to 32 g L⁻¹ at a depth of 15 m. The salinity then remain relatively constant increasing to 34 g L⁻¹ at a depth of 33 m (local seawater is 36.5 g L⁻¹).

As expected, the deep temperature of ca. 26 °C is close to the mean annual surface temperature of the area (ca. 27 °C on the east coast) and it appears stable between years (Humphreys et al. 1999). The substantially cooler surface water probably results from evaporation from the exposed surface into the dry atmosphere. While thermal

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**Figure 2.** Profiles of Bundera Sinkhole in 1997 where the grey bands denote the zones of elevated hydrogen sulphide concentration (see Fig 3F), the descent rate was 0.05 m s⁻¹ (sd 0.028, n=21). A; Temperature (° C) and salinity (g L⁻¹ TDS) profiles. B; the salinity-temperature relationship of the vertical profiles where the dotted line denotes the conservative mixing hypothesis. C; pH profile. D; the salinity-pH relationship of the vertical profiles where the dotted line denotes the conservative mixing hypothesis. E; dissolved oxygen (mg L⁻¹ DO) and oxidation reduction (redox) potential (mV) profiles. F; the vertical distribution of H₂S (as total acid soluble sulphides, µM).
exchange with the surface is continuous, the sparse freshwater input at the surface is spasmodic and unpredictable (Humphreys et al. 1989) hence there is ample time for a broad zone of mixing (Fig 2A) to develop between the lower sea water and upper brackish water increasing in salinity with time owing to evaporation from the surface. The non-conservative mixing (Fig 2B) between temperature and salinity indicates that the water had warmed since entering the ground-water.

Temperature and salinity, the parameters most commonly measured to examine the physico-chemical profiles of water, had a fairly simple profile with an inverted thermocline but the salinity and temperature profiles were not concordant, with the halocline being much broader than the thermocline (Fig 2A). However, together these parameters provide a markedly oversimplified picture of the complexity of the vertical stratification present in Bundera Sinkhole.

**Hydrogen ion concentration.** pH declined from greater than 7.5 at the surface to ca. 6.9 at ca. 8 m at the bottom of the thermocline and then remains between ca. 6.9-7.0 to the bottom of the water column (Fig 2C). Below the thermo-halocline the pH was fairly constant (6.90-6.96; Fig 2D). Non-conservative mixing is revealed by plotting pH against salinity (Fig 5) and due to hydrogen ion production at the halocline. This pattern is similar to that reported for the Mayan Blue Cave system, Mexico (Pohlman et al. 1997), but the details are different possibly due to the high photosynthetic activity in the surface waters of the sinkhole.

**Dissolved oxygen and redox.** DO fell rapidly from supersaturated at the surface to a minimum at about 7.5 m depth followed by additional minima at ca. 17 m and 27 m depth (Fig 2E). The complex DO curve is associated with the zones of increased [H₂S] although they are not entirely concordant. Stratification in the redox potential (Eh) was markedly polyphasic ranging from -125 mV to +175 mV. The redox minima correlated with oxygen minima but did not exactly map them; while each oxygen minimum occurred through a broad depth range (ca. 5 m), a redox minimum occupied only a narrow depth range in depth (Fig 2E).

**Hydrogen sulphide.** Two layers of elevated hydrogen sulphide, also detected by divers, were recorded at ca. 5 m and 16 m (Fig 2F) loosely concordant with the upper two

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**Figure 3.** A: Vertical profile with depth of the dissolved inorganic nitrogen species (DIN µM) N-NH₄, N-NO₂, N-NO₃, shading denotes the region of elevated H₂S concentration (see Fig 2F). B: Vertical distribution of sightings of the fauna in Bundera Sinkhole reported by divers, a) the closed circles denote the vertical distribution of the taxon named at the head of the column, b) the open circles denote the number of blind gudgeons (*Milyeringa veritas*) counted at one occasion at each of three depths (second bottom scale), c) the curve denotes the DO concentration (bottom scale), d) the grey bands denote the zones of elevated hydrogen sulphide concentration (see figure 9). These data are illustrative only as no regular sampling has been conducted through time. C: scatter plot of ¹³C ‰ and ¹⁵N ‰ values for samples from Bundera Sinkhole. If the food source is hypothesised to be some mix of C₃ and C₄ plants and algae then the average ¹³C and ¹⁵N values would lie in the polygon linking these data (shaded area). Data for plants with C₃ and C₄ metabolism, and algae are outlined, as are predators (left group, deep cave *Milyeringa*; right group, shallow cave *Milyeringa* [X]), gerrid [X1], molluscs and worms. D: scatter plot of ¹³C ‰ and ¹⁵N ‰ values for samples from Bundera Sinkhole. If the food source is hypothesised to be some mix of C₃ and C₄ plants and algae then the average ¹³C and ¹⁵N values would lie in the polygon linking these data. This is represented as a shaded area but it has been displaced by one (heavy shading) and two (light shading) trophic increments of respectively 2 ‰ for ¹³C and 4 ‰ for ¹⁵N.
DO and redox minima (Figs 2F, 3A). Owing to the sampling resolution at depth in the sinkhole an additional layer of elevated H₂S associated with the deepest redox minimum may have been missed. In sediments, also, there can be a similar close correlation between the redox and S²⁻ ion activity (Fenchel & Finlay 1995:11). This result at Bundera contrasts with the finding of Pohlman et al. (1997, in press) who recorded no dissolved sulphides (<2 µM) in the water column of an anchialine cave.

The maximum recorded H₂S was 15.6 µM. This compares with 250 µM H₂S in freshwater of Movile cave (Sarbu in press; Table 1), the most notable sulphide driven subterranean ecosystem, but other chemooautotrophic sulphide caves systems are being recognized (Sarbu et al. in press; Pain 1998).

**Dissolved inorganic nitrogen species.** Large changes in the vertical distribution of the DIN species are associated with the thermocline (Fig 3A). N-NO₃ is elevated in the upper H₂S layer that lay on the thermocline, while N-NO₂ increases markedly at the thermocline. It is not known whether a similar stratification of the DIN species occurs associated with the deeper hydrogen sulphide layer (Fig 2F), or the even deeper redox minimum (Fig 2E). The upper layer of hydrogen sulphide in the sinkhole is directly related with the changes in [N-NH₃] (MS = 10.56, P = 0.009), an association previously unreported for an anchialine system.

The greatest [N-NO₃] recorded was 24 µM, similar in magnitude to that found by Pohlman et al. (1997, in press), but the concentration was still increasing with depth at the lowest sample. However, data from 1994 (Yager & Humphreys 1996) suggest that it will decline to ca. 4 µM at a depth of 14 m (similar levels of N-NO₃ were recorded at 6 m in 1994 and 1997, respectively 1.07 and 1.43 µM). The [N-NH₃] is inversely related to the [N-NO₃] (Fₛ₋₁₀ = 14.82, P = 0.003). These data are consistent with nitrification (Ward 1986), a chemooautotrophic process when ammonium (NH₄⁺) is successively oxidised to nitrate (NO₃⁻) and nitrite (NO₂⁻), an exclusively aerobic process that takes place in the oxic-anoxic boundary layer (Fenchel & Finlay 1995:219). Similar data have been recorded in an anchialine system in Quintana Roo, Mexico (Pohlman et al. 1997, in press:11).

An inverse linear relationship between nitrate concentration and salinity was found in an anchialine system by Lifle et al. (1984) and this generally implies conservative mixing and a single freshwater source (Burton & Liss 1975). The opposite relationship between N-NO₃ and salinity was recorded in Bundera Sinkhole (Fₛ₋₁₀ = 112.1, P < 0.001). As the concentration of N-NO₃ in seawater is less than in the sinkhole (data in Yager & Humphreys 1996; Table 1) then the increased concentration of N-NO₃ from 6 m to 14 m depth suggests that NO₃⁻ is accumulating in the anchialine system. This finding implies that nitrogen fixation is occurring in the anchialine system and is consistent with the data in Fig 3A and with the findings of Pohlman et al. (1997, in press:11).

**Microbiotic environment.** Much of the surface of Bundera Sinkhole was covered by algal mats at low tide, but at high water these became submerged and the water appeared clear. So, light penetration into the cave will vary both diurnally and with a superimposed lunar periodicity. In the water column immediately above the thermocline are globular veils characteristic of *Thiovulum* bacteria, but not identified, and which typically enclose regions of elevated sulphide concentration (Fenchel & Finlay 1995). On the sediments are motile filaments (characteristic of *Beggiatoa* spp), which occur mainly in sediments, and there were attached filaments which occur as ‘aufwuchs’ on solid surfaces (commonly *Thiotrix* spp).

White filamentous bacteria were photographed moving out of sediments, collected from around the thermocline in Bundera Sinkhole, up the walls of the collecting vessels, presumably tracking the vertical position of the oxygen–sulphide boundary (Fenchel & Finlay 1995:188). Observations on the bacteria samples from Bundera Sinkhole (PD Franzmann, Centre for Ground-water Studies, Wembley; pers. comm.) showed that in samples of globular bodies in the near surface waters there were some short rods, and a number of dinoflagellates (*Gymnodinium*-like) but no filamentous bacteria. At a depth of 8.3 m (at the upper oxygen minimum and where the redox value again just becomes positive) wall samples contained many unsheathed bacteria. There were also many filamentous bacteria (*Sphaerotilus*-like: Clamydobacteriales; *Beggiatoa*-like: *Beggiatoaceae* and some sulphur crystals) including many large segmented filaments (*Thioploca chileae*-like: *Beggiatoaceae*), and some eukaryote cysts and diatoms. At a depth of 10.4 m (near the second oxygen maximum) there were limited numbers of rod bacteria, large segmented filaments (*Thioploca chileae*-like >10 µm in diameter), and some larger eukaryote cysts. At a depth of 30.1 m (at the lower oxygen maximum) there were many sheathed bacteria, *Sphaerotilus*-like (some with Fe-III on sheaths), and many rods and some spore-formers. *Sphaerotilus* spp are able to deposit iron on the sheath but sulphur is not deposited in the cells. However, they are considered to be chemo-organotrophic, not lithotrophic, organisms utilizing readily biodegradable substrates.

*Beggiatoa*, the sister taxon to *Thioploca* (Teske et al. 1995), and *Thiothrix* (which was not identified in these samples) are large white gliding filamentous sulphur oxidizing bacteria (chemolithoautotrophic) that may form mats in some anchialine systems as well as at deep sea hydrothermal vents and cold seeps. *Beggiatoa* are profuse in areas that are high in sulphide and combined nitrogen but low in dissolved oxygen (Ahmad et al. 1999) and their presence can be an indication of advanced eutrophication (Rosenberg & Diaz 1993). They are mixotrophic and some strains harvest sulphide oxidizing it to sulphate using oxygen as the electron acceptor (Hagen & Nelson 1997) but, as high concentrations of nitrate may occur in the vacuole (McHatton et al. 1996), it has been suggested that the oxidation of hydrogen sulphide, or endogenous stores of elemental sulphur, are used for the respiratory conversion of nitrate to ammonia.

*Thioploca* (and the recently described giant bacterium *Thiomargarita namibiensis*; Schulz et al. 1999) are common on continental shelves (Huettel et al. 1996) and belong in a small class of bacteria known to link the sulphur and nitrogen cycles. *Thioploca* spp and *Beggiatoa* spp inhabit aquatic environments at the oxygen-sulphide transition and where the negative chemotactic reactions to oxygen
and sulphide are thought to keep both Beggiatoa and Thioploca at the optimal position for growth (Huettel et al. 1996).

There is a similarity between the upper 8 m of the profile in Bundera Sinkhole and that found at a microscale (4 cm) in marine sediments. In the sediments nitrate is continually replenished in the oxic zone by nitrifying organisms oxidizing the outward (up and down) diffusion of ammonia from the centre of the anoxic (H$_2$S) zone. In the dark, sulphide, thiosulphate and elemental sulphur are oxidized primarily by chemolithotrophic sulphur bacteria, most of which store sulphur in the oxic layers (Fenchel & Finlay 1995:167). Nonetheless, the fauna in the vicinity of aerobic environments where nitrate and sulphide ecosystems (sulphureta) often contain the oxic layers (Fenchel & Finlay 1995:170). Below these areas were black sulphidic sediments on which the blind gudgeon Milyperringa were observed.

Concentrations of bacteria have also been reported from Cenote Angelita in Mexico, an anchialine sinkhole that, like Bundera Sinkhole, lacks significant water circulation. Degradation of organic matter, falling from the forest above, led to the production of H$_2$S that was utilized by the bacteria floating above the layer of 250 mM H$_2$S at the halocline (Pohlan et al. in press).

**Anaerobiosis and biotic effects**

Sulphide is very soluble in water and its dissociation characteristics are such that, at the pH values typical for natural waters, most of it is in the form HS-. Since the characteristics are such that, at the pH values typical for Anaerobiosis and biotic effects, mM H$_2$S at the halocline (Pohlman (Hagerman & Vismann 1995). However, the fauna in Bundera Sinkhole probably covers the full range of oxic conditions; supersaturated by photosynthesis near the surface, through dysoxic (2.0-0.2 mL L$^{-1}$), suboxic (0.2-0.0 mL L$^{-1}$) to anoxic (0.0 mL L$^{-1}$) conditions associated with the hydrogen sulphide layers.

It would appear that the anaerobiosis in Bundera Sinkhole is caused by mechanisms typical of those found to occur in the upper few centimetres of sediments or to other illuminated aquatic microbiotic communities that contain steep gradients of oxygen and sulphide, such as those associated with black band disease in corals (Richardson 1998). I hypothesise that in the upper photic part of the sinkhole, photosynthesis by the algae and cyanobacteria oxidizes the environment during the day and the organic material they produce is broken down by aerobic heterotrophs, restoring the redox balance. However, light intensity falls with depth and the temperature increases markedly at the thermocline resulting in both a reduction in oxygen production and an increase in reaction rate. Furthermore, this is associated with a local stability as oxygen transport will be reduced in the absence of convection across the thermo-halocline. Together, these result in local depletion of oxygen. As degradation by anaerobic microbes takes over, the metabolic end-products (e.g. sulphide, hydrogen) will render the surroundings chemically reducing. These changes are most likely to occur in places where there is a large import of dead organic material — the photic zone of Bundera Sinkhole is eutrophic producing large amount of organic matter — and protection from turbulent or advective transport of water, respectively by the sinkhole walls and the thermo-halocline. While this process is well supported by the data for the upper sulphide layer, the uniquely repeating and sulphidic (and roughly concordant redox and oxygen minima) layers in Bundera Sinkhole (Fig 2E) is poorly documented and less obvious in origin. Nonetheless, it would seem that the typical remipede crustacean community is only found below the second sulphide layer (Fig 3B), and which itself is not associated with a thermo-halocline (Fig 2A).

**Stable isotope analysis**

The data for the stable isotope enrichment of both carbon and nitrogen are given in Fig 3C. They are
grouped according to C₃ and C₄ plant metabolism (Acoutia and Triodia respectively), algae, shrimps (Atyidae), predators (the blind gudgeon, Milyeringa veritas, and gerrids [one pooled sample]). Analysis is shown of pooled samples of tubificid worms and the mollusc Iravadia sp that inhabit the algal layer. Finally, the ‘line sediment’ represents the accumulation of particulate organic matter settling over more than a year onto the guide line at depths of 17.5 m and 21 m.

The overt organic input is determined by the local vegetation which in this arid area is hummock grassland (C₃ plants) with few scrub and annuals (C₄ plants) at the time of sampling. Hence, the vast majority (>90%) of local vegetation coverage comprises C₃ plants on the shallow sandplain overlying a marine terrace cut into limestone which deeply covers the cave (Fig 1). Given the scant and episodic rainfall in this region the downward percolation of organic matter through the sand and limestone overlying the cave is likely to be sparse. There are a few plants in the sinkhole of both C₃ and C₄ plants but the predominant vegetation is the thick algal community in the water of the sinkhole and which sometimes covers the surface and extends to several metres below the water level. It mainly comprises the algae Rhizoclonium ?tortuosum (Dilliw) Kuetz (Chlorophyta: Cladophoraceae) and Leprothamnium papulosum (Wallr) J Gr (Charophyta: Characeae) and is thickly populated by the mollusc Iravadia sp. Large colonies of bacteria occur near the thermo-halocline both in the water column and on the walls and floor of the cave.

Carbon. The δ¹³C values for the C₃ and C₄ plants are within normal limits which have median values respectively of -27 ‰ and -11 ‰ (Litjha & Marshall 1994). While the values of δ¹³C for algae in Bundera Sinkhole are heavy compared with most values in the literature, they are not outside recorded limits. Values of δ¹³C in marine algae are between -19 to -24 ‰ and are affected by the uptake of inorganic carbon in planktonic photosynthesis (Peterson & Fry 1987). In fresh water values of δ¹³C vary widely depending on the source of dissolved CO₂ where respiration inputs are strong, the δ¹³C values for dissolved inorganic carbon may approach -20 ‰, with algae reaching -45 ‰ owing to fractionation during carbon uptake (Peterson & Fry 1987). In algal cultures δ¹³C variations ranging from -29.7 ‰ to -5.5 ‰ have been reported (Falkowski 1991), with about one third of this variation attributed to changing water values of δ¹³C in the water column and on the walls and floor of the cave.

Nitrogen. The values of δ¹⁵N for the C₃ and C₄ plants are within normal bounds (Peterson & Fry 1987) but the δ¹⁵N values for algae are high, for unknown reasons. While the fractionation of nitrogen isotopes is rarely greater than 2 ‰ in natural systems, it is often large (10-20 ‰) for nitrate or ammonium uptake by aquatic algae and microbes (Macko & Ostrom 1994). Where nitrate is abundant, as in the surface water of Bundera Sinkhole (Yager & Humphreys 1996), fractionation during assimilation by phytoplankton may lead to a δ¹⁵N sufficiently heavy (Peterson & Fry 1987) to reach the level found in Bundera Sinkhole. Study of the isotopic fractionation in the cascade of DIN species in the upper water profile of the cave would help resolve these uncertainties.

SIA and trajectories. The line sediment, the tubificid worms and molluscs lie within the envelope of an hypothesised source of carbon and nitrogen derived from a mix of C₃ and C₄ plant material and algae (see Figs 3C, 3D). The predators form two groups depending whether they originate from superficial parts of the cave or the deep zone, with the former being ‘heavier; the gerrid data are consistent with this pattern. The atyid shrimps lie outside the hypothesised food mix envelope, being lighter. The trophic trajectories (Fig 3D) suggest that the two predator populations are derived from C₃ plants (two trophic steps removed), or atyid shrimps (one trophic step removed); Milyeringa veritas is known to eat the shrimps (Humphreys & Feinberg 1995).

As shown above, there is evidence from the stratification of the DIN species that nitrification was occurring in Bundera Sinkhole. Furthermore, visual observations in the main water body, observation of the characteristic motile behaviour of sulphur bacteria observed in vitro, and the presence of H₂S, suggest that sulphur bacteria are widely present at and below the thermo-halocline and these are likely to be involved in chemooautolithotrophy. Such chemosynthetic biomass is characteristically lighter (more negative δ¹⁵N) than photosynthetically derived carbon (Conway et al. 1994). While no isotope determinations were made on bacteria, these observations could be verified by stable isotope determinations of the bacteria.

One explanation for the lightness of the atyid shrimp data is that they are utilizing chemooautotrophic bacteria for part of their food source: atyid shrimp have a brush-shaped tuft of setae in the first two pereiopods with which they brush the substrate while feeding (Bailey-Brock & Brock 1993). Evidence in support of chemooautolithotrophy in anchialine ecosystems is presented by Pohlman et al. (1997 in press) for an anchialine cave in Mexico.

Comparison with other areas

The high surface salinity and the complexity of the profile in Bundera Sinkhole may be associated with the aridity of the area and hence minimal surface input and low ground-water flow of ca. 0.003 mm s⁻¹ on the eastern side of the same peninsula. This results in a broad zone of diffusion, 20-30 m thick, between the overlying freshwater lens and the underlying seawater (Exmouth water supply borefield; Consultative Environmental Review; Water Corporation, unpublished, 1996). Bundera Sinkhole probably lies at the extreme seaward side of the system where only the diffusion zone is represented and thus exhibits only the brackish/seawater interface seen in the sinkhole.

Most anchialine systems that have been examined have a freshwater lens overlying sea water (Iliffe in
press); the freshwater lens may be thick and increase in depth inland, for example, on the Yucatan Peninsula 2 km inland it is 10 m thick at sinkhole Abejas and 10 km inland it is 20 m thick at sinkhole Vaca Ha (Iliffe in press). At Wonderland Cave, Bermuda, brackish water overlies seawater, and this cave is most comparable to Bundera Sinkhole in its salinity profile (Iliffe in press: Fig 3).

A summary of published physico-chemical profiles of anchialine systems is given by Humphreys et al. (1999); Bundera Sinkhole is the deepest, and probably the smallest, of these. In Bundera Sinkhole there is a polynodal DO depth profile, a form shown in none of the published DO depth profile, all of which exhibit a unimodal DO depth profile. By inference it suggests that the other systems probably have a single H2S layer should one be present.

The DIN species data for Bundera Sinkhole (Fig 3A) are very similar to those reported by Pohlman et al. (in press) in a cave where they obtained strong evidence, using stable isotope analysis, that chemoautotrophic energy fixation was occurring. This means that such systems, rather than being totally dependent on imported (allochthonous) energy sources, as is the general model for cave systems, they can derive part of their energy in situ from autochthonous energy fixation. This has considerable implications to anchialine systems because it means that energy may be available broadly through anchialine systems, even where the ground-water is covered by impermeable ceilings. In this context, it is worth noting that sulphide-oxidising mixotrophic Thiothrix and Beggiatoa bacteria, which may be primary producers using sulphide-based chemosynthesis (Brigmon et al. 1994; cf. Kane et al. 1994), occur widely in anchialine and other ground-waters (Yager 1991a; Brigmon et al. 1994), as well on deep-sea hydrothermal vents (Belkin & Jannasch 1989).

The physico-chemical environment in Bundera Sinkhole is very complex and this complexity is associated with biogeochemical processes that are likely to be of fundamental importance to the maintenance of the unique community contained in this anchialine cave. SCUBA diving alters the vertical stratification of the physico-chemical environment in the sinkhole (Humphreys et al. 1999) and the effects of this may be disruptive to the unique anchialine community inhabiting the cave. As some of the fauna associated with such anchialine systems have only ever been collected by divers, remipedes for example, then diver access is essential for biological work. However, this should be limited and where possible be conducted using rebreathing equipment. There is a lack of basic data on the gross physico-chemical environment in Bundera Sinkhole, on ground-water movement, temporal changes in the profile resulting from the effects of episodic rainfall on surface input as well as ground-water flow. A diver-free profiling system needs to be established to determine long term changes, particularly the establishment and maintenance of the complex redox profile, its associated chemolithotrophic organisms and the significance of these processes to the remipede community.

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